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The high ascorbic acid content is the main cause of the low stability of anthocyanin extracts from acerola

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Abstract

Acerola is considered to be one of the best natural sources of ascorbic acid (AA) and, for this reason, the influence of this component on the stability of anthocyanins from acerola extracts was determined and compared to those from açai, which have no detectable AA. The addition of three different levels of AA to the solution of açai anthocyanins resulted in a 110-fold increase in the degradation rate (k_{obs}) at the highest fortification level (276 mg/ml). The fact that the flavonoid concentration of the açai anthocyanin extract was 10 times higher than that of the acerola was probably responsible for the three times higher stability of the AA-fortified açai system compared to the acerola system, both at the same AA concentration and similar total polyphenol levels. The higher the level of AA addition to açai anthocyanin solutions, the greater was the colour fading, indicated by increase of L^* and decrease of a^* and C^* values.

Keywords: Anthocyanins; Ascorbic acid; Stability; Acerola; Açai; Colour

1. Introduction

The anthocyanins belong to the flavonoid class of compounds, and are responsible for the attractive colours, varying from red to blue, found in flowers and fruits (Strack & Wray, 1993). Due to the great variety of hue colours and their solubility in water, the anthocyanins represent an alternative with good potential to substitute the red-bordeaux artificial dyes in foods. However, many commercial limitations exist for the application of anthocyanin extracts in food products, including their low stability, which is influenced by pH, temperature, oxygen, light, polymeric forms, concentration, the presence of phenolic compounds and the chemical structure.

The low stability of the acerola anthocyanins represents a problem during the storage of the pasteurized juice and frozen pulp of this fruit. Due to the colour fading that occurs in these products, dyes, such as cochineal, tartrazine yellow and Bordeaux red have been added to these products in Brazil.

The presence of ascorbic acid (AA) has shown a negative impact on anthocyanin stability, leading to the mutual degradation of these compounds (Rodriguez-Saona, Giusti, & Wrolstad, 1999; Garzón & Wrolstad, 2002; Brenes, Del Pozo-Insfran, & Talcott, 2005). The mechanism proposed by Jurd (1972), and later reinforced by Poei-Langston and Wrolstad (1981), for the degradation of anthocyanins in the presence of AA consists of direct condensation of AA on the carbon 4 of the anthocyanin molecule, causing the loss of both. On the other hand, according to Iacobucci and Sweeny (1983), the loss of anthocyanin colour, caused by AA, occurs due to oxidative cleavage of the pyrilium ring by a free radical mechanism in which the AA acts as a molecular oxygen activator, producing free radicals. In addition, Shrikhande and Francis (1974) reported that the presence of flavonol exerted a protective effect with respect to the degradation of anthocyanins in the presence of AA, probably by competition with

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the anthocyanins in the preference for condensation reactions. The negative effect caused by the AA-fortification on colour of grape anthocyanins was also verified by instrumental colour measurement, leading to an increment in lightness (L^*) and decrease in red colour (a^*) (Brenes et al., 2005).

The number of hydroxyl and methoxyl groups, and the nature and number of sugars and acids attached to the sugars, have a great effect on the anthocyanin stability (Mazza & Miniati, 1993). Diglycosidic substitution confers higher stability to the molecule than does monoglycosidic (Markakis, 1982; Mazza & Miniati, 1993). The presence of sugar residues acylated with acids promotes greater stability, preventing hydration reactions (Asen, Stewart, & Norris, 1972; Giusti & Wrolstad, 1996). It must be mentioned that, independently of the amount of natural or added AA, the relative stability of the various anthocyanins, due to their different structures, remains as cited above (Hrazdina & Franzese, 1974; Garcia-Viguera & Bridle, 1999; Del Pozo-Insfran, Brenes, & Talcott, 2004).

Considering that acerola is recognized as one of the best sources of AA (Vendramini & Trugo, 2000; Assis, Lima, & Faria-Oliveira, 2001), the objective of the present study was to evaluate the effect of high levels of AA on the colour and stability of acerola anthocyanin solutions and compared to that of açai, another tropical fruit, but one containing no detectable AA in its composition. In addition, the contribution of other compounds, environmental conditions and the chemical structures of the anthocyanins found in these fruits were also considered.

2. Materials and methods

2.1. Materials

High performance liquid chromatography (HPLC) grade standards of catechin (Fluka, Steinheim, Germany), gallic acid (Extrasynthèse, Genay, France) and solvents (Merck, Darmstadt, Germany) were used. The other reagents used were all of analytical grade, citric acid and sodium phosphate (dibasic) were from Labsynth (Diadema, Brazil), and the Folin-Ciocalteau reagent and ascorbic acid from Merck. The water was purified by the Milli-Q system (Millipore, Bedford, United States) and the samples and solvents were filtered through Millipore membranes of 0.22 and 0.45 μm, respectively.

2.2. Anthocyanin extracts

Acerola fruits *in natura* (4 kg) from the region of Campinas, São Paulo State, Brazil, were pulped in a knife pulper to remove skins and seeds. The açai was obtained in the form of commercial frozen pulp (1 kg) in Campinas. The fruit pulps were exhaustively extracted with 1% HCl in methanol, with agitation provided by a homogenizer (Metabo, Nurtingen, Germany). The solution obtained was then filtered and vacuum-concentrated ($T < 38$ °C) to complete evaporation of the methanol. This concentrated crude extract of anthocyanins (CE) was stored at −18 °C under nitrogen.

2.3. Model-systems

The anthocyanin systems were prepared with the acerola and açai CE in citrate-phosphate buffer, pH 2.5, plus 0.1% sodium benzoate to prevent microbial growth. Since the buffer solution prepared with the acerola CE presented an AA concentration of 276 mg/100 ml, the açai model-system solutions were fortified with AA to the same level (276 mg/100 ml) and also at half this concentration (138 mg/100 ml). A further sample was prepared with an addition of 30 mg/100 ml, a value used previously in blood orange juice (Choi, Kim, & Lee, 2002).

For each fruit, the same ratio of CE mass to buffer volume was used in order to give an initial absorbance (A_0) of *ca.* 0.8, measured at the maximum absorption wavelength in the visible region (λ_{max}). The solutions were allowed to rest for 3 h in the absence of light, to attain the equilibrium among the different forms of anthocyanin, the absorbance being monitored every hour. The solutions were then distributed in screw-capped tubes with a nominal volume of 10 ml. The solutions contained in part of the tubes were aspersed with nitrogen (99.99% purity), and the tubes were then sealed. The tubes were placed in a support between two 32 W fluorescent lamps, corresponding to 850 lux, in a room free of other light source at a temperature of 20 ± 1 °C. Tubes of each solution were also maintained in the same room in the dark, to serve as control.

2.4. Monitoring of the model-systems

The stability of the anthocyanins was monitored using a Beckman DU-70 spectrophotometer (Fullerton, United States), measuring the solution absorbance at λ_{max} up to approximately 75% loss as compared to the initial absorbance.

In addition, the colour changes of the anthocyanin solutions were determined from the CIELAB parameters, using the Color Quest XE colorimeter (Hunter Lab., Reston, United States) equipped with the light source D_{65} and observation angle of 10°. Using the parameters L^* (lightness), a^* (red) and b^* (yellow), the values for C^* (chroma), h (hue angle) and ΔE^* (total colour difference) were calculated using Eqs. (1)–(3).

$$C^* = \left[(a^*)^2 + (b^*)^2\right]^{1/2} \quad (1)$$

$$h = \arctan\left(b^*/a^*\right) \quad (2)$$

$$\Delta E^* = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2} \quad (3)$$

The relative loss of each anthocyanin in the various systems was evaluated by HPLC, taking aliquots at the beginning, middle and end of the experiment period. Immediately,

solvent and water were removed from the aliquots, under nitrogen with addition of absolute alcohol. The dried anthocyanin extracts were re-dissolved in acidified methanol immediately before the HPLC analysis. A high efficiency liquid chromatography apparatus (Waters, Milford, United States), consisting of a quaternary solvent pumping system, Rheodyne injector with a 20 μl loop, external oven with temperature control, on-line degasser, diode array detector (PDA) and Millenium data collection and processing system, was used. The anthocyanins were separated on a C_{18} Shim-pak CLC-ODS column (5 μm, 250 × 4.6 mm) from Shimadzu (Canby, United States), using, as mobile phase, a linear gradient of 5% aqueous formic acid/methanol, going from 85:15 (v/v) to 20:80 in 25 min, the isocratic proportion being maintained for a further 15 min, at a flow rate of 1 ml/min and column temperature maintained at 25 °C. The chromatograms were processed at 280, 320 and 520 nm, and the spectra acquired between 200 and 600 nm.

2.5. Phytochemical analysis

The total anthocyanin contents of the acerola and açai pulps and of their respective CE were determined using the differential pH method (Lee, Durst, & Wrolstad, 2005) and quantified using the absorption coefficient of $A^{1\%}_{1\,cm} = 982$, corresponding to the anthocyanin extract of cranberry (Francis, 1982). The total polyphenols and flavonoids were extracted according to Singleton and Rossi (1965), the polyphenols being quantified using the Folin-Ciocalteau reagent and the flavonoids by the Zhinshen, Mengcheng and Jianming method (1999), the results being, respectively, expressed in equivalents of gallic acid (GAE) and catechin (CCE). The contribution of AA in the Folin-Ciocalteau reaction was established in the solutions prepared from the acerola CE. The AA was determined by HPLC, using the equipment and column described above and, as mobile phase, an isocratic elution of aqueous sulphuric acid solution at pH 2.5, flow rate at 0.7 ml/min, column temperature of 25 °C and chromatogram processing at 254 nm (Souza, Benassi, Meneghel, & Silva, 2004). Each sample was analyzed in duplicate and quantified with an external calibration curve, the area of the sample peak being compared to that of the AA standard.

2.6. Kinetic calculations and statistical analysis

The residual anthocyanins (%) were plotted against time (h) and a linear regression analysis was used to determine the adequacy of the anthocyanin degradation kinetic model. The degradation rate constant (k_{obs}) was determined from the first-derivative of the curves plotted (Eq. (4)) and the half-life time was calculated from Eq. (5).

$$[\text{anthocyanin}] = [\text{anthocyanin}]_0 * \exp(k_{obs} \cdot t) \quad (4)$$

$$t_{1/2} = \frac{\ln 2}{k_{obs}} \quad (5)$$

The ANOVA analyses were carried out using the Student's *t*-test at a significance level of 95%. The software Microcal Origin 5.0 was used for all the analyses, both statistical and kinetic.

3. Results and discussion

3.1. Influence of AA on the anthocyanin extract stability

In all systems evaluated, the degradation of the anthocyanins followed first-order kinetics. Independently of the condition, the açai CE showed higher stability than did that from acerola (Table 1).

The addition of 276 mg AA/100 ml to the açai anthocyanin solution caused a 109- to 116-fold increase in the

Table 1
Rate constants (k_{obs}) and half-life times ($t_{1/2}$) for anthocyanin degradation in acerola and açai systems in citrate-phosphate buffer at pH 2.5 and at 20 °C

Model-systems	k_{obs} (h^{-1})[a]	$t_{1/2}$ (h)[a]	*r*
Acerola-CE air/light	5.05×10^{-2}	13.7	0.998
Acerola-CE air/dark	3.95×10^{-2}	17.5	0.996
Acerola-CE N_2/light	3.70×10^{-2}	18.7	0.991
Acerola-CE N_2/dark	3.18×10^{-2}	21.7	0.990
Açai-CE air/light	7.64×10^{-4}	909.0	0.987
Açai-CE air/dark	1.07×10^{-4}	6456.2	0.926
Açai-CE N_2/light	5.43×10^{-4}	1275.6	0.993
Açai-CE N_2/dark	8.41×10^{-5}	8235.0	0.914
Açai-CE + AA (276 mg/100 ml) air/light	1.65×10^{-2}	42.0	0.997
Açai-CE + AA (276 mg/100 ml) air/dark	1.25×10^{-2}	55.2	0.994
Açai-CE + AA (276 mg/100 ml) N_2/light	1.04×10^{-2}	66.6	0.971
Açai-CE + AA (276 mg/100 ml) N_2/dark	9.18×10^{-3}	75.5	0.984
Açai-CE + AA (138 mg/100 ml) air/light	1.46×10^{-2}	47.6	0.999
Açai-CE + AA (138 mg/100 ml) air/dark	1.18×10^{-2}	58.4	0.981
Açai-CE + AA (30 mg/100 ml) air/light	1.03×10^{-2}	66.9	0.991
Açai-CE + AA (30 mg/100 ml) air/dark	8.87×10^{-3}	78.1	0.989

[a] Average of 2 sets of experiments.

degradation rate (k_{obs}) when compared to non-fortified açai, respectively, under nitrogen and air, both in the absence of light (Fig. 1A and Table 1). With the fortification level decreasing to 138 and 30 mg/100 ml, a non-linear decrease in the k_{obs} values was observed for the degradation of açai anthocyanins (Fig. 1A); in other words, the anthocyanin degradation was not dose-dependent on the AA concentration. Under dark conditions, at 37 °C and pH 3.5, Del Pozo-Insfran et al. (2004) verified a 30-fold increase in the açai anthocyanin degradation rate after AA addition at a concentration of 45 mg/100 ml; however, a 83-fold increase was verified with 30 mg/100 ml AA fortification in the present study. This difference can either be related to the greater AA degradation at higher temperature and pH values or to the different compositions of the açai samples.

The k_{obs} value obtained for the acerola-CE solution was three times faster than that of the açai solution fortified at 276 mg AA/100 ml, although the two solutions presented the same AA concentration (Fig. 1B, Table 1). Since the total polyphenol contents of both fruit extracts were similar, this difference could be attributed to the fact that the flavonoid concentration in the açai anthocyanin extract was 10 times higher than that found in the acerola one (Table 2). The flavonoids can protect the anthocyanins by inter-molecular co-pigmentation, due to decreased carbinol pseudobase production and increased quinonoidal anhydrobase stabilization (Mazza & Brouillard, 1990). Shrikhande and Francis (1974) also reported such a protective effect in the presence of flavonoids, such as quercitin and quercitrin, which retarded the degradation of cranberry anthocyanins by 20% in the presence of AA.

Table 2
Phytochemical characteristics of pulps and anthocyanin extracts from acerola and açai

Phytochemical characteristics	Acerola	Açai
Total anthocyanins (mg/100 g)[a]	7.21	282.5
Monomeric anthocyanins (%)[b]	75.0	62.0
Total polyphenols (mg GAE/100 g)[b]	3300	3783
Total flavonoids (mg CCE/100 g)[b]	53.3	537.0
Ascorbic acid (mg/100 g)[b]	1921	Nd

Average of duplicate analysis.
n.d., not detected; GAE, gallic acid equivalents; CCE, catechin equivalents.
[a] Determined in the acerola and açai pulps.
[b] Determined in the acerola and açai anthocyanin crude concentrated extract.

The influence of the anthocyanin structures on the systems stability can be discarded since the major anthocyanins in acerola were reported to be cyanidin-3-rhamnoside and pelargonidin-3-rhamnoside (Hanamura, Hagiwara, & Kawagishi, 2005) and cyanidin-3-glucoside and cyanidin-3-rutinoside were found in açai (Gallori, Bilia, Bergonzi, Barbosa, & Vincieri, 2004; Lichtenthäler et al., 2005). None of the anthocyanins, from both sources, presented acylated groups which could promote greater stability of the anthocyanins, such as was observed in red grape and hibiscus anthocyanins (Del Pozo-Insfran et al., 2004). In addition, both acerola and açai fruits presented cyanidin as the major aglycone in their composition, and thus the contributions of hydroxyl and methoxyl groups were identical. In the same way, since the major anthocyanins from both fruits presented monoglycosidic substitution, there was no difference in the stability due to glycosidic substitution.

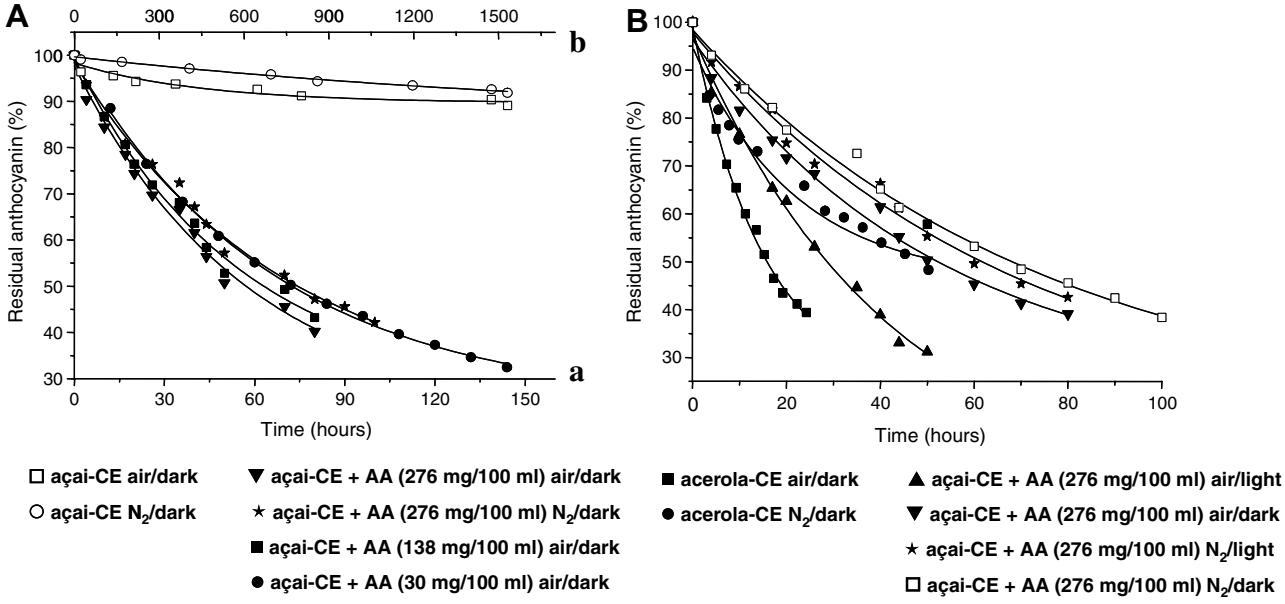

Fig. 1. Degradation kinetic curves of anthocyanins from acerola and açai fortified with AA at different levels; in citrate-phosphate buffer at pH 2.5. In (A), scale a: açai-CE + AA, scale b: açai-CE.

3.2. Effect of oxygen and light

The degradation rates found for both the acerola and açai anthocyanin solutions in an inert atmosphere were ca. 1.3 –1.4 times slower than in air, both in the presence and absence of light (Fig. 2, Table 1). For the AA-fortified (276 mg/100 ml) açai anthocyanin systems, the k_{obs} values under nitrogen were 1.8 and 1.4 times slower than under air, respectively, under light and dark (Fig. 1B) conditions. Our results were similar to those reported for cyanidin-3-glucoside degradation, present in an elderberry extract, with k_{obs} values 1.6 times faster in the presence of oxygen than under nitrogen (Kaack & Austed, 1998), as well as to those found by Chan and Yamamoto (1994) for the stability of anthocyanins in acerola juice, which were 1.8 times higher when stored under nitrogen than under oxygen.

The deleterious effect of light was more intense for the anthocyanins of açai than for those from acerola (Fig. 2). The k_{obs} values found for the açai anthocyanin systems in the dark were 7.1 times slower under air and 6.5 times lower under nitrogen than those found in the presence of light. On the other hand, for the acerola anthocyanin systems, the k_{obs} values found in the dark were only 1.3 and 1.2 times slower than in the light, respectively, under air and nitrogen. In addition, the k_{obs} values found for the 276 mg/100 ml concentration of AA-fortified açai model-systems in the dark were also only about 1.3 times slower than in the presence of light, either under air or nitrogen. These results show that the deleterious effect of the AA, naturally present or added to anthocyanin solutions, is much higher than that of any other factor, such as the presence of light or oxygen. Thus the presence of AA is the main cause of anthocyanin degradation in acerola.

The mutual degradation of anthocyanins and ascorbic acid (AA) has been demonstrated in various systems (Iacobucci & Sweeny, 1983; Skrede, Wrolstad, Lea, & Enersen, 1992; Iversen, 1999), although the reaction mechanism has not been completely elucidated. Since, in the absence of light, the k_{obs} value of the non-fortified AA açai system was 109 times slower than that observed for the fortified system under inert atmosphere and 116 times slower in the presence of oxygen, it is evident that the main degradation pathway occurred due to the condensation reaction between the AA and anthocyanins, with a small contribution from the free radical reaction. Skrede et al. (1992) also suggested direct condensation as the reaction leading to anthocyanin degradation in strawberry syrup after the addition of AA.

3.3. Changes in the relative composition of the anthocyanins

During storage of all açai and acerola anthocyanin systems, a gradual decrease in the peak area of each anthocyanin was verified, without the appearance of intermediate degradation compounds absorbing at 520, 320 and 280 nm. Rodriguez-Saona et al. (1999) also did not detect anthocyanin degradation products in a red radish juice type model-system after 26 weeks of storage at 25 °C, in either the presence or absence of light.

Independently of the environmental conditions, the acerola anthocyanins, cyanidin-3-rhamnoside, pelargonidin-3-rhamnoside and cyanidin-3,5-dirhamnoside, which represented, respectively, 77%, 14% and 7% of the total area at zero time, showed area losses of 70%, 85% and 92% after 24 h, whilst peonidin-3-rhamnoside, which presented 3% of the relative area at zero time, disappeared in 24 h (Fig. 3A).

However, the relative degradation of açai anthocyanins was dependent on the conditions of the system. For example, cyanidin-3-glucoside and cyanidin-3-rutinoside, which corresponded to, respectively, 13% and 87% of the total relative area, were degraded by 63% and 56% under light and oxygen (Fig. 3B), whereas these values decreased to 50% and 43% under light and nitrogen, both in 1532 h. On the other hand, the relative anthocyanin degradations were similar, under the different conditions, for each level of AA fortification of the açai systems.

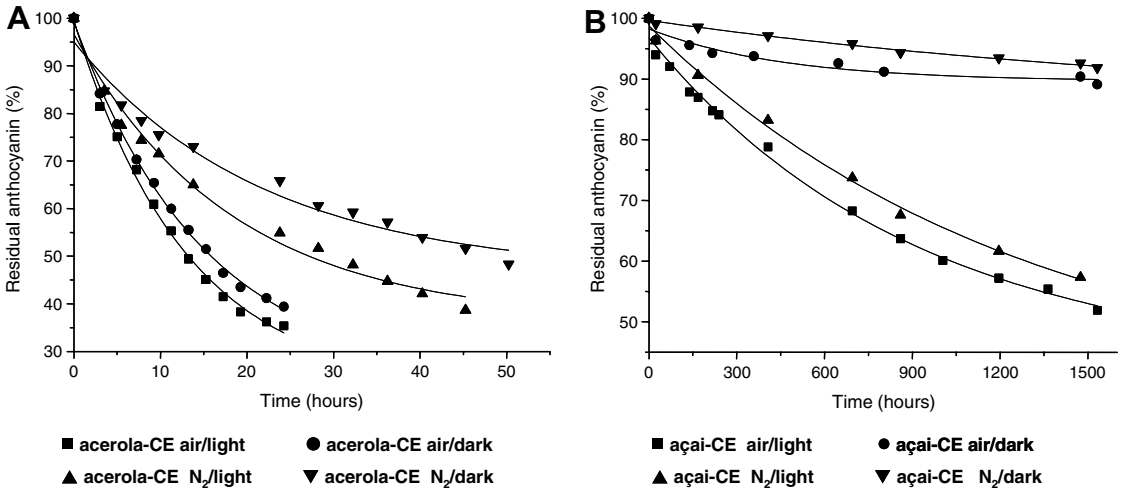

Fig. 2. Degradation curves of anthocyanin extracts from (A) acerola and (B) açai, in citrate-phosphate buffer at pH 2.5.

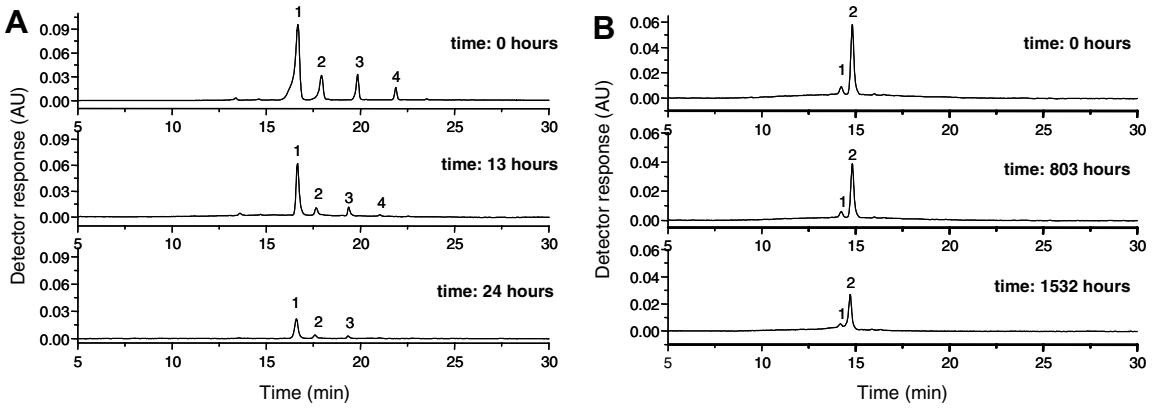

Fig. 3. HPLC-PDA chromatograms obtained during the degradation of (A) acerola and (B) açai anthocyanins in phosphate-citrate buffer at pH 2.5, in the presence of light and air. See text for chromatographic conditions.

Since the structures of the acerola and açai anthocyanins can be considered similar, the concentration of each anthocyanin in the systems was the factor most influencing the degradation, considering that the anthocyanin degradation follows first-order kinetics.

3.4. Changes in the ascorbic acid concentration

The losses of AA, either natural or added, verified by HPLC at the beginning, middle and end of storage, showed that the presence of light did not influence AA degradation (Table 3). The AA degradation was 1.2 and 1.9 times lower under nitrogen than under air, respectively, in the acerola and for the 276 mg/100 ml concentration of AA-fortified açai systems. Iversen (1999) also verified that light did not show a significant effect on AA degradation in blackcurrant nectar; however, de-aeration of the blackcurrant nectar did not increase the stability of the AA in relation to the nectar stored under oxygen.

Under the same conditions, AA degradation in the acerola system was slightly higher than that in the açai system fortified with 276 mg AA/100 ml (Table 3), probably due to the highest flavonoid concentration in the açai anthocyanin extract. In this type of interaction, the flavonoids can act in two ways: one by competition between the flavonoids and the AA for the condensation reaction with the anthocyanins, and the other by interfering in the free radical formation reaction, which would lead to AA auto-oxidation (Shrikhande & Francis, 1974). The influence of the ratio between the concentrations of the anthocyanins and flavonoids would be relevant in both mechanisms, apart from the chemical structures of the anthocyanins and flavonoids.

Table 3
Ascorbic acid degradation in acerola and AA-fortified açai anthocyanin systems in citrate-phosphate buffer at pH 2.5

Model-systems	Degradation (%)		
	t_0	t middle	t final
Acerola-CE air/light	0	4.6	8.1
Acerola-CE air/dark	0	4.5	8.0
Acerola-CE N_2/light	0	3.4	6.9
Acerola-CE N_2/dark	0	3.4	6.7
Açai-CE + AA (276 mg/100 ml) air/light	0	3.9	7.4
Açai-CE + AA (276 mg/100 ml) air/dark	0	3.9	7.4
Açai-CE + AA (138 mg/100 ml) air/light	0	6.8	12.6
Açai-CE + AA (30 mg/100 ml) air/light	0	10.3	18.8
Açai-CE + AA (276 mg/100 ml) N_2/light	0	2.5	3.9
Açai-CE + AA (276 mg/100 ml) N_2/dark	0	2.5	3.9

In AA-fortified açai model-systems, the higher the addition of AA, the lower is its degradation, indicating a first-order degradation during storage, as previously found (Skrede et al., 1992; Iversen, 1999).

In the present study, the anthocyanin degradation was between 5 and 10 times faster than the AA degradation. Iversen (1999) also observed this behaviour in blackcurrant nectar stored under light at 20 °C, where the anthocyanin degradation rate was four times faster than that of the ascorbic acid.

3.5. Colour stability

Differences in the colour parameters of the acerola and açai systems can be attributed to various factors, such as the different quantitative and qualitative compositions of the pigments, the nature of the phenolic compounds and the proportion between these compounds and the anthocyanins in the extracts.

The parameter L^* is associated with the intensity of luminosity that the solution transmits, and the linear behaviour of the $\ln L^*$ versus time curve (Fig. 4A) confirmed that the anthocyanin degradation, in all systems, followed first-order kinetics.

The parameter C^* is related to colour intensity and hence, as degradation occurred, the C^* values also decreased (Fig. 4B). The negative effect caused by AA-fortification on colour was easily distinguishable, resulting in colour fading (increased L^* and decreased C^* and a^* values) during storage.

The hue angle parameter (h) defines the species of colour, which can be yellow, red, blue, green or any combination of

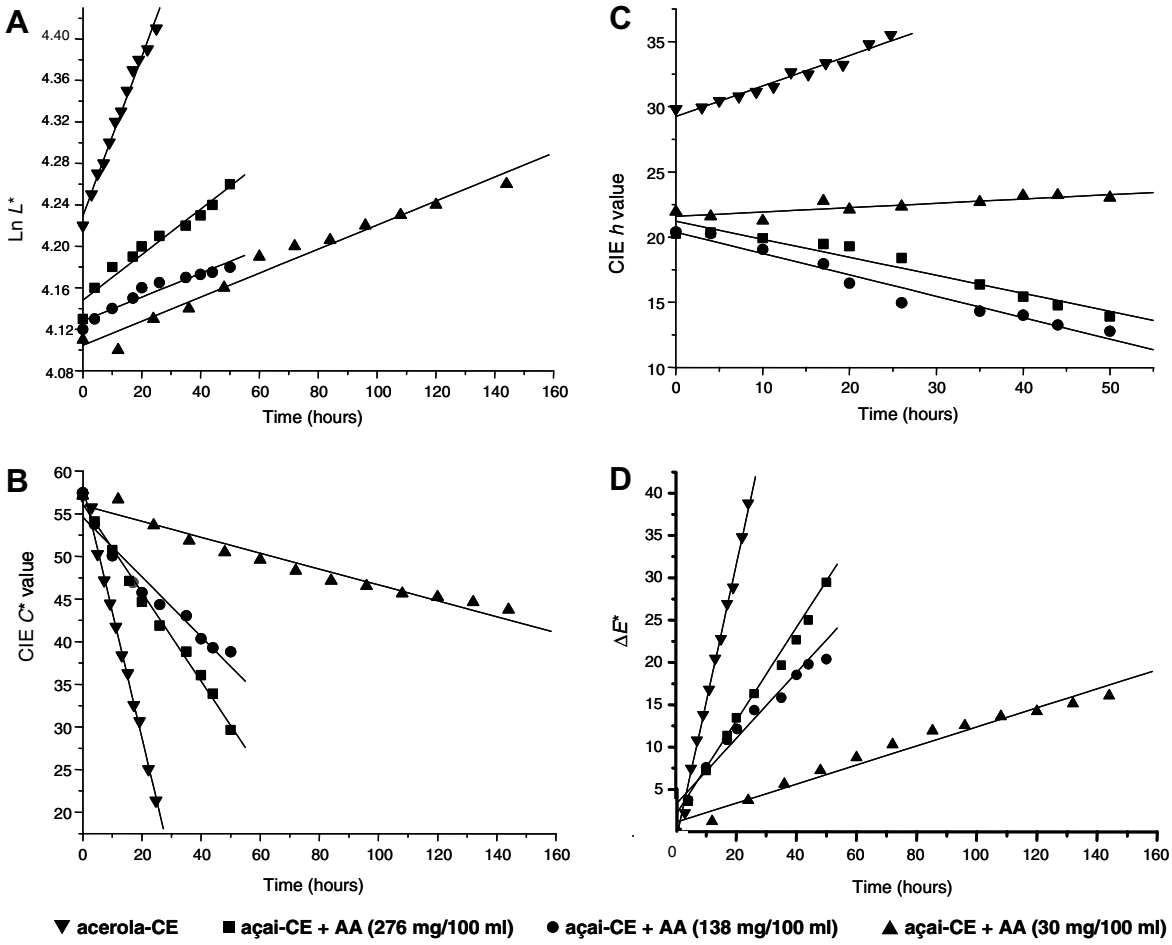

Fig. 4. Changes in the colour parameters values (A) ln L^*, (B) chroma (C^*), (C) hue angle (h) and (D) ΔE^* for acerola and AA-fortified açai systems of anthocyanin extracts in phosphate-citrate buffer at pH 2.5, in the presence of light and air.

these colours. During storage, the h values increased in acerola and in non-fortified and fortified (30 mg AA/100 ml) açai solutions, whilst h values decreased in açai solutions fortified with AA at higher levels (Fig. 4C). The different behaviour of the h values, depending on the AA-fortification level and anthocyanin source, is related to the changes of a^* and b^* values at different rates in all systems (Fig. 5), indicating that the colour was changing from orange-red to yellow during storage of acerola systems, whereas the tonality of the AA-fortified (276 mg/100 ml and 138 mg/100 ml) açai solutions remained red. Brenes et al. (2005) also reported that the h values of anthocyanin solutions fortified with AA at 45 mg/100 ml increased.

As can be seen in Fig. 5, a^* and b^* values decreased in all systems, with the exception of b^* values in the açai system, which increased during storage, most probably due to formation of yellow and brown polymerization compounds during the longest storage time (12:00 h). The initial a^* and b^* values found in acerola systems were 49.52 and 28.40, respectively, while the açai system fortified with AA at 276 mg/100 ml showed an initial a^* value of 53.71 and b^* value of 19.84. The initial higher b^* value in the acerola systems can be related to the highest carotenoid concentration in this fruit, 370–1881 μg/100 g (De Rosso & Mercadante, 2005), as compared to that in açai 223–307 μg/100 g (De Rosso, Zanatta, Silva, & Mercadante, 2005). At the final storage period under air and light, the values found in the acerola system were 17.39 for a^* and 12.41 for b^*, whilst for AA-fortified (276 mg/100 ml) açai solution, the corresponding values were 28.79 and 7.14. In acerola systems the values decreased 2.8 times for a^* and 2.3 times for b^*, whereas the opposite behaviour was observed in the AA-fortified (276 mg/100 ml) açai solution, the loss of b^* value being higher than that found for a^*, respectively, 2.8 and 1.9 times.

The overall colour difference (ΔE^*) was more intense in the acerola systems, followed by the açai in decreasing fortification order (Fig. 4D). The final values of ΔE^* were 29.4, 20.4 and 16.0 for the açai systems fortified with AA at concentrations of 276, 178 and 30 mg/100 ml, respectively, and the value for the acerola system was 38.8, all under light and air. In açai systems under the same conditions, the ΔE^* value was 26.7 at the final storage time (1200 h), whilst a much smaller value, $\Delta E^* = 4.3$, was found at 50 h, same storage time to that of the açai AA-fortified systems.

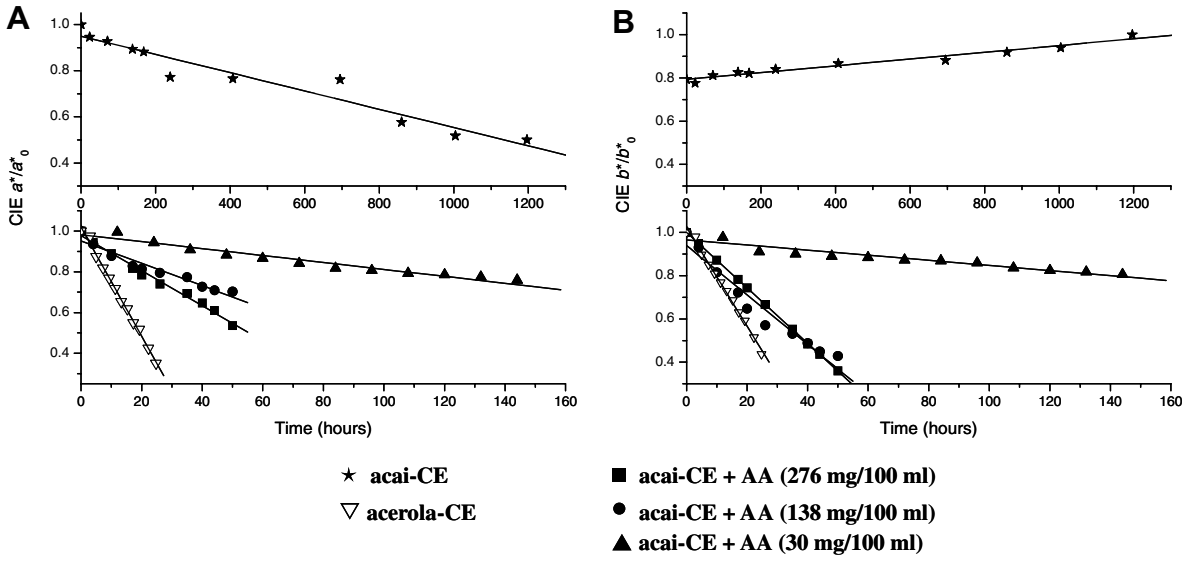

Fig. 5. Changes in the colour parameters (A) a^*/a_0^* and (B) b^*/b_0^* values for anthocyanin extracts from acerola and from açai in phosphate-citrate buffer solutions at pH 2.5, in the presence of light and air.

Since a threshold value of $\Delta E^* = 1$ is assumed as a basis for a colour difference noticeable by the human eye (Gonnet, 1998) and in the present study the final ΔE^* values were greater than 1, this fact proves that fortification at the higher AA level produced a greater fade. In addition, the final ΔE^* value of the acerola system was greater than that calculated for the açai system fortified with 276 mg AA/100 ml, indicating that the presence of other compounds in the anthocyanin extracts also had a considerable effect on the colour (Gonnet, 1998).

In summary, the results of the present study prove that the presence of elevated concentrations of ascorbic acid is the main cause of the low stability of the acerola anthocyanins, which occurs mainly due to the direct condensation of the AA on carbon 4 of the anthocyanin, resulting in losses of both components. Anthocyanin degradation is responsible for the loss in red colour of the frozen acerola pulp and processed juice, the main problem occurring during the commercial storage of these products.

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