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# UNDERSTANDING THE STRUCTURE OF MANNAN FROM AÇAÍ SEEDS FOR THE EFFICIENT BIOCONVERSION TO MANNOSE

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## ABSTRACT

Açaí is a typical fruit from the Amazon Forest, whose seed are 85% of the fruit's weight. Almost half of this agroindustrial waste weight is mannan, and 90% of the glycosil residues composition is mannose. However, the mannan from the seeds is water-insoluble and recalcitrant to sustainable deconstruction by enzymatic action. Therefore, evaluate the structure of this polysaccharide can help to understand the limitations of its bioconversion. In this study, the açaí seeds were fractionated to isolate the mannan. After, the NMR analysis of the isolated fraction resulted in δ which were in agreement with signals of 1,4-linked β-D-Man*p*. with β(1→4) glycosidic linkage between Man*p* units. The identified linear mannan is known to have crystal structures, polymorphs I and II, which were identified in the sample. Afterwards, the linear mannan structure was elucidated as a main chain β(1→4) linked Manp, with ramifications of α-1,6-D-Gal*p*. The first *in situ* analysis of the acai seed confirmed the presence of a linear mannan structure in the cell wall. Thus, the results obtained will allow the establishment of strategies to reduce the crystallinity of the mannan, as well as the selection of enzymes that are more suitable for the hydrolysis of this recalcitrant biomass.

**Keywords:** *Euterpe oleracea* Mart. Structural elucidation. Linear mannan. Crystalline. Recalcitrance.

## 1 INTRODUCTION

Açaí is a typical fruit from the Amazon Forest that has beneficial effects on health due to the variety of nutritional components and antioxidant properties. Considered a "superfruit", the popularity of açaí and, consequently, its production has been growing in the last years, driven by its versatility of integration into different market niches. However, its seeds, which represent 85% of the total weight of the fruit, are left over as waste from the edible pulp production process. Nowadays, more than1,600,000 tons of seeds are generated annually in Brazil, which, without proper disposal, accumulate in the producing area, causing environmental impacts. This agroindustrial waste has high mannan content, reaching up to 50% of its total dry weight[1]. Mannans are polysaccharides composed of mannose residues, a sugar with market value and potential as a functional ingredient and biological functions of great interest in the industry. Mannose is not much exploited, probably due to the scarcity of abundant natural sources, what makes açaí seeds a valuable material. For suitable industrial reuse of the seeds, the sustainable deconstruction of mannan from the endosperm of açaí seeds to produce mannose is an alternative. However, the mannan from the seeds is water-insoluble and recalcitrant to depolymerization by enzymatic action. Therefore, understanding the mannan structure could solve the challenges hindering the industrial conversion of açaí seeds into mannose, to identify the limitations of its processing. The valorization of açaí seeds can increase in income and the generation of jobs, resulting in local economic growth in the Amazon region and social development. Thus, this proposal is in line with the three pillars of sustainability for the development of a circular economy, being able to add value to the açaí production chain in Brazil. For this, this study aimed to investigate the structure of the mannan from açaí seed (*Euterpe oleracea* Mart.), helping to understand the limitations of the bioconversion of seeds into mannose.

## 2 MATERIAL & METHODS

The external fibrous mesocarpic layer was removed from the açaí seed, and the internal fraction was grounded and sieved between 20 and 80 mesh. The seed components were fractionated by successive extractions of an alcohol insoluble residue (AIR). For this, the ground seeds were suspended in ethanol and resuspended in chloroform/methanol. The resulting AIR was submitted to sequential steps to remove the specific components from plant cell walls. All the following extractions had the supernatant collected and the pellet washed with water to be collected for the next step. The resulting AIR was subjected to a starch removal step using amylases and amyloglucosidases. In a sequential step, the pectin was removed using ammonium oxalate. The next two extraction steps used KOH and NaBH4 to remove hemicelluloses (xylan and xyloglucan enriched). Then, the solid fraction was treated with sodium chlorite to remove lignin. Finally, the pellet was resuspended in KOH. The isolated water-insoluble fraction of mannan, obtained from the final residue was analyzed by 2D nuclear magnetic resonance (NMR) analysis (HSQC and HMBC), at 900 MHz and also at 500MHz with cryoprobe, with δ expressed in ppm with relation to DSS (sodium 2-(trimethylsilyl)-1-propane sulfonate) after solubilization in 50% w/w urea in $D_2O$. The isolated fraction was also analyzed by solid state NMR using $^{13}C$ CPMAS (Cross Polarization/Magic Angle Spinning), operating at 100.63 MHz ($U_{Larmor}$ $^{13}C$), for a total

assessment of the mannan, without losses. The crystallinity of the isolated fraction was analyzed by X-ray diffraction (XRD) at scan angles in the range of 10°≤2$\theta$≥40° with acquisition times of 0.2 s. The glycosyl residue composition of the isolated fraction of mannan was confirmed applying the alditol acetate method of sugar derivatization to be analyzed by gas chromatography coupled with a mass spectrometry detection (GC-MS). To confirm the components in the isolated fraction, the sample was submitted to a thermogravimetry analysis, varying the heating rate at 10 °C/min up to 600 °C in an inert nitrogen atmosphere. To complement the structural characterization of the mannan from the açaí seeds, the primary monoclonal antibody (mAb) BS-400-4 and the secondary mAb was used to identify the specific epitopes of linear mannan *in situ* in açai seed.

## 3 RESULTS & DISCUSSION

The mannan-type hemicellulose is a heterogeneous group with different structures and compositions that result in different biological functions. The compositional analysis of the açaí seed carried out in previous studies of our research group indicates that the endosperm of the mature seed would be rich in a linear type of mannan, with high mannose contents[1]. However, there is no studies of structural analysis of this mannan to confirm this hypothesis. Then, for a complete characterization of plant cell wall polysaccharides, it was necessary the use of different complementary analytical methods to have a definitive answer.

The açaí seeds were fractionated to obtain a water-insoluble fraction, which could be correlated to the mannan content. After isolating the açaí seed mannan, it was analyzed by NMR and the attribution of the signs was made by comparison with data from the literature. NMR analysis of this sample resulted in δ from $^{1}H/^{13}C$ HSQC spectrum which is in agreement with signals of 1,4-linked β-D-Man*p*, similar to the δ of mannan from other palm seeds, known to contain linear mannan, and also similar to unbranched β-D-Man*p* residues of the backbone of low-substituted seed galactomannans[2,3,4,5]. The $^{13}C$ spectra had six carbon signals assigned to a β-(1→4) D-mannan, as the $^{1}H/^{13}C$ HMBC confirms the presence of β(1→4) glycosidic linkage between Man*p* units by the cross peaks at 4.73/78.0 ppm (H-1/C-4) and 3.88/101.8 ppm (H-4/C-1).

Subsequent, given the difficulty in solubilizing the isolated fraction, it was decided to also analyze the sample by $^{13}C$ CPMAS ssNMR, for a total assessment of mannan. The identified linear mannan have crystal structures, polymorphs I and II, which were identified in the sample, since the two signals corresponding to carbon 5 of both forms were detected at 70.2 and 70.9 ppm, respectively, with the polymorph I in greater concentration[6].

As linear mannan is known to be a crystalline polysaccharide, the isolated fraction was evaluated by XRD. The evaluation of isolated sample had intensity peaks at angle 2θ equal to 16°, 18°, 20°, 24°, and 26°, which correspond to the crystalline planes 110, 111, 200, 210, and 211, respectively7. This profile is similar to a crystalline form of linear mannan, mannan I, also contained in ivory nuts (Figure 1a). This hydrophobic crystalline form has strong intermolecular interaction, which results in a highly compact structure. Hence, the crystallinity of the material may be one of the structural factors that justify the recalcitrance to the action of enzymes in the açaí seeds. Although a peak, corresponding to cellulose Iβ, was observed at 22.5° (200).

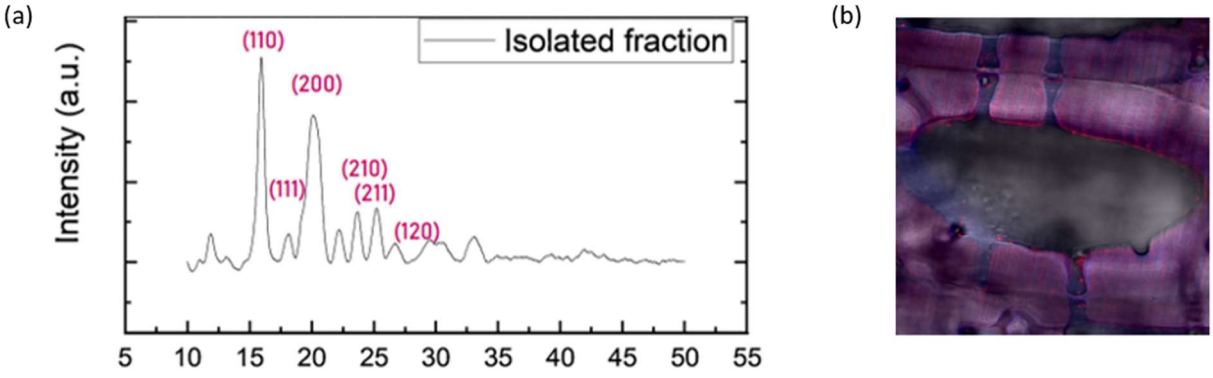


**Figure 1** (a) XRD profile of the isolated insoluble fraction; (b) Immunolocalization of linear mannan (red) filling the cell wall of the açaí seed endosperm.

Therefore, the compositional characterization of the isolated sample was confirmed by GC-MS, however, the molar ratio obtained for Man:Glc:Gal was 78:19:1, respectively, suggesting a possible contamination of the mannan with cellulose traces, undetected by NMR due to solvent selected to sample preparation. This hypothesis could be reinforced by the thermal degradation behavior of the isolated fraction. As a low galactomannan content was identified by GC-MS, adjusting the NMR acquisition parameters, it was possible to note the branch of galactose in the main chain of mannan, due to the presence of the residues Gal-(1→ and →4,6)-Man-(1 →. On the other hand, to overcome contamination issues, the mannan in açaí seeds was also analyzed directly in the plant cell wall, in situ, through immunolocalization (Figure 1b). The mAbs were used to obtain information about the *in situ* structure of mannan, for the evaluation of mature. Immunolocalization identified the concentration of linear mannan in the mature

endosperm cell wall. Linear mannan was detected filling the cell wall. Nevertheless, this unprecedented identification of the linear mannan structure from the açaí seed will allow the establishment of strategies to reduce the crystallinity of the mannan.

## 4 CONCLUSION

This work has shown the identification of the linear mannan structure from the açaí seed for the first time. The açaí seed mannan structure was elucidated as a main chain β(1→4) linked Manp, with ramifications of α-1,6-D-Gal*p* in a molar ratio of 50:1 man:gal, characterizing it as a linear mannan. The results highlight the need for strategies to reduce the crystallinity of the mannan, and/or the screening of mannanases that are more suitable for the hydrolysis of this crystalline mannan.

## REFERENCES


1 MONTEIRO, A. F., MIGUEZ, I. S. et al. 2019. Scientific Reports, 9(1), 1–12.
2 ALBUQUERQUE et al. 2014. Carb. Polym. 104, 127-134.
3 FENG, L. et al. 2018. Food Hydrocolloids. 76, 67–77.
4 GORIN, 1973, Can. Journal Chem. 51, 2375-2383
5 JARVIS, 1990, Carb. Res.. 197, 276-280
6 MARCHESSAULT et al., 1990, Can.J.Chem. 68, 1192-95
7 HORI et al., 2007, Carbohydrate Polymers, 70.


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