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Juice matrix composition and ascorbic acid fortification effects on the phytochemical, antioxidant and pigment stability of açai (*Euterpe oleracea* Mart.)

Lisbeth A. Pacheco-palencia [a], Palo Hawken [b], Stephen T. Talcott [c,*]

[a] *Department of Food Science and Human Nutrition, University of Florida, P.O. Box 110370, Gainesville, FL 32611-0370, USA*
[b] *Bossa Nova Beverage Group, 11965 Venice Blvd. Suite 409, Los Angeles, CA 90066, USA*
[c] *Department of Nutrition and Food Science, Texas A&M University, College Station, TX 77843-2254, USA*

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Abstract

The effects of juice matrix composition on the phytochemical stability of açai (*Euterpe oleracea* Mart.) were evaluated by contrasting natural clarified juice systems with isolated polyphenolic and anthocyanin fractions, in the presence or absence of ascorbic acid (500 mg/l) under accelerated storage conditions (37 °C). Polyphenolic (anthocyanin and non-anthocyanin polyphenolics) and anthocyanin fractions were isolated using C18 Sep-Pak columns and then re-dissolved in the original aqueous juice matrix (unbound fraction) or in a citric acid buffer (pH 3.5). The isolation of anthocyanins from the açai matrix improved their colour stability but a greater retention of non-anthocyanin polyphenolics and antioxidant properties was favoured by the initial juice composition. The presence of non-anthocyanin polyphenolics exerted a protective effect against ascorbic acid oxidation and enhanced polyphenolic and antioxidant stability in isolates fortified with ascorbic acid. However, all isolates obtained from açai experienced significant colour, polyphenolic, and antioxidant losses during storage and this indicated that optimization of early stages of industrial processing, storage and distribution are necessary to retain the functional properties of açai-containing products.

Keywords: Açai; Fractionation; Ascorbic acid; Polyphenolic; Anthocyanin; Stability

1. Introduction

Functional foods and beverages are finding global success, partially due to consumer trends toward health maintenance. Increased attention has been given to the protective effects of fruit and vegetables and their roles in the prevention of various degenerative diseases, including cancer and coronary heart disease (Burda & Oleszek, 2001; Franceschi et al., 1998; Van Poppel, Cardinal, Princen, & Kik, 1994). Açai (*Euterpe oleracea* Mart.), a palm fruit native to the Amazon region, has recently captured international interest, not only due to its perceived novelty and exotic flavour but also due to potential health benefits associated with its phytochemical composition. However, studies on the functional properties of açai are still lacking and compositional factors influencing the phytochemical and antioxidant stability of its matrix have not been determined. Factors such as the presence of ascorbic acid, polyphenolic copigments, sugars, organic acids and trace metals may significantly affect the phytochemical, antioxidant, and colour stability of açai-containing products. Ascorbic acid is commonly added to fruit juices to prevent enzymatic browning reactions and enhance nutritional properties (Freedman & Francis, 1984). According to Ozkan, Kirca, and Cemeroglu (2004), foods naturally low in ascorbic acid, such as açai juice (Rogez, 2000), are particularly good candidates for fortification. Unfortunately, the presence of ascorbic acid in many anthocyanin-rich systems has been

* Corresponding author. Tel.: +1 979 862 4056; fax: +1 979 458 3704.
E-mail address: stalcott@tamu.edu (S.T. Talcott).

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shown to promote their oxidation (Brenes, Del Pozo, & Talcott, 2005; Marti, Perez-Vicente, & Garcia-Viguera, 2001; Poei-Langston & Wrolstad, 1981; Skrede, Wrolstad, Lea, & Emresen, 1992) and to negatively impact colour retention, nutritional quality, and overall functional properties (Shrikhande & Francis, 1974). However, both positive (Kaack & Austed, 1998) and negative effects of ascorbic acid fortification have been reported in various fruit juices and nectars (Kirca, Ozkan, & Cemeroglu, 2006), indicating the role of the matrix composition and anthocyanin chemistry in overall pigment stability. Variables such as extent of glycosylation, presence of acylated moieties and other compositional factors, such as sugars, organic acids, metal ions, proteins, ascorbic acid and other polyphenolics can influence anthocyanin degradation. Therefore, this study was undertaken to investigate the effects of açai juice matrix composition and ascorbic acid fortification on the phytochemical, antioxidant and colour stability of açai juice and juice fractions during storage.

2. Materials and methods

2.1. Materials and processing

Pasteurized, frozen açai pulp was obtained from Bossa Nova Beverage Group (Los Angeles, CA) and shipped overnight to the Department of Food Science and Human Nutrition at the University of Florida. Pulp was thawed, centrifuged (2000*g* at 4 °C for 15 min) and passed through a 1 cm bed of diatomaceous earth to obtain a clarified juice (Fraction I) that was used for further preparation of experimental variables, following acidification to pH 3.5 with citric acid (Fig. 1). In three independent trials, polyphenolics were isolated from the clarified açai juice by two separate isolation procedures, using C18 Sep-Pak Vac 20 cm^3 mini-columns (Waters Corporation, MA) to remove sugars, organic acids and metal ions (C18 unbound) from the polyphenolic fraction (C18 bound). In the first isolation, polyphenolics were recovered from the cartridges by elution with acidified methanol (0.01% HCl), followed by solvent removal under reduced pressure (<40 °C). The polyphenolics included anthocyanin and non-anthocyanin polyphenolics that were re-dissolved in an equivalent volume of 0.1 M citric acid buffer pH 3.5 (Fraction II) or re-dissolved in the C18 unbound fraction, adjusted to pH 3.5 (Fraction III). In the second isolation, non-anthocyanin polyphenolics were removed with ethyl acetate and the remaining anthocyanins recovered with acidified methanol (0.01% HCl) and likewise re-dissolved in an equivalent volume of the citric acid buffer (Fraction IV) or in the C18 unbound fraction (Fraction V), all at pH 3.5. Each of the five isolates were further divided into two subfractions and fortified with either L-ascorbic acid (500 mg/l) or with an equal volume of citric acid buffer as a non-fortified control. All were sealed in screw-cap glass vials, following the addition of sodium azide (50 mg/l) to retard microbial growth, and stored in the dark at 37 °C for 12 days. Samples were collected every 48 h, held frozen (−20 °C), and analyzed within 2 weeks.

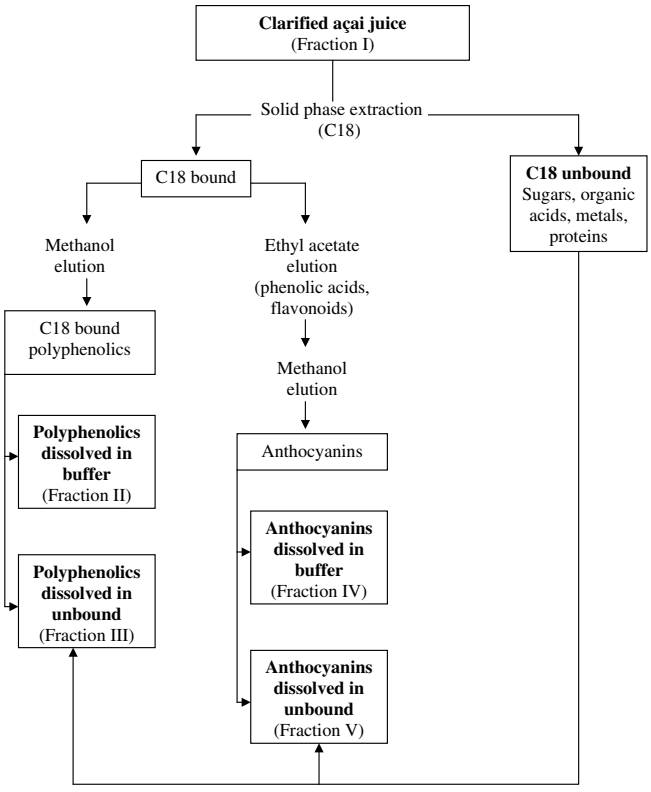

Fig. 1. Açai juice fractionation procedure.

2.2. Chemical analyses

The phytochemical stability of açai fractions was determined by monitoring changes in total anthocyanin content, polymeric anthocyanin concentration, total soluble phenolics, antioxidant capacity, and individual polyphenolics during short-term, accelerated storage. Total anthocyanin contents were determined spectrophotometrically by the pH differential method (Wrolstad, Durst, & Lee, 2005) and total anthocyanin concentration was calculated, using mg/l equivalents of cyanidin-3-glucoside with an extinction coefficient of 29,600 (Jurd & Asen, 1966). Polymeric anthocyanin concentrations were calculated, based on colour retention in the presence of sodium sulfite (Rodriguez-Saona, Guisti, & Wrolstad, 1999). Absorbance was read on a Beckman DU® 640 spectrophotometer (Beckman, Fullerton, CA) at a fixed wavelength of 520 nm. Total soluble phenolics were determined by the Folin–Ciocalteu assay (Singleton & Rossi, 1965) with absorbance read using a UV–Vis microplate reader (Molecular Devices Spectra Max 190, Sunnyvale CA) at 726 nm and quantified by linear regression against a gallic acid standard curve. Antioxidant capacity was measured by the oxygen radical absorbance capacity method of Cao, Wang, and Prior (1996) adapted to a 96-well Molecular Devices fmax® fluorescent microplate reader (485 nm

excitation and 538 nm emission) and regressed against a standard curve of trolox, a water-soluble analogue of vitamin E. Individual polyphenolic compounds were analyzed by reverse phase HPLC, using the modified chromatographic conditions of Talcott and Lee (2002) with a Waters 2695 Alliance system (Waters Corp., Milford, MA) equipped with a Waters 996 photodiode array detector. Separations were performed on a 250 × 4.6 mm Acclaim 120-C18 column (Dionex, Sunnyvale, CA) with a C18 guard column. Mobile phases consisted of water (phase A) and a 60:40 methanol and water (phase B), both adjusted to pH 2.4 with *o*-phosphoric acid. The gradient solvent program ran phase B from 0% to 60% in 20 min; 60% to 100% in 20 min, then 100% for 7 min, 100% to 0% in 3 min and final conditions were held for 2 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).

2.3. Statistical analysis

The study was designed as a 5 × 2 × 7 full factorial that included five açai juice fractions (100% açai juice and four polyphenolic fractions), two L-ascorbic acid levels, and seven storage times. For each treatment, the mean of three independent replicates is reported. Analysis of variance, multiple linear regression, Pearson correlations and means separation by Tukey's HSD test ($p < 0.05$) were conducted using JMP software (SAS Institute, Cary, NC).

3. Results and discussion

3.1. Polyphenolic stability

Major polyphenolic compounds present in açai juice and juice fractions were identified (Table 1) and their relative changes after 12 days at 37 °C determined (Table 2). Several phenolic acids, including protocatechuic acid, *p*-hydroxy benzoic acid, vanillic acid and ferulic acid, were found at concentrations that ranged from 0.94 to 27.2 mg/l in açai juice. In addition, two compounds sharing spectroscopic similarities with gallic acid were detected in concentrations of 30.1 and 32.9 mg/l (gallic acid equivalents), respectively. Açai juice also contained (+)-catechin (9.3 mg/l) and (−)-epicatechin (5.9 mg/l), as well as three flavan-3-ol derivatives spectrally identified as procyanidins at concentrations that ranged from 5.3 to 34 mg/l as (+)-catechin equivalents, in agreement with previous reports by Lichtenthaler et al. (2005). Actual polyphenolic concentrations varied slightly among the isolates due to independent preparations, yet polyphenolic recovery rates from the C18 cartridges in Fractions II and III were consistently >90%. Cartridge elution with ethyl acetate, to generate Fractions IV and V, consistently removed phenolic acids (protocatechuic, *p*-hydroxy benzoic, vanillic and *p*-coumaric), (+)-catechin, (−)-epicatechin and procyanidin-1 (Table 1). The resultant anthocyanin fractions contained the two gallic acid derivatives, ferulic acid, and procyanidins 2 and 3. Total polyphenolic contents decreased linearly during storage with maximum losses from 48% to 66% observed by the end of storage. However, the rates of change differed among individual compounds during storage. The majority of non-anthocyanin polyphenolics present (protocatechuic acid, *p*-hydroxy benzoic acid, (+)-catechin, vanillic acid, (−)-epicatechin, *p*-coumaric acid and procyanidin-1) underwent a relatively small decrease (<5%) during storage whereas the gallic acid derivatives, procyanidins 2 and 3, and ferulic acid experienced considerable degradation (>50%) over 12 days (Table 2). A decrease in procyanidin concentration may have reflected oxidative or polymerization reactions involving anthocyanins and other polyphenolics that have been associated with browning reactions and the formation of polymeric anthocyanins in other fruit juice systems (Akissoe, Mestres, Hounhouigan, & Nago, 2005; Es-Safi & Cheynier, 2004; Salas, Fulcrand, Meudec, & Cheynier, 2003).

Table 1
Non-anthocyanin polyphenolics present in açai juice and açai juice fractions (mg/l)

Polyphenolic	Concentration (mg/l)		
	100% Açai juice (Fraction I)	Polyphenolic fractions (Fractions II and III)	Anthocyanin fractions (Fractions IV and V)
Protocatechuic acid	0.94 ± 0.10	0.89 ± 0.11	–
p-Hydroxy benzoic acid	1.39 ± 0.11	1.33 ± 0.14	–
(+)-Catechin	9.31 ± 0.46	8.61 ± 0.43	–
Vanillic acid	7.74 ± 0.65	7.70 ± 0.55	–
(−)-Epicatechin	5.96 ± 0.73	5.38 ± 0.25	–
Procyanidin-1	5.32 ± 0.71	5.30 ± 0.50	–
p-Coumaric acid	1.57 ± 0.32	1.50 ± 0.29	–
Gallic acid derivative-1	30.1 ± 2.96	27.4 ± 2.29	24.9 ± 2.52
Gallic acid derivative-2	32.9 ± 3.15	29.9 ± 1.54	31.0 ± 2.87
Procyanidin-2	34.2 ± 2.25	33.7 ± 1.60	30.8 ± 1.06
Ferulic acid	27.2 ± 2.01	24.8 ± 1.27	20.3 ± 0.79
Procyanidin-3	11.9 ± 1.30	10.8 ± 0.90	9.01 ± 0.252

Table 2
Percentage retention of initial non-anthocyanin polyphenolic contents in açai juice and açai juice fractions following storage at 37 °C (12 days)

Polyphenolic	100% Açai juice (Fraction I)		Polyphenolic fractions (Fractions II and III)		Anthocyanin fractions (Fractions IV and V)	
	No AA (% retention[a])	AA (% retention[a])	No AA (% retention[a])	AA (% retention[a])	No AA (% retention[a])	AA (% retention[a])
Protocatechuic acid	103 ± 3.14	102 ± 2.74	101 ± 2.70	102 ± 2.99	–	–
p-Hydroxy benzoic acid	98.3 ± 2.50	100 ± 2.95	99.4± 2.62	101 ± 2.86	–	–
(+)-Catechin	103 ± 3.42	101 ± 3.35	104 ± 3.88	102 ± 3.21	–	–
Vanillic acid	102 ± 2.64	103 ± 3.71	103 ± 3.59	104 ± 3.43	–	–
(−)-Epicatechin	99.4 ± 3.89	102 ± 3.12	104 ± 2.89	98.1 ± 2.23	–	–
Procyanidin-1	98.9 ± 4.01	102 ± 3.44	100 ± 3.41	104 ± 2.58	–	–
p-Coumaric acid	103 ± 3.22	102 ± 3.61	104 ± 2.85	102 ± 3.72	–	–
Gallic acid derivative-1	32.7 ± 1.37	32.1 ± 1.44	30.8 ± 1.28	29.8 ± 1.32	30.6 ± 1.73	31.3 ± 1.12
Gallic acid derivative-2	16.2 ± 0.98	14.0 ± 1.17	14.4 ± 0.67	14.9 ± 0.71	13.9 ± 0.56	14.2 ± 0.65
Procyanidin-2	38.1 ± 1.42	37.9 ± 2.13	38.9 ± 1.55	38.1 ± 1.01	36.9 ± 1.03	37.3 ± 1.22
Ferulic acid	23.0 ± 1.03	24.0 ± 0.76	25.5 ± 0.84	21.6 ± 0.75	22.6 ± 0.85	22.1 ± 0.94
Procyanidin-3	103 ± 3.10	101 ± 2.74	101 ± 3.20	101 ± 3.06	99.4 ± 2.20	98.2 ± 2.80
Total	52.3 ± 1.54	51.6 ± 1.59	52.1 ± 1.41	51.7 ± 1.52	32.5 ± 1.25	32.7 ± 1.32

[a] Percentage retention based on initial polyphenolic concentrations reported on Table 1.

3.2. Anthocyanin stability

Total anthocyanin contents (457 ± 5.5 mg/l açai juice) were determined spectrophotometrically and were related to optical appreciations of anthocyanin colour (Wrolstad et al., 2005). In addition, HPLC analysis of açai juice revealed the presence of cyanidin-3-rutinoside (135 ± 1.8 mg/l) and cyanidin-3-glucoside (42.4 ± 0.45 mg/l), in agreement with the reports of Gallori, Bilia, Bergonzi, Barbosa, and Vincieri (2004), and Lichtenthaler et al. (2005). Differences between spectrophotometric and HPLC determinations of total anthocyanin concentrations are attributed to the influence of non-anthocyanin polyphenolics, polymeric anthocyanins and other matrix components that influence spectroscopic properties of the juice (Talcott, Brenes, Pires, & Del Pozo, 2003; Wrolstad et al., 2005). Moreover, anthocyanin recoveries following fractionation were >85% when using the spectrophotometric assay and >95% by HPLC analysis, reflecting the consistency of monomeric anthocyanin recovery from C18 cartridges for use in subsequent evaluations of pigment stability.

Anthocyanin degradation followed first order kinetics ($p < 0.05$), in agreement with previous reports for anthocyanins (Cemeroglu, Velioglu, & Isik, 1994; Garzon & Wrolstad, 2002; Kirca et al., 2006). Kinetic parameters included first order reaction rate constants (k) and half lives ($t_{\frac{1}{2}}$), or time needed for the degradation of 50% of the initial anthocyanin contents under the experimental conditions, were calculated using the equations: $\ln(A/A_0) = -kt$ and $t_{\frac{1}{2}} = \ln 0.5/k$, where A_0 was the initial anthocyanin absorbance at 520 nm and A was the absorbance after t days of storage at 37 °C (Table 3). Under the conditions of

Table 3
Kinetic parameters for total and individual anthocyanin degradation in ascorbic acid (AA) fortified and non-fortified açai fractions stored at 37 °C

	Total anthocyanins		Cyanidin-3-glucoside		Cyanidin-3-rutinoside	
	k[A]	$t_{\frac{1}{2}}$[B]	k	$t_{\frac{1}{2}}$	k	$t_{\frac{1}{2}}$
No AA						
100% Açai Juice (Fraction I)	0.279	2.48b[C]	0.342	2.02b	0.297	2.33bc
Polyphenolics in buffer (Fraction II)	0.221	3.14a	0.258	2.68a	0.241	2.88a
Polyphenolics in unbound (Fraction III)	0.261	2.65b	0.299	2.32b	0.288	2.41b
Anthocyanins in buffer (Fraction IV)	0.230	3.01a	0.267	2.60a	0.244	2.84a
Anthocyanins in unbound (Fraction V)	0.272	2.54b	0.336	2.06b	0.327	2.12c
AA						
100% Açai Juice (Fraction I)	0.291	2.38bc	0.578	1.20c	0.449	1.54d
Polyphenolics in buffer (Fraction II)	0.240	2.89a	0.529	1.31c	0.259	2.68a
Polyphenolics in unbound (Fraction III)	0.295	2.35bc	0.550	1.26c	0.442	1.57d
Anthocyanins in buffer (Fraction IV)	0.252	2.75b	0.546	1.27c	0.262	2.65a
Anthocyanins in unbound (Fraction V)	0.307	2.26c	0.563	1.23c	0.447	1.55d

[A] Reaction rate constant (k days^{-1}).
[B] Half life (days) of initial absorbance value for each treatment.
[C] Values with similar letters within columns are not significantly different (LSD test, $p < 0.05$).

storage, the polyphenolic isolates experienced rapid loss of total anthocyanins (67–78%) during the first 6 days of storage, and only minor changes occurred thereafter. Fractions dissolved in citric acid buffer (Fractions II and IV) exhibited greater anthocyanin stability ($t_{\frac{1}{2}} = 3.0$–3.1 days) than did açai juice alone (Fraction I) or those fractions dissolved in C18 unbound ($t_{\frac{1}{2}} = 2.5$–2.6 days; Fractions III and V). This enhanced stability over the course of the first 6 days was an indication that compounds present in the C18 unbound fraction, including metal ions, ascorbic acid, monosaccharides and degradation products, that formed during storage, were detrimental to anthocyanin stability. The combined presence of unbound fraction components, such as ascorbic acid (<10 mg/l), furfurals from sugar degradation, and trace metals, such as iron, found at 7.3 ± 0.4 mg/l in the açai juice, were likely responsible for the poor anthocyanin stability of isolates re-dissolved in the unbound fraction (Fractions III and V) and have also been implicated in other anthocyanin systems (Dyrby, Westergaard, & Stapelfeldt, 2001; Kirca et al., 2006; Maccarone, Maccarone, & Rapisarda, 1985; Rodriguez-Saona et al., 1999). The presence of metal ions is known to enhance the formation of reactive oxygen species (Pietta, 2000), while rapid anthocyanin degradation likely occurred due to the presence of furfurals (Es-Safi, Cheynier, & Moutounet, 2000), and other reducing sugar and/or ascorbic acid degradation products (Brenes et al., 2005; Choi, Kim, & Lee, 2003; Iversen, 1999; Ozkan, 2002) that generally form quickly at the pH and storage temperatures of this study. Individual anthocyanins followed a parallel trend during storage (Table 3), with cyanidin-3-glucoside degrading at a slightly faster rate ($t_{\frac{1}{2}} = 2.0$–2.7 days) than cyanidin-3-rutinoside ($t_{\frac{1}{2}} = 2.1$–2.9 days), consistent with the reports of Rubiskiene, Jasutiene, Venskutonis, and Viskelis (2005). Moreover, the presence of non-anthocyanin polyphenolics had only a minor influence on the overall stability of both cyanidin glycosides in fractions redissolved in buffer (Fractions II and IV, $t_{\frac{1}{2}} = 3.14$–3.01 days, respectively) or in the unbound part (Fractions III and V, $t_{\frac{1}{2}} = 2.65$–2.54 days, respectively).

Total anthocyanin stability in the presence of ascorbic acid was highly correlated ($r = 0.94$) to changes in cyanidin-3-rutinoside concentrations, while monomeric forms of cyanidin-3-glucoside were less stable, regardless of matrix composition ($t_{\frac{1}{2}} = 1.2$–1.3 days). As previously observed, ascorbic acid fortified fractions dissolved in buffer also had greater anthocyanin stability ($t_{\frac{1}{2}} = 2.7$–2.9 days) than had those dissolved in the C18 unbound ($t_{\frac{1}{2}} = 2.3$–2.4 days), further demonstrating the matrix effects of açai juice. In addition, anthocyanin fractions (Fractions IV and V) had decreased anthocyanin half-lives ($t_{\frac{1}{2}} = 2.7$ and 2.3 days, respectively) when compared with their polyphenolic counterparts (Fractions II and III, ($t_{\frac{1}{2}} = 2.9$ and 2.4 days), respectively), indicating a protective effect of non-anthocyanin polyphenolics on anthocyanin stability. Phenolic acids and flavonoids contained in the ethyl-acetate fraction of açai were previously shown to affect anthocyanin stability in the presence of ascorbic acid and hydrogen peroxide (Del Pozo, Brenes, & Talcott, 2004). Similar non-anthocyanin polyphenolic fractions have also been shown to enhance colour stability in various juice and model systems (Brenes et al., 2005; Ozkan et al., 2004; Rein & Heinonen, 2004). As a result, kinetics of anthocyanin degradation seemed to be strongly dependent on the nature of the system and be influenced, not only by composition, but also by protective interactions among anthocyanin and non-anthocyanin polyphenolic components.

3.3. Anthocyanin polymerization

Anthocyanins are labile compounds, subject to numerous detrimental reactions during processing and storage (Wrolstad et al., 2005), among which the transformation of monomeric forms into oligomeric or polymeric pigments gives rise to important colour changes toward brownish-red hues (Monagas, Gomez-Cordoves, & Begoña, 2006) that are generally more stable. The formation of polymeric anthocyanin compounds in açai juice and juice fractions was spectrophotometrically assessed and was found to increase linearly during storage (Fig. 2). No differences in polymeric anthocyanin contents were initially observed among treatments, and recoveries of monomeric anthocyanins of >95% during the isolation process demonstrated partition efficiency. During storage, slightly higher (~5%) anthocyanin polymerization was observed in açai juice than in juice fractions; however, no differences were detected among juice fractions, suggesting that major juice components involved in anthocyanin polymerization reactions were present in all fractions. While anthocyanin polymerization reactions in other juice systems have been attributed to oxidation reactions induced by the presence of other matrix components, such as polyphenolics, metal ions, sugars, ascorbic acid and their degradation products (Kirca et al., 2006; Maccarone et al., 1985; Rodriguez-Saona et al., 1999), results from this study further confirm their influence and suggest that non-anthocyanin polyphenolic components may also play a role in anthocyanin polymerization reactions during storage of açai juice.

In the presence of ascorbic acid, anthocyanin polymerization rates increased markedly in açai juice and juice fractions (Fig. 2). Fortification of anthocyanin-rich matrices with ascorbic acid was shown to promote polymerization in various fruit juices (Garzon & Wrolstad, 2002; Skrede et al., 1992), and has been proposed to result from an aldehyde-mediated anthocyanin-flavanol condensation reaction (Timberlake & Bridle, 1976). Interactions between polyphenolics and carbohydrate/ascorbic acid degradation products, such as furfurals and other aldehydes, will influence the formation of brown pigments during storage of fruit-based foods (Es-Safi et al., 2000). Aldehydes generally promote anthocyanin polymerization with flavonols, flavan-3-ols, and their derivatives, resulting in the formation of both colourless and yellow-coloured compounds that

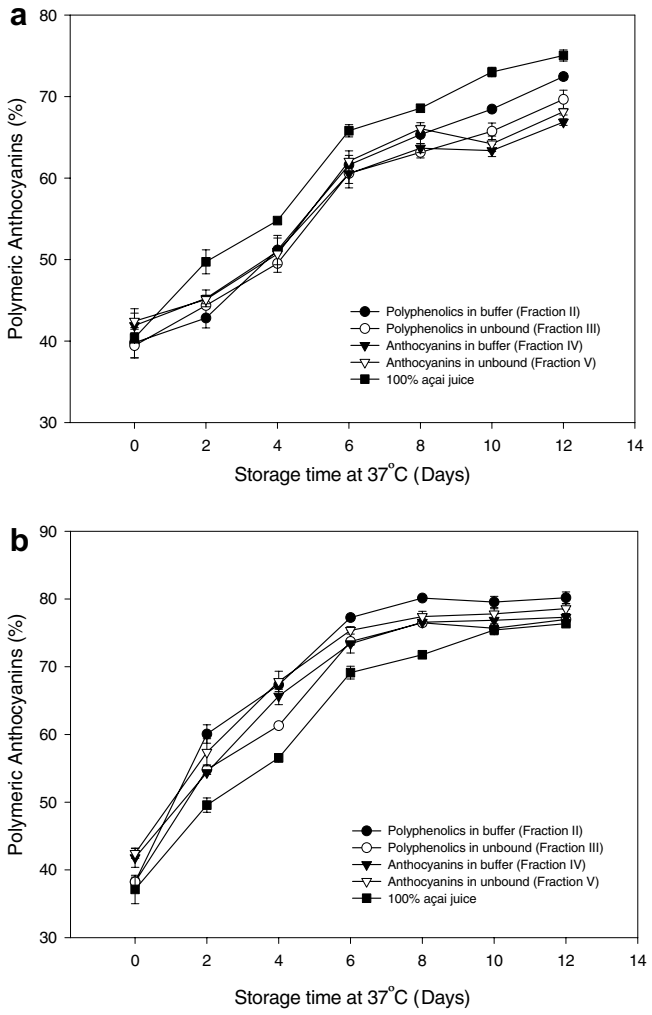

Fig. 2. Polymeric anthocyanin contents (%) in non-fortified (a) and ascorbic acid (500 mg/l)-fortified (b) açai fractions during storage at 37 °C. Error bars represent the standard error of the mean, $n = 3$.

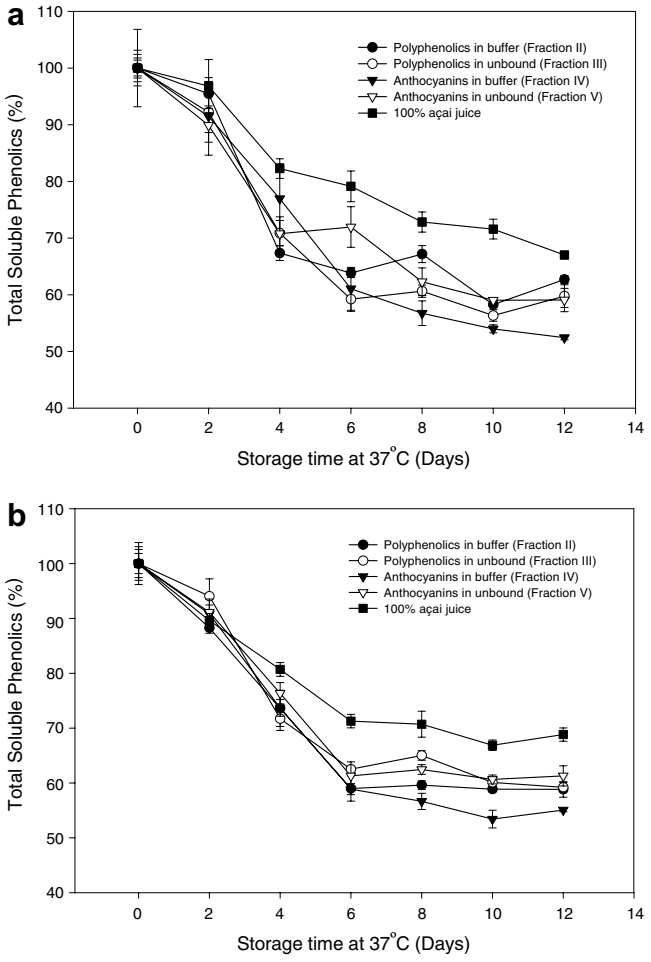

Fig. 3. Percent of initial total soluble phenolic contents in non-fortified (a) and ascorbic acid (500 mg/l)-fortified (b) açai fractions during storage at 37 °C. Error bars represent the standard error of the mean, $n = 3$.

contribute to browning reactions and decreased colour stability of anthocyanins (Es-Safi, Cheyner, & Moutounet, 2002).

3.4. Total soluble phenolics

Soluble phenolics were quantified based on the total reducing capacity of açai juice and fractions, according to the procedures of Singleton and Rossi (1965). As observed for anthocyanins, total phenolic contents decreased markedly (33–42%) during initial stages of storage (first 6 days), with only minor changes subsequently observed (Fig. 3). Initial phenolic contents varied among the juice fractions, depending not only on recovery rates from the solid phase cartridges, but also on the presence of components in the C18 unbound part that contributed 6–9% of the total reducing capacity of the system. Losses in soluble phenolics were correlated to total anthocyanin contents ($r = 0.88$) and to changes in both cyanidin-3-glucoside ($r = 0.76$) and cyanidin-3-rutinoside ($r = 0.87$). However, losses in total soluble phenolics could not be entirely attributed to changes in anthocyanin concentrations, given that different degradation patterns were observed. While monomeric anthocyanins in the juice system decreased by more than 80% after 12 days, only 40% of the total soluble phenolic content was lost during the same period and this indicated the influence of reducing agents in the system, including polymeric anthocyanin forms. Changes in soluble phenolics were likely due to oxidation reactions, involving both anthocyanin and non-anthocyanin polyphenolic components (Es-Safi et al., 2002; Santos-Buelga, Bravo-Haro, & Rivas-Gonzalo, 1995) that adversely impact the total reducing capacity of the system (Klopotek, Otto, & Bohm, 2005).

3.5. Antioxidant capacity

Açai juice had an antioxidant capacity of 61.5 ± 1.21 μmol trolox equivalents/ml and this was comparable to previously reported values for açai pulps (Lichtenthaler

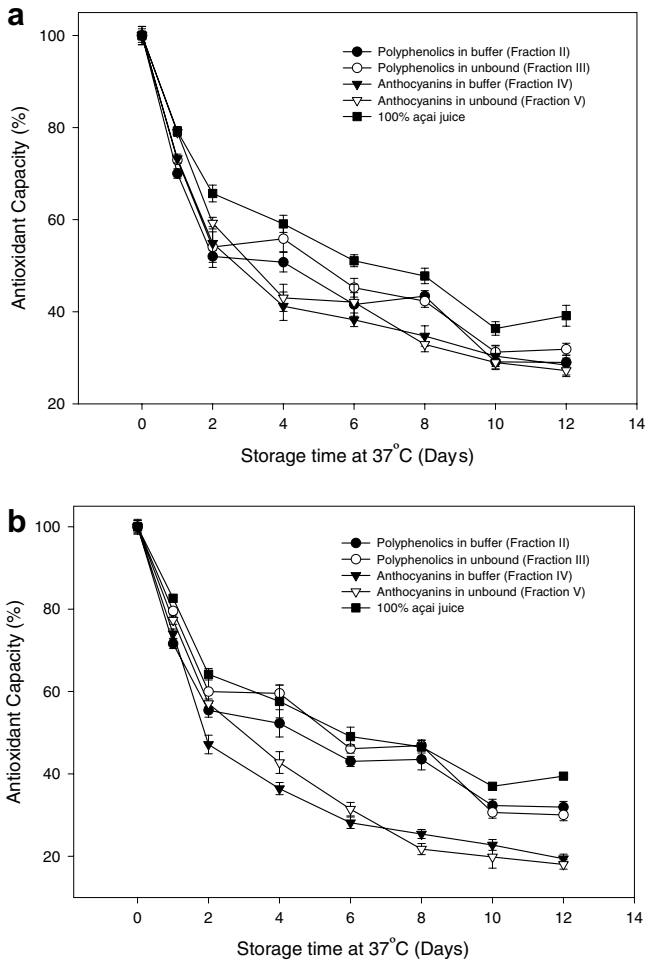

Fig. 4. Percent of initial antioxidant capacity in non-fortified (a) and ascorbic acid (500 mg/l)-fortified (b) açai fractions during storage at 37 °C. Error bars represent the standard error of the mean, $n = 3$.

et al., 2005). Only minor differences were initially detected between juice (Fraction I) and juice fractions (Fractions II–V), suggesting that the presence of non-anthocyanin polyphenolics had only a minor influence on the overall antioxidant capacity of fractions. Moreover, no antioxidant capacity was associated with components present in the unbound (trace sugars, metals, proteins). Therefore, polyphenolics present in all fractions, such as anthocyanins, gallic acid derivatives, and procyanidins, were likely major contributors to the antioxidant capacity of açai juice and juice fractions. Throughout storage, antioxidant activities of açai juice and juice fractions decreased appreciably (>60%) and paralleled losses in total and individual anthocyanins ($r = 0.85$–0.90), soluble phenolics ($r = 0.83$), and individual polyphenolics ($r = 0.72$–0.78). Açai juice and polyphenolic fractions (Fractions II and III) retained appreciably higher antioxidant capacity levels throughout storage than did anthocyanin fractions (Fractions IV and V) (Fig. 4), suggesting a protective effect of non-anthocyanin polyphenolics on the retention of antioxidant capacity as radical scavengers and as metal chelators (Robbins, 2003). In the presence of ascorbic acid, anthocyanin fractions (Fractions IV and V) underwent greater losses in antioxidant capacity than did juice or polyphenolic fractions (Fractions II and III), which further confirmed the protective role of non-anthocyanin polyphenolics in the prevention of additional oxidation reactions.

4. Conclusion

Polyphenolic, antioxidant, and colour stabilities of açai juice are dependent on interactions among its matrix components and are influenced by processing, storage, temperature and chemical composition. While colour stability was favoured by anthocyanin isolation, the retention of non-anthocyanin polyphenolics and antioxidant capacity was enhanced by the natural polyphenolic composition of the juice matrix. Following ascorbic acid fortification, the presence of non-anthocyanin polyphenolics favoured not only anthocyanin, but overall polyphenolic and antioxidant stability in açai juice and polyphenolic fractions. The majority of colour, phytochemical and antioxidant losses occurred during the first 6 days of storage; therefore, the maximum quality retention and functional properties of açai-containing products can be achieved by proactive monitoring of processing, storage and distribution conditions.

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