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Antioxidant Compounds Recovery from Juçara Residue by Thermal Assisted Extraction

Leilson O. Ribeiro[1] · Ricardo N. C. Pereira[2] · Renata V. Tonon[3] · Lourdes Maria C. Cabral[3] · Manuela Cristina P. A. Santiago[3] · António A. Vicente[2] · José António C. Teixeira[2] · Virgínia M. Matta[3] · Suely P. Freitas[1]

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Abstract

This study aimed to recover bioactive compounds by solid-liquid extraction from the agro-industrial residue obtained during juçara fruits processing into pulp. A preliminary study using different solvents (methanol, ethanol and water) indicated ethanol in aqueous solution as the best solvent for antioxidants recovery. Then, a Box-Behnken design was applied considering as independent variables the solvent composition (30–70% ethanol in water), temperature (30–70 °C) and time (30–60 min), in order to evaluate the effects of these factors on antioxidant activity in juçara extract. Results showed that the extracts with higher antioxidant activity were obtained using 30% ethanol at 70 °C for 60 min; measurements included ABTS and DPPH assays, determination of total phenolic content and total monomeric anthocyanins. Furthermore, the effect of pH in antioxidants recovery was evaluated. For this purpose, the 30% ethanol solution was acidified to pH 1 and 2 with HCl. Principal component analysis showed the formation of three distinct groups: one characterized by high bioactive compounds content (pH 1.0), another with superior antioxidant activity (pH 5.75, non-acidified), and finally the group at pH 2 presenting the worst concentrations in the evaluated responses. HPLC analysis showed the presence of cyanidin-3-*O*-rutinoside and cyanidin-3-*O*-glucoside in the extracts. Therefore, the conventional solid-liquid extraction using renewable solvent can be successfully applied to recover bioactive compounds from juçara residue, which can be used by different food industries.

Keywords *Euterpe edulis* Martius · ABTS · DPPH · HPLC · Box-Behnken design

Abbreviations

ABTS	2,2′-azino-bis-[3-ethylbenzothiazoline-6-sulphonic acid] diammonium salt
C3OG	Cyanidin-3-*O*-glucoside
C3OR	Cyanidin-3-*O*-rutinoside
DPPH	2,2-Diphenyl-1-picrylhydrazyl
GAE	Gallic acid equivalents
HPLC	High performance liquid chromatography
PCA	Principal component analysis
TE	Trolox equivalent
TMA	Total monomeric anthocyanins
TPC	Total phenolic compounds
RCF	Relative centrifugal force

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✉ Leilson O. Ribeiro
leilson@eq.ufrj.br

[1] School of Chemistry, Federal University of Rio de Janeiro, Avenue Athos da Silveira Ramos 149, Rio de Janeiro, RJ 21941-909, Brazil

[2] Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

[3] Embrapa Agroindústria de Alimentos, Avenida das Américas 29501, Rio de Janeiro, RJ 23020-470, Brazil

Introduction

Euterpe edulis Martius palm is typical of the Atlantic Forest and can be found in the northeastern, southern and southeastern regions from Brazil. This palm presents just one stem, which can reach 15 m height in the adult age [1]. Due to this characteristic, the extraction of the palm heart, the main product from juçara palm, implies in cutting down the tree, which makes that juçara palm had been officially included in the endangered species list.

Due to their rich nutritional composition, juçara fruits gained more attention and became an economic and

sustainable alternative for small farmers, once the harvesting of the fruit does not affect the perennial palm tree. It is estimated that each palm tree produces about 3 to 5 kg of fruits [1, 2].

Juçara palm fruits are similar to those produced by the açaí palm (*Euterpe oleraceae*), mainly in the physical aspects. However, regarding their bioactive composition, the fruits of juçara palm have shown to be richer and present higher antioxidant potential. Rufino et al. [3] observed higher total anthocyanin content in juçara fruits (192 mg C3OG.100 g^{-1}) when compared to açaí palm fruits (111 mg C3OG.100 g^{-1}). Juçara pulp also presented anthocyanin content superior to those observed in mulberry, grape, strawberry and açai pulps, as reported by Kuskoski et al. [4].

Borges et al. [5] cited the cytoprotective effect of juçara in terms of oxidative damage induced by radical *terc*-butyl hydroperoxide in mice Vero cell culture. According to the authors, the fruit extract composed by phenolic acids and flavonoids was more effective in protecting the cells than the gallic acid standard. A positive synergistic effect of the phenolic compounds was observed, highlighting the importance of consumption of a food rich in bioactive compounds instead of purified compounds.

Juçara fruits are mainly commercialized as frozen pulp. This product has been used as an ingredient in drink formulations such as juices blends, ice creams and others. However, the industrial processing used to obtain the pulp generates solid residues and effluents, rich in anthocyanins and other phenolic compounds, which are generally discarded to the environment [6]. Garcia-Mendoza et al. [6] recovered anthocyanins of a juçara residue, composed of peel and pulp adhered to the seeds, by several extraction methods focusing on those in which pressurized liquids and supercritical fluids were applied. Their results showed extracts with antioxidant potential, but literature is still scarce regarding the use of thermal solid-liquid extraction to recover antioxidant compounds from this residue, only one operational condition was evaluated (50% ethanol; pH 2; 60 °C; 45 min). Furthermore, the thermal assisted extraction, in relation to the pressurized liquid extraction techniques present as main advantage the low operational cost, being thus relevant more studies on it.

Anthocyanins are considered natural antioxidant and colorant compounds, which justifies the relevance of this study from the industrial point of view, since these recovered compounds could be used by the food and/or cosmetic industries as potential bioactive ingredients in different formulations. Thus, this work addresses the recovery of bioactive compounds from the industrial juçara residue by solid-liquid thermal extraction.

Material and Methods

Raw Material

The residue was obtained from juçara pulp industry (Juçaí, Serrinha do Alambarí, Rio de Janeiro, Brazil). The raw material corresponded to the solid fraction in the aqueous stream, after the equipment washing, separated by filtration in 100 μm nylon filter. These solids were small fragments from fruits, resulting from mechanical abrasion during the pulp extraction process, being therefore different of juçara residue used in the Garcia-Mendoza et al. [6] work. The residue was dried at 60 °C for 10 h in an air circulation dryer (Rio de Janeiro, Brazil). Residual moisture was about 14%, measured gravimetrically using AOAC methodology [7].

Solid–Liquid Extraction

In the first part of the study, preliminary solid–liquid extractions were performed by mixing 1 g of juçara residue with 10 mL of two different organic solvent separately [methanol and ethanol, both at concentrations of 30 and 70% (*v*/v)] and distilled water. The extractions were performed in 50 mL flasks, which were duly covered to avoid solvent loss, and heated during 60 min in a water-bath with stirring at 60 °C. The extracts were centrifuged (2500 rcf, 10 min) and stored at −20 °C until further analysis.

The solvent selected in this stage, was then used in the experimental assays carried out with different solvent concentrations (30–70%, v/v), extraction temperature (30–70 °C) and extraction time (30–60 min), which were combined according to a Box-Behnken design. Each extract obtained was centrifuged at the same conditions used in the initial experiments and stored until analyses.

After this step, the effect of pH on the antioxidant compounds recovery from juçara residue was also evaluated. Thus, new extractions were carried out using the best processing condition with HCl acidified solvent solutions at pH 1 and 2.

Analytical Methods

A more detailed description of the used methods (TPC, TMA, ABTS and DPPH assays and anthocyanins by HPLC) can be found as supplementary material (see Online resource 1). All results were expressed in wet basis.

Total Phenolic Compounds (TPC) This determination was performed using the Folin-Ciocalteu reagent (Merck®, Germany) according to the method described by Singleton

and Rossi [8] modified by Georgé et al. [9], adapted to a 96-well microplate reader (Synergy HT, Bio-Tek, USA).

Total Monomeric Anthocyanins (TMA) These compounds were quantified by differential pH method, using C3OG as reference, molar extinction coefficient of 26,900 M^{-1} cm^{-1} and molecular weight of 449.2 $g.gmol^{-1}$ [10].

ABTS Assay The antioxidant activity was determined by the reduction method of the $ABTS^{\bullet+}$ radical (Sigma-Aldrich®, Spain) according to Gião et al. [11] adapted to a 96-well microplate reader (Synergy HT, Bio-Tek, USA).

DPPH Assay The DPPH (Sigma-Aldrich®, Spain) radical scavenging activity of extracts was determined according to method described by Hidalgo et al. [12].

Anthocyanins by HPLC This determination was performed according to Gouvea et al. [13].

Statistical Analysis

In the first part of this study, a statistical analysis was carried out for the evaluation of the effect of each solvent type on the extraction of antioxidant compounds from juçara residue. Difference among the samples was verified by Tukey's test considering a significance level at $p < 0.05$, using Statistic 7.0 software (StatSoft Inc.). For determination of the extraction operational parameters that would improve antioxidant activity, TMA and TPC in the juçara extract, the desirability function was applied. The data used for it are available as supplementary material (Online resource 1 - Table S1). All determinations were performed in triplicate and analyzed using Statistic 7.0 software (StatSoft Inc.).

Results and Discussion

Selection of the Extraction Solvent

According to the data available as supplementary material (Online resource 1 - Table S2), extracts with antioxidant potential were obtained in all the evaluated conditions, including when distilled water was used. The anthocyanins are only one of many classes of bioactive compounds able to promote antioxidant activities. These compounds were mainly evaluated in this work due to their antioxidant and colorant potential. Besides of this, the anthocyanins are the major compounds in juçara fruits [2, 14]. The *in vitro* antioxidant activity of extracts rich in anthocyanins and other phenolic compounds has been reported in various studies, mainly those about berry fruits [14, 15].

According to Nijveldt [16], the antioxidant activity of anthocyanins is related to the presence of hydroxyl group in their structure, which promotes the capture of radical species. This action is very important for human health, since it can decrease the risk of chronic diseases such as metabolic syndrome and cancer [17, 18].

By both ABTS and DPPH assays, the ethanolic or methanolic extracts had higher antioxidant activity ($p < 0.05$) than the extracts obtained with distilled water. This behavior was also observed by Ballesteros et al. [19] when evaluated the antioxidant compounds recovery from coffee Silverskin by solvent extraction. These results confirm that the polarity of the solvent had strong influence in the antioxidant compounds recovery [20]. The use of solutions lightly more apolar than distilled water favored the extraction of the antioxidant compounds. Despite of the major antioxidant compounds of juçara are water-soluble [2, 14], the use organic solvents in small amount may have improved the extraction efficiency due to easier rupture of the vegetables cell wall [20].

Taking into account the metabolites analyzed, the solution of ethanol at 30% concentration was the best solvent condition for bioactive compounds recovery from juçara residue. This result is relevant because ethanol presents low toxicity in relation to methanol, being a more environmentally friendly option [20]. Therefore, this solvent was selected for using in the next steps of this study.

Experimental Design to Obtain Ethanolic Extracts from Juçara Residue

The operational variables exerted strong influence on the extraction of antioxidant compounds from juçara residue (Online resource 1 - Table S3). The content of extracted phenolic compounds, for example, varied between 591.80 (Trial 3) and 1915.66 mg GAE.100 g^{-1} (Trial 8), while the antioxidant activity, measured by ABTS assay, increased from 34.47 (Trial 3) to 79.92 μmol TE.g^{-1} (Trial 8). The anthocyanin content presented the same behavior, where the lowest and highest values were also observed in trials 3 and 8, respectively.

It is interesting to highlight that the TPC value of the extract from trial 8 was higher than those verified by Inada et al. [21] in juçara pulp (1200 mg GAE.100 g^{-1}) and by Borges et al. [22] in juçara extracts obtained using several solvents and pH (292.56 to 684.00 mg GAE.100 g^{-1}). These differences among TPC values may be related to soil, weather, agricultural practices and ripeness stage of fruits, as well as losses during harvest, transport, storage and depulping process.

Response surface methodology showed that the data (Online resource 1 - Table S4) were well fitted to the models by regression analysis. A good estimation of the experimental data was also confirmed by the high determination coefficients (R^2) for antioxidant activity by ABTS assay, TPC and TMA (0.997, 0.999, and 0.993, respectively). According to the statistical data (see Online resource 1- Table S4), the linear terms of independent variables tested, *i.e.*, temperature, ethanol content and time presented statistical significance for almost all responses ($p < 0.05$).

Among linear terms, temperature was the independent variable that most affected the evaluated responses. The increase of temperature resulted in a better recovery of antioxidant compounds measured by TPC and TMA. The ethanol content linear term was also significant for TPC and ABTS responses, showing a negative effect ($p < 0.05$) on them. As can be observed in supplementary material (Online resource 1 - Table S3), high ethanol content in solvent (above 50%, approximately) decreased the concentration of bioactive substances in the extracts. As cited above, phenolic compounds have better solubility in intermediate polar conditions. The same behavior was reported by Borges et al. [22], which performed the anthocyanins extraction from juçara pulp using acetone, methanol, and ethanol solutions and water. According to the authors, ethanol aqueous solution acidified with HCl presented the highest TMA content among the evaluated solvents.

Extraction time showed a positive effect on antioxidant activity of the extracts (Online resource 1 - Table S4). On the other hand, this behavior was not observed for TPC and TMA responses. This possibly occurs due to the presence of different class of bioactive compounds in the substrate such as flavonoids and phenolic acids, for example [2, 14].

The linear interaction between temperature and ethanol content was significant for ABTS response, as well as the linear interaction between ethanol content and time for TPC and ABTS ($p < 0.05$). However, this interaction presented negative effect (Online resource 1 - Table S4). It shows that the simultaneous increasing of time and ethanol content in solvent reduces the release of phenolic compounds from juçara residue, since the medium becomes more apolar with increasing of ethanol concentration in solvent solution being it less effective for antioxidant recovery over processing time.

By analyzing the desirability contour surfaces (see Online resource - Fig. S1), it can be seen the optimum conditions for extraction of antioxidant compounds from juçara residue. A desirability value equal to 1 means that the best operational condition for recovering of bioactive compounds was found. Thus, regarding the evaluated conditions, the use of 70 °C during 60 min and 30% ethanol content can be considered the best condition for obtaining extracts rich in antioxidant compounds from juçara residue.

Effect of pH on the Characteristics of Juçara Residue Extracts Obtained in the Selected Condition

Principal component analysis (PCA) was performed to identify a global behavior regarding pH effects on extracts from juçara residue, as well as to identify relationships between measured variables (see Online resource 1 - Fig. S3). According to PCA, the horizontal axis (first component) and vertical axis (second component), accounted for 97% of the variability found in measured data, which confirms that the two first components are adequate to describe the variation among all data. The 70.7% of variability found on the first component is explained by the differences observed in contents of TPC, TMA, C3OR and C3OG, while the remaining variability (26.2%) of the second component is due to the different antioxidant activity of the extracts.

The pH was the driving force of PCA patterns, affecting TPC and TMA contents, antioxidant response by both ABTS and DPPH methods, as well as C3OR and C3OG contents. Through PCA is possible to observe three distinct groups, highlighted with 95% confidence ellipses (see Online resource 1 - Fig. S3). Samples extracted with 30% acidified ethanol (pH 1) presented higher TPC and TMA contents, as well as C3OR and C3OG contents, but less antioxidant activity in relation to sample extracted in not-acidified 30% ethanol (pH 5.75). The pH 2 extracts presented lower contents of TPC, TMA when compared with extracts from pH 1, and also lower antioxidant activity when compared with non-acidified samples (Fig. 1 and Online resource 1 - Table S5).

Rodrigues et al. [23] evaluated the recovery of antioxidants from jabuticaba peel by ultrasound extraction using ethanol as solvent and varying pH (0.98 to 6.02) on extracting solution. Their results showed that the highest C3OG content was obtained at pH 0.98, confirming the pH influence on phenolics extraction process. PCA analysis also show that contents of TPC and TMA are positively correlated with C3OR and C3OG contents determined by HPLC. Results from antioxidant activity of the extracts (measured by ABTS and DPPH methods) were also positively correlated with each other, but followed a distinct pattern when compared with TPC, TMA, C3OR and C3OG contents. This means that the effects of pH used during extraction should not be overlooked once it may affect in different ways the properties of the extracts obtained.

The presence of acid in the extracting solution makes easier the rupture of cell wall, increasing the extraction yield as well as the rate of bioactive compounds diffusion into the

solvent. However, the acidity of the extraction solvent may also affect the chemical composition of extracts by release of other bioactive compounds or structural changes [22]. Once the anthocyanins can be used as natural colorant in food preparations and differently extracted depending upon pH, major attention was given for them in this study. Concerning anthocyanin pigments, the pH has a strong influence, since their structures can undergo transformation when the pH of the solution varies. According to Castañeda-Ovando et al. [24], at pH 1 the cation flavylium is the predominant specie, while at pH values between 2 and 4, the quinoidal species are predominant. At pH values between 5 and 6, a carbinol pseudobase and a chalcone can be observed, and at pH values above 7 degradation of these pigments may occur.

This knowledge is important for a better understanding of the values of antioxidant activity of the extracts evaluated in this study. Results showed that the extracts obtained at pH 5.75 presented higher antioxidant power measured by both ABTS and DPPH assays, although they have presented lower TPC, TMA, C3OR and C3OG contents. These results are in accordance with previous ones from Sui, Dong and Zhou [25]. These latter authors have evaluated the effects of pH and temperature on antioxidant activity of two anthocyanins in aqueous solution, observing an increase of activity with increasing pH. It was suggested that this occurred due to the presence of quinoidal base, pseudo base and chalcone forms in samples with pH above 4, which may be more effective in scavenging free radicals, thus resulting in higher antioxidant activity.

Regarding anthocyanins detected by HPLC (see Online resource 1 - Fig. S3), the C3OR and C3OG contents, that are the main anthocyanins in juçara fruits [14], were higher in the extract at pH 1 than in the extracts at pH 5.75 (not acidified) and pH 2 (see Online resource 1 - Table S5), which corroborates the effects observed in PCA analysis.

Conclusions

The 30% ethanol aqueous solution was the best solvent condition for antioxidant compounds recovery from juçara residue. The Box-Behnken experimental design and linear regression analysis allowed the estimation of operational conditions for extraction of compounds with antioxidant activity from juçara residue using a solid–liquid thermal extraction. Furthermore, the pH of solvent showed to be an important variable that affects the bioactive compounds, mainly the anthocyanins, and antioxidant activity of extracts from juçara residue.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights This article does not contain any studies with human or animal subjects.

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