OPTIMIZATION OF SOLVENTS FOR THE EXTRACTION OF PHENOLIC COMPOUNDS FROM AÇAÍ (*Euterpe oleracea*)

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ABSTRACT

The objective of this study is to investigate the use of different solvents as a parameter to optimize the extraction of phenolic compounds from açaí (Euterpe oleracea). To this end, fourteen solvents and mixtures of solvents were prepared from methanol and ethanol, varying in concentrations (% v/v), addition of water, and acidification with acetic acid. Extractions were performed with lyophilized açaí pulp and each solvent or solvent mixture, followed by shaking and centrifugation. Total phenolic content quantifications were performed for all extractions. The results showed that both the addition of water and the acification of solvents significantly increase the extraction of phenolic compounds from açaí, and that a mixture of solvents improves extraction when compared to the use of isolated solvents. The three most efficient solvents were ethanol/water/acetic acid (49/49/2 %; v/v/v), methanol/ethanol/water/acetic acid (24/24/50/2 %; v/v/v/v) and methanol/ethanol/water (25/25/50 %; v/v/v), with total phenolic contents of 59.50 ± 0.60, 58.18 ± 1.51 and 57.49 ± 1.24 mg GAE/g d.w, respectively. They are therefore recommended for extraction of phenolic compounds from açaí, aiming its biotechnological application in food, cosmetics and pharmaceutical products.

Keywords: Açaí. Bioactive compounds. Extraction. Optimization. Phenolic compounds.

1 INTRODUCTION

The Amazon Basin is one of the most biodiverse regions in the world and is home to a vast range of valuable genetic resources, which the economic exploitation crucial to the region[1]. Many Amazonian species are known for their high concentrations of bioactive compounds, arousing growing interest in their exploration for the sustainable development of biotechnological products and processes. Among these species, açaí (Euterpe oleracea) stands out. Phenolic compounds are the main bioactive compounds in açaí and have been related to health-promoting effects such as antioxidant, anti-proliferative and anti-inflammatory[2].

Extraction is the first and one of the most important steps in the recovery and purification of phenolic compounds from plant matrices. However, there is no general extraction procedure that is applicable to all phenolic compounds. Phenolic compounds derived from plant materials include a variety of substances with different structures and physicochemical properties, and these particularities must be taken into account for the correct performance of extraction. Appropriate selection of extraction parameters is critical for accurate quantification and evaluation of the biological properties of phenolic compounds. Thus, a specific extraction procedure must be developed and optimized for each plant material[3].

With the increasing adoption of sustainable approaches to the development of products and processes and the consequent boost in the natural products market, there is great interest in bioactive compounds from plant sources, aiming to develop products that deliver benefits to health and the environment, making it necessary to adequately explore plant matrices, which includes the knowledge about their characteristics of interest. In view of the above, the objective of this study is to investigate the use of different solvents as a parameter to optimize the extraction of phenolic compounds from açaí (*Euterpe oleracea*).

2 MATERIAL & METHODS

Açaí (*Euterpe oleracea*) pulp was collected in the municipality of Muaná/PA. The pulp was ground, sieved and lyophilized to remove the water.

To evaluate the influence of solvent in the extraction of phenolic compounds from the lyophilized açaí pulp, fourteen solvents and mixtures of solvents were prepared from methanol and ethanol, varying in concentrations (% v/v), addition of water, and

acidification with acetic acid. Extractions were performed with 0.1 g of açaí pulp and 1900 µL of each solvent or solvent mixture. The mixtures were shaken for 30 minutes and then centrifuged for 20 minutes.

The total phenolic contents (TPC) of the açaí pulp extracts were determined using the Folin-Ciocalteu method[4]. The reactions were performed by mixing 500 µL of each extract appropriately diluted, 250 µL of Folin-Ciocalteu reagent (50 % v/v) and 1250 µL of sodium carbonate (7,5 % m/v), followed by incubation for 30 min. The absorbance was measured at the wavelength of 760 nm against a blank on a UV-Vis spectrophotometer. Gallic acid was used as standard, with a concentration range of 3,34–69,0 mg/L. The total phenolic contents were expressed in mg gallic acid equivalents per g of fruit dry weight (mg GAE/g d.w.).

All assays were performed in triplicate. Statistical analyses were performed at a significance level of 5 % ($p \leq 0.05$) using Microsoft Excel software.

3 RESULTS & DISCUSSION

The solvents or solvent mixtures prepared for the extraction of phenolic compounds from açaí pulp and the total phenolic content obtained are shown in Table 1.

Table 1 Total phenolic contents (TPC) of açaí pulp obtained by extraction with different solvents or solvent mixtures.

Solvent type	TPC (mg GAE/g d.w.)
Methanol	15.95 ± 0.62
Ethanol	6.77 ± 0.94
Methanol/ethanol (50/50 %; v/v)	12.41 ± 0.76
Water	48.93 ± 0.45
Methanol/water (50/50 %; v/v)	47.79 ± 2.03
Ethanol/water (50/50 %; v/v)	47.85 ± 0.42
Methanol/ethanol/water (25/25/50 %; v/v/v)	57.49 ± 1.24
Methanol/acetic acid (98/2 %; v/v)	21.78 ± 0.65
Ethanol/acetic acid (98/2 %; v/v)	6.67 ± 0.60
Methanol/ethanol/acetic acid (49/49/2 %; v/v/v)	8.45 ± 0.83
Water/acetic acid (98/2 %; v/v)	55.40 ± 0.69
Methanol/water/acetic acid (49/49/2 %; v/v/v)	53.84 ± 0.47
Ethanol/water/acetic acid (49/49/2 %; v/v/v)	59.50 ± 0.60
Methanol/ethanol/water/acetic acid (24/24/50/2 %; v/v/v/v)	58.18 ± 1.51

Based on the results obtained, it was possible to observe that the isolated use of ethanol or methanol as solvents results in a low extraction of phenolics from the açaí, as verified by the lower TPC values obtained by the extracts. The isolated use of water resulted in better extraction, however, the extracted phenolic content by water still remains below other solvent mixtures tested. In the case of açaí, a combination of different solvents showed greater efficiency in the extraction of phenolic compounds, as observed by the higher TPC content performing extraction with ethanol/water/acetic acid (49/49/2 %; v/v/v), of 59.50 mg GAE/g d.w., methanol/ethanol/water/acetic acid (24/24/50/2 %; v/v/v/v), of 58.18 mg GAE/g d.w., and methanol/ethanol/water (25/25/50 %; v/v/v), of 57.49 mg GAE/g d.w. Statistical analysis showed that there is no significant difference between the three higher results obtained, so the choice of the mixture of solvents for extraction can be given considering its availability.

The results obtained for the evaluation of the addition of water to solvents for extraction are shown in Table 2.

Table 2 Extraction of phenolics from açaí with addition of water to the solvents.

Solvent type	TPC (mg GAE/g d.w.)	Influence in TPC	Statistical significance ($p \leq 0.05$)
Methanol Methanol/water (50/50 %; v/v)	15.95 ± 0.62 47.79 ± 2.03	Positive (increase)	Yes
Ethanol Ethanol/water (50/50 %; v/v)	6.77 ± 0.94 47.85 ± 0.42	Positive (increase)	Yes
Methanol/ethanol (50/50 %; v/v) Methanol/ethanol/water (25/25/50 %; v/v/v)	12.41 ± 0.76 57.49 ± 1.24	Positive (increase)	Yes

For all solvents tested, the addition of water positively influenced the extraction of phenolic compounds from açaí pulp. The addition of water to organic solvents improves extraction, as it causes swelling of the plant material, increasing the contact area between the plant matrix and the solvent. This facilitates the penetration of the organic solvent into the sample matrix and intensifies mass transfer by molecular diffusion. In addition, it extracts the highly hydrophilic fraction of glycosylated phenolic compounds[3]. From a statistical point of view, the TPC of all samples increased significantly. Therefore, it is recommended to add water to solvent mixtures in order to extract the highest amount of phenolic compounds from açaí.

The results obtained for the evaluation of the acidification of solvents for extraction are shown in Table 3.

Table 3 Extraction of phenolics from açaí with acidification of the solvents.

Solvent type	TPC (mg GAE/g d.w.)	Influence in TPC	Statistical significance ($p \leq 0.05$)
Methanol	15.95 ± 0.62	Positive	
Methanol/acetic acid (98/2 %; v/v)	21.78 ± 0.65	(increase)	Yes
Ethanol	6.77 ± 0.94	Negative	
Ethanol/acetic acid (98/2 %; v/v)	6.67 ± 0.60	(decrease)	No
Methanol/ethanol (50/50 %; v/v)	12.41 ± 0.76	Negative	
Methanol/ethanol/acetic acid (49/49/2 %; v/v/v)	8.45 ± 0.83	(decrease)	Yes
Water	48.93 ± 0.45	Positive	
Water/acetic acid (98/2 %; v/v)	55.40 ± 0.69	(increase)	Yes
Methanol/water (50/50 %; v/v)	47.79 ± 2.03	Positive	
Methanol/water/acetic acid (49/49/2 %; v/v/v)	53.84 ± 0.47	(increase)	Yes
Ethanol/water (50/50 %; v/v)	47.85 ± 0.42	Positive	
Ethanol/water/acetic acid (49/49/2 %; v/v/v)	59.50 ± 0.60	(increase)	Yes
Methanol/ethanol/water (25/25/50 %; v/v/v)	57.49 ± 1.24	Positive	
Methanol/ethanol/water/acetic acid (24/24/50/2 %; v/v/v/v)	58.18 ± 1.51	(increase)	No

For 5 of the 7 conditions tested, acidification of the solvent resulted in a higher extraction of phenolic compounds from the açaí pulp. In 4 of them, the difference in phenolic extraction was significant. In two conditions, the extracted phenolic content was lower in the acidified solution, but only in the methanol/ethanol/acetic acid solution (49/49/2 %; v/v/v) the decrease was significant. Considering, in isolation, the 3 solvents added to water, a condition that showed to have greater efficiency in extraction, in all of them it was possible to observe an improvement in the extraction of phenolics with acidification, with a significant increase in the extraction with ethanol/water/acetic acid (49/49/2 %; v/v/v) and methanol/water/acetic acid (49/49/2 %; v/v/v).

The results showed that, in general, acidifying the solvent mixture improves the extraction of phenolic compounds from açaí. One reason for these results may be the high anthocyanin content in this species[5]. Anthocyanins are a class of phenolics that are more stable under low pH conditions, where they are present in the form of flavylium ions[6]. The use of an acidified solvent for the extraction can therefore help to better preserve these compounds for quantification.

4 CONCLUSION

Based on the results showed by the different solvents tested, it can be concluded that the combination of solvents showed greater efficiency in the extraction of phenolic compounds from açaí pulp when compared to the individual use of solvents. The addition of water and the acidification of solvents has also resulted in significant improvements in extraction. Based on the total phenolic contents obtained, the three most efficient solvents were ethanol/water/acetic acid (49/49/2 %; v/v/v), methanol/ethanol/water/acetic acid (24/24/50/2 %; v/v/v/v) and methanol/ethanol/water (25/25/50 %; v/v/v), with contents of 59.50 ± 0.60, 58.18 ± 1.51 and 57.49 ± 1.24 mg GAE/g d.w, respectively. They are therefore recommended for extraction. The optimization of the extraction parameters of phenolic compounds from açaí represents a promising step towards the sustainable exploitation of this species, aiming to bring advances to its biotechnological application in food, cosmetics and pharmaceutical products.

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