Extracción con Líquido Presurizado de Compuestos Fenólicos de Semillas de Açai (*Euterpe precatoria Mart.*)

Pressurized Liquid Extraction of Phenolic Compounds from Acai seeds (*Euterpe precatoria Mart.*)

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Resumen

In the Amazon region there are two predominant species: Euterpe oleracea Mart. and Euterpe precatoria Mart, these are palm trees whose fruit is known as açai. However, unlike E. precatoria, E. oleracea has received special attention for its incomparable taste, energy properties and for being an important source of antioxidants. Recently, studies have been conducted on E. precatoria demonstrate its bioactive superior to that reported for E. oleracea, however, these studies are rare and focus only on the pulp leaving aside açai seed. On the other hand, businessmen have shown great interest on the obtaining the responsible compounds for their antioxidant activity such as phenolics, for its incorporation in cosmetics, pharmaceutical and food products. However, the use of large amounts of mostly toxic solvents, long extraction times, low extract yield, low antioxidants-concentration of the extracts and presence of solvent in the final product are disadvantages of the current extraction processes that reduce the possibility of having high quality açai extracts in the market. In this paper, the obtaining of phenolic compounds and antioxidants from açai seeds by pressurized liquid extraction (PLE) was studied. Extractions were made by using ethanol as the solvent extraction at flow rate of 2.0×10^{-5} kg of ethanol/s, temperatures of 313 to 333 K, pressures of 2 to 6 MPa with and solvent to feed mass ratio (S/F) of 2.3. Total phenolics and antioxidant activity of açai extracts were

evaluated while the extraction yield was determined to evaluate the PLE performance. Rich-extracts in phenolic compounds with antioxidant activity from açai (*E. precatoria*) seeds were obtained by PLE. The highest phenolic content in extracts was 33 % and it was obtained at 313 K/ 4 MPa while the highest phenolic extraction yields were quickly produced at 4 MPa and low temperatures (313 to 333 K) using only small amounts of a GRAS.

Keywords: solvents, antioxidants, secondary metabolite.

Abstract

En la región amazónica hay dos especies predominantes: *Euterpe oleracea Mart*. y *Euterpe precatoria Mart*. Estas son palmeras, cuya fruta es conocida como açai. Sin embargo, a diferencia de *E. precatoria*, la *E. oleracea* ha recibido especial atención por su incomparable sabor, propiedades energéticas y por ser una importante fuente de antioxidantes. Recientemente, se han realizado estudios sobre la *E. precatoria* que demuestran su bioactividad superior a la reportada para *E. oleracea*. Sin embargo, estos estudios son raros y se enfocan solo en la pulpa, dejando de lado la semilla de açai. Por otro lado, los empresarios han mostrado un gran interés en la obtención de compuestos responsables por su actividad antioxidante, como los fenólicos, para su incorporación en cosméticos, productos farmacéuticos y alimentos. Sin embargo, el uso de grandes cantidades de solventes, mayormente tóxicos, largos tiempos de extracción, bajo rendimiento de extracto, bajos antioxidantes-concentración de los extractos y presencia de solvente en el producto final son desventajas de los procesos de extracción actuals, que reducen la posibilidad de tener alta calidad de los extractos de acai, en el mercado. En este trabajo, se estudió la obtención de compuestos fenólicos y antioxidants, a partir de semillas de acai, mediante extracción líquida presurizada (PLE). Las extracciones se realizaron mediante el uso de etanol como extracción con solvente a una velocidad de flujo de 2.0 × 10-5 kg de etanol / s, temperaturas de 313 a 333 K, presiones de 2 a 6 MPa, con una relación de masa de disolvente y alimentación (S / F) de 2,3. Se evaluaron los compuestos fenólicos totales y la actividad antioxidante de los extractos de açai, mientras que el rendimiento de extracción se determinó para evaluar el rendimiento de PLE. Los extractos ricos en compuestos fenólicos con actividad antioxidante de semillas de açai (*E. precatoria*) se obtuvieron por PLE. El contenido fenólico más alto en extractos fue 33 % y se obtuvo a 313 K / 4 MPa, mientras que los rendimientos de extracción fenólica más altos se produjeron rápidamente a 4 MPa y bajas temperaturas (313 a 333 K), utilizando solo pequeñas cantidades de GRAS.

Palabras clave: solventes, antioxidantes, metabolitos secundarios.

Introduction

The food, pharmaceutical and cosmetic industries have shown recent interest in Amazonian native plants, which are widely recognized for their nutritional value and potential bioactivity. Phenolic compounds have specific biological activities in humans and can be found in small quantities in plants and fruits (Sasidharan et al., 2010). Several properties have been attributed to phenolic compounds, including antioxidant, anticancer, and anti-inflammatory properties (Liu, 2013).

Açai (*Euterpe precatoria Mart.*) is an Amazonian fruit of Brazil and is well known as an energetic fruit and an important source of phenolics among other bioactive compounds. These phenolics and flavonoids consist mainly of anthocyanins, which are responsible for the fruit's antioxidant effects (Rosso et al., 2008; Bataglion et al., 2015; Rufino et al., 2011). Most of the published studies on açai only consider the pulp of the fruit. Few studies consider the açai seeds which represent up to 90 % of the fruit's weight. Açai seeds contain saturated and unsaturated fats. However, in contrast to pulp, açai seeds do not contain cyaniding-3-glucoside and cyanidin-3-rutinoside (Wycoff et al., 2015). On the other hand, açai seed extracts have antioxidant activity that can be attributed to the presence of procyanidins and other unidentified compounds (Rodrigues et al., 2006).

Despite the potential biological activity of this Amazonian fruit, the wastes from fruit consumption and processing are currently discarded and are not considered to be source of bioactive compounds that are feasible for recovery. Moreover, efficient and environmentally friendly extraction techniques which aim to efficiently obtain bioactive compounds by producing highly concentrated extracts in short periods of time by using small amounts of non-toxic solvents, have not been applied to Amazonian fruit waste.

Pressurized liquid extraction (PLE) has been used to generate value-added compounds from several raw materials and has demonstrated higher efficiencies than conventional extraction techniques such as soxhlet and maceration (Kaufmann and Christen, 2002). PLE uses a liquid solvent at high temperature and high pressure. High temperatures enable the interactions between the solid matrix and target compounds to be disrupted, thus increasing the solubilities of the target compounds in the solvent (Mustafa and Turner, 2011). Because increasing temperature decreases the surface tension and viscosity of the solvent, mass transfer is improved. In addition to increased temperature, pressure is applied to keep the solvent in the liquid state, thus improving the solvation power of the solvent (Xynos et al., 2012). Therefore, PLE can be performed in short periods of time with small amounts of solvent (Osorio-Tobón et al., 2014). Because PLE is conducted in a closed extractor, the degradation of oxygen- and light-sensitive compounds is avoided. PLE has been mainly applied for extracting polar compounds and is preferentially performed with water and ethanol (green solvents) as extraction solvents.

PLE has been successfully used to obtain polyphenols (Machado et al., 2015; Veggi et al., 2014; Paes et al., 2014; Osorio-Tobón et al., 2014; Garcia-Mendoza et al., 2015), carotenoids (Cardenas-Toro et al., 2015; Garcia-Mendoza et al., 2015; Zaghdoudi et al., 2015) and thiosulfinates (Farías-Campomanes et al., 2014). The objective of this work was to evaluate the abilities of PLE to extract bioactive compounds from açai seeds.

Material and Methods

Raw materials and characterization. Açai (*Euterpe precatoria Mart.*) was collected in the Amazonas state (Brazil) in March 2013. The fruit were cleaned in the Laboratory of Plant Products Technology at the Federal University of Amazonas (UFAM). Açai seeds were manually separated from the pulp. The seeds were dried in an oven at 308 K (model SP-100/27-A, Novatecnica, Sao Paulo, Brazil) for 30 h, then açai seeds were milled using a knife grinder (model SP31, SP Labor, Sao Paulo, Brazil), packed in plastic bags, labeled and stored in a domestic freezer at 255 K until their transport to Campinas (Sao Paulo, Brazil). At the Laboratory of Supercritical Technology: Extraction, Fractionation and Identification of Extracts (LASEFI) at the University of Campinas (UNICAMP), the geometric mean diameter of the particles was determined according to ASAE (ASAE, 2008). The particle size distribution was determined using a vibratory system (Bertel, model 1868, Sao Paulo, Brazil) equipped with 16- to 80- mesh sieves (Tyler series, Wheeling, USA). The moisture contents of the raw materials were determined according to a gravimetric method based on water removal upon heating in an oven (Tecnal, model TE 395-1, Sao Paulo, Brazil) at 378 K. The bed's apparent density was calculated by dividing the mass of the raw material used in the experiments by the volume occupied by the material in the extractor vessel.

Pressurized liquid extraction. PLE was conducted in a homemade PLE system previously described by Rodrigues et al. (2014). This system was slightly modified by replacing the back-pressure valve with blocking and micrometric valves. The PLE system was equipped with a 5.7 cm^3 extraction vessel (Thar Designs, CL 1373, Pittsburg, USA) that was completely filled with açai seeds. Ethanol (99.5 % purity, Dinamica, Campinas, Brazil) was used as the extraction solvent at flow rate of 2.0 × 10^{-5} kg/s. PLE experiments were performed at temperatures of 313, 323 and 333 K, pressures of 2, 4 and 6 MPa in static mode followed by dynamic mode. Static extraction was performed for 10 min to equilibrate the system with respect to temperature and pressure. After filling with açai seeds, the extraction vessel was brought to the operating temperature and then filled with solvent until the operating pressure was reached. Static extraction began when the operating conditions (temperature and pressure) were reached. During static mode, solvent was not fed into or removed from the extraction vessel. Dynamic extraction involved continuously flowing the solvent at the operating flow rate through the extraction bed within the vessel at the operating temperature and pressure. Dynamic

extraction was performed until to reach the solvent mass-to-feed mass ratio (S/F) of 2.3 ± 0.2. After the PLE experiments, the ethanol in the açai seed extracts was immediately removed by vacuum evaporation at 0.01 MPa (Laborota, model 4001, Viertrieb, Germany) using a thermostatic bath at 313 K. Finally, the collecting flasks with the extract inside weighed in a semi-analytical balance (Sartorius, model A200S, Gottingen, Germany) to determine the extract masses. PLE experiments were performed in duplicate.

Extract yield (EY) was calculated as the ratio of extract mass (M_E) to the total dry mass of the raw material (M_R) used to form the extraction bed, as shown in Eq. (1).

$$EY(\%) = \frac{M_E}{M_R} \times 100 \qquad (1)$$

Extract Characterization

Total phenolic content. The total phenolic content was determined according to the method proposed by Singleton et al. (1999). The Folin-Ciocalteau method is based on the reduction of phosphomolybdic-phosphotungstic acid to a blue complex in an alkaline solution, a reaction that occurs in the presence of phenolic compounds. Quantification was done based on a standard curve of gallic acid. Analyses were performed in triplicate, and the results are expressed in g of gallic acid equivalent (GAE) per 100 g of extract (%) and g of GAE per 100 g of raw material in dry basis (%).

DPHH radical scavenging activity. The antioxidant activity of the extracts was determined using the method proposed by Kordali et al. (2005). This method involves measuring the radical scavenging ability of samples using the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Analyses were performed in triplicate, and the results are expressed as percentages of inhibition (PI) according to Eq. (2).

$$PI(\%) = \frac{A_C - A_S}{A_C} \times 100 \qquad (2)$$

Where A_C is the absorbance of the control and A_S is the absorbance of the sample. The antioxidant activity of an extract was expressed as a PI when the extract concentration used in the analysis did not scavenge 50 % of the DPPH free radicals.

Results and Discussion

The characterization data of açai seeds are presented in Table 1. Açai seeds used in the PLE had a moisture content of 7.1 ± 0.1 %. Also, the particle size of açai seeds employed in the experiments was lower than 16 mesh.

Table 1. Physical and chemical characteristics of açai seeds.

			Açai seeds
Moisture	(%)		7.1 ± 0.1
Extraction bed	(g, w.b.)		5.1 ± 0.3
Apparent density	(g/cm^3)		0.90 ± 0.01
Geometric mean diameter of particle	(mm)		0.99 ± 0.01
Retained mass	(%)	16 mesh	68 ± 1
		24 mesh	15.5 ± 0.3
		32 mesh	5.3 ± 0.3
		48 mesh	3.9 ± 0.2
		80 mesh	5 ± 1
		Plate	3 ± 1

Note: Values are reported as the means ± standard deviation

The extract yield is shown in Figure 1. As expected, an increase in temperature increased the extract yield, since high temperature favors solubility. High temperatures decrease the solvent surface tension and therefore enable the formation of solvent cavities; thereby allowing a faster dissolution of analytes in the solvent, which results in the increasing of the extract yield (Mustafa and Turner, 2011). However, increased temperature is also associated with decreasing extraction selectivity, i.e. non-target compounds are also dissolved; therefore, extract characteristics must be considered to determine the optimal extraction temperature.

In PLE, pressure keeps the extraction solvent in the liquid state also helps the solvent to efficiently penetrate the solid matrix, thus maximizing the contact between target compounds and solvent (Mustafa and Turner, 2011). In this study, the extract yield increased when the pressure was raised from 2 to 6 MPa; however, this behavior was only observed in extractions performed at 323 and 333 K. At 313 K, an interaction between temperature and pressure may have occurred. Because solvent viscosity is high at low temperature and density solvent increases as pressure is increased, then these solvent properties may have hindered its passage through the extraction bed, resulting in a decreased extract yield.

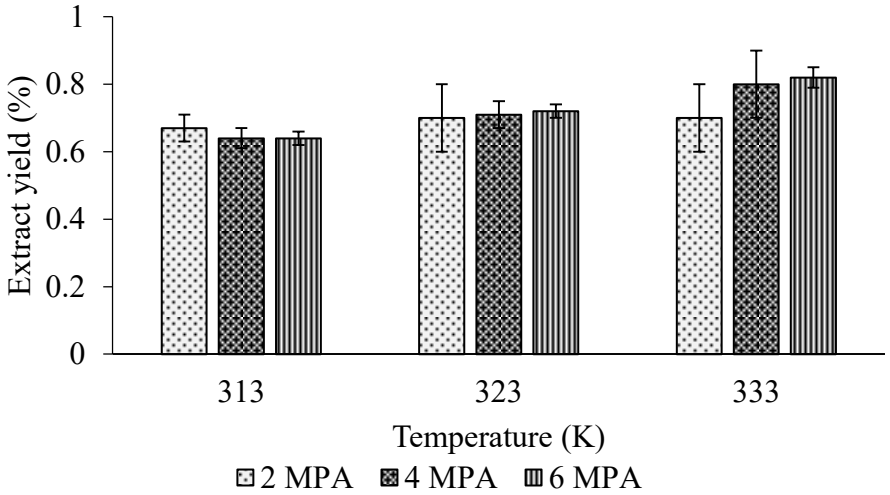

Figure 1. Extract yield from pressurized liquid extraction of açai seeds.

The highest extract yield obtained in this study from *E. precatoria* seeds was 0.82 ± 0.03 % and it was achieved at *333* K/ 6 MPa. This result was lower than that reported by Wycoff et al. (2015) for white and purple açai seeds from E. oleracea. Wycoff et al. (2015) reported extract yields of *3.4* % for white açai seeds and 4.7 % for purple açai seeds using an accelerated solvent extractor at *373* K / 10.3 MPa with methanol as the extraction solvent. The lower extract yields obtained in this study can be attributed to the operating conditions, the extraction solvent and the raw material source. However, it should be noted that unlike ethanol, methanol is a toxic solvent; therefore, the use of methanol as extraction solvent represents a no green extraction process, besides reducing the field of application of the extracts. Also, the use of elevated temperature may promote the bioactive compounds degradation including the decrease of the extraction selectivity.

Recently, the food, cosmetic and pharmaceutical industries have shown a special interest in açai because of its antioxidant capacity. This antioxidant capacity has been partially attributed to the fruit's phenolic content (Pacheco et al., 2009). Thus, the phenolic yield of açai seeds using PLE was determined. Due to the lack of studies on the phenolic content of açai seeds, açai pulp was considered in this discussion of results. According to Figure 2, the highest phenolic extraction yields were obtained at 4 MPa, where these varied from 0.19 to 0.21 % as temperature was decreased. These yields were higher than the yields reported for E. oleracea pulp (0.018 and 0.027 %) whose extracts were obtained with methanol and water, respectively, after maceration, agitation, sparging, filtration and freeze-drying stages (Pacheco-Palencia et al., 2009; Bataglion et al., 2015). However, the results obtained in this work are comparable with the phenolic extraction

yield reported for defatted Euterpe edulis pulp (0.22 %) whose extracts were obtained in an ultrasonic bath with methanol 0.1 M HCl (338 K for 15 min), centrifugation and filtration (Borges et al., 2011). The small white bars that are superimposed represent the phenolic content in the extracts and these are expressed as g of GAE/ 100 g of extract. It is observed that phenolic extract content decreased as the temperature increased, a trend likely caused by a decrease in extraction selectivity. High temperatures reduce the dielectric constant and polarity of a solvent (Mohsen-Nia et al., 2010), potentially favoring the extraction of less polar compounds such as fat. Wycoff et al. (2015) reported a saturated and unsaturated fat content of 0.22-0.33 % in açai seeds.

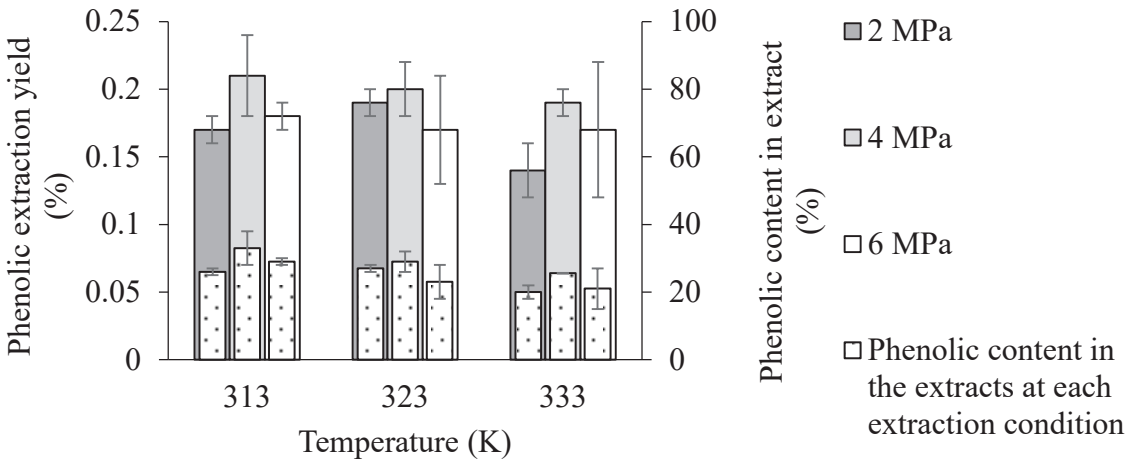

Figure 2. Phenolic extraction yield and phenolic content in the açai seeds extracts.

Açai seed extracts with a minimum phenolic content of 20 % (dry basis) were produced at 333 K/ 2 MPa while the maximum phenolic content was 33 ± 5 % and it was obtained at 313 K/ 4 MPa. The phenolic concentration in the ethanolic extracts prior to solvent removal was 0.7 ± 0.1 g GAE/ L of extract. This result agrees with the results reported by Rodrigues et al. (2006), who produced açai seed extracts with polyphenol concentrations of 0.68 g/L by exhaustive Soxhlet extraction with ethanol.

High DPPH radical inhibition percentages (PI) were obtained despite the low concentrations of the extract solutions (0.25 mg/ mL of ethanol). Extracts produced at 2 and 4 MPa showed similar antioxidant activities, although the phenolic content in the extracts differed. This finding suggests that is possible the co-extraction of non-phenolic but antioxidant compounds. To determine if the observed antioxidant activity is related to the phenolic content in the extracts, a correlation between inhibition percentage and phenolic content in the extracts was analyzed (Figure 3). According to Figure 3 there is a strong correlation between phenolics in the extracts and its antioxidant activity since the calculated Pearson correlation coefficient (r) were 0.962, 0.973 and 0.980 for the extracts obtained at 2, 4 and 6 MPa respectively.

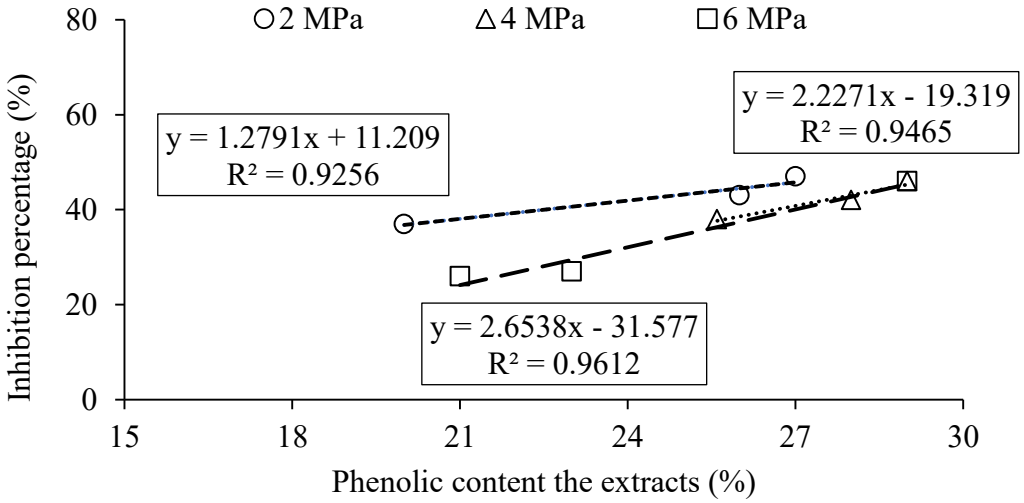

Figure 3. Antioxidant activity versus phenolic content in the açai seeds extracts.

As seen by the obtained results, PLE is a promising and environmentally friendly technique for producing açai seed extracts with high phenolic content and antioxidant activity. Passing a small amount of ethanol (S/F of ratio of 2.3 ± 0.2) at low temperature and pressure (313 to 333 K/ 4 MPa) through the extraction bed for a short time (10 min) was required to obtain the highest extract yields and to produce extracts with the highest phenolic content. Due to the features of açai seed extracts generated by PLE, these extracts may be used in the food industry to replace synthetic antioxidants or as ingredients in cosmetic and drug formulation.

Conclusion

Rich-extracts in phenolic compounds with antioxidant activity from açai (E. precatoria) seeds were obtained by PLE. The highest phenolic content in extracts was 33 % and it was obtained at 313 K/ 4 MPa while the highest phenolic extraction yields were quickly produced at 4 MPa and low temperatures (313 to 333 K) using only small amounts of a GRAS.

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