Food Chemistry 133 (2012) 256–263

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Chemical characterization and evaluation of antioxidant properties of Açaí fruits (*Euterpe oleraceae* Mart.) during ripening

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ARTICLE INFO

Article history:
Received 6 April 2011
Received in revised form 7 November 2011
Accepted 23 November 2011
Available online 12 January 2012

Keywords:
Açaí (*Euterpe oleracea* Mart.)
Anthocyanins
Ripening
Flavones
TOSC
Antioxidant capacity
LC–MS
TEAC

ABSTRACT

Consumption of açaí fruits has been linked to positive health effects due to its phenolic content and nutritive value. The objective of this study was to characterize açaí fruits chemically and to determine the antioxidant capacity at three different maturity stages. With the exception of fat, amounts of macronutrients, minerals and titratable acids decreased during the ripening process. The same trend was observed for most of the phenolic constituents identified by HPLC–ESI-MS/MS. A consistent decline was shown for flavones and hydroxycinnamic acids. The concentration of the anthocyanins increased in the course of ripening. In accordance with the total amount of the identified phenolic compounds, the antioxidant capacity, measured by TEAC and TOSC, also decreased. However, the contribution of the main phenolic compounds to the overall antioxidant capacity evaluated by TOSC was estimated to be low.

1. Introduction

Açaí (*Euterpe oleracea* Mart.) is a tropical palm tree that occurs naturally in the Amazon region. Its spherical grape-sized fruits are green when young and ripen usually to a dark purple (Strudwick & Sobel, 1988). An important reloading point for açaí is the city of Belém in Pará State, Brazil, where fruits can be collected throughout the year. However, a major harvesting period exists during the "dry-months" from August to December. Usually, the fruits are used to prepare a liquid with creamy texture by macerating the pericarp and mixing it with different amounts of water, yielding commercially available açaí pulp (Lichtenthäler et al., 2005). In the production region, açaí is integrated in the daily dietary habits of the native people and is normally used in main meals for lunch or dinner. In modern Brazilian society, it has gained interest as a nutritionally valuable wellness product (Strudwick & Sobel, 1988). Meanwhile, açaí is favoured as an ingredient in fruit beverages beyond the Brazilian borders and is exported mainly to the USA or to Europe (Sabbe, Verbeke, Deliza, Matta, & Van Damme, 2009).

Interest in açaí has arisen especially due to its high *in vitro* antioxidant activity, explained by the considerably high content of phenolic compounds, e.g., different anthocyanins, flavones, and phenolic acids (Lichtenthäler et al., 2005; Pacheco-Palencia, Duncan, & Talcott, 2009). Phenolic constituents are generally associated with health-promoting properties and the prevention of several degenerative diseases (Xia, Deng, Guo, & Li, 2010). Because of legislators and consumers' growing concern over the use of artificial food additives, there is a growing demand for additives from natural resources (Giusti & Wrolstad, 2003). For instance, anthocyanins can be used as food colourants (Pazmino-Duran, Giusti, Wrolstad, & Gloria, 2001). Moreover, plant extracts containing a broad range of polyphenols may act as antioxidants or antimicrobial agents (El-Hela & Abdullah, 2010).

Generally, the ripening process of fruits is indicated by intensive metabolism of primary and secondary plant compounds. This study gives information on quantitative changes of macronutri-

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0308-8146/$ - see front matter © 2012 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2011.11.150

ents, individual polyphenols and the antioxidant activity in açaí fruits during ripening. In addition, the influence of major individual phenolic compounds on the overall antioxidant capacity was estimated.

2. Materials and methods

2.1. Chemicals

Ultrahigh quality (UHQ) water was prepared with a Direct-Q 3 system (Millipore, Billerica, USA). Protocatechuic acid (⩾97%) and *p*-hydroxybenzoic acid (⩾99%) were purchased from Merck (Darmstadt, Germany) and caffeic acid (purum) from SERVA Feinbiochemica (Heidelberg, Germany). Gallic acid (⩾97.5%), vanillic acid (⩾97%), syringic acid (⩾95%), chlorogenic acid (5-*O*-(3,4-dihydroxycinnamoyl)-L-quinic acid) (⩾97%), taxifolin (⩾85%), diethylenetriaminepentaacetic acid (DTPA) (⩾99 %), α-keto-γ-methylbutyric acid (KMBA) (⩾97%), 2,2′-azobis(2-methylpropionamidine) dichloride (ABAP) (⩾97%), 3-morpholinosydnonimine *N*-ethylcarbamide (SIN-1), 2,2-azinobis-(3-ehtylbenzothiazoline-6-sulphonic acid)-diammonium salt (ABTS), Folin–Ciocalteu reagent, and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®) were obtained from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Cyanidin 3-*O*-glucoside (⩾96%), cyanidin 3-*O*-rutinoside (⩾96%), peonidin 3-*O*-glucoside (⩾95%), luteolin (⩾99%), isovitexin (⩾99%), chrysoeriol (⩾99%), orientin (⩾99%) and homoorientin (⩾99%) were purchased from Extrasynthèse (Genay, France). Luteolin 7-*O*-glucoside and chrysoeriol 7-*O*-glucoside standards were a gift from Professor Dr. Galensa (University of Bonn). They were self-isolated and had a purity grade of ⩾84% and ⩾70%, respectively.

2.2. Raw material, sampling and preparation of the pulp

Açaí fruits of three different maturity stages (unripe – green fruits, intermediate – reddish-brown fruits, ripe – deep purple fruits) were collected at the experimental station of the Federal Rural University of Amazon in Castanhal, PA, Brazil. Fruits of each maturity stage were processed separately, following the specifications that are usually applied by the industry. After reception, the fruits were selected, washed, weighed, and sanitized in chlorinated water. The subsequent maceration step consisted of steeping the fruits in hot water (50 °C) for 30 min to facilitate the separation of the exo- and mesocarp from the seeds. This separation was carried out in an açaí specific extractor by adding water in a proportion of 0.6 l/kg fruit. The obtained pulp was stored at −30 °C.

2.3. Determination of ash, minerals, lipids, protein, carbohydrates and titratable acids

Minerals were quantified by using flame atomic absorption spectrometry according to official AOAC methods (2005). Proximal composition of açaí pulp samples at three maturity stages was determined according to official AOAC methods (1998). Ash content was measured by a gravimetric assay. Lipids were determined by acid hydrolysis and gravimetric measure of the final ether solution. Protein content was measured following Kjeldahl method. Total protein was calculated by multiplication of the obtained nitrogen content with the fruit-specific conversion factor of 6.25. Carbohydrates were calculated as centesimal difference of the previous determinations. All results are referred to dry matter (DM) basis. Titratable acids were determined by a titration method using an automatic titrator with sodium hydroxide. Results are expressed in malic acid equivalent/100 g of DM.

2.4. Identification and quantification of phenolic compounds by HPLC–ESI-MS/MS

2.4.1. Extraction of phenolic compounds

Açaí pulp was lyophilized and defatted by Soxhlet extraction with petroleum ether. The extraction of phenolic compounds was performed using a modified sample preparation according to a method described in Pacheco-Palencia et al. (2009). For two times, sample of each maturity stage (500 mg) was dissolved in 10 ml of acetone–water–formic acid (70% + 29% + 1%; v/v/v), sonicated for 10 min, then centrifuged for 10 min with 10,000 rpm at 10 °C. The residue was extracted once more with 10 ml acetone–water–formic acid. Afterwards the supernatants were combined. To get rid of the organic solvent, the extract was vapourized using a rotary evaporator (Rotavapor R-210, Büchi, Essen, Germany) at 30 °C and the aqueous supernatant was shaken with 10 ml ethyl acetate. The received ethyl acetate fraction was vapourized to dryness. The residue was solubilized in 1 ml methanol–water–formic acid (50% + 49% + 1%; v/v/v) and filtered through a 1.0/0.45 μm syringe filter (Chromafil GF/PET-45/25, Macherey–Nagel, Düren, Germany) prior to application to HPLC–ESI-MS/MS.

Anthocyanins were extracted using a modified method explicitly described in Wu, Gu, Prior, and McKay (2004). Briefly, freeze-dried sample (250 mg) of each maturity stage was extracted in duplicate with 4 ml of methanol–UHQ water–acetic acid (MeOH–H_2O–HAc) (50% + 49.5% + 0.5%; v/v/v). After vortexing, sonication and centrifugation, the supernatant was removed and the sample once more extracted but with 2.5 ml of MeOH–H_2O–HAc. Both sample solutions were combined and filtered through a 1.0/0.45 μm syringe filter (Chromafil GF/PET-45/25, Macherey–Nagel, Düren, Germany) prior to application to HPLC–ESI-MS/MS.

2.4.2. Analysis of phenolic compounds

Quantification of the phenolic compounds was performed following a method described in Gordon, Schadow, Quijano, and Marx (2011). HPLC instruments consisted of a pump system and a UV-detector of the HP 1050 series (Hewlett Packard, Waldbronn, Germany), a degasser Degasys Populair DP3010 (Uniflows, Tokyo, Japan) and an analytical column Aqua 3 μm C18, 150 mm, 2 mm i.d., with a guard column Security Guard, C18, 4 mm, 2 mm i.d. (both Phenomenex, Aschaffenburg, Germany). The solvents were UHQ water with 1% (v/v) formic acid (mobile phase A) and 1% (v/v) formic acid in acetonitrile (mobile phase B). The HPLC gradient, using a flow rate of 0.2 ml/min started at 5% B and rose to 35% B after 55 min, 100% B after 65 min and re-equilibrated for 15 min at 5% B. Another gradient was used to obtain a better separation for quantification of the luteolin derivatives, orientin and homoorientin. Starting at 5% B, the gradient rose to 17.5% B after 50 min with a subsequent washing and re-equilibration procedure. Twenty microlitres of each sample extract were injected for analysis. The coupled API 2000 HPLC–ESI-MS/MS system was controlled with Analyst 1.5 Software (both Applied Biosystems, Darmstadt, Germany). Mass spectra for the determination of anthocyanins were obtained by using positive ionization, whereas all other phenolic compounds were detected in negative ionization mode.

Identification of phenolic compounds was performed by comparing fragmentation patterns in multiple reaction mode and retention times with those of authentic standard substances. Standards were also used to create calibration curves for quantification. Results were recalculated to the non-defatted material and expressed in mg/100 g DM.