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Phytochemical composition and thermal stability of two commercial açai species, *Euterpe oleracea* and *Euterpe precatoria*

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ABSTRACT

Açai fruit are native to the Amazon region of South America and two predominant species are commercially exported as fruit pulps for use in food and beverage applications. Detailed characterisation of the polyphenolic compounds present in the de-seeded fruits of *Euterpe oleracea* and *Euterpe precatoria* species were conducted by HPLC–ESI–MS^n analyses and their thermal stability and overall influence on antioxidant capacity were determined. Anthocyanins were the predominant polyphenolics in both *E. oleracea* (2247 ± 23 mg/kg) and *E. precatoria* (3,458 ± 16 mg/kg) species, and accounted for nearly 90% of the trolox equivalent antioxidant capacity in both *E. oleracea* (87.4 ± 4.4 μmol TE/g) and *E. precatoria* (114 ± 6.9 μmol TE/g) fruits. Various flavones, including homoorientin, orientin, taxifolin deoxyhexose and isovitexin; various flavanol derivatives, including (+)-catechin, (−)-epicatechin, procyanidin dimers and trimers, and phenolic acids, including protocatechuic, *p*-hydroxybenzoic, vanillic, syringic and ferulic acids, were also present in both species. Thermal stability of these compounds was evaluated, following a thermal holding cycle (80 °C for up to 60 min) in the presence and absence of oxygen. Both species experienced only minor changes (<5%) in non-anthocyanin polyphenolic contents during all thermal processes whereas 34 ± 2.3% of anthocyanins in *E. oleracea* and 10.3 ± 1.1% of anthocyanins in *E. precatoria* were lost under these conditions, regardless of the presence of oxygen. Proportional decreases (10–25%) in antioxidant capacity accompanied the anthocyanin changes. Results suggest that both açai species are characterised by similar polyphenolic profiles, comparable antioxidant capacities, yet only moderate phytochemical stability during heating.

1. Introduction

Açai fruit, *Euterpe oleracea* Mart. and *Euterpe precatoria* Mart., are an important economic palm fruit export from the Brazilian Amazon (Galotta & Boaventura, 2005) and constitute the majority of açai pulp exported for food and ingredient applications. The predominant species of açai exported from Brazil is *E. oleracea*, locally referred to as "açai-do-Pará", and frequently found in mono-specific populations common in the Amazon River estuary floodplains (Muñiz-Miret, Vamos, Hiraoka, Montagnini, & Mendelsohn, 1996). The less available *E. precatoria* or "açaí-do-terra-firma" is widely distributed in the central and western regions of the Brazilian Amazon and is commonly found in non-flooded, upland fields (Clay & Clement, 1993). The palms of *E. oleracea* are multi-stemmed, monoecious, and may reach heights of >25 m to produce small, round (1.0–1.4 cm in diameter), dark purple fruits that are mainly harvested between July and December (Muñiz-Miret et al., 1996). By contrast, the palms of *E. precatoria* are single-stemmed and may grow to a maximum height of 22 m to produce spherical, dark purple fruits (1.0–1.8 cm in diameter) harvested from December to August (Clay & Clement, 1993). Fruits from both species are characterised by a single seed that constitutes approximately 80% of the total volume, covered by fibrous layers and a slight oily coating under a thin (0.5–1.5 mm. thick), edible mesocarp (Clay & Clement, 1993; Muñiz-Miret et al., 1996). Fruits of both species ripen from green to a deep purple colour and are commercially pulped with added water to prepare a thick, dark purple pulp with an oily surface appearance and distinctive flavour. Locally known in Brazil as "açai" and popular in the eastern regions of South America (Brondizio, Safar, & Siqueira, 2002), açai pulp has gained in commercial export markets for use in a variety of food and beverage applications, greatly favoured by international consumer trends towards health, wellness, novelty and exotic flavours. Particular attention has been given to the potential health benefits of açai, associated with its *in vitro* antioxidant properties (Gallori, Bilia, Bergonzi, Barbosa, & Vincieri, 2004), attributed to its polyphenolic composition (Lichtenthaler et al., 2005). Polyphenolics from *E. oleracea* have only been recently characterised (Gallori et al., 2004; Lichtenthaler et al., 2005; Pacheco-Palencia, Hawken, & Talcott, 2007a, 2007b; Schauss et al., 2006), and no previous reports on the phytochemical composition comparing *E. oleracea* or *E. precatoria* fruits are

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available. Moreover, factors affecting polyphenolic stability have not been determined. Therefore, this study was conducted to assess the phytochemical composition, antioxidant properties, and thermal stability of de-seeded *E. oleracea* and *E. precatoria* fruits to determine the contribution of major polyphenolic fractions to the overall antioxidant capacity of these fruits. Results from these studies are aimed to assist the food industry in determining potential uses of these fruits for a variety of food and supplement applications.

2. Materials and methods

2.1. Materials and processing

Frozen, manually de-seeded, fully ripe açai fruits from *E. oleracea* and *E. precatoria* were kindly donated by Everything Nature, Inc. (Orlando, FL, USA) and Turiya Corp. (Vero Beach, FL, USA) and shipped overnight to the Department of Nutrition and Food Science at Texas A&M University. Only the edible portion of the fruit pulp was retained, and the inedible seed discarded. A composite, from approximately 250 fully ripe fruits, was used in these trials. Replications were made from these de-seeded fruit pulps by macerating with a known volume of water (1:5 w/v fruit/water ratio) adjusted to pH 3.5 with citric acid. Treatments were prepared by loading 10 ml of the prepared fruit puree into screw-cap tubes and sparging for 5 min with either nitrogen or air (as an oxygen source) until complete saturation. Dissolved oxygen content was monitored using a YSI-57 dissolved oxygen metre (Yellow Springs, OH, USA). Purees were then heated in a water bath to an internal temperature of 80 °C for 1, 5, 10, 30 or 60 min and compared to a non-heated control. Treatments were held frozen at −20 °C and analysed within 1 week after processing. Prior to analysis, purees were centrifuged (Eppendorf centrifuge 5810R, Eppendorf North America, Westbury, NY, USA) at 290*g* and 4 °C for 15 min to separate insoluble solids and lipids from the aqueous juice fraction, which was collected and filtered through Wattmann #4 filter paper to clarify it. For antioxidant analyses, these aqueous extracts were partitioned into two polyphenolic fractions using ethyl acetate, which separated most phenolic acids and flavonoids (non-anthocyanin fraction) from the remaining aqueous juice (anthocyanin fraction). Ethyl acetate was removed under reduced pressure at <40 °C, and the isolate re-dissolved in 0.05 M citric acid buffer (pH 3.5) and, along with the anthocyanin fractions, subjected to acid hydrolysis for 90 min at 95 °C in 2 M HCl and immediately assessed for antioxidant capacity and phytochemical composition.

2.2. Phytochemical analyses

Polyphenolic compounds present in açai were analysed by reverse phase HPLC with a Waters 2695 Alliance system (Waters Corp., Milford, MA, USA), using previously described chromatographic conditions (Pacheco-Palencia et al., 2007a). Polyphenolics were identified and quantified by spectroscopic characteristics and retention time, as compared to authentic standards (Sigma Chemical Co., St. Louis, MO, USA). Unidentified flavonoid compounds were quantified as rutin equivalents, while procyanidin concentrations were expressed in (+)-catechin equivalents. Mass spectrometric analyses were performed on a Thermo Finnigan LCQ Deca XP Max MS^n ion trap mass spectrometer equipped with an ESI ion source (ThermoFisher, San Jose, CA, USA). Separations were conducted, using the Phenomenex (Torrace, CA, USA) Synergi 4μ Hydro-RP 80A (2 × 150 mm) with a C18 guard column. Mobile phases consisted of 0.5% formic acid in water (phase A) and 0.5% formic acid in 50:50 methanol:acetonitrile (phase B) run at 0.25 ml/min. Polyphenolics were separated with a gradient elution programme where phase B changed from 5% to 30% in 15 min, from 30% to 65% in 25 min, and from 65% to 95% in 10 min and was then held isocratic for 20 min. Electrospray ionisation was conducted in the negative ion mode under the following conditions: sheath gas (N_2), 60 units/min; auxiliary gas (N_2), 5 units/min; spray voltage, 3.3 kV; capillary temperature, 250 °C; capillary voltage, 1.5 V; tube lens offset, 0 V. Total anthocyanin contents were determined spectrophotometrically (Helios, Thermo Electron, San Jose, CA, USA) at 520 nm and quantified using mg/kg equivalents of cyanidin-3-glucoside with a molar extinction coefficient of 29,600 (Pacheco-Palencia et al., 2007b). Antioxidant capacity was determined using the oxygen radical absorbance capacity assay, adapted to be performed in a BGM Labtech FLUOstar fluorescent microplate reader (485 nm excitation and 520 nm emission), as previously reported (Talcott & Lee, 2002). Results were quantified in micromol trolox equivalents per gram of de-seeded açai fruit.

2.3. Statistical analyses

Data for each chemical analysis were the means of three replicates, prepared independently. Analysis of variance, parametric correlations, and means separations (Tukey–Kramer HSD post-hoc test, $p < 0.05$) were conducted using SPSS version 15.0 (SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Polyphenolic characterisation

From historical observations and repeated evaluations of both açai species for characteristics such as total polyphenolics, total anthocyanins and radical scavenging activity, it was observed that *E. precatoria* had consistent and appreciably higher values of these attributes than had *E. oleracea*. Such observations led to a more in-depth study, comparing these two commercial species of açai. Commercial açai pulps can vary considerably, depending on processing methods, the ratio of fruit pulp to water, fruit quality and level of fruit ripeness, yet the fruit selected for these evaluations were chosen by industrial processors to be most representative of the fruit commercially available. Both açai species shared similar polyphenolic profiles, yet significant variations among individual polyphenolic concentrations were detected. Anthocyanins were the predominant polyphenolics in both species, and accounted for more than 90% of their total polyphenolic contents. Spectroscopic and mass spectrometric data, under both positive and negative ionisation modes, along with individual anthocyanin concentrations for each species, are presented in Table 1. Cyanidin glycosides were characterised by major ion signals at $m/z = 287.1$, $[M-H]^+$, and $m/z = 285.2$, $[M-H]^-$, corresponding to cyanidin aglycone. Similarly, peonidin-3-glucoside resulted in peonidin aglycone ion signals at $m/z = 301.1$, $[M-H]^+$, and $m/z = 299.0$, $[M-H]^{-\cdot}$ while pelargonidin-3-glucoside produced analogous aglycone ion signals at $m/z = 271.1$, $[M-H]^+$, and $m/z = 269.0$, $[M-H]^-$. Total anthocyanin concentrations were over 50% higher in *E. precatoria* than in *E. oleracea* fruits, mainly due to the abundance of cyanidin-3-rutinoside (3,135 ± 47 mg/kg), although pelargonidin-3-glucoside (319 ± 1.2) and cyanidin-3-sambubioside (4.6 ± 0.8 mg/kg) were also present. The lower anthocyanin concentrations detected in *E. oleracea* fruits included cyanidin-3-rutinoside (1,256 ± 38 mg/kg), cyanidin-3-glucoside (947 ± 29 mg/kg), and peonidin-3-rutinoside (44.0 ± 3.1 mg/kg). These findings confirm previous investigations that report anthocyanins in *E. oleracea* pulp and freeze-dried concentrates (Gallori et al., 2004; Lichtenthaler et al., 2005; Pacheco-Palencia et al., 2007a, 2007b; Schauss et al., 2006) and are the first report of the anthocyanin profile of *E.*

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Table 1
HPLC–ESI–MS$^-$ analyses of anthocyanins present in *Euterpe oleracea* and *Euterpe precatoria* fruits.

RT (min)	Compound	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	MS/MS$^-$ (*m/z*)	$[M-H]^-$ (*m/z*)	MS/MS$^-$ (*m/z*)	Concentration (mg/kg)[a]
Euterpe oleracea							
25.4	Cyanidin-3-glucoside	520	449.1	287.1	447.1	285.2	947 ± 29.0
26.5	Cyanidin-3-rutinoside	520	595.1	449.1, 287.1	593.1	447.1, 285.2	1256 ± 38.1
29.1	Peonidin-3-rutinoside	520	609.0	463.0, 301.1	607.0	461.1, 299.0	44.0 ± 3.1
Euterpe predatoria							
25.7	Cyanidin-3-sambubioside	520	581.2	371.1, 287.1	579.2	369.2, 285.2	4.6 ± 0.8
26.5	Cyanidin-3-rutinoside	520	595.1	449.1, 287.1	593.1	447.1, 285.2	3135 ± 47.1
27.9	Pelargonidin-3-glucoside	515	433.2	271.1	431.2	269.1	319 ± 1.2

[a] Values represent the means and standard errors from three replicates.

precatoria. Spectrophotometric determinations of total anthocyanin content of *E. oleracea* (2,056 ± 83 mg/kg) and *E. precatoria* (4227 ± 104 mg/kg) fruit purees also revealed major differences in pigment colour intensities. Additional differences between spectrophotometric and chromatographic measurements might have originated from copigmentation reactions among anthocyanins and other non-anthocyanin polyphenolics naturally present in both açai species, which are known to enhance visual colour and result in higher estimates of total anthocyanin contents in spectrophotometric assays (Boulton, 2001; Wilska-Jeszka & Korzuchowska, 1996).

Non-anthocyanin polyphenolics of *E. oleracea* (Fig. 1, Table 2) and *E. precatoria* (Fig. 2, Table 3) fruits included a diversity of phenolic acids and flavonoids. Phenolic acids detected in both species included protocatechuic, *p*-hydroxybenzoic, vanillic, syringic and ferulic acids, with vanillic and syringic acids being equally predominant. Both individual and total phenolic acid concentrations were higher in *E. precatoria* than in *E. oleracea* fruit. Flavonoids were among the most abundant non-anthocyanin polyphenolics in both species, and included various flavone-*C*-glycosides of apigenin and luteolin. Flavone-*C*-glycosides were identified by their spectral and mass spectrometric characteristics, and particularly by their distinctive fragmentation patterns, yielding product ion signals at $[M-H-60]^-$, $[M-H-90]^-$ and $[M-H-120]^-$ in negative ion mode MS^n analyses (Caristi, Bellocco, Gargiulli, Toscano, & Leuzzi, 2006; Ferreres, Silva, Andrade, Seabra, & Ferreira, 2003; Gattuso, Barreca, Gargiulli, Leuzzi, & Caristi, 2007; Pereira, Yariwake, & McCullagh, 2005; Voirin et al., 2000. Thus, isovitexin (apigenin-6-*C*-glucoside) identification was based on its distinctive molecular ion at *m/z* = 431.1, $[M-H]^-$, and subsequent fragmentation to product ions at *m/z* = 341.2, $[M-H-90]^-$, and *m/z* = 311.1, $[M-H-120]^-$, while scoparin (chrysoeriol 8-*C*-glucoside) was characterised by a precursor ion at *m/z* = 461.3, $[M-H]^-$, and fragment ions at *m/z* = 371.2, $[M-H-90]^-$ and *m/z* = 341.1, $[M-H-120]^-$. Both orientin (luteolin-8-*C*-glucoside) and isoorientin (luteolin-6-*C*-glucoside) gave predominant molecular ions at *m/z* = 447.2, $[M-H]^-$. Loss of water resulted in ion signals at *m/z* = 429.2, while fragmentation of the attached glycoside was likely responsible for ions at *m/z* = 357.1, $[M-H-90]^-$, and *m/z* = 327.2, $[M-H-120]^-$. Finally, cleavage of the *C*-sugar bond allowed detection of the luteolin aglycone at *m/z* = 285.1. Identification of taxifolin deoxyhexose was based on spectral (λ_{max} = 295, 340) and mass spectrometric (*m/z* = 449.1, $[M-H]^-$ and *m/z* = 269.1, $[M-H-180]^-$) characteristics, as compared to previous reports using authentic standards (Rijke et al., 2006; Schauss et al., 2006). Additional luteolin and apigenin glycosides were also detected in both species, and tentative identifications were based on their typical spectral characteristics (absorption at 350–360 nm) and mass fragmentation patterns

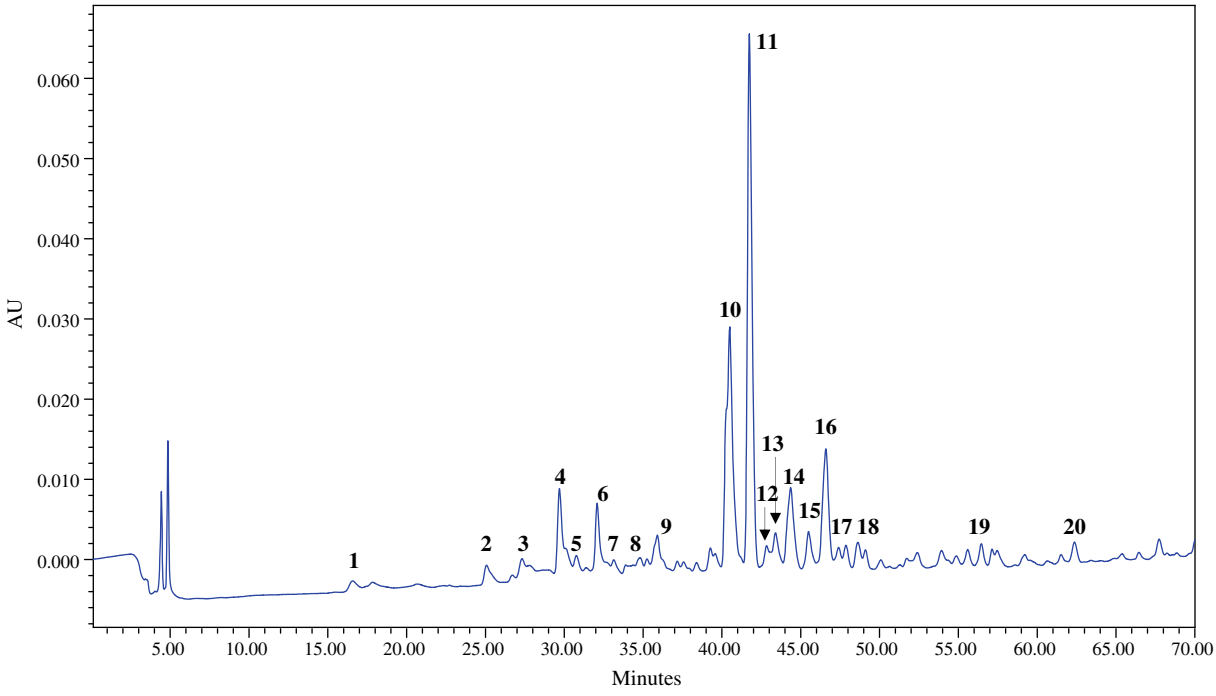

Fig. 1. HPLC chromatogram of non-anthocyanin polyphenolics present in *E. oleracea* juice at 280 nm. Peak identifications and their MS data are shown in Table 2.

(ion signals at $[M-H-60]^-$, $[M-H-90]^-$, and $[M-H-120]^-$), along with fragment ions corresponding to luteolin (m/z = 285.2, $[M-H]^-$) and apigenin (m/z = 269.1, $[M-H]^-$) aglycones. Orientin and isoorientin were the predominant flavonoids in both species, accounting for over 50% of the total flavonoid concentration. Isovitexin, scoparin, taxifolin deoxyhexose, two isovitexin and taxifolin derivatives, and two luteolin and apigenin glycosides were also present in *E. oleracea* fruits at concentrations between 3.7 and 10.6 mg rutin equivalents/kg. Of these, only isovitexin and taxifolin deoxyhexose were also detected in *E. precatoria* fruits (4.2 and 7.5 mg rutin equivalents/kg respectively), along with four apigenin glycosides, a taxifolin derivative, and an unidentified flavone, likely a glycoside, in concentrations ranging from 4.6 to 9.9 mg rutin equivalents/kg. Results were in agreement with previous HPLC–MS characterisations of flavonoids in *E. oleracea* (Gallori et al., 2004; Schauss et al., 2006), but this is the first report with quantitative information. In addition to flavonoids, procyanidin dimers and trimers were among the most predominant non-anthocyanin polyphenolics in both açai species. Procyanidin dimers were identified by precursor ion signals at m/z = 577.1, $[M-H]^-$ and fragments corresponding to (+)-catechin or (−)-epicatechin units (m/z = 289.1 and m/z = 287.1, $[M-H]^-$), probably resulting from

Table 2
Characterisation of non-anthocyanin polyphenolics present in *Euterpe oleracea* fruits.

Peak no.	RT (min)	Compound	λ_{max} (nm)	$[M-H]^-$ (m/z)	MS/MS (m/z)	Concentration (mg/kg)[a]
1	16.5	Protocatechuic acid	263, 292	153.2	109.2, 91.0	1.77 ± 0.11
2	24.9	*p*-Hydroxybenzoic acid	253.9	137.3	112.9	1.80 ± 0.13
3	26.7	(+)-Catechin	277.5	289.2	245.2, 203.2, 187.2, 161.3	5.11 ± 0.22
4	29.5	Vanillic acid	263, 291	167.3	140.9, 108.0, 95.2	5.05 ± 0.27
5	31.1	Luteolin di-glucoside[b]	266, 352	609.2	489.1, 369.2, 285.2	7.33 ± 0.68
6	33.1	Syringic acid	271	196.9	182.2, 153.1, 138.1	4.02 ± 0.36
7	32.4	Apigenin di-glucoside[b]	266, 356	593.1	575.1, 502.9, 473.1, 353.1, 269.1	8.13 ± 0.68
8	34.1	(−)-Epicatechin	277.5	289.2	245.2, 203.2, 187.2, 161.3	1.07 ± 0.10
9	36.3	Taxifolin derivative[b]	272, 356	449.4	327.1, 269.2, 225.1	7.89 ± 0.57
10	41.3	Isoorientin	272, 352	447.2	393.1, 357.1, 327.2, 299.2, 285.1	34.8 ± 1.19
11	41.9	Orientin	272, 348	447.2	429.2, 357.1, 327.3, 299.2, 285.1	53.1 ± 1.84
12	42.5	Isovitexin derivative[b]	267, 338	431.1	341.2, 311.1, 283.0, 269.1	3.71 ± 0.22
13	43.0	Ferulic acid	323.7	193.2	149.1, 134.1, 117.0	0.98 ± 0.10
14	44.6	Taxifolin deoxyhexose	295, 340	449.1	269.1	7.91 ± 0.19
15	45.7	Procyanidin dimer	235, 282	577.1	425.0, 407.2, 289.1, 287.1	4.37 ± 0.47
16	47.1	Isovitexin	267, 338	431.1	341.2, 311.1, 283.0, 269.1	10.6 ± 0.39
17	47.8	Scoparin	257, 352	461.3	371.2, 341.1, 231.1	5.83 ± 0.23
18	48.7	Procyanidin dimer	235, 282	577.1	425.0, 407.2, 289.1, 287.1	4.85 ± 0.61
19	56.5	Procyanidin trimer	235, 291	865.1	577.2, 451.0, 425.0, 407.3	5.74 ± 0.48
20	63.7	Procyanidin trimer	235, 291	865.1	577.2, 451.0, 425.0, 407.3	5.44 ± 0.36

[a] Values represent the means and standard errors from three replicates.
[b] Tentative identification based on similarities of spectral characteristics and ESI–MS^- fragmentation patterns.

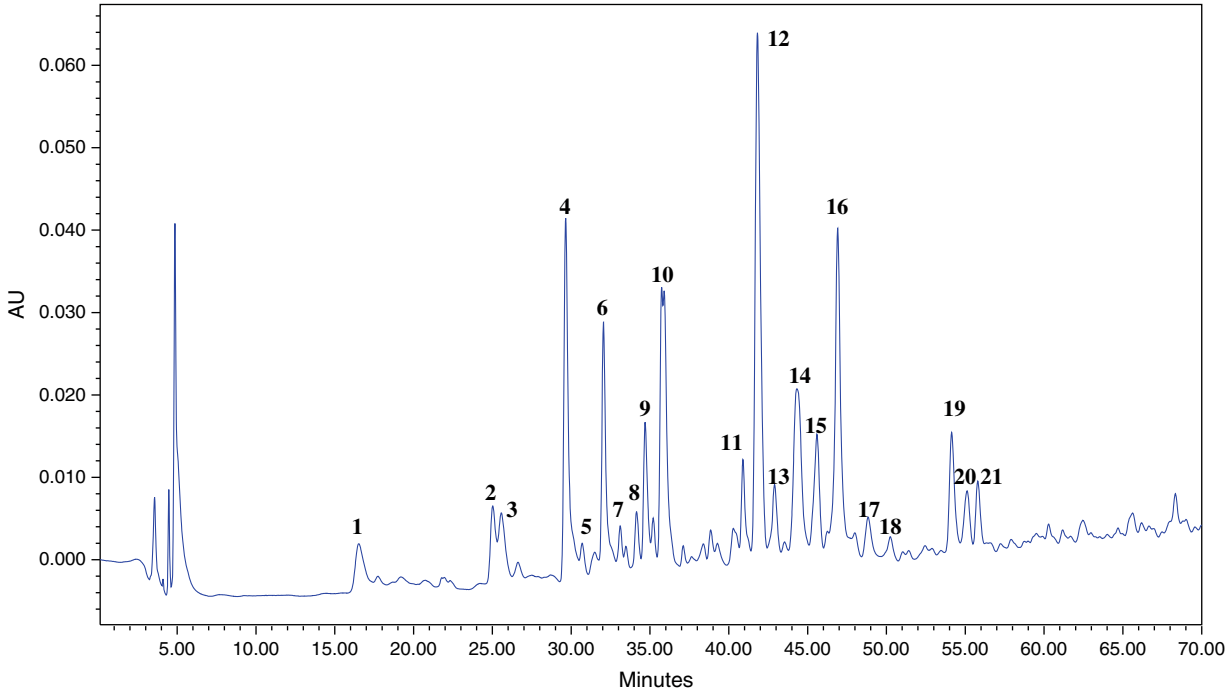

Fig. 2. HPLC chromatogram of non-anthocyanin polyphenolics present in *E. precatoria* juice at 280 nm. Peak identifications and their MS data are shown in Table 3.

cleavage of the interflavanoid bond, and characteristic of B-type procyanidin dimers (Friederich, Eberhardt, & Galensa, 2000; Gu et al., 2003). Procyanidin trimers (m/z = 865.1, $[M-H]^-$) were characterised by predominant product ion signals at m/z = 577.2, $[M-H-288]^-$, likely due to the loss of a (+)-catechin or (−)-epicatechin unit, yielding dimeric procyanidin fragment ions (Friederich et al., 2000; Gu et al., 2003). Further fragmentation of ions at m/z = 577.2 occurred in a manner similar to those in the previously identified B-type procyanidins, confirming their identity. Procyanidin dimers were particularly abundant in *E. precatoria* fruits, accounting for more than 25% of the total non-anthocyanin polyphenolic content, while procyanidin trimers accounted for just over 5%. Conversely, procyanidin dimers and trimers accounted for just over 10% of the total non-anthocyanin polyphenolics in *E. oleracea*. Flavonol monomers, such as (+)-catechin and (−)-epicatechin, also represented less than 5% of the total non-anthocyanin polyphenolic content, for both species. Higher molecular weight compounds, likely polymeric procyanidins (Schauss et al., 2006), were also detected; however, quantification was not possible, due to their poor resolution under these chromatographic conditions (Santos-Buelga & Williamson, 2003).

3.2. Antioxidant properties

Both species were characterised by an initially high antioxidant capacity, 87.4 ± 4.4 μmol trolox equivalents (TE)/g for *E. oleracea* and 114 ± 6.9 μmol TE/g for *E. precatoria* (Fig. 3), in relation to other fruits and vegetables. Variations in antioxidant capacity between species were attributed to simple differences in phytochemical composition and concentration, primarily associated with the

Table 3
Characterisation of non-anthocyanin polyphenolics present in *Euterpe precatoria* fruits.

Peak no.	RT (min)	Compound	λ_{max} (nm)	$[M-H]^-$ (m/z)	MS/MS (m/z)	Concentration (mg/kg)[a]
1	16.5	Protocatechuic acid	263, 292	153.2	109.2, 91.0	2.38 ± 0.24
2	24.9	*p*-Hydroxybenzoic acid	253.9	137.3	112.9	2.42 ± 0.44
3	26.7	(+)-Catechin	277.5	289.2	245.2, 203.2, 187.2, 161.3	5.46 ± 0.57
4	29.7	Vanillic acid	263, 291	167.3	140.9, 108.0, 95.2	13.4 ± 1.07
5	31.1	Apigenin glucoside[b]	258, 352	563.3	545.1, 473.2, 443.2, 353.1, 269.1	9.91 ± 0.84
6	33.1	Syringic acid	271	196.9	182.2, 153.1, 138.1	10.1 ± 0.93
7	32.6	Apigenin glucoside[b]	258, 352	563.3	545.1, 473.2, 443.2, 353.1, 269.1	7.82 ± 0.61
8	34.1	(−)-Epicatechin	277.5	289.2	245.2, 203.2, 187.2, 161.3	2.35 ± 0.28
9	34.7	Unidentified flavone	272, 352	521.3	359.1, 344.2	5.11 ± 0.36
10	36.7	Taxifolin derivative[b]	272, 356	449.4	327.1, 269.2, 225.1	9.20 ± 0.72
11	41.3	Isoorientin	272, 352	447.2	393.1, 357.1, 327.2, 299.2, 285.1	23.6 ± 1.07
12	41.9	Orientin	272, 348	447.2	429.2, 357.1, 327.3, 299.2, 285.1	47.7 ± 2.04
13	43.0	Ferulic acid	323.7	193.2	149.1, 134.1, 117.0	1.22 ± 0.13
14	44.7	Taxifolin deoxyhexose	295, 340	449.1	269.1	7.50 ± 0.49
15	45.7	Procyanidin dimer	235, 282	577.1	425.0, 407.2, 289.1, 287.1	52.9 ± 3.16
16	47.1	Isovitexin	267, 338	431.1	341.2, 311.1, 283.0, 269.1	4.21 ± 0.18
17	49.2	Apigenin glucoside[b]	272, 352	533.4	443.2, 425.2, 383.1, 353.1	6.31 ± 0.57
18	50.4	Apigenin glucoside[b]	272, 352	533.4	443.2, 425.2, 383.1, 353.1	4.59 ± 0.41
19	53.2	Procyanidin dimer	235, 282	577.1	425.0, 407.2, 289.1, 287.1	15.5 ± 1.22
20	54.9	Procyanidin trimer	235, 291	865.1	577.2, 451.0, 425.0, 407.3	7.11 ± 0.44
21	56.3	Procyanidin trimer	235, 291	865.1	577.2, 451.0, 425.0, 407.3	7.23 ± 0.38

[a] Values represent the means and standard errors from three replicates.
[b] Tentative identification based on similarities of spectral characteristics and ESI–MS^- fragmentation patterns.

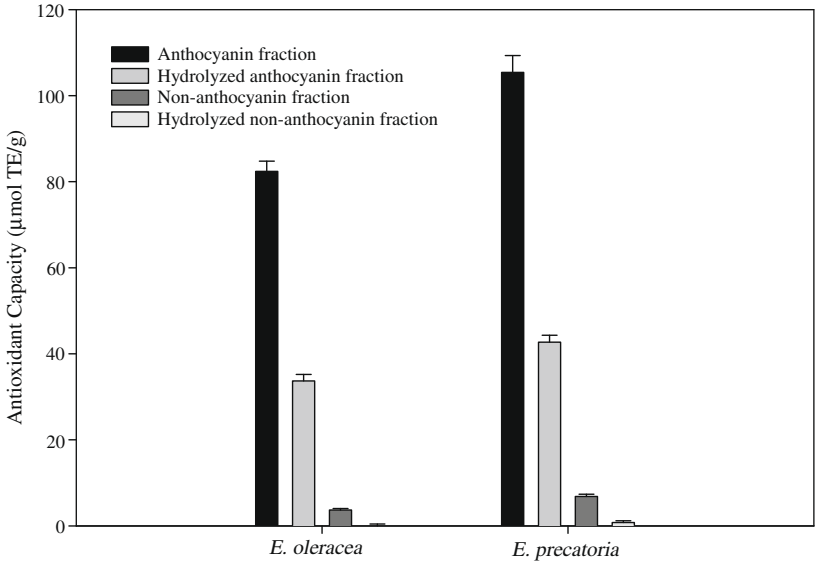

Fig. 3. Antioxidant capacity of non-hydrolysed and hydrolysed *E. oleracea* and *E. precatoria* phytochemical isolates (non-anthocyanin and anthocyanin fractions). Bars represent the standard error of the mean (n = 6). Antioxidant capacity quantified in μM trolox equivalents (TE)/g.

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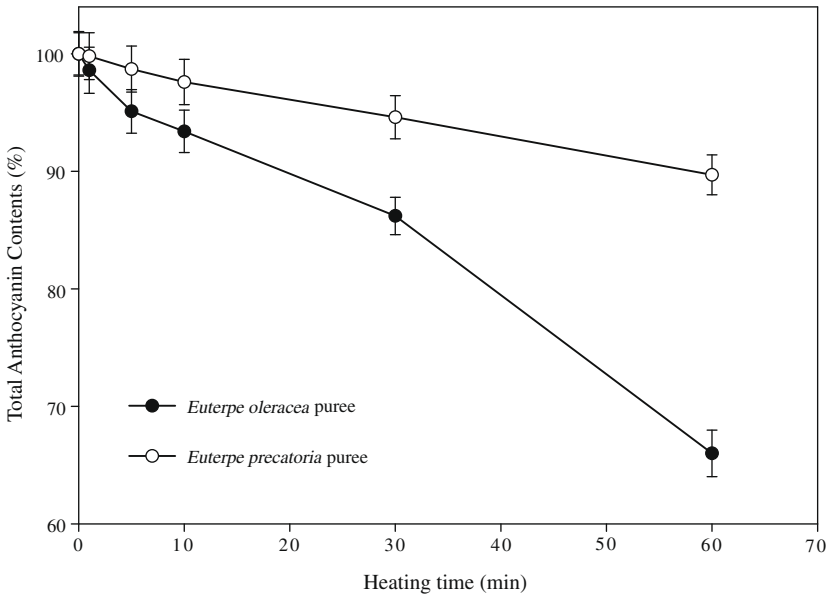

Fig. 4. Percent changes in total anthocyanin contents in *E. oleracea* and *E. precatoria* fruit purees following heating (80 °C), as a function of heating time.

higher anthocyanin and procyanidin contents of *E. precatoria*. Fractionation of anthocyanins from remaining polyphenolics, followed by acid hydrolysis, provided additional detail that related to those compounds most responsible for radical-scavenging contributions from each species. Anthocyanin-containing fractions from both species exhibited the highest antioxidant capacity, ranging from 82.4 ± 2.3 μmol TE/g in *E. oleracea* to 105 ± 3.9 μmol TE/g in *E. precatoria*, confirming that anthocyanins were the major contributors to antioxidant capacity at over 90% of the total. The remaining antioxidant capacity was due to non-anthocyanin compounds and represented from 3.7 ± 0.34 μmol TE/g in *E. oleracea* to 6.8 ± 0.52 μmol TE/ml in *E. precatoria*. Acid hydrolysis resulted in a significant reduction in the antioxidant capacities of both species, equivalent to 59.1% to 59.5% decrease for the anthocyanin isolate compared to 88.9% to 100% for the remaining polyphenolics (Fig. 3).

Chromatographic analyses of polyphenolics in the hydrolysed, anthocyanin fractions revealed the presence of cyanidin (m/z = 287.1, $[M-H]^-$, 99%) and peonidin (m/z = 301.1, $[M-H]^-$, 1%) aglycones in *E. oleracea* and cyanidin (m/z = 287.1, $[M-H]^-$, 94%) and pelargonidin (m/z = 271.1, $[M-H]^-$, 6%) aglycones in *E. precatoria* fractions, further confirming their presence. Moreover, luteolin (m/z = 285, $[M-H]^-$) and apigenin (m/z = 269, $[M-H]^-$) aglycones were also detected in the hydrolysed non-anthocyanin polyphenolic fraction of both species, along with trace concentrations of a cyanidin aglycone (<1 mg/kg) derived from polymeric procyanidins.

3.3. Thermal stability

The overall thermal stability of polyphenolics in açai was evaluated by holding açai pulps at 80 °C for 1, 5, 10, 30, and 60 min, in the presence and absence of oxygen, as compared to a non-heated control. No significant differences ($p < 0.05$) were observed between the presence or absence of oxygen on polyphenolic degradation during heating. Non-anthocyanin polyphenolics, including flavone glycosides, flavonol derivatives and phenolic acid concentrations, remained constant during heating for up to 60 min, demonstrating appreciable thermal stability of these compounds in both açai species. However, extensive anthocyanin degradation occurred under these heating conditions, likely due to accelerated chalcone formation with prolonged anthocyanin exposure to high temperatures (Delgado-Vargas, Jiménez, & Paredes-Lopez, 2002). Anthocyanin degradation rates were directly related to thermal exposure times (Fig. 4), yet were highly variable between species, ranging from 10.3 ± 1.1% in *E. precatoria* to 34.0 ± 2.3% in *E. oleracea* purees. Variations in overall anthocyanin stability were attributed to differences in anthocyanin composition and variations in non-anthocyanin polyphenolics, which likely conferred additional stability. Cyanidin-3-rutinoside consistently showed a higher thermal stability (7.0 ± 0.6% loss following heating at 80 °C for 1 h) than did cyanidin-3-glucoside (up to 72 ± 5.3% loss under identical heating conditions) in both açai species. Therefore, an overall higher anthocyanin thermal stability in *E. precatoria* purees was likely due to higher concentrations of cyanidin-3-rutinoside (~90% of total anthocyanins, Table 1), compared to *E. oleracea* purees (~55% of total anthocyanins, Table 1). Results were in agreement with previous investigations on the storage stability of anthocyanins in *E. oleracea* juice, where cyanidin-3-rutinoside half-lives were double those of cyanidin-3-glucoside, in both the presence and absence of ascorbic acid (Pacheco-Palencia et al., 2007a). Similar observations have also been reported for other cyanidin-3-glucoside- and cyanidin-3-rutinoside-containing fruits, such as blackcurrants (Rubiskiene, Jasutiene, Venskutonis, & Viskelis, 2005), where cyanidin-3-rutinoside was found to be the most thermally stable anthocyanin (35% loss after 150 min at 95 °C). Variations in total anthocyanin contents during heating were highly correlated (r = 0.98) with changes in total antioxidant capacity, which decreased by up to 10 ± 0.8% in *E. precatoria* and by 25 ± 2% in *E. oleracea* purees, evidencing a major contribution of anthocyanins to the overall antioxidant capacity of both açai species.

4. Conclusions

E. oleracea and *E. precatoria* species shared similar polyphenolic profiles, characterised by the predominant presence of anthocyanins, which accounted for nearly 90% of the total antioxidant capacity in both açai fruits. Moreover, changes in antioxidant activity during heating were highly correlated with anthocyanin losses, while phenolic acids, flavone glycosides, and flavanol derivatives, present in both species, were not significantly altered by thermal exposure. Thus, both açai species are comparably suitable for food

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and beverage applications involving mild exposure to high temperatures.

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