SEEDS FROM AÇAÍ-DO-AMAZONAS AND JUÇARA PALM AS NEW SOURCES OF MANNOSE

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ABSTRACT

Over the past 20 years, açaí pulp production in Brazil has increased, with emphasis on *Euterpe oleracea* Mart (açaí-do-pará). However, there is a growing interest in exploring other palm species that have fruits with sensory and organoleptic characteristics similar to those of açaí-do-pará, such as *E. precatoria* Mart. (açaí-do-amazonas) and *E. edulis* Mart. (juçara). This study aims to investigate the seeds of açaí-do-amazonas and juçara, seeking a better understanding through chemical processing of their recalcitrant biomass, containing over 60% of mannan in both seeds. Despite the chemical compositions of both seeds being very similar to each other, the açaí-do-amazonas seed was identified as more recalcitrant than the juçara seed. For that, we investigated the parameters for dilute acid hydrolysis of those seeds, including dilute sulfuric acid concentration, reaction temperature, and solid loading. Through the utilization of the central composite rotatable design (CCRD) across distinct assays, we achieve a maximum mannose recovery of 87% and 100% and mannose concentrations reached 137 g/L and 160 g/L, respectively, after açaí-do-amazonas and juçara seeds processing.

Keywords: *Euterpe precatoria*. *Euterpe edulis*. mannan. mannose production. (CCRD).

1 INTRODUCTION

The pulp production process from açaí-do-amazonas and juçara generates large quantities of seeds, as it has been observed that approximately 80% of the fruits' weight corresponds to the seeds[1,2]. These seeds are typically discarded at the pulp processing sites, lacking a proper disposal method. However, these seeds may contain valuable polysaccharides that can be utilized in cosmetics production, animal feed, and as platform molecules for producing other bioproducts. Therefore, studies are needed to understand their composition and develop more sustainable and environmentally friendly processing methods for better utilizing these byproducts. Due to the limited data in the literature concerning the chemical composition of açaí-do-amazonas and juçara seeds, this study aims to characterize these biomasses and to investigate hydrolysis methods using dilute acid to obtain monosaccharides. Therefore, this study aims to add value to agro-industrial waste and contribute to the environmental preservation.

2 MATERIAL & METHODS

The chemical characterization of *E. precatoria* and *E. edulis* seeds was performed by a standard method for the determination of carbohydrates content[1]. To understand physico-chemical properties of the seeds that could influence in their recalcitrance, different techniques were used, such as: i) Rigaku Miniflex X-ray diffraction, with Cu Kα radiation (λ = 0.15418 nm), nickel filter (0.02 mm), 40 kVA voltage, and 40 mA current, scanning in the range of 5 to 50°; ii) thermogravimetry, using the SDT Q600 equipment, where 10 mg of the material was weighed, varying the heating rate at 10 °C/min up to 600 °C in an inert nitrogen atmosphere; iii) rupture test, with parameters of 10 kN with a displacement rate of 5 mm per minute in the Instron® 3382 Floor Model equipment iv) enzymatic hydrolysis of *in natura* milled seeds using BGM 'Amano' 10 with an enzymatic load of 400 UI/g of biomass. The dilute acid hydrolysis of the seeds was conducted under various conditions considering the following varibles: i) sulfuric acid concentration, (ii) temperatures, and (iii) biomass solid loading. These different conditions were optimized in an experimental design of CCRD with 2 levels and 3 variables, as shown in Table 1. This resulted in a total of fourteen different reaction combinations (each performed in duplicate), plus a center point (performed in triplicate), for each seed.

Table 1 Levels of factorial points, axial and central, both actual and encoded, of the parameters used in the optimization of dilute sulfuric acid hydrolysis of Amazonian açai (*E. precatoria*) and juçara (*E. edulis*) seeds in the central composite rotational design (CCRD).

Parameters	Açaí-do-amazonas					
	Coded	-1,68	-1	0	+1	+1,68
Acid concentration (%)	X1	0.25%	1.01%	2.13%	3.24%	4.00%
Temperature (°C)	X2	130.0	140.1	155.0	169.9	180.0
Solid load (%)	X3	5.00%	9.05%	15.00%	20.95%	25.00%

Parameters	Juçara					
	Coded	-1,68	-1	0	+1	+1,68
Acid concentration (%)	X1	0.50%	1.01%	1.75%	2.49%	3.00%
Temperature (°C)	X2	121.0	131.1	146.0	160.9	171.0
Solid load (%)	X3	5.00%	10.07%	17.50%	24.93%	30.00%

3 RESULTS & DISCUSSION

The chemical characterization of both seeds revealed that their carbohydrate content corresponded to over 70% of total mass, with their main polysaccharide corresponding to the mannan, and containing lower amounts of glucan, xylan and galactan. This high carbohydrate content is consistent with our previous results of carbohydrate mapping directly on the surface of slices of both seeds using the MALDI-imaging technique[3]. More specifically, Figure 1a presents the mannan content in the seeds, revealing that mannan represents 73% and 62% of all content of açaí-do-amazonas and juçara seeds, respectively. Corroborating with the chemical analysis, results from the thermogravimetric assays of both seeds showed that the highest mass loss during their thermal decomposition occurred around 272°C, which corresponds to the average temperature for mannan degradation[4], confirming that this polysaccharide is the principal component of these biomasses.

The structure of those seeds was analyzed by X-ray diffraction. When we compared the seeds with the standard crystalline mannan structure from ivory nut, the XRD data from both seeds exhibited crystalline peaks that matched the standard, indicating that their structure corresponds to linear mannan type 1[5]. Additionally, the seeds' recalcitrance was evaluated based on their hardness, measured by rupture assays. The force necessary to rupture the açaí-do-amazonas and juçara seeds was measured, with their rupture points of 1190 N, and 987 N, respectively, indicating high resistance. Thus, these results suggest that the açaí-do-amazonas seed structure is slightly more organized and compact. In fact, the enzymatic hydrolysis using a commercial mannanase of açaí-do-amazonas resulted in lower mannan conversion than the hydrolysis of juçara seeds, corresponding to 7.02% and 14.66% of mannan to mannose, respectively, within 72 hours. Thus, these results also support the hypothesis that mannan in açaí-do-amazonas is more recalcitrant than in juçara, possibly requiring more severe parameters for its deconstruction, which was further evaluated during the dilute acid hydrolysis processing.

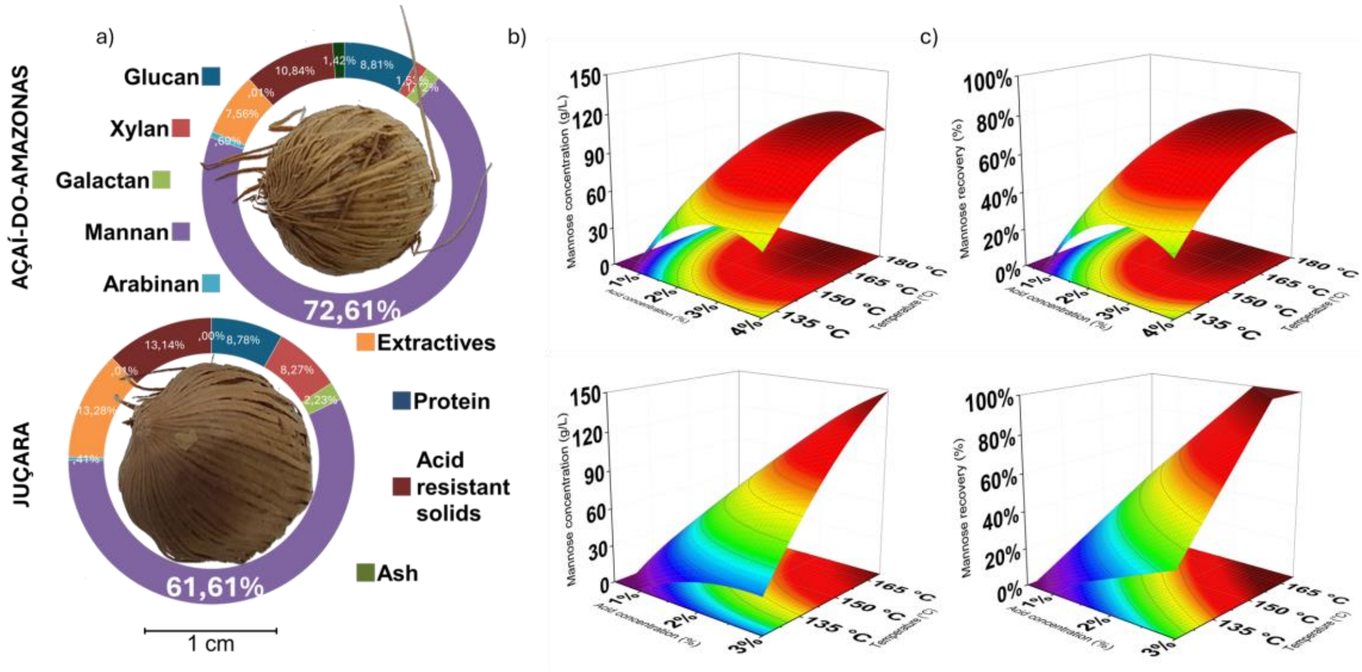

Figure 1a) corresponds to the chemical composition of açaí-do-amazonas (line above) and juçara (line below) seeds; Figure 1b) corresponds to the response surface of mannose concentration (g/L); Figure 1c) corresponds to the response surface of mannose recovery (%).

Therefore, more severe conditions for the açaí-do-amazonas seed were necessary due to its higher recalcitrance compared to that of the juçara seed. To achieve higher mannose recovery and concentration, an optimized method was evaluated studying acid concentration, temperature, and biomass solid loading as parameters. The model allowed for generating a response surface, as represented in Figures 1b and 1c, for açaí-do-amazonas and juçara seeds. Subsequently, a region for model validation was selected, in order to evaluated conditions that could promote high mannan conversion (>70%), high mannose concentration (>100 g/L), while maintaining acid concentration as low as possible. The results, including the experimental and predicted values are shown in Table 2.

Table 2 The predicted values and the experimental values of the concentrations and mannose recovery obtained from dilute acid hydrolysis of the seeds of açaí-do-amazonas and juçara. The same letters represent statistically equal values (p-valor < 0,05).

Biomass seed (Acid concentration; temperature; solid load)	Responses	Predicted values	Experimental values	Relative difference
Açaí-do-Amazonas (2.04%; 169.1 °C; 15.43%)	Mass loss (%)	85.6[a]	88.2 ± 4.3[a]	2.9%
	Mannose concentration (g/L)	103.63[b]	104.2 ± 5.6[b]	0.6%
	Mannose recovery (%)	70.37[c]	70.8 ± 3.8[c]	0.6%
Juçara (2.02%; 171.0 °C; 19.13%)	Mass loss (%)	86.9[d]	82.5 ± 3.4[d]	-5.4%
	Mannose concentration (g/L)	122.11[e]	121.9 ± 2.9[e]	-0.1%
	Mannose recovery (%)	85.17[f]	85.1 ± 2.1[f]	-0.1%

Predicted values were found to be in good agreement with experimental values, with R^2 values higher than 0.9 in all cases. The highest R^2 value was 0.98, corresponding to mannose concentration in juçara. This study has shown that the CCRD can be efficiently applied to model the responses of mass loss, mannose concentration, and mannose recovery, obtaining predicted values statistically equal to the experimental results. These conditions were evaluated with the goal of achieving high concentrations and recoveries of mannose while using the lowest possible concentrations of sulfuric acid to minimize environmental impact. The experimentally evaluated and validated conditions correspond to concentrations greater than 100 g/L, with the conversion of mannan to mannose being greater than 70%, promoting high expectations for economically viable processing of the seeds. Thus, experimental values were very close to the predicted ones, indicating that the mathematical model generated was validated for processing these seeds and is adequate to represent the experimental results.

4 CONCLUSION

The seeds of E. precatoria (açaí-do-amazonas) and E. edulis (juçara) palm trees exhibit a high polysaccharide content, with mannan being the predominant component, comprising over 60% of their structures. The low release of mannose during enzymatic hydrolysis can be attributed to the crystallinity of linear mannan type 1 in the biomass and its mechanical resistance, which indicates organized and compacted polysaccharides of mannan. This recalcitrance underscores the need for more rigorous processing to achieve higher yields of monosaccharides. The mathematical model developed for processing has proven effective in its predictions, enabling the optimization of parameters for the complete hydrolysis of polysaccharides into monosaccharides. Thus, the seeds of these palm species have been demonstrated to be an excellent source of mannan. These findings aim to enhance the understanding of these seeds and potentially stimulate the valorization of these underexplored agro-industrial residues in Brazil.

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