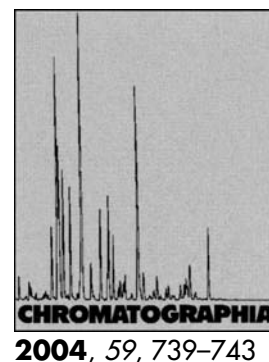


Polyphenolic Constituents of Fruit Pulp of *Euterpe oleracea* Mart. (Açaí palm)



S. Gallori¹, A. R. Bilia¹, M. C. Bergonzi¹, W. L. R. Barbosa², F. F. Vincieri¹

¹ Department of Pharmaceutical Science, University of Florence, Polo Scientifico di Sesto Fiorentino, Via Ugo Schiff 6, Sesto Fiorentino, Florence, Italy; E-Mail: sandra.gallari@unifi.it

² Phytochemical Laboratory, Federal University of Pará, Belém, Pará, Brazil

Received: 8 December 2003 / Revised: 18 February 2004 / Accepted: 24 February 2004
Online publication: 3 June 2004

Abstract

The polyphenolic composition of *Euterpe oleracea* Mart. (Açaí palm) fruit was investigated by HPLC-DAD-UV-Vis and HPLC-MS. Açaí palm is widely diffused and cultivated in Amazon regions and especially in the Pará state (Brazil), because the pulp of the fruit is largely consumed as food. This study confirms the presence of cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside as major anthocyanic compounds. Moreover, four main compounds were also identified for the first time, i.e. homoorientin, orientin, taxifolin deoxyhexose, and isovitexin. Traces of a methyl-derivative of homoorientin were also detected. The amount of total anthocyanins was 0.5 mg g⁻¹ of the dried pulp weight and the amount of the other flavonoids was 3.5 mg g⁻¹ of the dried pulp weight. No other reports on the presence of non-anthocyanic flavonoids in *Euterpe oleracea* Mart. have been found so far.

Keywords

Column liquid chromatography – DAD-mass spectrometry
Flavonoids
Anthocyanins
Euterpe oleracea Mart.
Açaí palm

Introduction

The aim of this work was to contribute to the phytochemical analysis of the polyphenolic content of the fruit of *Euterpe oleracea* Mart. (Açaí palm), a palm of the Arecaceae family.

The plant is widely diffused and cultivated in Amazon regions especially in the Pará State of Brazil. Açaí is commonly also called cabbage palm because its terminal buds or “cabbages”, the hearts of palm, are eaten pickled and as a salad.

The fruit is a purple-black berry at complete maturity with a diameter of 10–12 mm and an unusual flavour similar to raspberries or blackberries as well as having a nutty taste. Especially in the Pará state, the pulp of the fruit has a large consumption (about 180 tons/year): it is used fresh to prepare “vinho de açaí” (watery emulsion of the oily fruit pulp with sugar and manioc flour) or it is used fermented as a refreshing beverage. It is also employed to prepare desserts and ice creams or it is made into a syrup

to be used with other foods. Furthermore, the juice represents an ingredient of the traditional “cassava meal” [1–2].

To our knowledge a few papers [3–7] have been reported in the literature on the constituents of *Euterpe oleracea* fruits and only three of them are focused on the anthocyanin content, i.e. cyanidin 3-*O*-arabinoside, cyanidin-3-*O*-arabinosylarabinoside, cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside. However, the identification of these constituents was based on their hydrolysis and TLC analyses.

In the present study, HPLC analysis was carried out on the hydroalcoholic extract of the raw fruit pulp and on the fraction by liquid-solid purified extraction (LSE) procedure. A qualitative and quantitative analytical HPLC-DAD-MS method was developed and constituents were identified by means of comparison of a combination of R_f , UV-Vis and MS data of the peaks with those of authentic samples and with literature data [8–10].

This is the first report on the presence of non-anthocyanic flavonoids in *Euterpe oleracea* Mart.

Experimental

Sample Preparation and Extraction of Polyphenols

Chemicals

All the solvents used were HPLC grade purity; CH₃CN and MeOH for HPLC

Table 1. The linear gradient program used for analytical HPLC-DAD and HPLC-MS analysis of total hydroalcoholic extract and EtOAc fraction from LSE (Samples **1a** and **2a** respectively) of *Euterpe oleracea* Mart. (Analysis was carried out at flow rate of 0.8 mL min⁻¹ using a 250 × 4.6 mm Luna RP-18 (5 μm) column (Phenomenex, Germany), equipped with a 4 × 3.0 mm precolumn of the same phase, operating at 26 °C

H ₂ O/H ⁺	CH ₃ CN	MeOH	Time (min)
100	0	0	0.1
85	15	0	5.0
75	25	0	25.0
75	25	0	40.0
0	25	75	47.0
0	25	75	52.0

were from E. Merck, (Darmstadt, Germany). 85% formic acid was provided by Carlo Erba (Milan, Italy). Water was purified by a Milli-Q plus system from Millipore (Milford, MA, USA).

Orientin, homoorientin, taxifolin, vitexin, isovitexin, and keracyanin standards were from Extrasynthese S.A. (Lyon, Nord Genay, France). Extrelut® NT 20 cartridges were from E. Merck, PTFE membrane filters 0.45 μm were from Waters Co. (Milford, MA).

Plant Materials

The *Euterpe oleracea* fruits were purchased by one of the authors (A.R.B.) in 2001 in a local market of Belém (Pará State, Brazil). The fruits were identified by Dr. P. Luzzi of the University of Florence and a voucher specimen is deposited at the Department of Pharmaceutical Sciences of Florence.

Extraction of Plant Material

From 20.0 g of purple-black mature fruit, 4.30 g of pulp were manually separated. The pulp was lyophilised (Lyovac GT2, Leybold Heraeus) to obtain 2.60 g. The lyophilised pulp was finely ground and extracted under stirring three times with 450 mL of EtOH-H₂O 7:3 (pH = 2 by HCOOH) and filtered through a Buckner funnel. The raw hydroalcoholic extract was concentrated under vacuum (Rotavapor 144 R, Büchi, Switzerland) (at 25 °C) to reach a final volume of 165 mL. The sample was defatted with *n*-hexane to eliminate lipophilic compounds such as chlorophylls and carotenoids. 20 mL of this solution was evaporated to dryness under vacuum (at 25 °C) and then rinsed

with 2 mL of ultra pure water acidified with HCOOH (5%). The solution was filtered through a 0.45 μm PTFE filter and analysed by HPLC-DAD and HPLC-MS (Sample **1**).

A portion of the concentrated raw hydroalcoholic extract was subsequently fractionated with a liquid-solid extraction (LSE) procedure. Thus, 60 mL of this solution were evaporated to dryness under vacuum (at 25 °C) and then rinsed with 5 mL of ultra pure water acidified with HCOOH (5%) and applied to a 20 mL Extrelut® cartridge. After 20 min the column was eluted with 200 mL of *n*-hexane to remove the lipophilic compounds and then with 300 mL of EtOAc to collect the phenolic derivatives. Finally, 250 mL of acidic MeOH (pH = 2 by HCOOH) was used to collect the anthocyanins and other hydrophilic compounds, such as sugars and organic acids. The EtOAc fraction was evaporated to dryness and then solubilised with 1 mL of a solution of H₂O (pH = 2 by HCOOH)/MeOH/CH₃CN 20:60:20 v/v (Sample **2a**). The MeOH fraction was concentrated to 10 mL and 5 mL of this solution was concentrated to 1.7 mL exactly, (Sample **2b**). All three samples (**1**, **2a** and **2b**) were subsequently used for phenol analysis by HPLC-DAD and HPLC-MS.

Analytical Techniques and Equipment

HPLC-DAD Analysis of the Raw Hydroalcoholic Extract and its EtOAc Fraction from LSE

HPLC-DAD analyses of the raw hydroalcoholic extract (Sample **1**) and its EtOAc fraction from LSE (Sample **2a**) were performed on an HP 1090L Series II liquid chromatograph equipped with a diode array detector (all from Agilent Technologies, Palo Alto, CA, USA). Column was a 250 × 4.6 mm Luna RP-18 (5 μm) (Phenomenex, Germany) equipped with a 4 × 3.0 mm precolumn of the same phase operating at 26 °C. Flow rate was 0.8 mL min⁻¹. The eluents were H₂O (pH = 3.2 by HCOOH), CH₂CN and MeOH; the applied three-step linear gradient program is reported in Table 1. UV-Vis spectra were recorded in the range λ 190–450 nm, and the chromatograms were acquired at λ 230, 254, 280, 330 and 350 nm. Typical chromatograms at λ 350 nm of Samples **1** and **2a** are reported in Fig. 1a and 2a, respectively.

HPLC-DAD Analysis of the Raw Hydroalcoholic Extract and its MeOH Fraction from LSE

HPLC-DAD analyses of the raw hydroalcoholic extract (Sample **1**) and its MeOH fraction from LSE (Sample **2b**) were performed on a HP 1090L Series II liquid chromatograph equipped with a diode array detector (all from Agilent Technologies, Palo Alto, CA, USA). Column was a 4.6 × 250 mm Phenomenex® Synergy MAX RP-12 (4 μm) operating at 26 °C. Flow rate was 1.0 mL min⁻¹. The anthocyanins were separated using the following mobile phases: Solution A- H₂O/HCOOH 95:5, Solution B - H₂O/CH₃CN/MeOH/HCOOH 50:22.5:22.5:5; the applied five-step linear gradient program is reported in Table 2. The UV-Vis spectra were recorded in the range λ 190–600 nm, and the chromatograms were acquired at λ 520 nm. Typical chromatograms at λ 520 nm of Samples **1** and **2b** are reported in Fig. 1b and 2b, respectively.

HPLC-MS Analysis

MS spectra were performed using a HP 1100 MSD with API-electrospray interface coupled with an HP 1100L liquid chromatograph equipped with a DAD detector (Agilent Technologies). The same column, mobile phase, time period and flow rate were used. Mass spectrometry operating conditions were as follows: negative and positive ionisation mode (scan spectra from *m/z* 100 to 800), gas temperature of 350 °C, nitrogen flow rate of 10.0 L min⁻¹, nebulizer pressure 30 psi, quadrupole temperature 30 °C, and capillary voltage 3500 V. The applied fragmentors were in the range 80–150 V.

Analysis of flavonoids was optimised by using negative ion mode with a fragmentation of 120 and 150 V while analysis of anthocyanins was optimised by using positive ion mode with a fragmentation of 120 V.

The orthogonal position of the nebulizer with respect to the capillary inlet allowed the use of the same conditions of HPLC-DAD analysis with H₂O adjusted to pH 3.2 by HCOOH.

Results and Discussion

Identification of constituents was carried out by HPLC-DAD and HPLC-MS analysis, and/or by comparison and

Table 2. The linear gradient program used for analytical HPLC-DAD and HPLC-MS analysis of total hydroalcoholic extract and MeOH fraction from LSE (Samples **1b** and **2b** respectively) of *Euterpe oleracea* Mart. (Analysis was carried out at flow rate of 1.0 mL min^{-1} using a $4.6 \times 250 \text{ mm}$ Phenomenex® Synergy MAX RP-12 ($4 \mu\text{m}$) operating at $26 \text{ }^\circ\text{C}$)

Sol.A	Sol.B	MeOH	Time (min)
98.0	0.0	2.0	0.1
73.0	25.0	2.0	8.0
68.0	30.0	2.0	10.0
68.0	30.0	2.0	13.0
63.0	35.0	2.0	20.0
63.0	35.0	2.0	22.0
58.0	40.0	2.0	32.0
43.0	55.0	2.0	37.0
0	0	100.0	47.0

combination of their retention times, UV-Vis and mass spectra of the peaks with those of authentic samples. Quantitative data were calculated by HPLC peak areas compared with those of external standard calibration curves. The evaluation was performed using four-point linear standard calibration curves ($r^2 > 0.99$) calculated by HPLC-DAD at the maximum absorbance wavelength for each compound (280 nm for taxifolin, 350 nm for homoorientin, orientin and vitexin, and 520 nm for the anthocyanin).

HPLC analysis was performed on the raw hydroalcoholic extract of the fruit pulp and on the EtOAc and MeOH fraction obtained by liquid-solid extraction (LSE) procedure. The performed LSE method with Extrelut® NT 20 cartridge allowed the separation of polyphenolic subclasses present in the hydroalcoholic extract of fruit, i.e. flavonoids and anthocyanins as confirmed from data of acquired chromatograms at different wavelengths.

Identification of Flavonoids

The chromatographic profiles reported in Fig. 1a and 2a, acquired at 280 and 350 nm, relative to the total hydroalcoholic extract (Sample **1**) and EtOAc fraction from LSE (Sample **2a**) respectively, revealed the occurrence of trace amounts of several flavonoid derivatives together with three main components identified by means of R_f , UV-Vis and MS data as homoorientin (1), orientin (2), and isovitexin (4).

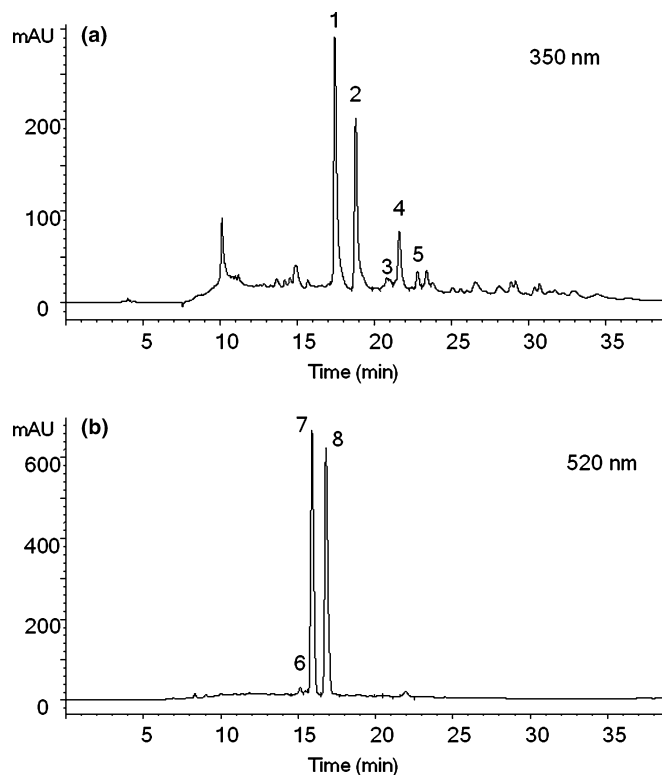


Fig. 1. HPLC chromatograms acquired at 350 nm (a) and 520 nm (b) of total hydroalcoholic extract of *Euterpe oleracea* Mart. fruits. Polyphenolic compounds: 1 = homoorientin, 2 = orientin, 3 = taxifolin deoxyhexose, 4 = isovitexin, 5 = homoorientin derivative, 6 = cyanidin glyc. derivative, 7 = cyanidin 3-*O*-glucoside, 8 = cyanidin 3-*O*-rutinoside

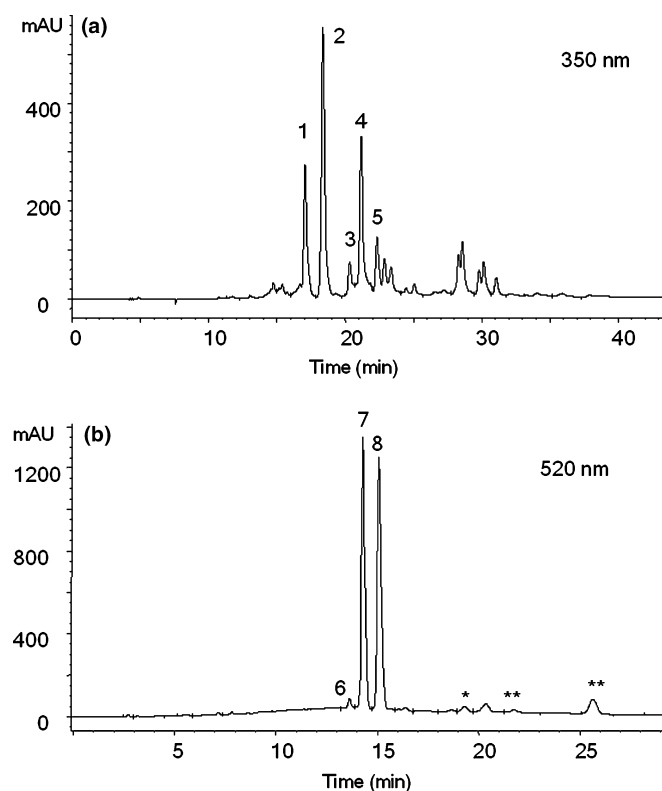


Fig. 2. HPLC chromatograms acquired at 350 nm (a) and 520 nm (b) of EtOAc and MeOH fractions, respectively, from LSE of *Euterpe oleracea* Mart. fruits. Peak identification as in Fig. 1. * Peonidin derivatives, ** cyanidin derivatives

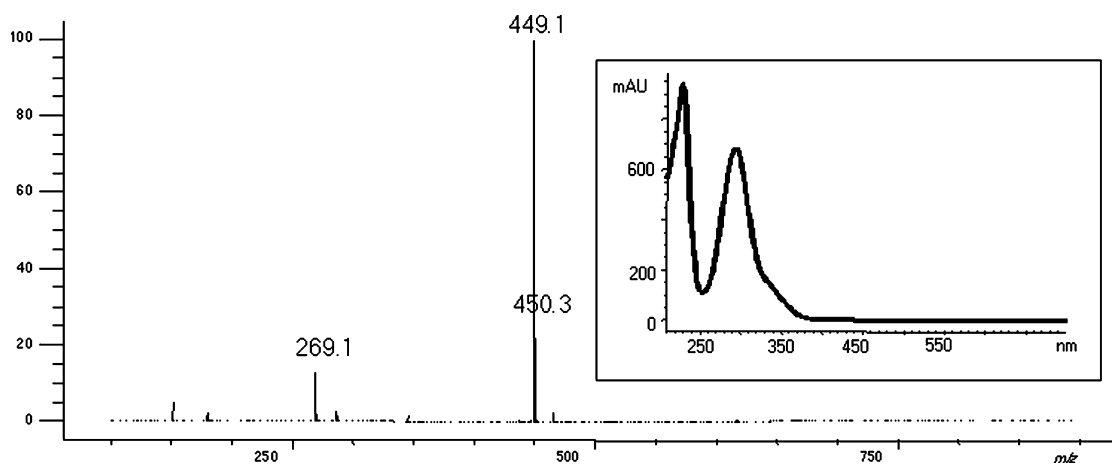


Fig. 3. UV-Vis and negative ion mass spectra of peak 3 acquired during the API-electrospray HPLC-MS analysis. Operating conditions: gas temperature of 350 °C, nitrogen flow rate of 10.0 L min⁻¹, nebulizer pressure 30 psi, quadrupole temperature 30 °C, and capillary voltage 3500 V. The applied fragmentor was 120 V

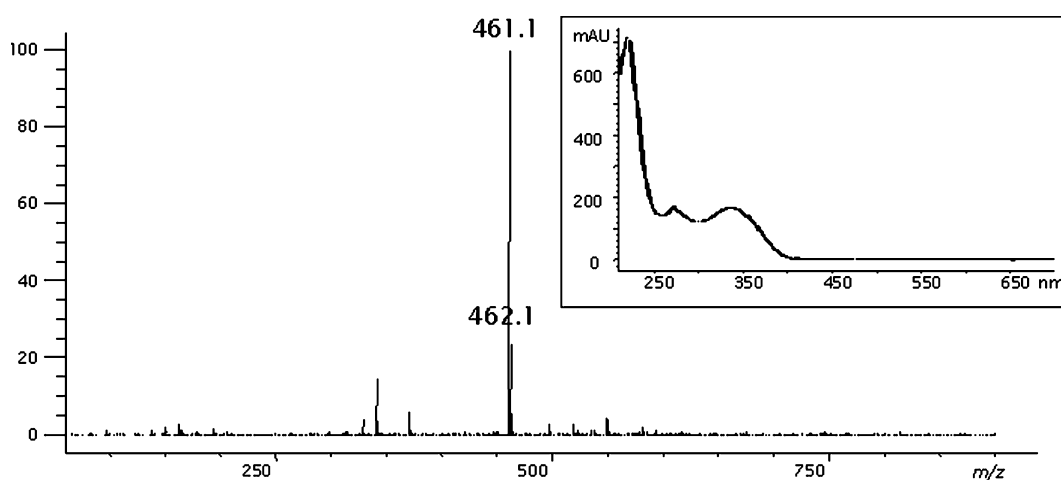


Fig. 4. UV-Vis and negative ion mass spectra of peak 5 acquired during the API-electrospray HPLC-MS analysis. Operating conditions as in Fig. 3

Peak 1 (Fig. 1a and 2a) was identified as the C6-glucoside of luteolin, i.e. homoorientin, by comparison of the R_f , UV-Vis, and MS spectra with those of an authentic sample.

Peak 2 (Fig. 1a and 2a) was identified as the C8-glucoside of luteolin, i.e. orientin, by comparison of R_f , UV-Vis, and MS spectra with those of an authentic sample.

Peak 3 in the chromatograms of Fig. 1a and 2a displays UV-Vis spectra with maxima at 295 and 340sh nm, typical of dihydroflavonols. The presence of a taxifolin derivative was evidenced by comparison with the UV-Vis spectra of taxifolin standard. In the MS profile, the peak shows a quasi-molecular ion at m/z 450. From these data, peak 3 was probably taxifolin deoxyhexose C-linked due to the absence of the fragment corresponding to the aglycon (Fig. 3).

Peak 4 (Fig. 1a and 2a) was identified as a C6-glucoside of apigenin, i.e. isovitexin. R_f , UV-Vis and the mass spectra of an authentic standard confirmed this data.

Peak 5 in the chromatogram of Fig. 1a displays UV-Vis spectra with maxima at 272, 287sh and 335 nm similar to those of orientin derivatives and shows a quasi-molecular ion at m/z 462, 14 amu more than orientin (Fig. 4). Thus, this peak was identified as a methyl-derivative of homoorientin, probably scoparin.

The absence of the characteristic $[M-162]^-$ ions confirmed that peaks 1, 2, 3, 4, 5 were all C-glucosides.

The amount of total non-anthocyanic flavonoids was quantified using homoorientin authentic sample as reference standard and it was about 3.5 mg per gram of dried pulp weight.

Chemical structures of the identified molecules are reported in Fig. 5.

Identification of Anthocyanins

Extrelut® NT 20 cartridge fractionation gave a purified fraction of anthocyanins, as confirmed from data of acquired chromatograms at different wavelengths because no characteristic absorptions of the other classes of flavonoids and phenolic acids were present.

The chromatographic profiles reported in Fig. 1b and 2b, acquired at 520 nm, relative to the raw hydroalcoholic extract (Sample 1b) and the methanolic fraction obtained from LSE purification (Sample 2b) respectively, evidenced the presence of two main compounds, cyanidin-3-O-glucoside (7) and cyanidin-3-O-rutinoside (8) previ-

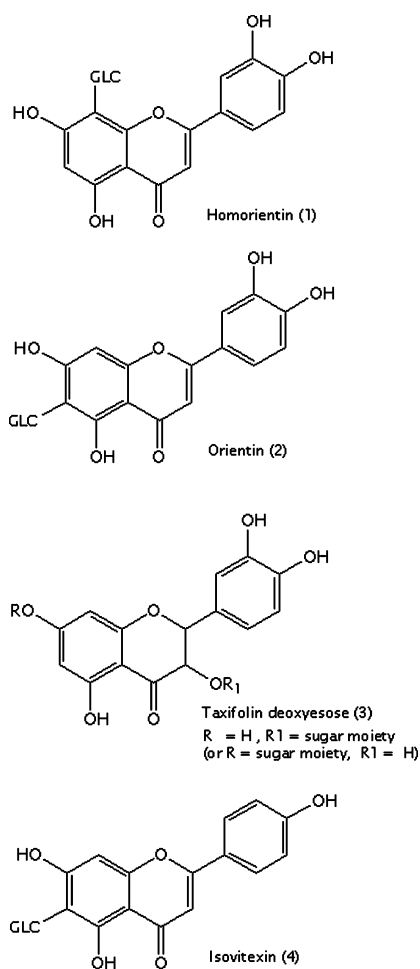


Fig. 5. Structures of flavonoids detected in *Euterpe oleracea* Mart. fruits

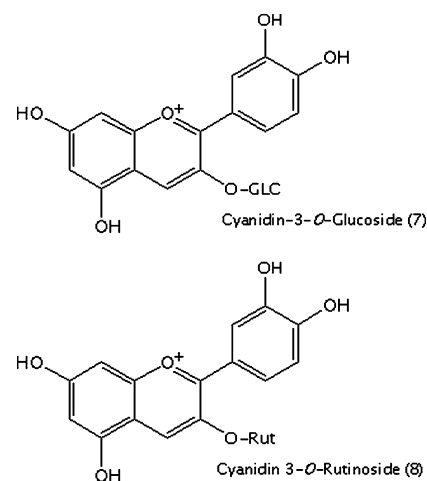


Fig. 6. Structures of anthocyanins detected in *Euterpe oleracea* Mart. fruits

ously identified in Açai fruit [3]. In addition, other minor anthocyanin derivatives were present.

Table 3. Chromatographic characteristics of flavonoids and anthocyanins from *Euterpe oleracea* Mart

Peak	Compounds	λ_{\max} (nm)	$[M]^-/[M]^+$ (m/z)	Fragments (m/z)
<i>Major constituents</i>				
1	homoorientin	255, 271, 350	448	–
2	orientin	255, 267, 293, 346	448	–
4	isovitexin	271, 336	432	–
7	cyanidin 3-O-glucoside	520	449	287
8	cyanidin 3-O-rutinoside	520	595	287, 449
<i>Other constituents:</i>				
3	taxifolin deoxyhexose	295, 340sh	450	–
5	Homoorientin derivative	272, 287sh, 335	462	–
6	cyanidin glyc. derivative	520	581	287

Peak 6 in the chromatograms of Fig. 1b and 2b shows a quasi-molecular ion at m/z 581 and the fragment ion at m/z 287 of cyanidin aglycon derived from the loss of two sugar molecules. Peak 6 was identified as a pentose-hexose derivative of cyanidin.

Peak 7 (Fig. 1b and 2b) shows two major signals in the mass spectra a peak at m/z 448 corresponding to the quasi-molecular ion and the fragment ion at m/z 287 corresponding to the aglycon cyanidin. Thus, peak 7 was identified as cyanidin-3-O-glucoside [8–10].

Peak 8 (Fig. 1b and 2b) was identified as cyanidin-3-O-rutinoside (keracyanin) by comparison of R_f , UV-Vis and MS spectra with those of an authentic sample.

Traces of other anthocyan derivatives of peonidin (Peak *) and cyanidin (Peaks **) moieties are also present in the chromatograms of Fig. 2b.

The amount of total anthocyanins was quantified using keracyanin as reference standard and it was about 0.5 mg per gram of dried pulp weight.

Chemical structures of the characterised molecules are reported in Fig. 6.

Conclusions

This study represents the first report of the analysis of all flavonoids in *Euterpe oleracea* Mart. Three principal flavones were identified for the first time: homoorientin (1), orientin (2), and isovitexin (4). Traces of taxifolin deoxyhexose (3) and homoorientin methyl-derivative (5) are also present. Moreover, the presence of cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside as major anthocyanic

compounds was confirmed in *Euterpe oleracea* fruit as previously reported [4]. Other cyanidin, pelargonidin and peonidin derivatives are found in *Euterpe oleracea* fruit, but the amount is very small and elucidation of their structures is still in progress.

Acknowledgements

The authors thank Dr. P. Luzzi of Botanical Garden “Giardino dei Semplici”, University of Florence, for identification of the plant material.

This work was supported by M.I.U.R. (Ministero Istruzione Università Ricerca, Roma).

References

- The New York Botanical Garden Illustrated Encyclopedia of Horticulture, Everet T H, (1982) (vol.4) Garland Publishing, Inc. New York & London, p.272
- Genera Palmarum. Uhl N W, Dransfield J, (1987), Allen Press, Inc., Lawrence, KS 66044, p.355
- Iaderoza M, Baldini VLS, Draetta I, Bovi MLA (1992) Tropical Science 32:41–46
- Bobbio FO, Druzian JI, Abrao PA, Bobbio PA, Fadelli S (2000) Cienc Tecnol Aliment 20(3):388–3902
- Bobbio FO, Bobbio PA Oliveira, PA Fadelli S (2002) Acta Alimentaria 31(4):371–377
- Yuyama L, Kiyoko O Acta Amazonica (2002) 32(3):521–525
- Lubrano C, Robin JR, Khaiat A (1994) Oleagineux 49(2):50–65
- Baldi A, Romani A, Mulinacci N, Vincieri FF, Casetta B (1995) J Agric Food Chem 43:2104–2109
- Gallori S, Giaccherini C, Bilia AR, Mulinacci N, Vincieri FF (2004) Chromatographia *in press*
- Mullen W, Lean MEJ, Crozier A (2002) J Chromatogr A. 966:63–70