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Anthocyanins and Antioxidant Properties of Juçara Fruits (*Euterpe edulis* M.) Along the On-tree Ripening Process

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ABSTRACT

Juçara (Euterpe edulis M.) fruits are an interesting source of phenolic compounds, mainly anthocyanins, making them valuable to the food and pharmaceutical industries. Juçara fruits were harvested along the on-tree ripening process between March and June as practiced in Paraná state, Brazil and examined for their total anthocyanin content (TAC) and total antioxidant capacity (TAA). The results showed that TAC increased (91.52– 236.19 mg cyanidin-3-glucoside equivalent/100 g dm) during ripening of juçara fruits. Use of tandem mass spectrometry allowed the identification of cyanidin-3,5-diglucoside, peonidin-3-glucoside and peonidin-3-rutinoside for the first time in juçara fruits. The high antioxidant capacity using DPPH radical scavenging capacity (655.89–745.32 µmol TE/g dm) and ORAC assays (1088.10–2071.55 µmol TE/g dm) showed that juçara fruits have potential as a source of novel natural antioxidants for disease prevention and health promotion, and also as natural food additives for developing new functional food products.

Keywords: Juçara, *Euterpe edulis* M., fruit ripening, anthocyanins, antioxidant capacity.

INTRODUCTION

Juçara (*Euterpe edulis* Martius, Arecaceae) is a palm tree found mainly in the Brazilian states of the Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Rio de Janeiro, Minas Gerais and Bahia (LORENZI, 2006). Juçara palm tree produces a non-climacteric round fruit which grows in bunches and has a pericarp covering a hard seed. The berries are small with a diameter of about 1 to 1.5 cm and the seed constituting 85% of the fruit. During ripening, the epicarp evolves from green to dark purple or almost black. Juçara is usually used in the form of pulp or juice.

Anthocyanins are an important group of phenolics in juçara fruits; they are responsible for their pigmentation. Moreover, anthocyanins are interesting for their use as natural water-soluble colorants thereby reducing the use of synthetic colorants in foods (CLIFFORD, 2000). In general, the ripening process of fruits involves biochemical and metabolic changes of primary and secondary compounds, resulting in nutritional, palatable and potentially health-promoting phytochemicals. Since fruit maturation has impact on its commercialization and on human nutrition, it is important to investigate changes in bioactive compounds occurring during maturation of juçara fruits. Thus, the aim of this study was to evaluate anthocyanins and total antioxidant capacity of juçara fruits (*Euterpe edulis*) along the on-tree ripening process.

MATERIALS AND METHODS

Juçara fruits were harvested at the experimental station of the Agricultural Research Institute of Paraná at Antonina City, Parana, Brazil. Fruits were harvested every two weeks between March and June 2012 and sorted into three stages of maturation according to external colour. Fruits of each harvesting stage (HS) were processed separately. The separation of the exo- and mesocarp from the seeds was carried out in a juçara specific extractor by adding water in a proportion of 0.6 l/kg fruit. Then, the obtained pulp was freeze-dried, milled to pass through 0.5 mm screen and stored at −20 °C prior to extraction. Samples were defatted with hexane (3×10 ml) using sonication for 30 min and centrifuged at 8,500 × g (RC6+ Sorvall Instruments, Newtown, CT, USA) for 15 min at 20 °C and the moisture content of samples was determined by AOAC official method 925.10 (AOAC, 2003) before extraction of compounds.

Defatted juçara powder (1 g) was extracted by methanol/1 M hydrochloric acid (85:15, v/v, MeOH/HCl) as reported previously (BICUDO; RIBANI; BETA, 2014). The supernatant was separated and used as a crude extract to determine total anthocyanin content and total antioxidant capacity.

The chromatographic separation of anthocyanins was carried on a HPLC (Waters 2695) system equipped with a photodiode array detector (Waters 996) and autosampler (Waters 717 plus) (Waters Corporation, Milford, MA, USA). The analytical column used was a Gemini 5 µm RP-18 (150 mm×4.6 mm) (Phenomenex, Torrance, CA, USA). A 10-µl volume of each sample crude extract was injected for analysis. The anthocyanin composition was eluted with a gradient mobile phase consisting of A (0.1 % formic acid in water) and B (0.1 % formic acid in methanol). The flow rate was 0.5 mL/min. A linear gradient was programmed as follows: 0–5 min, 10 % B; 5–8 min, 10–15 % B; 8–10 min, 15–20% B; 10–13 min, 20–25 % B; 13–18 min, 25–30 % B; 18–25 min, 30–35 % B; 25–30 min, 35–45 % B; 30–33 min, 45–60 % B; 33–35 min, 60–95 % B; 35–42 min, 95 % B; 42–44 min, 95–10 % B; 44–50 min, 10 % B. The quadrupole time-of-flight mass spectrometer (Q-TOF-MS) (Micromass, Waters Corp., Milford, MA, USA) was calibrated by using sodium iodide for positive mode through the mass range of 100–1500. MS parameters were set as follows: capillary voltage: 2100 V, sample cone voltage: 30 V, source temperature: 120 °C, desolvation temperature: 250 °C, desolvation gas (nitrogen gas) flow rate: 900 l/h. The MS/MS spectra were acquired by using collision energy of 30 V.

All the experiments were performed in triplicate, and the results were reported as mean ± SD (standard deviation). Data were subjected to one-way analysis of variance for comparison of means using SAS Software 9.3 version (SAS Institute Inc., Cary, NC, USA) and significant differences were calculated according to Tukey test at the 5 % level.

RESULTS AND DISCUSSION

There is growing interest in the possibility that consuming a diet rich in antioxidants may reduce the risks of many common chronic diseases (SZAJDEK; BOROWSKA, 2008). The TAA of juçara fruits measured using DPPH radical scavenging capacity and ORAC assays are shown in Table 1. Juçara pulp was found to have a relatively high antioxidant capacity with respect to other anthocyanin rich fruits such blackberries and açai (WANG; LIN, 2000). Our results suggest that anthocyanins may be a major contributor to the TAA in juçara fruits in addition to other phytochemicals.

Table 1 – Antioxidant capacity by DPPH radical scavenging capacity (DPPH) and ORAC assays in juçara fruits at six harvesting stages

	Antioxidant capacity (µmol TE/g dm)	
	DPPH	ORAC
HS1: March 29th – immature violet	655.89 ± 5.50^{D}	1088.10 ± 10.26^{E}
HS2: April 12th – mature purple	726.30 ± 8.70^{B}	1178.48 ± 15.93^{D}
HS3: April 26th – mature purple	709.51 ± 4.53^{C}	1564.20 ± 13.37^{B}
HS4: May 10th – mature purple	724.92 ± 1.35^{B}	1266.36 ± 25.40^{C}
HS5: May 24th – ripe dark purple	745.32 ± 5.32^{A}	1285.09 ± 22.59^{C}
HS6: June 7th – ripe dark purple	703.32 ± 1.21^{C}	2071.55 ± 37.03^{A}

TAC of juçara fruits at six harvesting stages is shown in Table 2 ranging from 91.52 to 236.19 mg C3G equivalent/100 g dm. Fruits from HS5 had the highest TAC and fruits from HS3 showed higher TAC than that of fruits from HS4 ($p<0.05$), which suggests a transient accumulation of anthocyanins in juçara fruits. Juçara palm tree grows in open fields under sun irradiation and temperature effects; moreover, fruits located in the external parts of the bunch receive more sunlight than those located under the shade. Thus, the variations in TAC observed among the harvesting stages can be attributed to genetic factors and environmental conditions as growth temperature and light intensity (ŠAVIKIN et al., 2009).

Table 2 – Total anthocyanin content (TAC) and anthocyanin composition in juçara fruits at six harvesting stages

	$[M+H]^+$ product ion m/z	HS1	HS2	HS3	HS4	HS5	HS6
TAC[a]	–	91.52 ± 3.46^{E}	95.67 ± 2.89^{E}	122.68 ± 1.14^{C}	113.56 ± 2.26^{D}	236.19 ± 3.88^{A}	210.42 ± 1.75^{B}
C3,5dG[a]	611/287	0.17 ± 0.01^{B}	0.17 ± 0.02^{B}	0.19 ± 0.01^{B}	0.11 ± 0.01^{C}	0.18 ± 0.01^{B}	0.33 ± 0.02^{A}
C3G[a]	449/287	29.09 ± 0.17^{E}	33.94 ± 0.21^{D}	30.51 ± 0.64^{E}	42.95 ± 0.15^{C}	85.61 ± 0.51^{B}	108.97 ± 1.07^{A}
C3R[b]	595/287	43.92 ± 0.24^{E}	42.77 ± 0.68^{E}	74.26 ± 0.67^{D}	76.59 ± 0.82^{C}	137.27 ± 1.22^{A}	87.08 ± 0.81^{B}
P3R[a]	579/271	nd	0.04 ± 0.01^{A}	0.06 ± 0.01^{A}	nd	nd	0.07 ± 0.03^{A}
PN3G[a]	463/301	0.33 ± 0.02^{B}	0.38 ± 0.01^{B}	0.42 ± 0.01^{B}	1.27 ± 0.06^{A}	1.21 ± 0.02^{A}	1.24 ± 0.03^{A}
PN3R[a]	609/301	0.47 ± 0.01^{C}	0.55 ± 0.03^{C}	0.71 ± 0.04^{B}	0.46 ± 0.01^{C}	0.81 ± 0.05^{AB}	0.83 ± 0.06^{A}

[a] The results are represented as mg C3G/100g dm; [b] The results are represented as mg C3R/100g dm; nd: Not significant quantity; Values in the same row with different letters are significantly different ($p<0.05$).

Anthocyanin composition as detected by HPLC-QTOF-MS/MS is displayed in Table 2 and Figure 1. Two main peaks were detected comprising cyanidin-3-glucoside (C3G) (RT=21.65 min) and cyanidin-3-rutinoside (C3R) (RT=23.03 min). Low levels of cyanidin-3,5-diglucoside (C3,5dG) (RT=20.73 min), pelargonidin-3-rutinoside (P3R) (RT= 24.47 min), peonidin-3-glucoside (PN3G) (RT=25.17 min), and peonidin-3-rutinoside (PN3R) (RT=26.40 min) were also found in juçara fruits. C3,5dG, PN3G and PN3R were identified in juçara fruits for the first time. C3G ranged from 29.09 to 108.97 mg/100 g dm and C3R ranged from 42.77 to 137.27 mg/100 g dm. C3R was significantly higher than C3G at HS5, and C3G was significantly higher than C3R at HS6. BRITO et al. (2007) identified six anthocyanins in juçara fruits from São Paulo state, Brazil; three of them (cyanidin 3-sambubioside, pelargonidin 3-glucoside and cyanidin 3-rhamnoside) were different to those found in our study, suggesting the influence of planting

location, even different metabolic pathways between fruits cultivated in São Paulo and Paraná states.

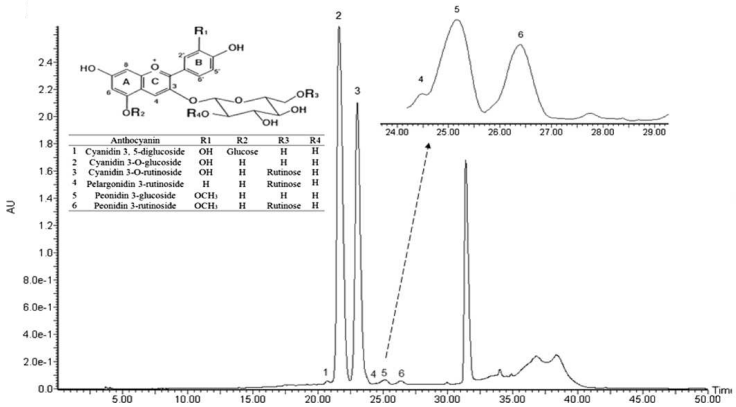

Figure 1 – The chromatogram of anthocyanins at HS6 (detected at 520 nm), compounds were identified as follow: 1A = cyanidin 3, 5-diglucoside, 2A = cyanidin 3-O-glucoside, 3A = cyanidin 3-O-rutinoside, 4A = pelargonidin 3-rutinoside, 5A = peonidin 3-glucoside, 6A = peonidin 3-rutinoside.

CONCLUSIONS

The quantification and identification of anthocyanins in juçara fruits along the on-tree ripening process were evaluated by various chemical assays for the first time. The results suggest that juçara fruits offer higher levels of potentially health-promoting compounds from late May to early June, the optimal harvesting stage according to this study. The demonstration of high anthocyanin content and high antioxidant activity confirms juçara fruit as promising and an excellent source of dietary phytochemicals.

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