Phytochemical Composition and Pigment Stability of Açai (*Euterpe oleracea* Mart.)

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Anthocyanin and polyphenolic compounds present in açai (*Euterpe oleracea* Mart.) were determined and their respective contribution to the overall antioxidant capacity established. Color stability of açai anthocyanins against hydrogen peroxide (0 and 30 mmol/L) over a range of temperatures (10−30 °C) was also determined and compared to common anthocyanin sources. Additionally, stability in a model beverage system was evaluated in the presence of ascorbic acid and naturally occurring polyphenolic cofactors. Cyanidin 3-glucoside (1040 mg/L) was the predominant anthocyanin in açai and correlated to antioxidant content, while 16 other polyphenolics were detected from 4 to 212 mg/L. Red grape anthocyanins were most stable in the presence of hydrogen peroxide, while açai and pigments rich in acylated anthocyanins displayed lower color stability in a temperature-dependent manner. In the presence of ascorbic acid, acylated anthocyanin sources generally had increased color stability. Açai was recognized for its functional properties for use in food and nutraceutical products.

KEYWORDS: Açai; anthocyanins; polyphenolics; copigmentation; stability

INTRODUCTION

Açai (*Euterpe oleracea* Mart.) is a palm plant widely distributed in northern South America with its greatest occurrence and economic importance in the flood plains of the Brazilian Amazonian state of Pará (*1−3*). Açai is a slender, multi-stemmed, monoecious palm that can reach a height of over 30 m. A wide variety of marketable products are produced from this palm, but the spherical fruits that are mainly harvested from July to December are its most important edible product. Each palm tree produces from 3 to 4 bunches of fruit, each bunch having from 3 to 6 kg of fruit. The round-shaped fruits appear in green clusters when immature and ripen to a dark, purple-colored fruit that ranges from 1 to 1.5 cm in diameter. The seed accounts for most of the fruit size and is covered by thin fibrous fibers under which is a small edible layer. A viscous juice is typically prepared by macerating the edible pulp that is approximately 2.4% protein and 5.9% lipid (*3*). The juice is currently used to produce energetic snack beverages, ice cream, jelly, and liqueur and is commonly blended with a variety of other juices.

A steady increase in the development of natural food colorants and functional food sources has been observed in recent years, not only due to consumer preferences for natural pigments but also for their health-related benefits and nutraceutical properties (*4−6*). Anthocyanins are a viable replacement for synthetic colorants due to their bright, attractive colors and water solubility, which allows their incorporation into a variety of food systems (*7*). However, limitations exist for their commercial application due to high raw material costs and their poor stability that is affected by their chemical structure, environmental factors, and the presence of additional phytochemicals in solution. Due to these constraints, a need exists to find stable, inexpensive anthocyanin pigments with a diverse array of functional properties, food, and nutraceutical applications.

Anthocyanin intermolecular copigmentation reactions are common in nature and result from association between pigments and cofactors such as polyphenolics and/or metal ions, or other anthocyanins (self-association). Preferably formed under acidic conditions, these weak chemical associations can augment anthocyanin stability and increase antioxidant properties (*8−10*). Studies have suggested that the copigmentation phenomenon is the main anthocyanin stabilizing mechanism in plants (*8, 9*). Polyphenolics are the predominant cofactors present in anthocyanin-containing fruits and vegetables and increased anthocyanin stability has been attributed to their high concentrations in foods (*8−10*). Malien-Aubert et al. (*10*) described how the diversity of polyphenolic compounds among different anthocyanins sources might affect anthocyanin stability, yet additional research on how these polyphenolics influence anthocyanin stability via copigmentation reactions has not been conducted.

The objective of this study was to characterize the major polyphenolics and anthocyanins present in açai pulp and to

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determine their contribution to the overall antioxidant capacity of this palm fruit. Color and pigment stability against hydrogen peroxide, ascorbic acid, and the presence/absence of naturally occurring cofactors was also determined and compared to other commercially available anthocyanin sources. Results of these studies can be used to determine application and functional properties of açai polyphenolics in a variety of food products.

MATERIALS AND METHODS

Materials. Pasteurized, frozen açai pulp was kindly donated by Amazon Energy LLC (Greeley, CO) and was shipped overnight to the Department of Food Science and Human Nutrition at the University of Florida. The pulp was thawed, centrifuged ($2000g$) at 4 °C for 15 min to separate lipids from the aqueous slurry, and subsequently filtered through Whatman #1 filter paper. The aqueous portion was then partitioned into lipophilic and hydrophilic extracts by the addition of petroleum ether and acetone, respectively. The upper petroleum ether phase was removed and evaporated under a gentle stream of nitrogen and redissolved in a known volume of acetone and ethanol (1:1). Acetone was removed from the lower aqueous phase under reduced pressure at temperatures <40 °C, and the resultant fraction containing hydrophilic compounds was diluted to a known volume with acidified water (0.1% HCl). Polyphenolics from the aqueous phase were subsequently concentrated using C_{18} Sep-Pak Vac 20 cm³ mini-columns (Waters Corporation, Mass.). Residual sugars and organic acids were removed with water (0.01% HCl), and polyphenolic compounds were recovered with acidified methanol (0.01% HCl). Methanol was removed from the polyphenolic fraction using vacuum evaporation at <40 °C, and the resulting isolate was redissolved in a known volume of acidified water.

Commercially available anthocyanin extracts from black carrot (*Daucus carota*; Exberry, Tarrytown, NY), red cabbage (*Brassica oleracea*) (Exberry), red grape (*Vitis vinifera)* (San Joaquin Valley Concentrates, Fresno, CA), purple sweet potato (*Ipomea batata*) (Food Ingredients Solutions, New York, NY), and a noncommercial extract from red hibiscus flowers (*Hibiscus sabdariffa*) were used for color stability evaluation. Each pigment source was dissolved in citric acid buffer (pH 3.5; 0.1M), and polar compounds were removed with C_{18} columns, as previously described. Color and anthocyanin stability were then assessed against açai for comparison.

Color Stability. Anthocyanin color stability of each pigment source was assessed in the presence of hydrogen peroxide (0 and 30 mmol/L) at 10, 20, and 30 °C, respectively. Stock solutions of each anthocyanin source were diluted with citric acid buffer (pH 3.5) to give a final absorbance value of 1.5 at their respective wavelength of maximum absorbance. Samples were placed into a water bath or refrigerated storage and allowed to reach the desired temperature at which a hydrogen peroxide solution was added. Loss of absorbance was measured periodically over time and percent color retention calculated as a percentage of the initial absorbance reading. Insignificant changes in absorbance values were observed for control treatments (no hydrogen peroxide) over 360 min of incubation.

Effect of Copigmentation. The effect of naturally occurring intermolecular copigmentation on anthocyanin stability in the presence and absence of ascorbic acid was also evaluated using in vitro model systems. Naturally occurring cofactors were removed by additionally loading each anthocyanin source onto C_{18} cartridges as previously described. Following elution of polar compounds with water, the cartridge was first washed with ethyl acetate to elute phenolic acids and flavonoids, followed by acidified methanol to remove anthocyanins. Ethyl acetate and methanol isolates were then evaporated under vacuum at <40 °C and redissolved in a known volume of citric acid buffer. Anthocyanin recovery was >96% for all sources. Anthocyanin color stability was evaluated using an in vitro model simulating a soft drink beverage system that contained anthocyanins (absorbance value of 1.5) dissolved in citric acid buffer, sucrose (100 g/L), and sodium azide (50 mg/L) to control microbial growth. Stock solutions were sub-divided and evaluated with and without polyphenolic cofactors, and again sub-divided for evaluation with and without ascorbic acid (450 mg/L). Data

were compared to a control that contained an equivalent volume of citric acid buffer. Each treatment was individually sealed into screw-cap vials (10 mL) and stored in the dark at 37 °C for 30 days. Samples were collected every day during the first 8 days of the study and subsequently every other day until the end of the study.

Phytochemical Analyses. Individual anthocyanin 3-glycosides present in açai were quantified according to the HPLC conditions of Skrede et al. (*6*) using a Dionex HPLC system and a PDA 100 detector. Compounds were separated on a 250- × 4.6-mm Supelcosil LC-18 column (Supelco, Bellefonte, PA) and quantified using a cyanidin standard (Polyphenols Laboratories AS, Sandnes, Norway). Anthocyanins were also characterized based on PDA spectral interpretation from 200 to 600 nm, and identification additionally confirmed following acid hydrolysis into their respective aglycones with 2N HCl in 50% v/v methanol for 60 min at 90 °C.

Major flavonoids and phenolic acids present in açai were separated by HPLC using modified chromatographic conditions of Talcott et al. (*11*). Separations were performed on a 250- × 4.6-mm i.d. Acclaim 120-C_{18} column (Dionex, Sunnyvale, CA) with a C_{18} guard column. Mobile phases consisted of water (phase A) and 60% methanol (phase B) both adjusted to pH 2.4 with *o*-phosphoric acid. A gradient solvent program ran phase B from 0 to 30% in 3 min, 30−50% in 5 min, 50−70% in 17 min, 70−80% in 5 min, and 80−100% in 5 min and held for 10 min at a flow rate of 0.8 mL/min. Polyphenolics were identified by spectroscopic interpretation, retention time, and comparison to authentic standards (Sigma Chemical Co., St. Louis, MO).

Six isolates were obtained from the extraction of açai pulp that included whole pulp, lipophilic fraction, C_{18} nonretained, C_{18} bound phenolics and anthocyanins, ethyl-acetate soluble polyphenolics, and anthocyanins. Each fraction was evaluated for antioxidant capacity using the oxygen radical absorbance capacity assay against a standard of Trolox as described by Talcott et al. (*11*). Each isolate was appropriately diluted in pH 7.0 phosphate buffer prior to pipetting into a 96-well microplate with corrections made for background interference due to phosphate buffer and/or extraction solvents.

Anthocyanin content in each in vivo model system was determined with the pH differential method of Wrolstad (*13*) and quantified using equivalents of the predominant anthocyanin present (cyanidin 3-glucoside for açai and hibiscus, cyanidin 3-sophoroside for black carrot and red cabbage, malvidin 3-glucoside for red grape, pelargonidin 3-rutinoside for purple sweet potato) (*10*, *13*, *14*). Percentage of polymeric anthocyanins was determined based on color retention in the presence of potassium metabisulfite (*13*), while instrumental CIE color characteristics (chroma, and hue angle) were measured using a Minolta Chroma Meter CR-300 Series (Minolta Co., Ltd., Osaka, Japan).

Statistical Analysis. Anthocyanin stability against hydrogen peroxide was designed as a 6 × 2 × 3 full factorial that included six anthocyanin sources, two hydrogen peroxide concentrations, evaluated at three temperatures. Anthocyanin stability in the presence of cofactors and ascorbic acid was designed as a 6 × 2 × 2 full factorial that included six anthocyanin sources, two ascorbic acid levels, in the presence or absence of native cofactors. Data for these evaluations and those for açai characterization represent the mean of three replicates at each sampling point. Multiple linear regression, analysis of variance, and Pearson correlations were conducted using JMP software (*15*) and mean separation using the LSD test (P < 0.05).

RESULTS AND DISCUSSION

Anthocyanin and Polyphenolic Characterization. Due to recurrent issues associated with the instability of anthocyanins during processing and storage, the food industry is constantly looking for novel, inexpensive and stable sources of pigments. Anthocyanins present in açai may offer a new source of these pigments; however, their stability has yet to be determined. Furthermore, the characterization of the major polyphenolic compounds in açai and their overall contribution to the antioxidant capacity has not been previously investigated. Therefore, this study examined the polyphenolic composition
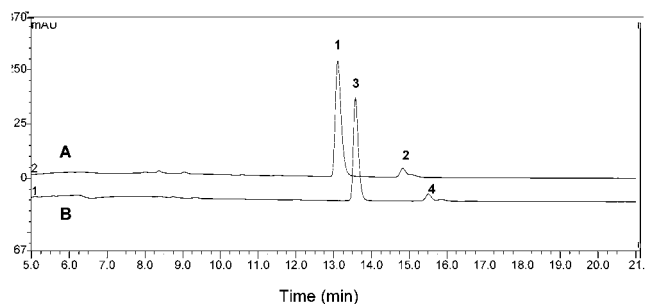
Figure 1. HPLC chromatogram of (**A**) anthocyanin 3-glucosides monitored at 520 nm (peak assignments: 1, cyanidin 3-glucoside; 2, pelargonidin 3-glucoside) and their (**B**) aglycones (peak assignments: 3, cyanidin; 4, pelargonidin) present in açai (*Euterpe oleracea* Mart.).

Table 1. Anthocyanin and Polyphenolic Content (mg/L Fresh Pulp) of Açai (*Euterpe Oleracea* Mart.)

polyphenolic	content (mg/L fresh pulp)
cyanidin 3-glucoside	1040 ± 58.2
pelargonidin 3-glucoside	74.4 ± 2.90
ferulic acid	212 ± 5.29
(−)-epicatechin	129 ± 3.28
p-hydroxy benzoic acid	80.5 ± 2.00
gallic acid	64.5 ± 1.64
protocatechuic acid	64.4 ± 1.64
(+)-catechin	60.8 ± 0.98
ellagic acid	55.4 ± 1.39
vanillic acid	33.2 ± 1.39
p-coumaric acid	17.1 ± 1.23
gallic acid derivative-1	47.3 ± 1.40
gallic acid derivative-2	18.4 ± 0.89
gallic acid derivative-3	17.3 ± 1.25
gallic acid derivative-4	13.3 ± 0.96
gallic acid derivative-5	3.9 ± 0.18
ellagic acid derivative	19.5 ± 0.40

and the anthocyanin stability of açai under a variety of experimental conditions as compared to other commercially available anthocyanin sources.

Figure 1 shows a typical HPLC chromatogram of anthocyanin 3-glycosides extracted from açai that when hydrolyzed yielded cyanidin (1040 mg/L pulp) and pelargonidin (74 mg/L pulp) as the only compounds detected. Spectroscopic analysis before and after acid hydrolysis confirmed the presence of each anthocyanidin and tentative identification of a monoglycoside attached to the C-3 position, presumably a glucose derivative, was made based on A_{440}/A_{max} ratios (∼33%) as described by Hong and Wrolstad (*14*). Presence of hydroxy-substituted aromatic acids attached to the glycoside (acylated moieties) was not found for either compound, as shown by the absence of their typical absorption spectrum in the 310−340 nm range.

The predominant polyphenolics present in açai pulp were ferulic acid > epicatechin > p-hydroxy benzoic acid > gallic acid > protocatechuic acid > (+)-catechin > ellagic acid > vanillic acid > p-coumaric acid at concentrations that ranged from 17 to 212 mg/L, as reported in **Table 1**. Additionally, five compounds were identified with spectroscopic characteristics comparable with gallic acid and were tentatively identified as gallotannins, while one compound shared spectroscopy similarities with ellagic acid and was tentatively identified as an ellagic acid glycoside (**Table 1**, **Figure 2**). Additional confirmation of these compounds was made following acid hydrolysis, as these compounds were no longer detected and a corresponding increase in either gallic acid or ellagic acid concentrations was observed.

Antioxidant Capacity. Açai pulp was found to have a relatively high antioxidant content (48.6 μmol Trolox equivalents/mL) with respect to other anthocyanin-rich fruits such as highbush blueberries (4.6−31.1 μmol TE/g) (*16*), strawberries (18.3−22.9) (*17*), raspberries (19.2−22.6) (*17*), blackberries (13.7−25.1) (*18*), cranberries (8.20−145) (*19*), and muscadine grape juice (18.2−26.7) (*20*). Fractionation of açai phytochemicals based on solubility and affinity characteristics was conducted to determine the distribution of antioxidant compounds among the isolates. Similar antioxidant content was observed for the whole pulp, C_{18}-retained phenolics (phenolic acids and anthocyanins), and the anthocyanins alone, while ethyl-acetate-soluble phenolics, the liphophilic, and C_{18} nonretained isolates had appreciably lower contributions to the total antioxidant content (44, 8, and 1.2%, respectively) (**Figure 3**). Results indicated that when ethyl-acetate-soluble phenolics and anthocyanin fractions were evaluated alone for antioxidant capacity, their sum was higher than values obtained for the total C_{18} bound polyphenolics. Although these fractions were not recombined again for analysis, there is indication that physical and/or chemical interactions among constituents in these fractions unfavorably impacted radical-scavenging properties. Previous studies have demonstrated antagonistic interactions between polyphenolics such as quercetin and caffeic acid (*21*), or cyanidin in combination with catechin and ellagic acid (*22*), all of which are present in açai. However, the effectiveness of an antioxidant compound is generally dependent on the polarity of the testing system, the nature of the radical, and type of substrate protected by the antioxidant (*23*). The diversity of antioxidant polyphenolics present in açai create a complex matrix from which evaluations can be made, but it was apparent that anthocyanins were the predominate contributors to the antioxidant capacity, and their presence with other polyphenolics resulted in an underestimation of the overall antioxidant capacity of açai pulp.

Color Stability as Affected by Hydrogen Peroxide and Temperature. The anthocyanin color stability of açai was assessed spectrophotometrically in the presence of hydrogen peroxide (0 and 30 mmol/L) at 10, 20, and 30 °C and compared to the five other anthocyanins sources. Regression analysis was used to determine adequacy of the model describing kinetics of color degradation over time and confirmed that degradation rates followed first-order kinetics ($P < 0.05$) in agreement with previous reports (*24−25*). Degradation rate constants (β_1) and half-life ($t_{1/2}$) values of anthocyanin color were calculated according to Taoukis et al. (*24*): $\ln A_t/A_o = -\beta_1 \times time$, and $t_{1/2} = \ln 0.5/\beta_1$; where A_o is the initial absorbance value, and A_t is the absorbance value at a given time. Increments in storage temperature allowed for calculation of a temperature quotient (Q_{10}) for each anthocyanin source (*24*), which is presented in **Table 2**.

Compared to açai and the other anthocyanin sources, greater color stability ($t_{1/2}$) was observed for red grape anthocyanins, results that were attributed to their high polymeric anthocyanin content (**Table 3**). The predominantly acylated anthocyanins from black carrot and red cabbage displayed reduced color stability at each temperature when compared to the nonacylated açai and hibiscus anthocyanins and to the acylated anthocyanins from purple sweet potato. Differences in half-life values (Y) between red grape and other anthocyanin sources increased linearly with reaction temperature ($Y = m \times temp$, $R^2 = 0.99$), with similar values obtained for these differences for hibiscus, purple potato, and açai ($m = 0.135$), and more pronounced for red cabbage and black carrot ($m = 0.2$). Increasing the reaction
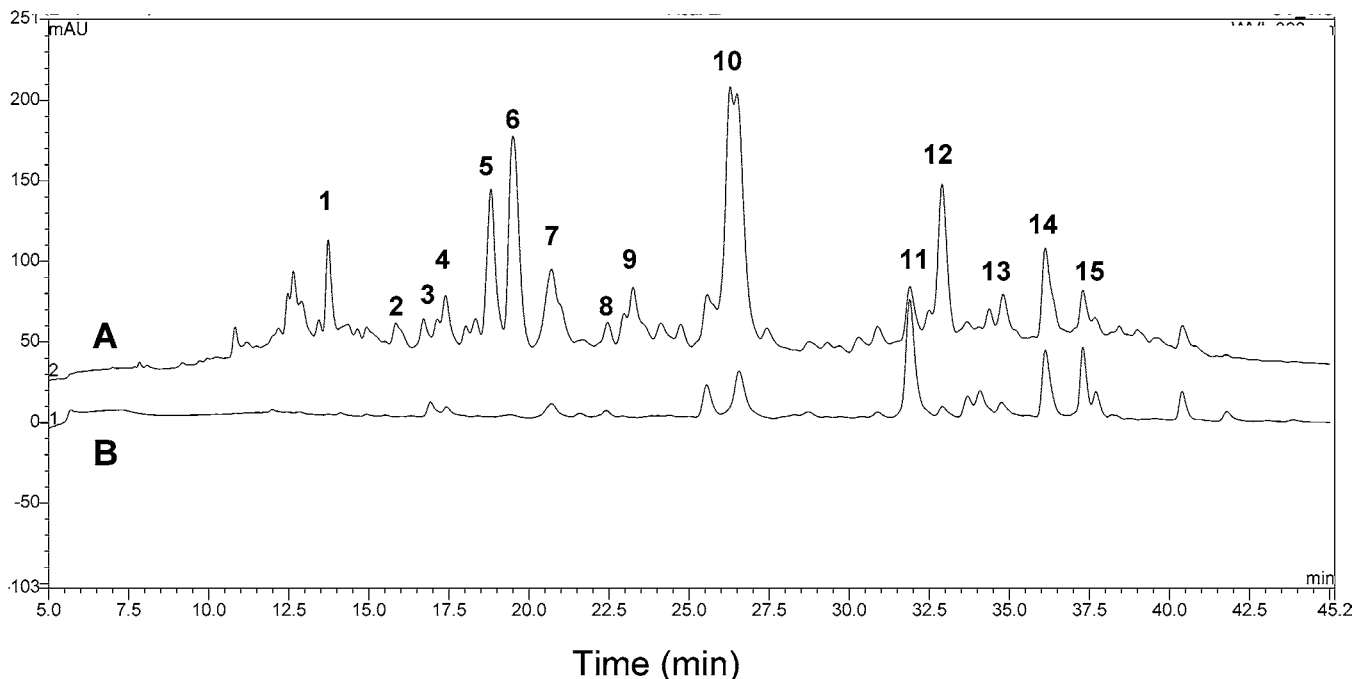
Figure 2. HPLC chromatogram of (**A**) phenolic acids monitored at 280 nm and (**B**) flavonoids monitored at 360 nm present in açai (*Euterpe oleracea* Mart.). Peak assignments: 1, gallic acid; 2, *p*-coumaric acid; 3, protocatechuic acid; 4, (+)-catechin; 5, *p*-hydroxybenzoic acid; 6, vanillic acid; 7, gallic acid derivative-2; 8, gallic acid derivative-5; 9, gallic acid derivative-3; 10, gallic acid derivative-1; 11, ferulic acid; 12, (−)-epicatechin; 13, gallic acid derivative-4; 14, ellagic acid; and 15, ellagic acid derivative.
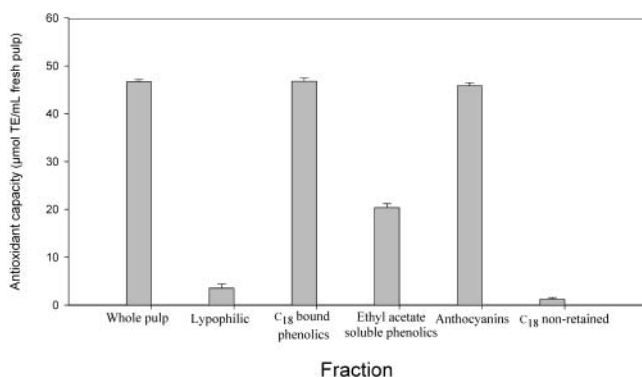

Figure 3. Antioxidant capacity of different phytochemical fractions (whole pulp, liphophilic extract, C$_{18}$-bound polyphenolics, ethyl-acetate-soluble phenolics, anthocyanins, and C$_{18}$ nonretained) of açai (*Euterpe oleracea* Mart.). Bars represent standard error of the mean (*n* = 6). Antioxidant capacity quantified using Trolox equivalents.

Table 2. Effect of Hydrogen Peroxide (30 mmol/L) and Temperature (°C) on Kinetic Parameters of Color Degradation for Different Anthocyanin Sources

	$\beta_1{}^a$			$t_{1/2}{}^b$			$Q_{10}{}^c$	
pigment	10	20	30	10	20	30	10–20	20–30
acai	7.7	11.3	13.9	90 c^d	61 c	50 c	1.5	1.2
hibiscus	6.3	9.8	11.7	110 b	71 b	59 b	1.6	1.2
purple potato	5.8	9.5	12.4	120 b	73 b	56 b	1.6	1.3
black carrot	8.7	14.4	18.7	80 d	48 d	37 d	1.7	1.3
red cabbage	8.4	12.5	15.9	83 d	55 c	44 d	1.5	1.3
red grape	2.2	4.2	5.6	315 a	165 a	124 a	1.9	1.3

^{a} Reaction rate constant ($\beta_1 \times 10^3$, min^{-1}). ^{b} Half-life (min) of initial absorbance value for each pigment source. ^{c} Temperature dependence quotients of color degradation as affected by increments in reaction temperature from 10 to 20 °C and 20 to 30 °C, respectively. ^{d} Values with similar letters within columns of each reaction temperature are not significantly different (LSD test, $P < 0.05$).

temperature from 10 to 20 °C significantly increased color degradation ($Q_{10} \sim 1.6$) for all sources except red grape, were a 1.9-fold increase was observed. This was in contrast to the relatively slower rate of color loss ($Q_{10} = 1.3$) observed for all the anthocyanin sources when the reaction temperature was increased from 20 to 30 °C.

Rates of anthocyanin degradation during storage significantly varied among sources and likely occurred due to factors such as varying molar ratios between reactants (anthocyanins and/or polyphenolics with peroxide), nonanthocyanin polyphenolic concentration, secondary free radical formation, or other oxidative reactions such as *o*-quinone formation involving phenolics and anthocyanins (*8, 20, 24*). Results of this study indicate that acylated anthocyanins were not more stable than their nonacylated counterparts in the presence of hydrogen peroxide. This observation may have been influenced by the presence of additional nonanthocyanin polyphenolics in solution, emphasizing the importance of conducting color stability evaluations with

pigment sources used industrially. These polyphenolics also form copigment complexes with anthocyanins, resulting in a more intense color that may be severalfold higher in color intensity due to hyperchromic and bathochromic spectroscopic shifts. Therefore, color comparisons among diverse pigments sources are difficult since molar ratios between reactants (hydrogen peroxide and anthocyanins) vary between sources for a given color intensity. Despite these varying ratios, industrial use of anthocyanins is based on color shade and intensity and their relative color stability under oxidizing conditions is very important for many food and beverage applications.

Color Stability in the Presence of Ascorbic Acid and Natural Cofactors. A primary concern regarding the use of anthocyanins in the food industry is their inherent instability during processing and storage. Moreover, a growing trend in the food industry is to fortify juices with various phytonutrients for both quality and health-promoting benefits. Ascorbic acid is among the most common fortificants used for this purpose; however, when present together with anthocyanins, their

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Table 3. Percent Monomeric Anthocyanins and CIE Color Attributes of a Juice Model System (pH 3.5, 100 mg/L sucrose) Prepared with Different Pigment Sources, along with Their Correspondent Hyperchromic and Bathochromic Shifts Due to the Presence of Naturally Occurring Polyphenolic Cofactors

pigment	% monomeric anthocyanins	chroma	hue	λ_{max}[a]	hyperchromic shift[b]	bathochromic shift[b]
acai	76.2 c[c]	20.1	18.2	515 nm	6%	1 nm
hibiscus	80.3 b	31.2	35.2	521 nm	19%	0 nm
purple potato	77.5 c	23.1	13.6	526 nm	35%	0 nm
black carrot	77.8 c	22.9	10.2	521 nm	20%	0 nm
red cabbage	92.2 a	19.8	−13.9	526 nm	8%	0 nm
red grape	58.1 d	17.2	6.1	528 nm	49%	2 nm

[a] Wavelength of maximum absorption for each pigment source. [b] Difference in absorbance between anthocyanin solutions with and without naturally occurring polyphenolic cofactors. [c] Values with similar letters within columns of each reaction temperature are not significantly different (LSD test, $P < 0.05$).

combination will lead to mutual degradation that causes the loss of nutrients and color stability during processing and storage. Therefore, a need exists to find an inexpensive and stable anthocyanin pigment that possesses a diversity of functional properties for food and nutraceutical applications. The stability of açai anthocyanins was evaluated in the presence of ascorbic acid (0 and 450 mg/L) under accelerated storage conditions (37 °C) using an in vitro model system as compared to those of other common anthocyanin sources (hibiscus, black carrot, red cabbage, red grape, and purple sweet potato). A further examination of how naturally occurring cofactors affect color stability within a given pigment source was also investigated.

Differences in spectroscopic properties and color attributes among in vitro juice model systems prepared with the six anthocyanin sources were initially observed (**Table 3**). Despite model systems with the same initial color value (absorbance value of 1.5), color differences were apparent and due to the diversity of ring substitutions (hydroxy, sugar, or acyl-linked organic acids) among sources. As previously discussed, the nature of polyphenolic cofactors and their relative molar ratio to anthocyanin concentration were also influential on color characteristics of each source. Isolation of polyphenolic cofactors revealed not only the appreciable difference in color exhibited by each pigment but also their specific role in anthocyanin stability. Red grape anthocyanins had the largest hyperchromic shift (49%), followed by purple potato (35%), hibiscus and black carrot (19.5% on average), and açai and red cabbage (7% on average) due to the presence of these native cofactors with a slight bathochromic shift in wavelength observed for açai and red grape anthocyanins. These spectroscopic features translated into a more intense colored solution and were influential on overall color stability.

Results from objective color analysis concluded that chroma values only differed slightly within anthocyanin sources in accordance with those observed in previous studies (*26, 27*), except for hibiscus, which had an appreciably higher value than other sources. Hue angles significantly differed among pigment sources due to various ring substitutions and were generally lower for acylated anthocyanins (**Table 4**), giving the later anthocyanins a characteristic intense purple color in solution that corresponded to their longer wavelength of maximum absorbance. Red grape anthocyanins were a notable exception due to its high polymeric anthocyanin content in relation to the other sources. Polymeric anthocyanins typically have greater color stability over their monomeric counterparts (*28–30*), and the high content in red grape (58%) appreciably influenced its color stability during storage. The red grape extract used in this study was obtained as a byproduct of the wine industry and may contain anthocyanins polymerized with oligomeric fla-

Table 4. Effect of Ascorbic Acid (0 and 450 mg/L) and Naturally Occurring Polyphenolic Cofactors (Presence or Absence) on Kinetic Parameters of Anthocyanin Degradation during Storage at 37 °C of in Vitro Models Systems (pH 3.5, 100 mg/L sucrose) Prepared with Different Pigment Sources

	no ascorbic acid				ascorbic acid (450 mg/L)			
	with cofactors		no cofactors		with cofactors		no cofactors	
pigment	β_1[a]	$t_{1/2}$[b]	β_1	$t_{1/2}$	β_1	$t_{1/2}$	β_1	$t_{1/2}$
acai	1.8	385 d[c]	2.2	319 d*[d]	55	13 d	49	14 c*
hibiscus	2.2	315 e	5.3	131 f*	19	37 c	60	11 c*
purple potato	0.8	866 b	2.0	355 c*	18	38 c	52	13 c*
black carrot	1.3	533 c	1.4	486 b*	52	13 d	60	12 e*
red cabbage	0.3	2450 a	0.6	1150 a*	14	50 b	34	20 b*
red grape	1.3	533 c	2.9	243 e*	11	62 a	23	30 a*

[a] Reaction rate constants ($\beta_1 \times 10^3$ hours^{-1}). [b] Half-life (hours) of initial anthocyanin content. [c] Values with similar letters within columns are not significantly different (LSD test, $P < 0.05$). [d] Means with an asterisk (*) for each pigment source indicate a significant effect (LSD test, $P < 0.05$) due to presence of naturally occurring cofactors when compared to the same treatment with an equivalent ascorbic acid content.

vanols and/or acetaldehyde (*29–32*) which gives this extract remarkable color and storage stability.

Regression analysis found that anthocyanins under the accelerated storage conditions of the in vitro models, with and without native cofactors, followed first order kinetics ($P < 0.05$). Kinetic parameters were calculated as previously described, with anthocyanin content used as the independent variable. Acylated anthocyanin sources along with those from red grape were found to be more stable than their nonacylated counterparts, independent of ascorbic acid content. Naturally occurring cofactors were shown to be key elements to decrease anthocyanin degradation during storage, an effect that was more pronounced for nonacylated anthocyanin sources.

Half-life evaluation of pigment stability revealed that acylated anthocyanin sources generally had increased stability ($t_{1/2} > 823$ h) with respect to nonacylated sources in the absence of ascorbic acid. A notable exception was black carrot anthocyanins ($t_{1/2} = 515$ h), which showed reduced stability with respect to that of nonacylated red grape anthocyanins ($t_{1/2} = 540$ h). By comparison, the red grape anthocyanins had reduced stability in the absence of ascorbic acid; especially in relation to the high stability observed against hydrogen peroxide, yet in the presence of ascorbic acid, the stability was again the highest among anthocyanin sources. Red grape anthocyanins ($t_{1/2} = 62$ h) were the most stable compounds in the presence of ascorbic acid followed by red cabbage ($t_{1/2} = 50$ h), hibiscus and purple potato ($t_{1/2} = 37$ h), and last, açai and black carrot ($t_{1/2} = 13$ h).

Overall, anthocyanin degradation was significantly increased in the presence of ascorbic acid as compared to nonfortified controls, generally having a more pronounced effect on acylated anthocyanin sources (40 to 46-fold) than for nonacylated sources (8.4 to 30-fold). A notable exception was purple potato anthocyanins, where ascorbic acid increased color degradation by 23-fold. Red grape and hibiscus anthocyanins exhibited the smallest change with only a 8-fold increase in degradation rates. Naturally occurring polyphenolic cofactors were found to significantly increase anthocyanin retention by up to 2.4-fold in the absence of ascorbic acid, an effect that was less pronounced for açai (1.2-fold) and black carrot (1.1-fold) anthocyanins. A similar protective effect conferred by inter-molecular copigmentation was observed in the presence of ascorbic acid for black carrot, açai, and red grape, yet additional increments in this protective effect was observed for hibiscus (+0.9-fold) and both purple potato and red cabbage (+0.4-fold).

The increased stability of acylated anthocyanins with respect to nonacylated pigment sources was likely related to the natural synthesis of acylated organic acids and diversity of glycosidic linkages in relation to these acylated moieties (*7*, *8*, *27*). The aromatic or aliphatic acyl groups covalently bound to these anthocyanins were shown to stack on the planar, polarizable nuclei of the anthocyanin, protecting the pyrylium nucleus from the nucleophilic attack of water at carbon 2 (*7*, *8*). Red cabbage and purple potato extracts typically contain cinnamic acid derivatives diacylated to their anthocyanins that can simulta-neously stack on both faces of the anthocyanin chromophore in a sandwich-type complex and thus offer greater color stability, while black carrots contain only monoacylated moieties that can only protect one face of the pyrylium ring (*7*–*10*, *26*, *27*, *30*). The observed differences in stability between the various sources of acylated anthocyanins in this study were likely related to the nature, number, and position of these substitutions.

For a given set of pH conditions, intramolecular copigmen-tation exerts a protective effect against anthocyanin degradation by keeping a larger proportion in their flavylium ion forms. Consequently, formation of intermolecular complexes will also take place with these acylated anthocyanins and thus give an additional protective effect against color degradation. Results of this study also demonstrated and confirmed that both forms of copigmentation (intra- and intermolecular) cooperatively acted to prevent anthocyanin color degradation, as demonstrated by similar pigment half-life values (12.5 days) in black carrot and purple potato after removal of naturally occurring cofactors.

The stabilization effect conferred by intermolecular copig-mentation has been attributed to hydrophobic interactions between anthocyanins and polyphenolic compounds, conse-quently protecting the pigment from further polymerization and degradation reactions (*7*–*9*, *30*–*32*). Previous studies have shown that not only ascorbic acid but also its degradation byproducts, including those from carbohydrates such as furfural and other aldehydes, can participate in anthocyanin degradation during processing or storage (*32*). Intermolecular copigmentation exerts a protective effect on anthocyanin degradation as cofactors compete with anthocyanins and preferentially react in the condensation reactions (*10*, *30*, *31*). The increased protection observed for a specific pigment source due to the presence of cofactors is most likely related to the type and content of polyphenolics present, as a higher copigment/pigment molar ratio could have occurred for a determined source. Moreover, specific polyphenolics or classifications of polyphenolics are more likely to form stable intermolecular complexes with anthocyanins than others (*8*, *10*, *32*).

Characterization of the major polyphenolic compounds present in açai and their contribution to the antioxidant capacity was determined for the first time. The effect of exogenously added cofactors on color enhancement and stability was previously evaluated in many food systems containing isolated anthocyanins, model juices, and wine, yet the effect of naturally occurring cofactors on color stability was not previously investigated prior to this study. The stability of açai anthocyanins as a new source of anthocyanin pigments was also established and can be used to determine application and functional properties of açai in a variety of food and nutraceutical products.

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