Evaluation of anthocyanin stability during storage of a coloured drink made from extracts of the Andean blackberry (*Rubus glaucus* Benth.), açai (*Euterpe oleracea* Mart.) and black carrot (*Daucus carota* L.)

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Abstract – Introduction. The effect of temperature on the stability of three purified anthocyanin sources in a soft drink (pH 3, 10 °Brix) stored at (4, 20, 30 and 50) °C for 60 days was investigated. **Materials and methods**. Anthocyanins from Andean blackberries (*Rubus glaucus* Benth.), açai *(Euterpe oleracea* Mart.) and black carrot (*Daucus carota* L.) were purified and concentrated on a laboratory scale by adsorption to a styrene divinylbenzene copolymer. Two classical empirical approaches (Arrhenius and Ball models) were used to describe the thermal degradation kinetic of these three anthocyanins. **Results**. No degradation was detected during the refrigerated storage (4 °C). At all temperatures, the degradation rate constant (k) for black carrot anthocyanins was less than those in açai and blackberry (0.42×10^{-2}, 0.77×10^{-2} and 1.08×10^{-2})·d^{-1}, respectively, at 30 °C). Anthocyanins in black carrot degraded less rapidly than those in açai and Andean blackberry. The activation energy (E_a) for degradation of black carrot anthocyanins was (63.2 ± 4.3) kJ·mol^{-1}, and (66.3 ± 2.7) kJ·mol^{-1} and (91.2 ± 0.4) kJ·mol^{-1} for açai and blackberry anthocyanins, respectively, at 20–50 °C. These higher E_a of blackberry anthocyanins as compared with those of black carrot and açai imply that a small temperature increase is sufficient to degrade them more rapidly. **Conclusion**. Our results clearly showed that anthocyanins from black carrot have a good stability during thermal storage (4 °C to 50 °C) with regard to blackberry and açai anthocyanins. Acylation of black carrot anthocyanins probably explains their greater stability. Acylated anthocyanins have shown to be promising alternatives to the use of synthetic dyes in drink systems.

France / *Rubus glaucus* / *Euterpe oleracea* / *Daucus carota* / fruits / soft drinks / plant extracts / storage / anthocyanins / degradation

Évaluation de la stabilité des anthocyanes au cours du stockage d'une boisson colorée par des extraits de mûres andines (*Rubus glaucus* Benth.), d'açaï (*Euterpe oleracea* Mart.) et de carottes noires (*Daucus carota* L.).

Résumé – Introduction. Nous avons étudié l'effet de la température sur la stabilité de trois sources d'anthocyanes purifiées, dans une boisson gazeuse (pH 3, 10 °Brix) stockées à (4, 20, 30, 50) °C pendant 60 jours. **Matériel et méthodes**. Des anthocyanes de mûre andine, d'açaï et de carotte noire ont été purifiées et concentrées en laboratoire par adsorption sur un copolymère de styrène divinylbenzène. Deux approches empiriques classiques (modèles d'Arrhenius et de Ball) ont été utilisées pour décrire la cinétique de dégradation thermique de ces trois anthocyanes. **Résultats**. Aucune dégradation n'a été détectée au cours du stockage réfrigéré (4 °C). A toutes les températures, la constante de la vitesse de dégradation (k) pour les anthocyanes de la carotte noire a été inférieure à celle de l'açaï et de la mûre ($0{,}42 \times 10^{-2}$, $0{,}77 \times 10^{-2}$ et $1{,}08 \times 10^{-2}$)·jour^{-1}, respectivement, à 30 °C. Les anthocyanes de la carotte noire se sont dégradées moins rapidement que celles de l'acaï et de la mûre andine. À 20–50 °C, l'énergie d'activation (E_a) a été de (63,2 ± 4.3) kJ·mol^{-1} pour la dégradation des anthocyanes de la carotte noire, de (66,3 ± 2.7) kJ·mol^{-1} pour l'acaï et de (91,2 ± 0.4) kJ·mol^{-}1 pour la mûre. Cette énergie d'activation E_a des anthocyanes de la mûre, supérieure à celle de la carotte noire et de l'açaï, implique qu'une faible augmentation de température est suffisante pour dégrader ces anthocyanes plus rapidement. **Conclusion**. Nos résultats ont clairement montré que les anthocyanes de carotte noire ont une bonne stabilité pendant le stockage thermique (4 °C à 50 °C) par rapport aux anthocyanes de la mûre et de l'açaï. L'acylation des anthocyanes de la carotte noire pourrait expliquer sa plus grande stabilité. Les anthocyanes acylées se sont révélées prometteuses en substitution à l'utilisation de colorants synthétiques dans les procédés de fabrication de boissons.

France / *Rubus glaucus* / *Euterpe oleracea* / *Daucus carota* / fruits / boisson non alcoolisée / extrait d'origine végétale / stockage / anthocyane / dégradation

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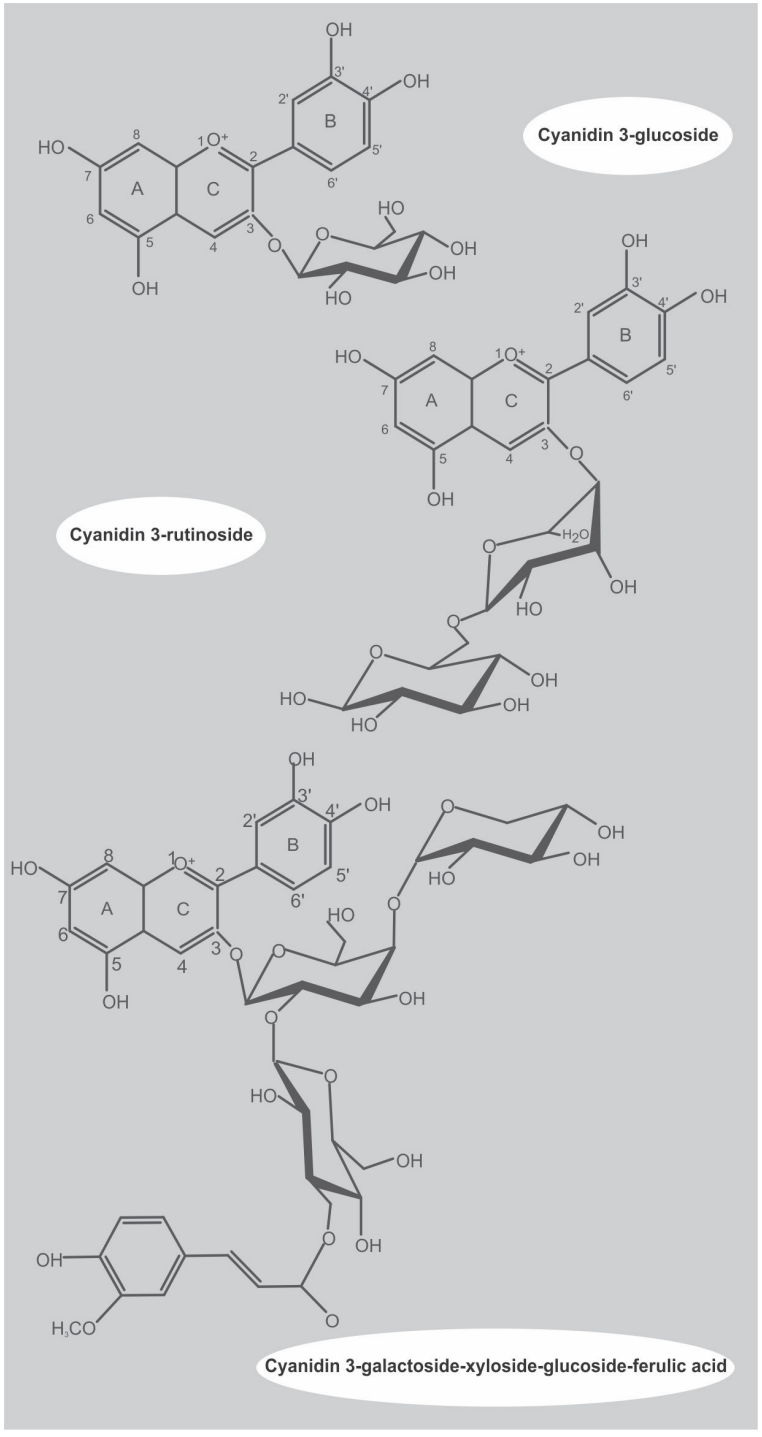

Figure 1.
Structure of the main anthocyanins found in Andean blackberry (cyanidin 3-glucoside), açai (cyanidin 3-rutinoside) and black carrot (cyanidin 3-galactoside-xyloside-glucoside-ferulic acid).

1. Introduction

Anthocyanins are a group of naturally occurring flavonoid heterosides in the plant kingdom. They are responsible for the attractive colour of many fruits and vegetables. Anthocyanins are glycosides of anthocyanidins and may have aliphatic or aromatic acids attached to the glycosidic residues [1]. The aglycone fraction (anthocyanidin) differs according to the B cycle substituent *(figure 1)*. Glycosylation most often appears in positions 3 and 5 [2] and acylation with aromatic acids including cinnamic, p-coumaric, caffeic, ferulic or even malonic acids has a significant stabilising effect on anthocyanins [3–5]. Anthocyanin colour is determined by the number of hydroxyl groups, their degree of methylation, the nature, number and position of the sugars bound to the anthocyanidin, and the nature and number of acids bound to the sugar, as well as the physico-chemical environment in which these anthocyanins are present. For example, the colour changes from pink to blue as the number of hydroxyls increases, and the other way round as the number of methyls increases [6]. The same anthocyanin may have different colours according to the pH or concentration of the solution, but also according to the presence of co-pigments. A co-pigment is generally not coloured, but can increase the colour intensity of a solution of anthocyanins [7]. Besides their antioxidant properties, anthocyanins have a high potential for use as natural colorants [8].

Thermal degradation of anthocyanins has been covered by numerous studies, such as for red cabbage [9], black carrot, blood orange [10–12], strawberry [13] and blackberry [14]. Indeed, knowing the degradation kinetic parameters is essential for predicting qualitative changes appearing during storage and during heat treatment processes such as pasteurisation. These studies have shown that anthocyanin degradation can be described using a first-order kinetic law.

The stability of anthocyanins during heat treatments depends on the composition and characteristics of the medium: presence of other solutes such as sugars, salts, ascorbic acid or co-pigments, dissolved oxygen

content and pH. The presence of sugars and salts increases the degradation rate of anthocyanins in red cabbage [9], açai and acerola [15]. Nonetheless, not all sugars appear to be equivalent in this respect: fructose, arabinose, lactose and sorbose, for example, seem to accelerate anthocyanin degradation more than glucose, maltose and saccharose [6]. The interaction mechanisms between sugar and anthocyanins have not been well explained. The reducing nature of sugar, for instance, is not critical. It is probable in this respect that certain products of hexose degradation in acid medium, such as furfural, are involved in anthocyanin degradation [16].

Ascorbic acid is frequently added to fruit-based products to limit oxidation reactions such as enzymic browning, but also for nutrition purposes. This compound appears to promote anthocyanin degradation [9, 17]. Nonetheless, the degradation constant k obtained in an acerola-based drink with 2.8 $g{\cdot}L^{-1}$ ascorbic acid is three times higher than that obtained in an açai-based drink to which ascorbic acid was added at the same concentration [17]. This difference could be attributed to the fact that the flavonoid concentration in açai is 10 times higher than in acerola, and they may protect anthocyanins by intermolecular co-pigmentation [8].

Other compounds may play a part in anthocyanin stability, such as dextrins, by preventing the transformation of the cationic form of anthocyanin into other less stable forms [18, 19]. Similarly, anthocyanins with at least one glycosyl residue acylated by a phenolic acid residue (generally via the primary hydroxyl group) are protected by the phenomenon of intramolecular co-pigmentation [1, 20, 21]. Acylated anthocyanins then adopt compact conformations, in which the flat aromatic part of the aromatic acyl residues stacks up (via hydrophobic interactions) on the positively charged pyrylium nucleus, thereby minimising its contact with water [4].

A number of anthocyanin-rich sources have been investigated for their potential as commercial pigment extracts. Research led to the discovery of anthocyanin molecules with patterns that exhibit remarkable stability in a wider variety of food products. Thus, in order to complete these investigations, we performed this study. Therefore, our objective was to observe the effect of thermal storage on purified anthocyanins in a matrix representing a non-alcoholic drink at different temperatures. The results from this study will give information about the stability of purified mono-acylated triglycosylated anthocyanins from black carrot, and monoglycosylated anthocyanins from açai and Andean blackberry used as drink colorants.

2. Materials and methods

2.1. Plant material

Fruits of Andean blackberries (*Rubus glaucus* Benth.) from Ecuador (Ambato), collected by a correspondent of the EPN (Escuela Politécnica National, Quito), were frozen whole upon harvesting, and kept at –18 °C. The açai (*Euterpe oleracea* Mart.) fruits were supplied to us by Bolthouse (Bélem, Brazil). Açai juice was extracted from fruits within 12 h of harvesting, and was then frozen and kept at –18 °C. For the black carrot (*Daucus carota* L.) studies, we used a purified anthocyanin powder extract (ColorFruit Carrot 12 WSP) supplied by Chr-Hansen (Prades-le-Lez, France). As a reference, a standard grape anthocyanin extract was selected (extract E163 supplied by Grap'Sud, Cruviers-Lascours, France).

2.2. Obtaining purified anthocyanin extracts

In our study we chose to work with purified anthocyanin extracts equivalent to those offered by food additive suppliers. The protocol used for obtaining the extracts from the raw products (blackberry and açai) is described below.

For blackberries, the juice was first extracted by pressing (Sakaya 12 hydraulic press, Bangkok). It was then stored for 12 h at 4 °C to precipitate some of the pectic compounds. After centrifuging at 8000 *g* for 15 min at 4 °C (Beckman Coulter, Ireland), the blackberry juice was enzyme-treated

Table I.
Main characteristics of açai, blackberry and black carrot anthocyanin extracts used in a coloured drink studied, and comparison with a standard grape extract (Grap'Sud grape extract E163).

Fruit studied	Anthocyanin content	-cyanidin 3-rutinoside	-cyanidin 3-glucoside	Total polyphenols[2] ($g \cdot kg^{-1}$)	Colorant intensity ($E^{1\%\ pH\,3}$)	Brown index	Violet index	pH
	($g \cdot kg^{-1}$ dry weight)							
Açai	226.4 ± 2.1	68.4	158	389	48.1 ± 1.1	0.45 ± 0.01	0.23 ± 0.02	3.1 ± 0.1
Blackberry	196 ± 2.3	58.8	137.2	340	31.7 ± 1.2	0.42 ± 0.01	0.12 ± 0.01	3.1 ± 0.1
Black carrot[1]	18	–	–	-	12	0.4	0.17	3.7

[1] Values supplied by Chr. Hansen, Prades-Le-Lez, France.
[2] Expressed as gallic acid equivalent.

(Pectinex Ultra SP-L, Novozymes, 1 $mL \cdot L^{-1}$, 12 h, 20 °C) then vacuum-filtered on sintered glass (porosity no. 1). For açai, we received the juice directly from Brazil.

Anthocyanins from blackberry juice were purified and concentrated on a laboratory scale by adsorption to a styrene-divinylbenzene copolymer with non-ionic and hydrophobic chemical properties, a specific surface of greater than 800 $m^2 \cdot g^{-1}$, and an average pore diameter of between (20 and 25) nm. This resin (XA 984) was supplied by Novacep (Epone, France). After optimisation, adsorption was performed at a flow rate of 8 $BV \cdot h^{-1}$ (BV = Bed Volume) and desorption with 65% ethanol (v/v) at 4 $BV \cdot h^{-1}$ [22]. Under these conditions, the overall anthocyanin recovery yield was 95.6% and the anthocyanin purification rate against the dry matter was 23. The purified anthocyanin extracts obtained were kept in pH 3-citrate buffer (46.5% 0.1 M citric acid, 3.5% 0.1 M dehydrated sodium citrate) at 4 °C. The same adsorption procedure was used to purify the anthocyanins in açai juice. The anthocyanin identification and concentration of these extracts were performed by HPLC by the method of Mertz *et al.* [23], as well as the total polyphenol content using the Folin Ciocalteu method [24]. The main characteristics of the three anthocyanin extracts used were summarised (*table I*).

2.3. Measuring chromatic characteristics

The colour unit equates to the colorant intensity $E^{1\%,\ pH3}_{(\lambda max,\ 1\ cm)}$ of 1 g of colorant, which, when diluted in 100 mL of buffer solution at pH 3, gives an absorbance of 1 [1]. This quantity therefore equates to an extinction coefficient in which concentration is expressed in g·100 mL, and the optical thickness in cm (Equation 1).

$$E^{1\%,\ pH3}_{(\lambda max,\ 1\ cm)} = \frac{A_{\lambda max} \times FD}{m} \qquad (1)$$

where $A_{\lambda max}$: absorbance at the maximum wavelength of diluted extract ($0.5 < A_{\lambda max} < 1$); FD: dilution factor; m: mass (g) of extract introduced into a 100-mL vial of pH 3 buffer.

The Brown Index [BI = ($A_{430\ nm}$ / $A_{520\ nm}$)] and Violet Index [VI = ($A_{580\ nm}$ / $A_{520\ nm}$)] are widely used for assessing colorant quality, but also for estimating the shade or hue [1, 20]. Hence, they reflect the quantity of brown ($A_{430\ nm}$) or blue ($A_{580\ nm}$) in anthocyanin-based red colorants.

2.4. Monitoring of degradation during storage

Anthocyanin degradation was monitored in a model solution simulating a non-alcoholic fruit-based drink. This aqueous solution comprised saccharose and citric acid. Potassium sorbate and sodium benzoate were used to prevent any microbe development (*table II*). Anthocyanin extracts from açai, blackberry and black carrot were added to the model solution until an absorbance of 2 was obtained (approximately 2 g·L^{-1}). A quantity of 0.5 g·L^{-1} of ascorbic acid (Sigma, L'Isle d'Abeau, France) was added to part of the preparation in order to evaluate its effect on anthocyanins at 30 °C. The solution was then transferred to 60-mL sterile food-quality polyethylene bottles. Each bottle was nitrogen-inerted to eliminate oxygen, and then sealed with wax paper. The number of bottles prepared equated to the number of samples to be taken.

The bottles were then placed in thermostatic chambers for 10 weeks. Three temperatures were chosen: 4 °C, representing the positive control; 20 °C, equating to an average temperate climate temperature; 30 °C, to simulate storage in a tropical environment; and 50 °C, to obtain accelerated ageing.

The total anthocyanin content was assessed by the pH differential method [25]. All absorbance readings were made against distilled water, which acted as the control. Spectrophotometric measurements were carried out using Shimadzu spectrophotometers (UV-1200 and UV-1605, Kyoto, Japan). Concentrations were expressed as cyanidin 3-glucoside (M = 449 g·mol^{-1}) equivalents for açai and blackberry. Based on the initial absorption spectra of the anthocyanin solutions from blackberries, black carrots and açai, the maximum absorption wavelength was determined: 517 nm for açai anthocyanin, 514 nm for blackberry and 524 nm for carrot anthocyanin (*figure 2*). The molar extinction coefficient at pH 1 used for calculation was 26,900 L·mol^{-1}·cm^{-1} for cyanidin 3-glucoside [25]. Every week, three bottles were removed to measure the absorbance at λ_{max} of the anthocyanin solutions and thrown out.

To verify possible oxygen entry, the dissolved oxygen content was measured on specially obtained standard samples using a dissolved oxygen probe (CellOx 325) at the start of, during, and at the end of storage.

The pH was measured at the start and end of storage on ten samples (Schott pH meter).

Table II.
Composition of the model solution (pH 3) simulating a non-alcoholic fruit-based drink, used for monitoring anthocyanin degradation in the case of a coloured drink made from Andean blackberry, açai and black carrot extracts.

Composition	Proportion (%)
Saccharose	11
Potassium sorbate	0.023
Sodium benzoate	0.018
Citric acid	0.215
Milli-Q water	100

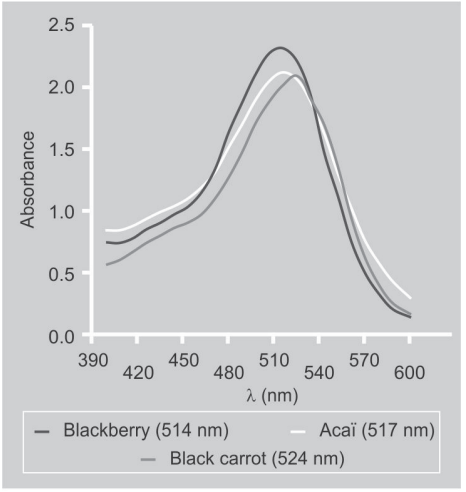

Figure 2.
Visible absorption spectrum of black carrot, Andean blackberry and açai extracts used for making a coloured drink, and maximum adsorption wavelengths.

The soluble dry extract was measured at the start of and during storage to monitor changes (Atago refractometer).

To detect any microbe developments, two indicators were used. For lactic bacteria, the titratable acidity of the solutions was measured by titrimetry (NaOH at 0.1 N to a pH of 8.2, Schott titrator). It was expressed as mass of lactic acid. For yeast, the ethanol content in the samples was analysed using an Agilent 6890 gas chromatograph (GC) in automatic injection mode on a DB-Wax column [26].

2.5. Modelling of degradation kinetics

According to previous studies on the black carrot [12], açai [15] and blackberry, Roselle and blood orange [14, 27], the thermal degradation of anthocyanins follows a first-order kinetic. Therefore, two models were chosen.

- The first is a conventional chemical kinetics model (Equation 2). It defines the degradation rate constant k, which depends on the temperature and is represented by the Arrhenius equation (Equation 3).

$$A = A_0^{e^{-kt}} \quad (2)$$

$$k = k_\infty e^{-(E_a/RT)} \quad (3)$$

where k_∞: pre-exponential factor (s^{-1}); E_a: activation energy ($J{\cdot}mol^{-1}$); R: perfect gas constant (= 8.32 $J{\cdot}mol^{-1}{\cdot}k^{-1}$).

- The second model (4), more often used in microbiology (Ball's model), defines a decimal reduction time which is related to temperature via a factor z (5).

$$D = \ln 10\ k \quad (4)$$

$$D = D_0 10^{-(T/z)} \ldots \quad (5)$$

where D: decimal reduction time at temperature T (s); D_0: value of D extrapolated at 0 °C; z is expressed in °C.

The model's parameters were identified, using linear regression on the logarithmic curves of experimental data. Although the z value could be estimated from E_a using the relationship ($z = \ln(10)RT^2/E_a$) in a narrow range of temperature, we chose to determine the z value graphically.

The Arrhenius equation represents the effect of temperature on the anthocyanin degradation rate. Indeed, the plot of the Napierian logarithm of the k as a function of the inverse temperature determines the activation energy (E_a), and the representation of $\log_{10}(D)$ as a function of temperature deduces the value of z.

All the analyses were repeated three times. The values given in the tables correspond to the average ± standard deviation.

3. Results

Regardless of the temperature, the pH of all the model solutions remained constant throughout the storage period (3.15 ± 0.007). The same applied to the soluble dry extract [(100 ± 1) $g{\cdot}kg^{-1}$]. The titratable acidity values measured at the end of storage revealed that there was no organic acid produced, and the production of ethanol measured by GC remained less than 3 $mg{\cdot}L^{-1}$. These results confirmed that the drinks used were indeed microbiologically stable, and that the changes observed were only chemical or biochemical. Finally, the dissolved oxygen content did not vary during storage [(3.2 ± 0.06) mg $O_2{\cdot}L^{-1}$].

3.1. Anthocyanin degradation

Thermal degradation of the anthocyanins followed a first-order kinetic model: the logarithm of absorbance was inversely proportional to the treatment time (*figure 3*). The kinetic parameters k and D of the anthocyanin extracts from açai, blackberry and black carrot were determined graphically. The Arrhenius law and the z factor fit the temperature dependence of k and D well considering the R^2 (*table III*). No anthocyanin degradation occurred in refrigerated storage (4 °C) during the 10 weeks of storage (*figure 3*).

At all temperatures, the lowest degradation rate constants k were for the black carrot drink followed by açai and then blackberry. So the black carrot anthocyanins degraded

Table III.
Kinetic parameters for thermal degradation of anthocyanins following the Arrhenius and Ball models for a coloured drink made from Andean blackberry, açai and black carrot extracts.

Extracts	°C	Arrhenius model			Ball's model		
		$K \cdot 10^{-2}$ (d^{-1})	E_a (kJ·mol^{-1})	R^2	D (d)	z (°C)	R^2
Açai	20	0.28 ± 5.2.10 $^{-03}$			835.5 ± 15		
	30	0.77 ± 8.2.10 $^{-03}$	66.3 ± 2.7	0.92	300.3 ± 3.2	26.6 ± 3.1	0.90
	50	1.55 ± 2.3.10 $^{-02}$			148.2 ± 2.2		
Blackberry	20	0.32 ± 1.4.10 $^{-02}$			720.3 ± 32.3		
	30	1.08 ± 1.7.10 $^{-02}$	91.2 ± 0.4	0.89	213.3 ± 3.5	18.2 ± 1.5	0.85
	50	2.46 ± 4.3.10 $^{-02}$			93.8 ± 1.6		
Black carrot	20	0.13 ± 7.2.10 $^{-03}$			1798.9 ± 104.4		
	30	0.42 ± 3.7.10 $^{-03}$	63.2 ± 4.3	0.96	550.9 ± 4.8	27.7 ± 2.0	0.93
	50	0.92 ± 8.7.10 $^{-03}$			251.7 ± 2.3		

more slowly than açai and Andean blackberry anthocyanins during this thermal storage (*table III*).

These results fall within the ranges usually reported in the literature for anthocyanin degradation [12, 14, 15]. However, these values were greater than ours were. This observation is probably due to the matrices used: juice, concentrate, buffer solution, soft drink, or fermented juice and their composition [9, 11, 13–15, 20, 28, 29]. In our study, we used anthocyanin extracts purified by resin adsorption, which was not the case with the previous work presented in the literature. In this respect, it is possible that purification of the anthocyanins improves their chemical stability by eliminating a lot of the non-phenolic solutes liable to promote degradation [30, 31].

Açai and Andean blackberry have two main anthocyanins - cyanidin 3-glucoside and cyanidin 3-rutinoside [22, 32]. Meanwhile, the black carrot has primarily a mono-acylated triglycosylated anthocyanin: cyanidin 3-galactoside-xyloside-glucoside-ferulic acid [33]. This ferulic acid is fixed on the glycosyl residue and can approach the flavylium nucleus to protect it from nucleophilic attack by water on C_2. This is the phenomenon of intramolecular co-pigmentation [5].

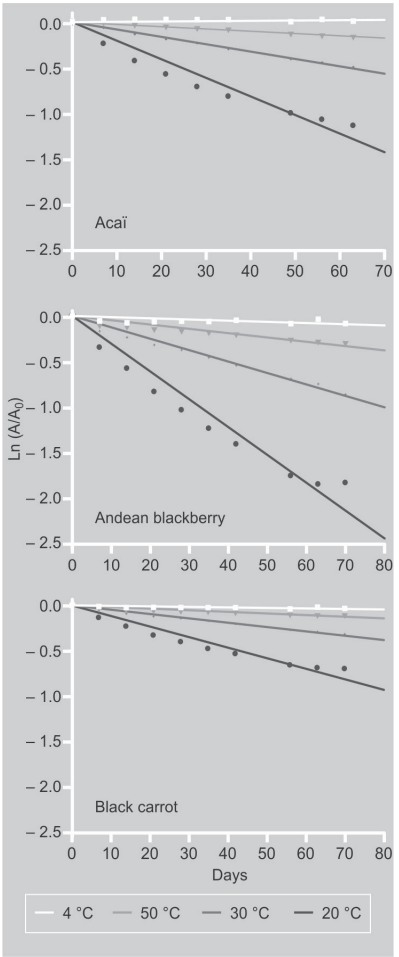

Figure 3.
Thermal degradation kinetics of açai, Andean blackberry and black carrot anthocyanins as a function of temperature over 10 weeks of storage at 4 °C, 20 °C, 30 °C and 50 °C.

The activation energies (E_a) ranged between (63 and 91) kJ·mol^{-1}, and the z factor ranged between (18 and 27) °C. E_a of black carrot anthocyanins (63.2 kJ·mol^{-1}) was less than açai anthocyanins (66.3 kJ·mol^{-1}) and Andean blackberry anthocyanins (91.2 kJ·mol^{-1}) (*table III*). Anthocyanins from blackberries and açai were therefore more sensitive to temperature increases than black carrot anthocyanins. Thus, purified acylated anthocyanins from black carrot are more stable in these conditions than blackberry and açai anthocyanins. These results agree with studies about the potential food applications of a number of acylated anthocyanin extracts [4, 33]. Cevallos-Casals *et al.* showed that acylated anthocyanins were more stable to pH and temperature change than non-acylated ones [34]. Such high stability may be attributed to the acylation on the anthocyanin structure [35–37].

However, the E_a of açai shows a good value compared with blackberry, although both have the same anthocyanin profile (*table III*): about 70% cyanidin 3-rutinoside and 30% cyanidin 3-glucoside. The polymeric resins used to purify the anthocyanins do not remove all other polyphenols. In fact, a purity index (anthocyanins / polyphenols) (% w/w) can be measured in order to evaluate the proportion of anthocyanins in contrast to the polyphenols. We found 60%. It means that there are 40% of polyphenol - non-anthocyanins in the extract. Thus, other flavonoids than anthocyanins are elute and can be found in the anthocyanin extract [38, 39].

Some flavonoids also appear to play an important role in the anthocyanin protection mechanism [8]. They appear to compete with water to interact with chromophore, which would displace the equilibrium between the coloured and colourless forms towards complexation of the coloured form. The literature highlights the effect of flavonoids and proanthocyanidin, contained in high level in açai [15, 40, 41]. According to Pacheco-Palencia's study [42], flavone-*C*-glycosides (schaftoside, vicenin-2, isoorientin, orientin, isovitexin) found in açai enhance anthocyanin stability, while no significant effects were attributed to the presence of phenolic acids and procyanidins. However, no flavone-*C*-glycosides were detected in Andean blackberry [22].

This result may suggest flavone-*C*-glycosides offer potential for their use as colour enhancers and stabilising agents in products rich in cyanidin glycosides.

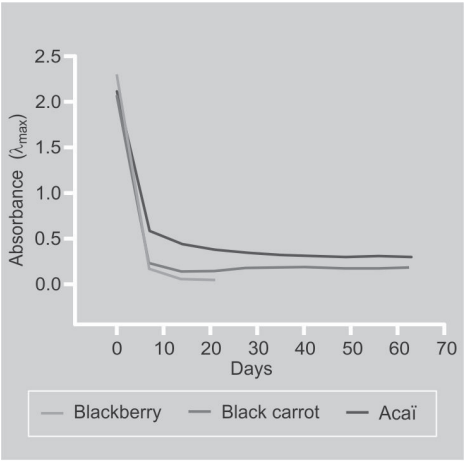

Figure 4.
Evolution of absorbance of açai, Andean blackberry and black carrot anthocyanins in the presence of ascorbic acid (50 mg·100 mL^{-1}) over 1 week of storage at 30 °C.

3.2. Effect of ascorbic acid on anthocyanin stability

Adding ascorbic acid caused a significant and rapid decrease in absorbance after one week of storage at 30 °C: 73% for acai, 89% for carrot and 93% for blackberry (*figure 4*).

This negative effect of ascorbic acid on anthocyanins agreed with previous work [8, 9, 13, 17, 43–45]. This decrease was immediate, since the absorbance of the açai, blackberry and carrot samples tended to stabilise after the first weeks of storage at 30 °C.

Work on anthocyanins from strawberry [13], açai [43] and blackcurrant [46] has suggested a possible interaction between these two compounds. The mechanism suggested by Poei-Langston *et al.* consists of direct condensation of ascorbic acid on carbon 4 of the anthocyanin molecule, thereby causing the loss of both [44]. Furthermore, according to Lacobucci and Sweeny (1983, quoted in [17]), anthocyanin colour loss caused by ascorbic acid could be due to oxidative cleavage of the pyrylium ring, with ascorbic acid acting as a molecular oxygen activator, producing free radicals [45].

Moreover, the presence of flavonols exerts a protective effect on anthocyanins in the presence of ascorbic acid, probably through competition with water in condensation reactions [47].

3.3. Indices for polymeric colour and browning

Anthocyanins are labile molecules that will undergo a number of degradative reactions. Enzymes such as polyphenoloxidase, peroxidase and glycosidase can have a devastating effect on anthocyanins [48]. These enzymes may be native to the plant tissue, or their source may be from mould contamination and cause brown oxidation pigments [1, 49].

An increase in the Brown Index ($BI = A_{430}/A_{520}$) indicates a degradation of red pigments (A_{520}) or an increase in yellow pigments (A_{430}) due to oxidative polymerisation of tannins by oxygen.

The stable BI values obtained at 4 °C indicate an absence of degradation at refrigerated temperatures, whereas, at higher temperatures, this index increased significantly (*figure 5*). Initially between 0.37 and 0.45, the BI was multiplied by a factor of 2.3 for açai and blackberry, and by 1.30 for black carrot at 50 °C. At 30 °C, the increase was smaller, with a multiplying factor of 1.37 for açai, 1.30 for blackberry and 1.08 for black carrot. At 20 °C, the increase ranged from 1.13 for açai, to 1.07 for blackberry and 1.04 for black carrot. These results confirmed that black carrot anthocyanins degraded much more slowly than blackberry or açai anthocyanins.

The violet index ($VI = A_{580}/A_{520}$) exhibited similar variation, with a smaller increase factor for the açai and black carrot at 50 °C, while the blackberry's index evolved more rapidly during storage (results not shown).

In terms of colorant intensity, anthocyanin extract from açai is four times stronger than extract from black carrot and grape, in comparison with extract from blackberry, which is two times higher. This difference in colorant intensity can be explained among other things by their composition.

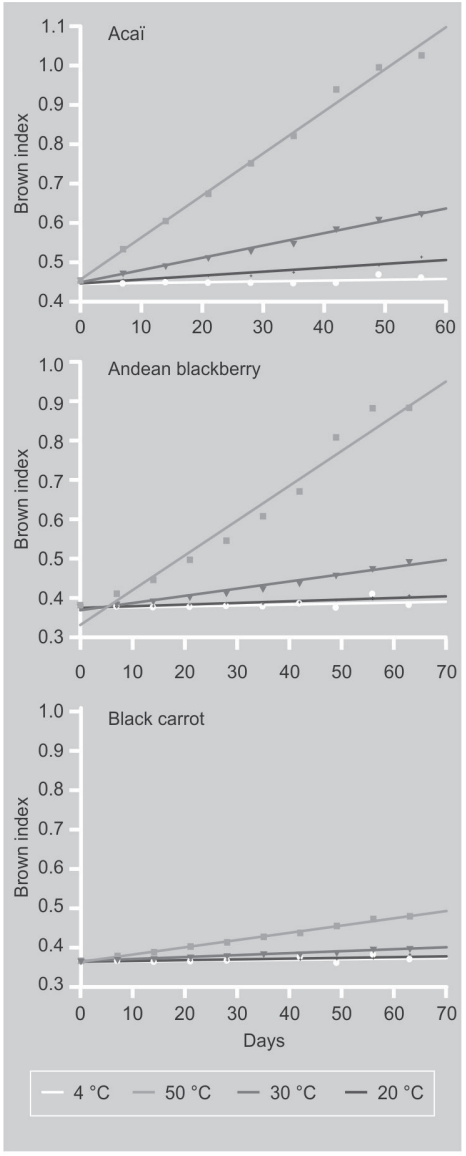

Figure 5.
Evolution of the Brown Index of açai, Andean blackberry and black carrot samples over 10 weeks of storage at 4 °C, 20 °C, 30 °C and 50 °C.

Indeed, blackberry and açai extracts primarily contain anthocyanins and other flavonoids, unlike carrot and grape extracts which contain texturising agents such as maltodextrin or even glucose syrup.

4. Conclusion

Our results clearly showed that anthocyanins from black carrot have a good stability during thermal storage (4 °C to 50 °C) with regard to blackberry and açai anthocyanins.

Acylation of black carrot anthocyanins probably explains its greater stability. Acylated anthocyanins have shown to be promising alternatives to the use of synthetic dyes in drink systems.

Under the tested conditions, no anthocyanin loss was revealed during storage of the model drinks in the refrigerated state (4 °C) for 10 weeks, and ascorbic acid at 0.5 $g \cdot L^{-1}$ considerably accelerated degradation of all anthocyanin studies.

The better stability of açai anthocyanins compared with those of blackberry may be explained by the presence of flavonols in açai, which may also play an important role in the anthocyanin protection mechanism.

Further studies are required to achieve better characterisation of anthocyanin behaviour in products such as drinks and to improve understanding of the phenomena involved. Indeed, it has been clearly demonstrated that the physico-chemical environment (pH, acidity, presence of enzymes, soluble dry extract, salts), nutritional environment (vitamins, sugars, proteins, phenolic acids, flavonoids) and external environment (light, temperature, ascorbic acid, maltodextrin) in which the anthocyanin is located affect its stability. It would first be necessary to define this environment precisely for each anthocyanin source. There should also be testing on a wider temperature range, in order to measure the kinetic parameters of degradation with greater precision. Similarly, a study of the various co-factors such as flavone-*C*-glycosides, which might improve and stabilise the colour of non-acylated anthocyanins, could expand the potential of anthocyanin-based colorants.

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Evaluación de la estabilidad de las antocianinas en el trascurso del almacenamiento de una bebida colorada por extractos de moras andinas (*Rubus glaucus* Benth.), de acaí (*Euterpe oleracea* Mart.) y de zanahorias negras (*Daucus carota* L.).

Resumen - Introducción. Estudiamos el efecto de la temperatura en la estabilidad de tres fuentes de antocianinas purificadas, en una bebida gaseosa (pH 3, 10 °Brix) almacenadas a (4, 20, 30, 50) °C durante 60 días. **Material y métodos**. Se purificaron y se concentraron antocianinas de mora andina, de acaí y de zanahoria negra en laboratorio por absorción en un copolímero de estireno divinilbenceno. Para describir la cinética de degradación térmica de estas tres antocianinas, se emplearon dos acercamientos empíricos clásicos (modelos de Arrhenius y de Ball). **Resultados**. No se detectó ninguna degradación en el trascurso del almacenamiento refrigerado (4 °C). A todas las temperaturas, la constante de la velocidad de degradación (*k*) para las antocianinas de la zanahoria negra fue menor que la del acaí y la de la mora ($0{,}42 \times 10^{-2}$, $0{,}77 \times 10^{-2}$ et $1{,}08 \times 10^{-2}$)·día^{-1}, respectivamente, a 30 °C. Las antocianinas de la zanahoria negra se degradaron menos rápidamente que las del acaí y las de la mora andina. A 20-50 °C, la energía de activación (E_a) fue (63,2 ± 4.3) kJ·mol^{-1} para la degradación de las antocianinas de la zanahoria negra, (66,3 ± 2.7) kJ·mol^{-1} para el acaí y (91,2 ± 0.4) kJ·mol^{-1} para la mora. Esta energía de activación E_a de antocianinas de la mora, superior a la de la zanahoria negra y a la del acaí, implica que un ligero aumento de la temperatura basta para degradar estas antocianinas más rápidamente. **Conclusión**. Nuestros resultados mostraron claramente que las antocianinas de la zanahoria negra tienen una buena estabilidad durante el almacenamiento térmico (4 °C a 50 °C) en relación con las antocianinas de la mora y del acaí. La acilación de las antocianinas de la zanahoria negra podría explicar su mayor estabilidad. Las antocianinas aciladas han resultado ser prometedoras en sustitución del uso de los colorantes sintéticos, en los procesos de fabricación de bebidas.

Francia / *Rubus glaucus* / *Euterpe oleracea* / *Daucus carota* / frutas / gaseosas / extractos vegetales / almacenamiento / antocianinas / degradación