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Total oxidant scavenging capacities of *Euterpe oleracea* Mart. (Açaí) fruits

RAMONA LICHTENTHÄLER[1], ROBERTA BELANDRINO RODRIGUES[1], JOSÉ GUILHERME S. MAIA[2], MENELAOS PAPAGIANNOPOULOS[1], HEINZ FABRICIUS[1], & FRIEDHELM MARX[1]

[1]*Institute of Nutritional and Food Sciences, University of Bonn, Bonn, Germany, and*
[2]*Department of Chemistry, Federal University of Pará, Belém, PA, Brazil*

Abstract
The antioxidant capacities of 11 commercial and non-commercial samples of *Euterpe oleracea* Mart. (açaí) fruit pulp were studied with the total oxidant scavenging capacity assay in a modified and automated version against three reactive oxygen species. The antioxidant capacities of all purple açaí samples were found to be excellent against peroxyl radicals, good against peroxynitrite and poor against hydroxyl radicals compared with common European fruit and vegetable juices recently analysed. In all cases the correlation between sample concentration and antioxidant capacities was non-linear. The antioxidant capacities against all three reactive oxygen species of the fruit pulp from one white açaí variety were very low. The phenolic compounds in purple açaí fruit pulp were identified by high-performance liquid chromatography–mass spectrometry, and the two major anthocyanins, cyanidin-3-glucoside and cyanidin-3-rutinoside, were quantified by high-performance liquid chromatography–visible spectrometry. The contributions of the anthocyanins to the overall antioxidant capacities of the fruit were estimated to be only approximately 10%. Obviously, compounds not yet identified are responsible for the major part of the antioxidant capacities of the açaí fruit pulp.

Keywords: Euterpe oleracea, *acai, TOSC assay, antioxidant, peroxyl radicals, hydroxyl radicals, peroxynitrite, anthocyanins, polyphenols*

Introduction

Euterpe oleracea Mart., Arecaceae, is a multistemmed palm widely distributed in the north of South America, locally called 'açaí'. It is one of the most naturally abundant species in the eastern Amazonian estuary floodplains (Cavalcante 1988; Strudwick & Sobel 1988). Their spherical grape-sized fruits are green when young and ripen usually to a dark purple colour, due to a high content of anthocyanins (Rogez 2000). The fruits of some varieties, however, remain green in their mature stage (Muñiz-Miret et al. 1996); they are called 'white açaí'. The fruits form bunches and are one-seeded, with the seed accounting for most of the size and being covered by thin but crude and stringy fibres under a small edible layer (Strudwick & Sobel 1988).

Correspondence: F Marx, Institute of Nutritional and Food Sciences, University of Bonn, Endenicher Allee 11–13, D-53115 Bonn, Germany. Tel: 49 228 733713. Fax: 49 228 733757. E-mail: f.marx@uni-bonn.de

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Fruits can be harvested throughout the year with higher yields and better organoleptic qualities during the 'dry months' (August–December in the area of the Amazon delta), the high harvesting season (Strudwick & Sobel 1988; Clay & Clement 1993; Rogez 2000).

The fruits are primarily used to prepare a beverage with the consistency of milkshake by macerating their pulp and mixing it with different amounts of water (Strudwick & Sobel 1988; Smith 1999). According to governmental regulations (Delegacia Federal de Agricultura 2000) the beverages are divided into three trading qualities: açaí grosso (>14% dry matter), açaí medio (11–14% dry matter) and açaí fino (8–11% dry matter).

Açaí pulp has a high nutritional value: lipids can account for up to 50% and proteins for about 10% of the dry matter (Rogez 2000). In its main extension region, their consumption is very popular throughout all socio-economic levels (Smith 1999). Recently, açaí pulp products have begun to become popular in the urban centres of Brazil. The taste of açaí pulp products is characterized as similar to beet and carrot with a weak odour (Bauer 2000); other sources classify it as unique and difficult to describe, metallic, slightly nutty and somewhat creamy with an oily appearance (Strudwick & Sobel 1988; Muñiz-Miret et al. 1996).

Sales promotions of açaí advertise the product to be rich in antioxidants, and to have several beneficial health effects especially for sportsmen (Strudwick & Sobel 1988). So far, with the exception of the energy-donating properties (Rogez 2000), none of these slogans has been proven scientifically.

Generally, anthocyanin-rich fruit juices have proved to possess high antioxidant capacities (Kähkonen et al. 2001; Zheng & Wang 2003). Therefore, in this study we examined the antioxidant capacities of anthocyanin-containing açaí fruit pulp products. The total oxidant scavenging capacity (TOSC) assay was chosen because it features several advantages in comparison with other antioxidant testing methods (Lichtenthäler et al. 2003). Furthermore, the main polyphenols of açaí fruit pulps were identified by high-performance liquid chromatography (HPLC)–mass spectometry (MS) and, additionally, the major anthocyanins were quantified with HPLC–visible spectrometry (Vis) for evaluation of their contributions to the overall antioxidant capacities.

Materials and methods

Materials

Chemicals. Ultra-high quality (UHQ) water was prepared with a UHQ-II system (ELGA, Ubstedt-Weiher, Germany) and was used for all solutions. Diethylenetriaminepentaacetic acid, 3-morpholinosydnonimine *N*-ethylcarbamide, α-keto-γ-methiolbutyric acid, gallic acid and Folin–Ciocalteu's phenol reagent were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). 2,2′-Azobis(2-methylpropionamidine) dichloride, ferric chloride hexahydrate and ethylenediamine tetraacetic acid were purchased from Acros Organics (Geel, Belgium). Ascorbic acid was from Kraemer & Martin (Sankt Augustin, Germany). Cyanidin-3-glucoside chloride and cyanidin-3-rutinoside chloride were from Extrasynthese (Genay, France).

Açaí samples. Non-commercial açaí samples were taken in the area of the river Aurá near Belém, Pará, Brazil during the high harvesting periods of 1998, 2000 and 2002

and during the low harvesting period of 2001 (March), as well as one sample of white açaí during the high harvesting period of 2002. The same trees served as sources for the samples of the different years. Samples were freeze-dried immediately after macerating the pulp with potable water and separation from the seeds.

One of the commercial samples (açaí medio I) was bought at a supermarket in Campinas, São Paulo, Brazil. The other commercial samples (açaí grosso I and II, açaí medio II as well as açaí fino I and II) were obtained from the import–export company Klaus Böcker GmbH (Buxtehude, Germany). All commercial samples were frozen (-20°C) during transport and stocking. For dry matter determination the samples were freeze-dried.

For TOSC analyses and anthocyanin quantification, 5 g freeze-dried non-commercial samples were suspended with UHQ water to a final volume of 50 ml. The suspensions were sonicated for 10 min and centrifuged for 10 min at 2800 *g* with a Heraeus Biofuge stratos (Kendro, Hanau, Germany) and filtrated through a folded filter (Schleicher & Schuell, Dassel, Germany). Sample solutions were diluted with UHQ water to at least five different concentrations for each of the three reactive oxygen species to cover the respective range from a low to a high antioxidant capacity as complete as possible. The dilutions were performed in duplicate and each solution was measured at least twice. The commercial samples were centrifuged, filtrated and diluted as already described.

Methods

TOSC assay conditions. The TOSC assay is based on the ethylene-yielding reaction of α-keto-γ-methiolbutyric acid with peroxyl radicals, hydroxyl radicals and peroxynitrite. Peroxyl radicals were generated by the thermal homolysis of 2,2′-azobis(2-methyl-propionamidine) dichloride. Hydroxyl radicals were formed during the iron+ ascorbate-driven Fenton reaction. Peroxynitrite was produced by the decomposition of 3-morpholinosydnonimine *N*-ethylcarbamide. Details of the assay conditions are published elsewhere (Winston et al. 1998; Regoli & Winston 1999; Lichtenthäler et al. 2003). The time course of ethylene formation during 1 h at 37°C was analysed and the data evaluation was done according to Lichtenthäler and Marx (2005).

Calculation of data for TOSC of 20, 50 and 80%. The experimental TOSC values for the standard compounds cyanidin-3-glucoside and cyanidin-3-rutinoside were plotted versus the corresponding concentrations of the studied solutions. For the açaí samples, the TOSC values were plotted versus the reciprocal value of the studied sample dilutions. Dose–response curves were fitted for best correlation. Based on the resulting equations of the curves, those dilutions of the açaí samples and the concentrations of cyanidin-3-glucoside and cyanidin-3-rutinoside were calculated that match TOSC values of 20, 50 and 80%, respectively. Curve fits and TOSC calculations were done with the software TableCurve 2D version 5.1 (SYSTAT Software Inc., Richmond, VA, USA).

Area under dose–response curves and first derivative at TOSC of 50% (DT_{50}). To give a more demonstrative overview of the curve progressions, the area under dose–response curves (ADRC) of the açaí samples from the zero point of the coordinate system up to a reciprocal dilution of 0.1 (i.e., a dilution of 1:10) were calculated as the DT_{50}. They

were normalized by defining the corresponding area and the first derivative of the diagonal of the coordinate system as 1. Calculations were made using TableCurve 2D version 5.1.

Identification and quantification of phenolic compounds in açaí fruits: Identification of individual phenolic compounds including anthocyanins by HPLC–MS. Individual polyphenols including anthocyanins were identified by multistep MS fragmentation after HPLC separation and ultraviolet (UV)–Vis diode array detection of açaí fruit pulp extracts prepared as described in the previous subsection 'Açaí samples'. This method was also used successfully for the identification of polyphenols in carob (Papagiannopoulos et al. 2004).

The liquid chromatograph system used was a Beckman System Gold (Beckman Coulter, Unterschleißheim, Germany) consisting of an Gastorr 154 degasser (SFD Schambeck, Bad Honnef, Germany), a 126-solvent module, a 507e Autosampler, a jetstream column oven (W.O.Electronics, Langenzersdorf, Austria) and a 168-diode array detector equipped with a micro cell. The system was controlled using the 32Karat Software package version 3 Build 937 (Beckman). The analytical column (Aqua 3μ C18, 150 mm × 2 mm i.d.) was equipped with a guard column (Security Guard, C18, 4 mm × 2 mm i.d.; both Phenomenex, Aschaffenburg, Germany) and kept at 35°C.

For the analysis of phenolic compounds other than anthocyanins, 1% v+v acetic acid in UHQ water (mobile phase A) and 1% v+v acetic acid in acetonitrile (mobile phase B) were used as solvents with a flow of 300 μl/min. A gradient elution program was used starting at 0% B with a linear gradient to 40% B after 80 min and to 100% B after 100 min. The column was washed with 100% B for 10 min and reequilibrated for 10 min with the initial conditions. Then 5 μl sample were injected for analysis and the chromatograms monitored at 200–595 nm with a track at 280 nm for comparison with MS data.

For the analysis of anthocyanins, another pair of solvents was used to force the complete protonation of the analytes, taking into account their low pKa value. 5% v+v formic acid in UHQ water (mobile phase A) and 5% v+v formic acid and 5% v+v acetonitrile in methanol (mobile phase B) were used as solvents with a flow of 200 μl/min. A gradient elution program was used starting at 10% B with a linear gradient to 65% B after 25 min. The column was washed with 100% B for 10 min and re-equilibrated for 15 min with the initial conditions. Then 5 μl sample were injected for analysis and the chromatograms monitored at 200–595 nm with a wavelength of 520 nm for comparison with MS data.

An LCQ classic ion-trap MS equipped with an electrospray interface and a metal needle kit was coupled to the HPLC and controlled with the Xcalibur Software version 1.2 (Thermo Finnigan, Dreieich, Germany). A flow of 100 μl/min methanol delivered by a System Gold Programmable Solvent Module 116 (Beckman) was added through a T-union before the HPLC eluent entered the ion source to enhance ionization of the polar analytes.

The settings for the MS analyses of phenolic compounds (in the negative mode) and anthocyanins (in the positive mode; mentioned in parentheses) were: source voltage, 4.5 kV in negative mode (4.0 kV in positive mode); sheath gas flow, 60 (60); auxiliary gas flow, 0 (25); capillary voltage, −45 V (+26 V); capillary temperature, 200°C (200°C); first octapole offset, +3 V (−3 V); interoctapole lens voltage, +22 V

(−16 V); second octapole offset, +7 V (−5 V); ion trap DC offset, +10 V (−10 V). In the negative mode, phenolic compounds are detected in the MS in the deprotonated form as the quasimolecular ion $[M\text{-}H]^-$ one mass unit below their molecular mass. In the positive mode, anthocyanins are detected in the MS directly in the oxonium form at their molecular mass.

Identification of individual compounds was conducted using UV-spectral and MS/MS fragmentation data. A UV spectral library set-up in the laboratory from standard compounds and the comparison of typical mass fragmentation patterns from the samples and standards were the basis of the identification of the individual sample compounds eluting from the HPLC system.

Quantification of anthocyanins by HPLC–Vis. Quantification of anthocyanins was performed on a 600 Multisolvent Delivery HPLC system (Waters, Eschborn, Germany) equipped with a LC 55 B UV–Vis detector (Perkin-Elmer, Norwalk, CT, USA). Separations were made on a MAX-RP 80A column (150 × 4.6 mm i.d., 4 μm particle size; Phenomenex, Aschaffenburg, Germany) equipped with a guard column (Security Guard, C18, 4 mm × 3 mm i.d.; Phenomenex) at room temperature. Gradient elution was performed using a 2% solution of formic acid in UHQ water (solution A) and a 2% solution of formic acid in acetonitrile (solution B): a linear gradient was used from 0 to 40 min from 0% B to 30% B followed by a linear gradient up to 60 min to 90% B at 0.8 ml/min. The column was washed for 10 min with 100% B and reequilibrated for 20 min with the initial conditions. Anthocyanins were detected at a wavelength of 525 nm. For quantification, external standards (cyanidin-rutinoside and cyanidin-glucoside) were used and the calibration curves were plotted for each standard compound on the basis of peak area. Peak integration was performed with EZChrom Elite version 2.8 (Scientific Software Inc., Pleasanton, CA, USA). All analyses were performed at least in duplicate.

Results and discussion

Tables I, II and III present the calculated dilutions and concentrations corresponding to TOSC values of 20, 50 and 80% for the açaí samples as well as for cyanidin-3-glucoside and cyanidin-3-rutinoside solutions. The ADRC and DT_{50} are only given for the açaí samples, because it is not suggestive to compare sample values with those of the standard compounds; a discussion in this regard is provided elsewhere (Lichtenthäler & Marx 2005).

For all analysed samples, a non-linear correlation between sample concentrations and antioxidant capacities was observed. As an example, typical dose–response curves for selected açaí samples against peroxyl radicals are shown in Figure 1.

TOSC of açaí samples

In comparison with recently studied European fruit and vegetable juices, all of the studied açaí samples with the exception of the white variety showed very high antioxidant capacity against peroxyl radicals. All purple açaí samples were in the top class of peroxyl radical scavengers together with, for example, lingonberry and beetroot juices. The white açaí ranked among the samples with the lowest antioxidant capacity, like tomato and sauerkraut juices. The antioxidant capacities for the purple

Table I. Peroxyl radicals: calculated dilution factors (for açaí samples) and concentrations (for cyanidin-3-glucoside and cyandin-3-rutinoside) for different TOSC values, DT_{50}, ADRC and fit correlation.

	TOSC					
	20%	50%	80%	DT_{50}*	ADRC*	r^2 of fit
	Dilution factors					
Açaí grosso I (13.9 g/100 ml DM)	1250	435	189	15.9	>1.9	0.9999
Açaí medio I (11.5 mg/100 ml DM)	1111	385	175	14.3	>1.9	1.0000
Açaí grosso II (13.4 g/100 ml DM)	833	286	128	10.4	>1.9	0.9998
Açaí pulp 2002 (10.0 g/100 ml DM)	769	256	120	9.7	>1.9	1.0000
Açaí pulp 1998 (10.0 g/100 ml DM)	588	238	106	9.2	>1.9	0.9998
Açaí medio II (10.0 g/100 ml DM)	714	227	98	7.8	>1.9	0.9998
Açaí fino I (7.7 g/100 ml DM)	667	217	103	8.0	>1.9	0.9999
Açaí pulp 2000 (10.0 g/100 g DM)	641	210	104	7.6	>1.9	0.9997
Açaí pulp 2001 low harvest season (10.0 g/100 g DM)	606	182	87	6.4	>1.9	0.9999
Açaí fino II (6.5 g/100 g DM)	500	145	61	4.8	>1.8	0.9998
White açaí (10.0 g/100 ml DM)	76	30	15	1.3	1.2	1.0000
	Concentrations (mg/l)					
Cyanidin-3-glucoside	4.4	11.2	21.8			0.9996
Cyanidin-3-rutinoside	5.7	15.1	30.3			0.9994

*Diagonal defined as 1.0. DM, dry matter.

açaí samples were so high that their ADRC values could only be estimated. A more detailed discussion about that fact is provided at Lichtenthäler and Marx (2005).

Against peroxynitrite, all purple açaí samples demonstrated high antioxidant capacity even though it was not as outstanding as against peroxyl radicals. Although the purple açaí samples showed higher results against peroxynitrite than those of most other analysed juices, they were not as high as, for example, lingonberry or beetroot

Table II. Peroxynitrite: calculated dilution factors (for açaí samples) and concentrations (for cyanidin-3-glucoside and cyandin-3-rutinoside) for different TOSC values, DT_{50}, ADRC and fit correlation.

	TOSC					
	20%	50%	80%	DT_{50}*	ADRC*	r^2 of fit
	Dilution factors					
Açaí grosso I (13.9 g/100 ml DM)	526	99	19	2.0	1.5	1.0000
Açaí medio I (11.5 g/100 ml DM)	588	100	13	1.7	1.4	1.0000
Açaí pulp 2002 (10.0 g/100 ml DM)	455	85	12	1.4	1.4	0.9999
Açaí grosso II (13.4 g/100 ml DM)	435	63	12	1.2	1.3	0.9998
Açaí medio II (10.0 g/100 ml DM)	400	67	10	1.2	1.3	1.0000
Açaí pulp 1998 (10.0 g/100 ml DM)	313	64	10	1.2	1.3	0.9999
Açaí fino I (7.7 g/100 ml DM)	357	59	10	1.0	1.3	1.0000
Açaí pulp 2000 (10.0 g/100 ml DM)	278	58	9	1.3	1.1	1.0000
Açaí pulp 2001 low harvest season (10.0 g/100 ml DM)	208	45	8	1.2	1.0	1.0000
Açaí fino II (6.5 g/100 ml DM)	175	35	6	0.7	1.1	0.9999
White Açaí (10.0 g/100 ml DM)	86	17	3	0.3	0.8	0.9996
	Concentrations (mg/l)					
Cyanidin-3-glucoside	11.6	50.9	259			1.0000
Cyanidin-3-rutinoside	15.8	58.1	362			0.9999

*Diagonal defined as 1.0. DM, dry matter.

Table III. Hydroxyl radicals: calculated dilution factors (for açaí samples) and concentrations (for cyanidin-3-glucoside and cyandin-3-rutinoside) for different TOSC values, DT_{50}, ADRC and fit correlation.

	TOSC					
	20%	50%	80%	DT_{50}*	ADRC*	r^2 of fit
	Dilution factors					
Açaí pulp 2001 low harvest season (10.0 g/100 ml DM)	111	43	7	1.2	1.0	0.9997
Açaí grosso II (13.4 g/100 ml DM)	161	39	9	1.3	1.1	0.9997
Açaí fino II (6.5 g/100 ml DM)	83	29	9	0.9	1.1	1.0000
Açaí pulp 2000 (10.0 g/100 ml DM)	94	29	5	0.5	1.0	0.9998
Açaí medio I (11.5 g/100 ml DM)	59	24	10	1.0	1.0	0.9994
Açaí medio II (10.0 g/100 ml DM)	84	26	6	0.7	1.0	0.9993
Açaí grosso I (13.9 g/100 ml DM)	57	23	10	0.9	1.0	1.0000
Açaí fino I (7.7 g/100 ml DM)	53	21	9	0.9	0.9	0.9994
Açaí pulp 2002 (10.0 g/100 ml DM)	57	22	8	0.8	0.9	0.9998
Açaí pulp 1998 (10.0 g/100 ml DM)	50	18	7	0.7	0.9	1.0000
White Açaí (10.0 g/100 ml DM)	27	7	3	0.2	0.5	0.9998
	Concentrations (mg/l)					
Cyanidin-3-glucoside	Activities too low to calculate					
Cyanidin-3-rutinoside	Activities too low to calculate					

*Diagonal defined as 1.0. DM, dry matter.

juice. Again, the antioxidant capacity of white açaí was low and was among the juices with the lowest results (Lichtenthäler & Marx 2005).

From the presented results (Tables I and II) conclusions on influencing parameters for the antioxidant capacities of the non-commercial samples against both reactive oxygen species can be drawn (only tentatively because of the small database). An important parameter is the plant variety—in both cases the white açaí sample presents the lowest capacities. Another relevant influencing factor is the harvest season—the samples harvested during the 'dry months' August–December, the main harvest season, consistently show the highest TOSC values. Finally, climatic differences between the considered harvesting years seem to play an important role as well—the

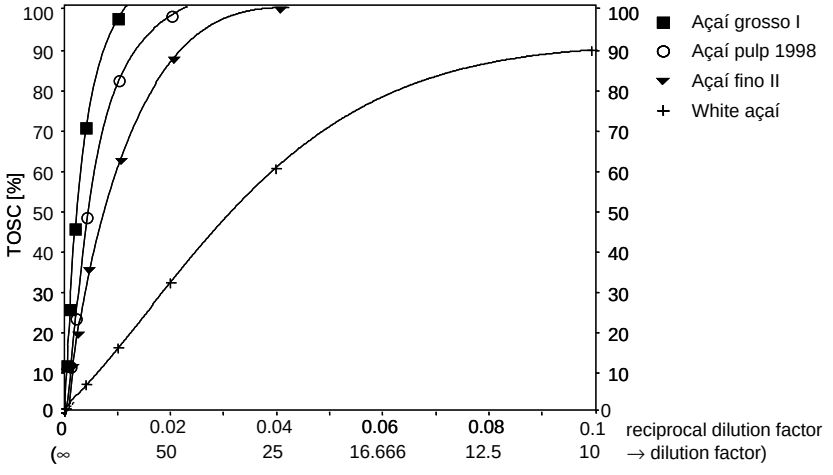

Figure 1. Characteristic dose–response curves of açaí samples and peroxyl radicals.

TOSC values against both reactive oxygen species are highest in 2002, followed by those from 1998 and finally from 2000.

Consequently, the differences of the TOSC values between the commercial samples are only in part explainable with the different dry matter contents. Additionally to the parameters discussed for the non-commercial samples, the antioxidant capacities may be influenced by different stocking and transport conditions. With the exception of one sample (açaí fino II), the TOSC values of the trade samples are in the same range as those for the self-harvested ones. Therefore it can be concluded that, as for the maintenance of the antioxidant capacity, the after-harvest treatment of the commercial samples generally was adequate.

In comparison with peroxyl and peroxynitrite the açaí sample antioxidant capacity against hydroxyl radicals was lower, by far. For all analysed purple açaí samples only small differences were observed (Table III). When compared with other types of fruit and vegetable juices (Lichtenthäler & Marx 2005), the TOSC values against hydroxyl are to be considered moderate. White açaí was even less effective than all other juice samples studied.

Identification of individual phenolic compounds including anthocyanins in açaí fruits by HPLC–MS

The TOSC assay in the form here utilized covers only water-soluble antioxidants; that generally means mainly ascorbic acid and polyphenols are to be considered responsible for the antioxidant capacities of the samples. As açaí is only a poor source of vitamin C (Rogez, 2000), the main attention has to be drawn on polyphenols in this case. Up to now, only a few studies about the polyphenols in açaí fruits have been published, with contradictory results. Iaderoza et al. (1992), Rogez (2000) and Gallori et al. (2004) detected cyanidin-3-glucoside and cyanidin-3-rutinoside in açaí fruits, whereas Bobbio et al. (2000, 2002) found cyanidin-3-arabinoside and cyanidin-3-arabinosylarabinoside.

Our HPLC–MS analyses prove the presence of two different anthocyanins in açaí fruits in appreciable amounts and some others in minor concentrations. The system settings used for the analysis of anthocyanins allowed a good ionization and selective MS detection of the molecular ions in the oxonium form, revealing their molecular weight directly. Subsequent MS fragmentation experiments showed the dissociation of the aglycon and the glycoside, delivering their molecular masses.

Thus, the two main anthocyanins were identified as cyanidin-3-glucoside and cyanidin-3-rutinoside, confirming the aforementioned findings of Iaderoza et al. (1992), Rogez (2000) and Gallory et al. (2004). Additionally, one of the minor anthocyanins was identified as peonidin-rutinoside (see Figure 2). Due to their low amount in the samples, the others could be assigned only tentatively to anthocyanins with pelargonidin, peonidin, delphinidin, petunidin and malvidin as aglycon.

Furthermore, protocatechuic acid, flavan-3-ols (catechin monomers through tetramers) and quercetin-rutinoside were identified in minute quantities in the açaí fruit samples. Table IV presents the MS data for the identified compounds. The pattern of these compounds corresponds to that of açaí seeds (unpublished results),

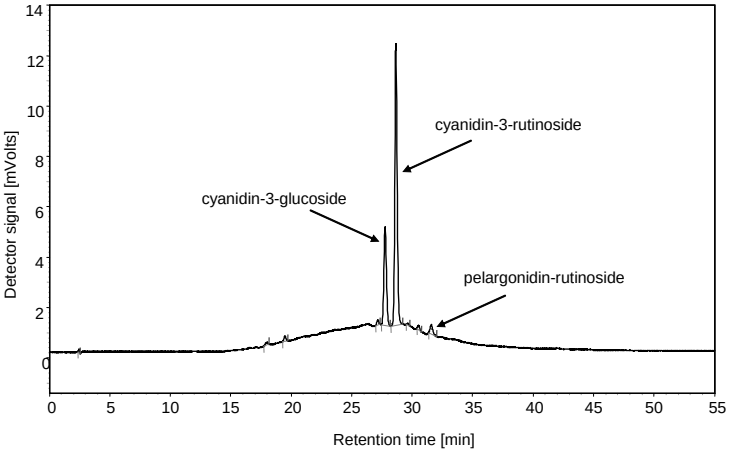

Figure 2. HPLC separation of anthocyanins with açaí pulp of 1998 taken as an example. Vis detection at 525 nm.

suggesting that these compounds were transferred from the seeds during the preparation of the açaí beverage.

Anthocyanin content and its relation to TOSC values

The concentrations of the two main anthocyanins in the analysed açaí samples are compiled in Table V.

As expected, the anthocyanin content of the white açaí sample was negligibly low—which combines with its low TOSC values against peroxyl and peroxynitrite. Concerning the purple açaí, cyanidin-3-rutinoside was the dominating anthocyanin in all samples, but to a varying extent. Its ratio to cyanidin-3-glucoside differs from 1.6 (pulp 2001, low season) to 65 (grosso I) in favour of cyanidin-3-rutinoside. The summarized anthocyanin contents of the purple samples vary between 13 and 456 mg/

Table IV. Mass spectrometric data for identified phenolic compounds in açaí.

Compound	Retention time (min)	Parent ion (m/z [polarity])	MS/MS fragments (m/z [neutral loss])
Cyanidin-glucoside	7.4	449 (+)	287 (-162 hexose$-H_2O$)
Cyanidin-rutinoside	8.3	595 (+)	449 (-146 desoxyhexose$-H_2O$) 287 (-308 hexose+desoxyhexose$-2\times H_2O$)
Peonidin-rutinoside	10.6	609 (+)	463 (-146 desoxyhexose$-H_2O$) 301 (-308 hexose+desoxyhexose$-2\times H_2O$)
Protocatechuic acid	11.1	153 (−)	109 (-44 CO_2)
Catechin	20.6	289 (−)	245, 205, 179
Procyanidin dimer*	18.5	577 (−)	425 (RDA), 451 ($-C_6H_6O_3$), 407, 289, 559
Procyanidin trimer*	20.2	865 (−)	713 (RDA), 739 ($-C_6H_6O_3$), 695, 577, 407
Procyanidin tetramer*	20.8	1153 (−)	1001 (RDA), 1027 ($-C_6H_6O_3$), 984, 575, 865
Quercetin-rutinoside (Rutin)	37.2	609 (−)	300/301 ($-308/309$ rutinose$-2\times H_2O$)

*For procyanidins, beside the structural informative fragment ions (Retro-Diels–Alder reaction [RDA], phloroglucinol [$-C_6H_6O_3$)], the masses of the three most abundant fragment ions are given.

Table V. Anthocyanin content of açaí samples.

	Anthocyanin content (mg/l)		
	Cyanidin-3-glucoside	Cyanidin-3-rutinoside	Σ Anthocyanins
Açaí grosso I (13.9 g/100 ml DM)	7	456	463
Açaí pulp 2002 (10.0 g/100 ml DM)	54	157	211
Açaí fino I (7.7 g/100 ml DM)	5	106	111
Açaí medio I (11.5 g/100 ml DM)	1	99	100
Açaí pulp 1998 (10.0 g/100 ml DM)	19	79	98
Açaí grosso II (13.4 g/100 ml DM)	19	76	95
Açaí pulp 2000 (10.0 g/100 ml DM)	27	61	88
Açaí medio II (10.0 g/100 ml DM)	7	67	74
Açaí fino II (6.5 g/100 ml DM)	6	24	30
Açaí pulp 2001 low harvest season (10.0 g/100 ml DM)	5	8	13
White Açaí (10.0 g/100 ml DM)	0	1	1

DM, dry matter.

l. That large variation is in accordance with the findings of Rogez (2000), who found anthocyanin contents between 71 and 1022 mg/kg for purple açaí samples with photometric measurements. It is striking that the sample with the highest anthocyanin content (açaí grosso I) also has the highest TOSC values against peroxyl and peroxynitrite, and that the low harvest season sample (pulp 2001) with low TOSC values also contains the lowest amount of anthocyanins (Tables I and II). However, general correlations of anthocyanin contents with the sample rankings for the TOSC values cannot be deduced.

In the case of the commercial samples, not even a general dependence from the dry mass content can be stated. That is not surprising because of the low stability of anthocyanins in aqueous solutions; different stocking conditions may lead to very different degrees of anthocyanin degradation.

TOSC values of cyanidin-3-glucoside and cyanidin-3-rutinoside standard solutions against peroxyl and peroxynitrite were determined and the concentrations calculated that are necessary to achieve a TOSC of 20, 50 and 80% (see Tables I and II). Comparison of the results with those of açaí samples with known anthocyanin contents should enable an estimation of the contributions of the individual anthocyanins to the overall antioxidant capacities. The calculation may be explained by means of açaí grosso I and peroxyl radical taken as an example. Açaí grosso I had a cyanidin-3-rutinoside content of 456 mg/l and had to be diluted 1:435 to achieve a TOSC value of 50%. Accordingly, the cyanidin-3-rutinoside concentration in the diluted sample was about 1 mg/l. To obtain the same inhibition from the standard cyanidin-3-rutinoside, a concentration of 11.2 mg/l was necessary. That means the antioxidant capacity of the açaí sample is about 11-fold higher than could be expected from the cyanidin-3-rutinoside content. For peroxynitrite, the calculation results are very similar. It turned out that all of the studied açaí samples showed far higher antioxidant capacities against peroxyl radicals and peroxynitrite (10-fold to 200-fold) in comparison with pure solutions with the same anthocyanin concentrations. For hydroxyl radicals, the antioxidant capacity of standard anthocyanin solutions was so low that an influence of anthocyanin content on sample TOSC values can be excluded completely.

Consequently, the main part of the antioxidant capacities of açaí fruit pulp must be due to other, not yet identified, compounds. This conclusion is supported by the results of different groups of researchers for other samples rich in anthocyanins. Miller and Rice-Evans (1997) found that there must be a significant unidentified antioxidant in blackcurrant drink, because the good results of this beverage could not be explained only by its vitamin C and anthocyanin content. Earlier conclusions (Harper et al. 1969) are confirmed as well. There, it was stated that not the anthocyanins are responsible for stabilization of ascorbic acid in blackcurrant juice. A survey of Wang et al. (1996) about the total antioxidant capacity of fruits revealed also that there may be some unknown antioxidants present in fruits that need to be identified. Actually, studies for their identification are in progress.

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