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Total Oxidant Scavenging Capacity of *Euterpe oleracea* Mart. (Açaí) Seeds and Identification of Their Polyphenolic Compounds

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The antioxidant capacity of methanol and ethanol seed extracts from *Euterpe oleracea* Mart. (açaí) against the reactive oxygen species (ROS) peroxyl radicals, peroxynitrite, and hydroxyl radicals was studied with the total oxidant scavenging capacity (TOSC) assay in a modified and automated version. Cold methanol digestion was the most efficient extraction method with respect to the antioxidant capacity. The extracts exhibit good antioxidant capacity against peroxyl radicals, similar to the capacity of the pulp. The antioxidant capacity against peroxynitrite and hydroxyl radicals is even higher. The main antioxidants identified by HPLC-MS and HPLC-CEAD are five different procyanidins (di- through pentamers); furthermore, protocatechuic acid and epicatechin were identified as minor compounds. Determination of TOSC values of HPLC seed extract fractions indicates that the procyanidins contribute substantially to the overall antioxidant capacity. In addition, however, other compounds that have not yet been identified are responsible for a large part of the observed antioxidant capacity.

KEYWORDS: ***Euterpe oleracea*; açaí; seed extract; TOSC assay; antioxidant; peroxyl radicals; peroxynitrite; hydroxyl radicals; procyanidins**

INTRODUCTION

Free radicals are implicated in several human illnesses such as arteriosclerosis, cancer, Alzheimer's and Parkinson's diseases, and also in the aging process. There is considerable evidence that the intake of antioxidants could help to maintain health and to prevent illnesses caused by oxidative stress (*1*). Because some artificial antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have demonstrated dose-dependent toxicological effects (*2*, *3*), the demand for alternative and safe antioxidants from natural sources is growing worldwide.

A promising new source for natural antioxidants is the *Euterpe oleracea* Mart. palm (Arecaceae), also known as "açaí". This plant is widely spread in northern South America with its greatest abundance in the Amazonian flood plains of Brazil. Actually, the açaí palm is the most important source for palm hearts. The other important nontimber product of açaí palms is the grape-sized, dark purple fruit that is used mainly for preparing a favored thickish beverage. There are also açaí varieties known with fruits that maintain a greenish to yellow color when they are ripe, locally called "açaí branco" or "white açaí" (*4*, *5*). Fruits can be harvested throughout the year, with higher yields and better organoleptic qualities during the "dry months" (August−December, the "high harvest season"). The "low harvest season" in the rainy months (January−July) gives fruits of lower quality (*5*). A survey of the remarkable antioxidant capacity of açaí pulp against peroxyl radicals, peroxynitrite, and hydroxyl radicals has previously been published by our research group (*6*).

Each açaí fruit contains one light brown seed that accounts for about 90% of the fruit's diameter (1−2 cm) and up to 90% of its weight (0.7−1.9 g). The seeds are covered with a layer of rough fibers under a thin edible violet pulp (*4*). Fibers such as cellulose and hemicellulose make up 63−81% of the seeds weight, followed by about 5−6% of proteins, 2−6% of minerals, and 2−3% of lipids (*5*). Other constituents have not yet been identified. For separating the seeds from the pulp, the fruits are macerated with warm water and spun in special grinding machines (*4*). It is estimated that in the city of Belém (PA), Brazil, alone, ∼110000 tons of fruit is worked up commercially every year, yielding ∼100000 tons of açaí seeds (*5*). Only a

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small portion of the seeds is utilized as pig food or, when rotten, for making a very rich potting soil for plantations or home gardens (*7*). Therefore, it would be of great economic interest to avoid waste production and, at the same time, find a new source of income from açaí seeds.

In a study of the antioxidant activities of extracts from tropical and oriental medicinal plants, *Euterpe oleracea* seed extracts showed strong antioxidant activities against the oxidation of linoleic acid as well as a potent scavenging capacity against DPPH radicals and superoxide anion (*8*). Thus, açaí seed extracts could possess benefits similar to those of, for example, grape seed or pine bark extracts, which are especially rich in oligomeric procyanidins. They have demonstrated not only in vitro radical scavenging capacity, similar to or better than that of BHT (*9*−*11*), but also, for example, cataract-preventing (*12*) and antibacterial properties (*13*).

The intention of this work was to survey the antioxidant capacity of açaí seeds against the three reactive oxygen species (ROS) peroxyl radicals, peroxynitrite, and hydroxyl radicals that cover a broad spectrum of different reactivities. Research was carried out with the total oxidant scavenging capacity (TOSC) assay (*14*, *15*) in a modified and automated version (*16*). In addition, the objective was to identify the main compounds responsible for the antioxidant capacities of the seeds.

MATERIALS AND METHODS

Chemicals. Ultrahigh quality (UHQ) water prepared with a UHQ-II system (ELGA, Siershahn, Germany) was used for all solutions. Diethylenetriaminepentaacetic acid (DTPA), 3-morpholinosydnonimine hydrochloride (SIN-1), α-keto-γ-methiolbutyric acid (KMBA), (+)-catechin, and (−)-epicatechin were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Protocatechuic acid was obtained from Merck (Darmstadt, Germany). 2,2′-Azobis(2-methylpropionamidine) dichloride (ABAP), ferric chloride hexahydrate, and ethylenediaminetetraacetic acid (EDTA) were purchased from Acros Organics (Geel, Belgium). Ascorbic acid was acquired from Kraemer & Martin (Sankt Augustin, Germany). All HPLC solvents were of HPLC grade obtained from J. T. Baker (Griesheim, Germany).

Açaí Seed Sampling and Extraction. Seeds from labeled trees of both the purple and white açaí varieties were sampled in the area of the river Aurá near Belém (PA), Brazil. Seeds from the purple açaí variety were collected during the low harvest season of 2001 and the high harvest season of 2002. Seeds from the white açaí variety were gathered during the high harvest season of 2002. For preliminary tests, an ethanol extract of seeds from the purple açaí variety of the high harvest season of 2000 was prepared by extracting ground seeds exhaustively with a Soxhlet extractor. For further analyses, 100 g of thoroughly crushed seeds was digested repeatedly with a total of 1 L (2 × 350 mL and 1 × 300 mL) of methanol at room temperature during an overall extraction period of 3 days. The solvent from the collected supernatant was removed by a rotary evaporator at 30 °C. Açaí seeds and extracts were stored at −28 °C until further analyses. Half a gram of each dried extract was suspended in UHQ water (final volume of 10 mL), sonicated for 10 min, and centrifuged for 10 min at 2800 *g* with a Heraeus Biofuge stratos (Kendro, Hanau, Germany), and the supernatant was filtered through a folded filter (Schleicher & Schuell, Dassel, Germany). For HPLC analyses, the sample solutions were filtered additionally through 0.45 μm cellulose membrane filters (Schleicher & Schuell). For TOSC analyses, the extract solutions were diluted with UHQ water to at least five different concentrations for each of the three ROS to cover the respective ranges of the antioxidative capacities as completely as possible. Dilution was done in duplicate in all cases, and each solution was measured at least twice.

TOSC Assay Conditions and Data Processing. The TOSC assay is based on the ethylene-yielding reaction of KMBA with either peroxyl radicals, hydroxyl radicals, or peroxynitrite. The capacity of different açaí seed extracts to inhibit this ethylene production from the three different ROS was analyzed by gas chromatographic ethylene quantification. The time course of the ethylene formation during 1 h at 37 °C was analyzed. Details of the assay conditions have been described previously (*14*−*16*).

For TOSC values, the time curves that provide the best fit for the experimental GC quantification of the ethylene production over a period of 60 min and the area beneath it were calculated with the data analysis software Root v3.02/07 (developed at the CERN particle physics center, Geneva, Switzerland). TOSC values were quantified by comparing the areas for the uninhibited control and the sample reactions. Thereby, a TOSC value of 0% means a sample without antioxidative properties; a solution that suppresses the ethylene formation completely has a TOSC value of 100% (*13*−*15*).

The experimental TOSC values were plotted versus the extract concentration (milligrams per liter) of the added sample solutions. Dose−response curves were fitted that showed the best correlation for the respective data. On the basis of the resulting equations, the concentrations (milligrams per liter) of the açaí seed extracts that match TOSC values of 20, 50, and 80% were calculated. Curve fits and TOSC calculations were done with the software TableCurve 2D v5.1 (SYSTAT Software Inc., Point Richmond, CA).

Identification of Polyphenols by HPLC-MS. Individual polyphenols were identified by multistep mass spectrometric fragmentation after HPLC separation and UV−vis diode array detection. The HPLC system used was a Beckman System Gold (Beckman Coulter, Unterschleissheim, Germany). The analytical column was an Aqua 3 μm C18, 150 mm × 2 mm i.d. (Phenomenex, Aschaffenburg, Germany), kept at 35 °C. One percent acetic acid in UHQ water (mobile phase A) and 1% acetic acid in acetonitrile (mobile phase B) were used at 300 μL/min starting at 0% B with a linear gradient to 40% B after 80 min followed by washing and reequilibration. Five microliters of each sample was injected, and the chromatograms were monitored at 200−595 nm.

An LCQ ion-trap mass spectrometer with an ESI interface (Thermo Electron, Dreieich, Germany) was operated in the negative mode as published earlier (*17*). The phenolic compounds were detected in their deprotonated form as the quasimolecular ion $[M - H]^-$ one mass unit below their molecular masses. Identification of individual compounds was conducted by comparison of their UV spectra and ion trap fragmentation patterns with a self-prepared library from standard substances and known compounds as described in detail previously (*18*).

Identification and Quantification of Polyphenols by HPLC-UV-CEAD. Quantification was performed on an ESA system (Chelmsford, MA) with an Aqua 3 μm C18, 150 mm × 4.6 mm i.d. column (Phenomenex). The detection system was an on-line coupling of a Beckman 168 diode array detector (Beckman Coulter) and a Coularray model ESA 5600. The diode array detector was set at 280 nm, and the six CEAD electrodes were set from 0 to 550 mV in steps of 110 mV. The column and the detector array were maintained at 30 °C.

The HPLC method is a modification of a previously reported method (*19*, *20*). In short, 0.02 M NaH_2PO_4, pH 3.4 (mobile phase A), and acetonitrile plus 0.05 M NaH_2PO_4, pH 3.0 (2+1, v+v) (mobile phase B), were used with a flow of 0.8 mL/min starting at 0% B with a linear gradient to 8% B after 5 min, 10% B after 25 min, 21% B after 40 min, and 35% B after 65 min followed by washing and reequilibration.

Polyphenols were quantified by HPLC-UV (280 nm) using catechin as external standard, because standard compounds are not commercially available. Lea (*21*) proved that the absorbance coefficients of procyanidins correspond to those of catechin. Their identity was confirmed by UV spectra and electrodynamic voltamograms and by comparison with the HPLC-MS data (*19*).

Seed Extract Fractionation by HPLC. Fractionation was performed on a 600 Multisolvent Delivery HPLC system (Waters, Eschborn, Germany) equipped with an LC 55 B UV−vis detector (Perkin-Elmer, Norwalk, CT) set at 210 nm. Separations were made on a MAX-RP 80 Å, 150 mm × 4.6 mm i.d., column with 4 μm particle size (Phenomenex) kept at room temperature. Linear gradient elution was performed using 2% formic acid in UHQ water (mobile phase A) and 2% formic acid in acetonitrile (mobile phase B) from 0% B to 30% B in 40 min followed by washing and reequilibration.

Fractions were collected starting directly after injection for a total collection time of 60 min with each fraction spanning 5 min. Each

Table 1. Açaí Seed Extracts: Calculated TOSC Values of 20, 50, and 80% for the Three Assayed ROS

		concn (mg/L) for TOSC of								
		peroxyl radicals			peroxynitrite			hydroxyl radicals		
	extract[a]	20%	50%	80%	20%	50%	80%	20%	50%	80%
1	white açaí (high harvest season)	5	18	56	5	45	431	27	52	284
2	purple açaí (high harvest season)	5	23	61	9	75	549	32	66	246
3	purple açaí (low harvest season)	5	23	85	11	79	676	30	71	1389
4	purple açaí (high harvest season)	20	72	187	28	258	1852	137	575	12500

[a] Extracts 1–3, prepared by cold digestion with methanol; extract 4, prepared by Soxhlet ethanol extraction.

sample fractionation was carried out twice. All collected samples were freeze-dried, dissolved in 500 μL of UHQ water, and ultrasonicated for 10 min before further analyses.

RESULTS AND DISCUSSION

Optimization of the Extraction Procedure. In the survey of Choi et al. (*8*), the highest antioxidant capacity was found for açaí seed extracts prepared with methanol at room temperature; less polar solvents, such as ether or chloroform, resulted in lower antioxidant capacity. These findings are in accordance with several other publications for the extraction of antioxidants from plant material with different solvents (*17*, *22*−*26*). The use of a Soxhlet extractor is also often recommended to obtain a high yield of antioxidants (*22*, *23*, *27*).

These different literature results were taken into account when extraction trials with methanol and ethanol at different temperatures were performed (data not shown). The results confirm the finding of Choi et al. (*8*)—extraction of açaí seeds with methanol at room temperature was the most effective with respect to the antioxidant capacity of the resulting extract. Thus, most of the work described in the following is based on that extraction method.

TOSC of Açaí Seed Extracts. In **Table 1**, concentrations of different açaí seed extracts for TOSC values of 20, 50, and 80% against the three assayed ROS are shown. Generally, the antioxidant capacity of a sample is higher when the concentration required to achieve a specific TOSC value is lower. Therefore, low concentrations indicate a high antioxidant capacity, whereas a high concentration means that more of the antioxidant is needed to achieve a certain percentage of inhibition.

The efficiency of the Soxhlet ethanol extraction with respect to the antioxidant capacity was low, resulting in the highest extract concentration for all three tested ROS. The highest TOSC values were found in extract 1 prepared from the white açaí variety with cold methanol digestion. Further studies with more seeds from both varieties and from different origins are necessary to confirm the generally higher antioxidant capacity from seeds of white açaí. Extracts 2 and 3 were prepared from seeds from the same tree (purple variety); however, extract 2 was taken during the high harvest season, whereas extract 3 was taken during the low harvest season. It can be suggested from the results that the antioxidant capacity of the açaí seeds is not substantially affected by seasonal influences. This is in contrast to açaí fruit pulp; its antioxidant capacity was found to be significantly higher when the fruits were harvested during the high harvest season (*6*).

The dose−response curves (**Figure 1**) demonstrate varying reaction behaviors of the extracts against the three assayed ROS.

For the inhibition of **peroxyl radicals**, extract concentrations from 5 to 500 mg/L were adequate to cover a TOSC range from a low to a nearly complete suppression of the ethylene production. The relationship between açaí extract concentration and TOSC for all analyzed seed extracts was clearly nonlinear (see **Figure 1a**). The highest antioxidant capacity against peroxyl radicals was found for the seeds of the white açaí variety (extract 1) followed closely by the two seed batches of the purple variety extracted with cold methanol (extracts 2 and 3).

The order of antioxidant capacity of the different extracts for **peroxynitrite** was the same (see **Figure 1b**). However, the antioxidant capacity of all extracts was lower than it was for peroxyl radicals. In addition, a broader concentration range from 5 to 5000 mg/L had to be applied for this ROS to cover a similar inhibition range due to a lower progression of the TOSC with increasing açaí extract concentration.

For **hydroxyl radicals**, the nonlinear relationship between extract concentration and antioxidant capacity was even more complex (see **Figure 1c**). It is striking that in the range of the low TOSC values the curve slopes of the methanol extracts are very steep. In the region of ∼70−80% TOSC the curves become very flat. This means that a further increase in the substrate concentration produces nearly no increase in the antioxidant activity. In contrast, the Soxhlet ethanol extract (extract 4) shows significantly lower antioxidant capacity against hydroxyl radicals. Also, the curve progress is different, less steep in the lower substrate concentration range and less flat in the higher concentration range.

The different behaviors of the extracts toward the three ROS can be explained by highly different reactivities and half-lives of the ROS (*13*, *28*). Peroxyl radicals are the least reactive of the three ROS with the highest life span. Therefore, they can be scavenged rather easily with lower amounts of antioxidants. For the more reactive peroxynitrite and hydroxyl radicals, higher amounts or more effective antioxidants are necessary for the same inhibition rate. However, from a certain range of the dose−response curves on, even the addition of much higher amounts of a compound does not lead to much higher protection from the ROS, thereby causing a plateau-like flattening of the chart.

From a comparison of the results of açaí seeds with açaí fruit pulps (in both cases based on dry matter), it can be concluded that the concentration of açaí seeds (320 mg/L) necessary for 50% inhibition against peroxyl radicals is in the same order of magnitude of açaí fruit pulp (300 mg/L). Against peroxynitrite and hydroxyl radicals it can be deduced that the seeds have a better antioxidative capacity than the fruits, because the concentrations necessary for 50% inhibition of peroxynitrite are 812 and 1150 mg/L, respectively, and for 50% inhibition of hydroxyl radicals the concentrations are 945 and 3000 mg/L, respectively.

Identification of Mono- and Oligomeric Polyphenols in Açaí Seeds. Although there has already been evidence that açaí seeds have a high antioxidant capacity (*12*), the compounds responsible for these properties have not yet been identified. The combination of multistep mass spectrometric fragmentation

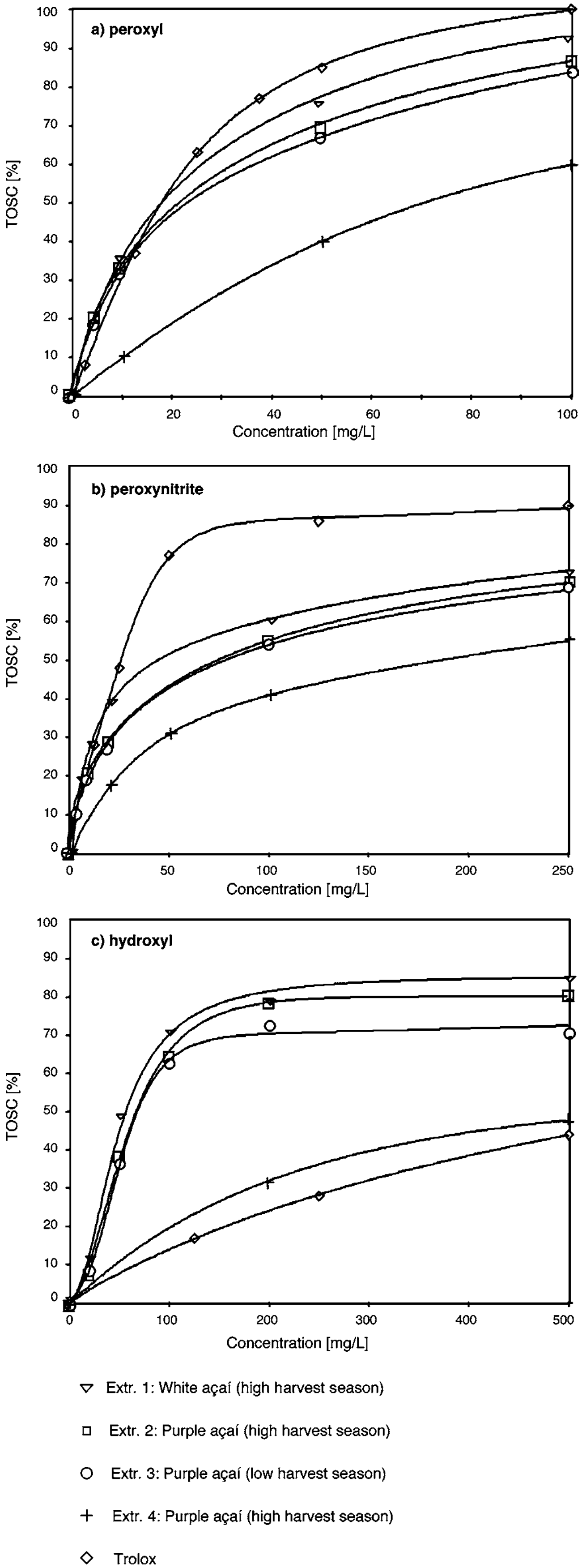

Figure 1. Dose–response curves of açaí seed extracts and Trolox against different ROS: (**a**) peroxyl radical; (**b**) peroxynitrite; (**c**) hydroxyl radical. Extracts 1–3 were prepared by cold digestion with methanol; extract 4 was prepared by Soxhlet with ethanol.

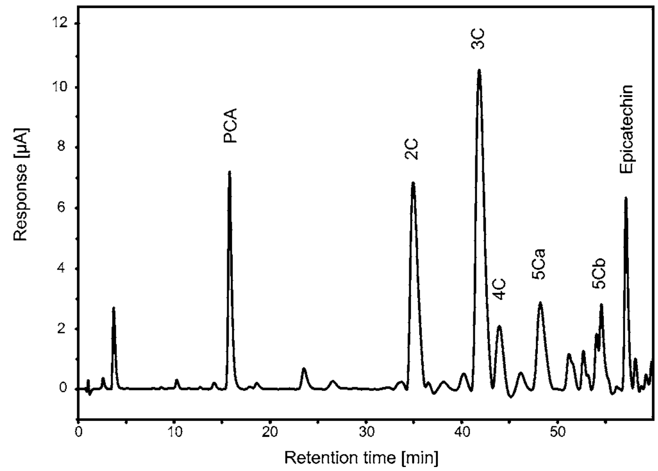

Figure 2. Characteristic CEAD chromatogram of a methanol extract of açaí seeds at 220 mV. For abbreviations see **Table 3**.

after HPLC separation, UV−vis diode array detection, and electrodynamic voltammograms allowed us to identify protocatechuic acid, epicatechin, and five procyanidins [one dimer (2C), one trimer (3C), one tetramer (4C), and two different pentamers (5Ca and 5Cb)] in açaí seeds. A characteristic HPLC chromatogram is displayed in **Figure 2**. In **Table 2**, the LC-MS data for the identified polyphenols are shown.

Concentration of Polyphenols in Seed Extracts. The concentrations of the identified polyphenols in the different seed extracts are summarized in **Table 3**. The quantification of epicatechin was not possible because of interference with other UV active compounds. However, from the small peak area of that multicompound peak it can be concluded that the epicatechin content in all extracts is low.

In all seed extracts, small amounts of protocatechuic acid and epicatechin and high amounts of oligomeric procyanidins (dimers up to pentamers) were detected. The lowest concentrations were found in the Soxhlet ethanol extract; in this case, dimeric procyanidins are predominant. However, due to the above-described overall low antioxidant capacity of that extract, the contributions of the main compounds of that extract have not been further examined.

Of the three cold methanol extracts, the highest overall contents were found in the seed extract of the white açaí variety followed by both extracts of the purple variety (slightly higher values were found for the seeds sampled in the high harvest season). The distribution patterns of the individual polyphenols are quite different from that of the ethanol extract; however, the patterns were very similar among themselves. All five quantified oligomers occur in similar concentrations with slightly higher values for the compounds of higher degree of polymerization. The differences in the polyphenol content of the four extracts fit the ranking of antioxidant capacity, as described before, which indicates that the identified procyanidins contribute substantially to the antioxidative properties of açaí seeds.

TOSC of HPLC Fractionated Samples. The identified di- to pentameric procyanidins are not commercially available as reference compounds. Therefore, it was not possible to determine the TOSC values of the individual compounds and to calculate their particular contribution to the overall antioxidant capacity of the extracts on that basis. Therefore, for an approximation, one of the methanol extracts was fractionated by HPLC, and each fraction was tested for its TOSC value against peroxyl radicals.

Extract 1 (white açaí variety) was chosen for the HPLC fractionation experiments because it demonstrated the highest

Table 2. MS Data for Identified Phenolic Compounds in Açaí

compound	retention time (min)	parent ion (*m*/*z*)	MS/MS fragments[a] [*m*/*z* (neutral loss)]
protocatechuic acid	11.1	153	109 (CO_2)
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
procyanidin pentamer	21.1	1441	1289 (RDA), 1315 ($-C_6H_6O_3$), 1272, 863, 1153
procyanidin pentamer	22.3	1441	1289 (RDA), 1315 ($-C_6H_6O_3$), 1272, 863, 1153
epicatechin	26.5	289	245, 205, 179

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

Table 3. Concentration of Individual Polyphenols in Açaí Seed Extracts

	extract	concn of individual polyphenols (mg/L)						
		PCA	2C	3C	4C	5Ca	5Cb	Σ polyphenols
1	white açaí (high harvest season)	10.6	775.2	768.5	1002.3	766.0	665.3	3988
2	purple açaí (high harvest season)	13.9	485.2	471.8	638.3	476.3	446.9	2532
3	purple açaí (low harvest season)	31.5	420.2	275.7	484.3	366.9	342.6	1921
4	purple açaí (high harvest season)	84.3	247.4	122.2	144.7	64.6	19.7	683

[a] PCA, protocatechuic acid; 2C, dimer; 3C, trimer; 4C, tetramer; 5Ca and 5Cb, two different pentamers.

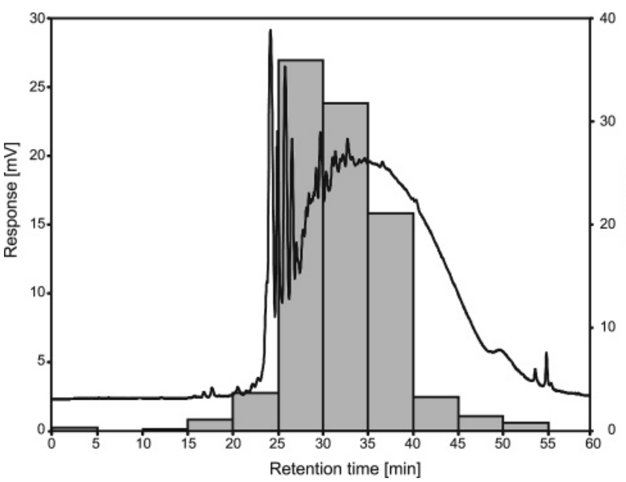

Figure 3. HPLC chromatogram at 210 nm and TOSC of the different fractions of açaí seed extract 1.

overall antioxidant capacities. The TOSC values of the fractions were studied against peroxyl radicals because all extracts demonstrated their highest antioxidant capacity against this ROS. We are aware that results from TOSC assays against peroxynitrite and hydroxyl radicals may be different.

The distribution of the measured TOSC values of the fractions follows roughly the UV course of the chromatogram (**Figure 3**). The TOSC values of the individual fractions varied markedly, so they had to be measured in different dilutions. For comparison with the UV absorption, the TOSC values of the individual fractions are expressed as the percentage of the fractions summarized antioxidant capacity (sum of TOSC of all fractions beeing 100%). The sum of the TOSC values from the individual fractions matches approximately the TOSC of the sample itself, so that there is no evidence so far for synergistic or inhibitory effects. Within the first 15 min, only very small peaks are registered and TOSC values of the corresponding fractions are negligibly low. With the appearance of the first significant peak (protocatechuic acid) in the segment of 15−20 min, the inhibition capacity increases. The main antioxidant capacity is found in the three fractions from 25 to 40 min where the main part of the identified polyphenols is eluted. Therefore, it can be concluded that the identified procyanidins are responsible for a significant portion of the extract's antioxidant capacity.

However, obviously other compounds in addition to the identified procyanidins contribute considerably to the inhibition activity of açaí extracts, because the antioxidant capacity cannot be explained by the procyanidins alone. Fractions from 35 min on do not contain detectable procyanidins in quantifiable amounts, but do exhibit considerable TOSC values, as well. It is likely that this is due to compounds which are responsible for the pronounced broad elevated baseline peak from 25 to 55 min of the chromatogram (**Figure 3**). From the late retention time it can be suggested that these compounds are rather nonpolar, as was similarly discussed for compounds responsible for the mountain-like shape of the baseline of açaí pulp extracts (*6*). The identification of these compounds is the focus of ongoing studies.

Counet and Collin (*29*) came to a similar conclusion for chocolate. They identified several procyanidins (up to decamers) in chocolate extracts and assigned part of the antioxidant activity of chocolate extracts to them, yet most of the compounds with considerable contributions to the antioxidant activity remained unidentified.

Implications. From the results it can be concluded that the açaí seed extracts, prepared by cold methanol digestion, exhibit antioxidant capacity, partially due to the content of oligomeric procyanidins. The concentration (18 mg/L) necessary for 50% inhibition against peroxyl radicals is in the same order of magnitude as that found for Trolox (21 mg/L) (*16*). Comparison with results obtained by Kolayli et al. (*30*) indicates that the generally recognized antioxidant BHT is even somewhat less effective. This explains also the observation that the color stability of açaí beverages is increased upon addition of açaí seed slices. Provided that the toxicological safety of açaí seed extracts will be confirmed by further studies, açaí seeds can be taken as a natural source, for example, for the preparation of a new powerful antioxidant for prolonging the shelf life of foods. Açaí seeds could change from waste to a valuable renewable raw material.

ABBREVIATIONS USED

ABAP, 2,2′-azobis(2-methylpropionamidine) dichloride; CEAD, coulometric electrode array detector; DTPA, diethylenetriaminepentaacetic acid; EDTA, ethylenediaminetetraacetic acid; EtOH, ethanol; KMBA, α-keto-γ-methiolbutyric acid; MeOH, methanol; ROS, reactive oxygen species; SIN-1, 3-morpholinosydnonimine hydrochloride; TOSC, total oxidant scavenging capacity; UHQ, ultrahigh quality.

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