INDUSTRIAL POTENTIAL OF AMAZONIAN FRUIT WASTE: MANNOSE EXTRACTION FROM AÇAÍ SEEDS (*Euterpe Oleracea*)

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ABSTRACT

This study aimed to valorize agricultural waste from Amazonian fruit cultivation, with a specific focus on extracting industrially relevant compounds, particularly mannose, from açaí seeds through hydrolytic processes. The strategy involved extracting mannose from the açaí seeds through two hydrolyses: (i) acidic (1:4 w/v ratio, 3% H_2SO_4, 60 min. at 121°C) and (ii) enzymatic (300 IU of the BGM enzyme "Amano" 10 per gram of dry biomass, at pH 4.8, 120 rpm, for 72 hours at 50°C). Acid hydrolysis yielded a mannose concentration of 40.48 g/L and enzymatic hydrolysis resulted in a mannose concentration of 25.11 g/L. Further optimization of the hydrolysis process and exploration of alternative acids or enzymes could enhance mannose extraction efficiency from açaí seeds, contributing to the valorization of agricultural waste and the production of valuable industrial compounds.

Keywords: Açaí seeds, Acid hydrolysis, Enzymatic hydrolysis, Mannose, Bioeconomy.

1 INTRODUCTION

The greenhouse gases have been increasing significantly in recent years due to the growing dependence on fossil hydrocarbons. During the 20th century, there was a recorded increase of 0.8°C in global temperature, with projections indicating a rise between 1.4°C and 5.8°C throughout the 21st century.[1] In response to this scenario, renewable energies have emerged as a solution to this environmental challenge. Notably, biomass derived from agricultural residues has emerged as a promising source for replacing these fossil fuels.[2] The palm tree *Euterpe oleracea*, commonly known as the açaí palm, exhibits a widespread distribution in the northwestern region of South America, predominantly inhabiting the estuarine areas of the Amazon River.[3] Brazil, the world's leading producer of açaí fruit, had a national agricultural production of 1,485,113 tons in 2021, with estimates projecting continued growth in the coming years.[4] However, increased productivity leads to a greater accumulation of agricultural residues, as the pulp represents only 5% to 15% of the fruit volume, while the açaí seed accounts for 85% to 95%.[5] Analysis revealed that açaí seeds have a higher content of total sugars, accounting for 67.70% of the dry biomass. Moreover, it was discovered that mannose constitutes 53.60% of the dry biomass, with glucose following at 8.66%.[2] Furthermore, compositional analyses of the various tissues of the seed has discovered that the endosperm has the highest concentration of mannose, accounting for approximately 74% of the dry biomass, while the fiber did not exhibit detectable quantities of mannose.[6] This study aims to valorize agricultural waste from Amazonian fruit cultivation, specifically açaí seeds, by developing hydrolytic processes for extracting mannose, contributing to sustainable waste management practices and the production of valuable industrial compounds.

2 MATERIAL & METHODS

Pre-treatment of the açaí seeds

Açaí fruits were acquired (10 kg) from traders in the city of Belém, Pará, during the harvest season (July to November 2023) and transported to the Centro de Valorização de Compostos Bioativos da Amazônia (CVACBA) for processing. The pulp was extracted according to the protocol recommended in the literature.[7] Subsequently, the seeds were washed and frozen (-22°C) for use in subsequent procedures. Then, açaí seeds were dried in an oven at 60°C until reaching a moisture percentage below 10%. Afterward, the seeds were comminuted in a knife mill to obtain a powder with particle size between 0.84 - 2.00 mm in diameter (mesh 10), which was then utilized in the hydrolysis process.

Acid Hydrolysis

The açaí seeds were subjected to an acid hydrolysis process employing 3% (w/w) diluted sulfuric acid, at a ratio of 1:4 (w:v), and heated to 121°C for one hour. Subsequently, the hydrolysate was cooled in an ice bath and filtered using Whatman filter paper nº1 (11µm). The liquid fraction's pH was then neutralized with 20% NaOH (to pH 7.0), filtered again, and stored at -22°C. The remaining solid fraction underwent washing cycles with distilled water (4 to 6 times) and was post-filtration through a 50 µm sieve (mesh 300) until reaching a pH close to neutral. Finally, the material was dried in an oven with air circulation at 60°C until its moisture content was below 10%, to be later employed in the enzymatic hydrolysis process.

Enzymatic hydrolysis

The enzymatic hydrolysis was conducted in a 50 mM citrate buffer, pH 4.8, with an enzyme concentration of 300 IU per gram of dry biomass (Amano BGM 10 from Amano® Enzyme), a solids concentration of 20% (w/w), at 120 rpm and 50°C, for 72 hours. After completion of the process, the catalytic activity was stopped by subjecting the flasks to a water bath at 100°C for 10 min. Finally, the liquid fraction was separated through filtration using Whatman filter paper nº1 (11 µm) and stored at -22°C.

Mannose quantification

The quantification of mannose was analyzed by high-performance liquid chromatography (HPLC) using a Thermo Fisher Scientific UltiMate™ 3000 series instrument. Compound separation was conducted on an HPX 87H 300 x 7.8 mm column (Aminex), operating at 25°C and using H_2SO_4 (5.0 mM) as the mobile phase in isocratic flow (0.6 mL/min) for 20 min. with an injection volume of 10 µL. Quantification of D-(+)-mannose was based on retention time and refractive index data, which were compared with the commercial standard (Sigma-Aldrich®, Brazil) and quantified through a calibration curve.

3 RESULTS & DISCUSSION

This study aimed to valorize agricultural waste from Amazonian fruit cultivation by extracting industrially relevant compounds, with a specific focus on mannose extraction from açaí seeds through hydrolytic processes. The summarized results are presented in Table 1.

Regarding acid hydrolysis, a concentration of 40.48 ± 0.83 g/L was obtained. According to the literature, 41.76 ± 1.09 g/L of mannose were obtained from acid hydrolysis under the same conditions using sulfuric acid.[8] In other studies, the substitution of sulfuric acid with maleic or oxalic acid has resulted in a hydrolysate with concentrations of 6.4 g/L and 24.4 g/L, respectively. Furthermore, the use of oxalic acid under more severe conditions (150°C for 40 min.), yielded a mannose concentration of approximately 49 g/L, which is higher than those obtained with sulfuric acid[6].

Table 1 Mannose extraction from açaí seeds under various hydrolysis methods.

Hydrolysis method	Conditions	Mannose [g/L]	Extraction yield [%]
Acid	3% w/w sulfuric acid concentration, at 121°C, 60 min.	40.48 ± 0.83	-
		41.76 ± 1.09[8]	~29[8]
	4% w/w oxalic acid, at 150°C, 40 min.	-	~49[6]
Enzymatic	300 U/gr, 20% (w/w), at 120 rpm and 50°C, for 72 hours[a]	25.11 ± 1.33	-
	400 U/gr, 20% (w/w), at 120 rpm and 50°C, for 72 hours[a]	146.3[8]	95[8c]
	400 U/gr, 2% (w/w), at 120 rpm and 50°C, for 72 hours[b]	-	47.4[6c]

[a] Enzymatic hydrolysis of açaí seeds subjected to acid hydrolysis with 3% w/w sulfuric acid concentration at 121°C for 60 min. [b] Enzymatic hydrolysis of açaí seeds subjected to acid hydrolysis with 4% w/w oxalic acid concentration at 121°C for 60 min. [c] Yield concerning the mannose content in açaí seeds after the acid hydrolysis process.

In terms of enzymatic hydrolysis, a mannose concentration of 25.11 ± 1.33 g/L was obtained. The literature reports a mannose yield of 146.3 g/L from acai seeds treated with acid hydrolysis (3% w/w H_2SO_4 at 121°C for 60 min.), followed by enzymatic hydrolysis using an enzyme concentration of 400 IU per gram of dry biomass. This process, conducted under similar conditions, achieved a 95% extraction efficiency from the mannose content in the biomass[8]. Other studies achieved a yield of approximately 47.4% mannose from açaí seeds previously hydrolyzed with 4.0% oxalic acid for 40 min. at 150°C. Possible explanations for this disparity may be attributed to the washing cycles with distilled water performed on the residual biomass after the acid hydrolysis process, where a portion of the solid fraction, possibly the mannose-rich tissues[6], was lost when using a 50 µm sieve (300 mesh). Another potential explanation is that the enzyme concentration used in this work was 100 IU/g biomass lower.

4 CONCLUSION

In conclusion, this study demonstrates the potential for valorizing agricultural waste from Amazonian fruit cultivation, particularly açaí seeds, through hydrolytic processes aimed at extracting mannose. While acid hydrolysis yielded satisfactory mannose concentrations, enzymatic hydrolysis showed promise for further optimization. Discrepancies in yields between different studies highlight the importance of process optimization and parameter standardization. Future research should focus on refining extraction methods, exploring alternative acids or enzymes, and minimizing losses during processing steps.

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