



Physicochemical characterization of açai seeds (*Euterpe oleracea*) from Colombian pacific and their potential of mannan-oligosaccharides and sugar production via enzymatic hydrolysis

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Abstract

The study aimed to characterize açai seeds and explore their potential for producing mannooligosaccharides (MOS) through enzymatic hydrolysis. According to characterization tests by XRD, FTIR, and chemical analysis, açai seeds, a waste material from açai fruit processing, contain significant hemicellulose content, with a main content based on mannan. The study utilized Rohalase®GMP enzyme for hydrolysis and monitored reducing sugars and MOS production (8–10 g MOS/L) hydrolysate with varying lengths, including di-, tri-, tetra-, and pentasaccharide over time. Results showed a concentration plateau of reducing sugars at 13 h and increased MOS until hour nineteen. Mannobiose (M2) was the predominant MOS produced. Comparative analysis with prior research indicated that the açai seed hydrolysate's MOS content aligns with that from other sources. Importantly, the study achieved % hydrolysis and MOS yield of $10.79 \pm 0.05\%$ and 51.39 ± 0.11 g M2-M5/g mannan, respectively. Açai seed is a promising source to produce enzymes, biofuels, or thermal energy, as well as highly valued chemical compounds in the industry. This work additionally demonstrates its potential as a sustainable source of valuable MOS, suggesting applications as prebiotics and functional food additives, with implications for various industries seeking eco-friendly alternatives.

Keywords Açai seeds · Enzymatic hydrolysis · Mannan-oligosaccharides · *Euterpe Oleracea* · Agro-waste valorization

Abbreviations

MOS	Mannan-oligosaccharides
M2	Mannobiose
M3	Mannotriose
M4	Mannotetraose
M5	Mannopentaose

1 Introduction

The açai fruit (*Euterpe Oleracea* Mart.) stands as a well-recognized superfood, renowned for its multifaceted health benefits, including high levels of antioxidants, anti-inflammatory agents, analgesics, antimicrobial compounds, and even anti-cancer properties [1]. Widely consumed in the form of frozen pulp, this fruit has also inspired the creation of diverse products such as atomized or freeze-dried powders, ice creams, and chocolate additives. Furthermore, the cosmetic industry has shown keen interest in harnessing açai, incorporating it into items such as soaps, creams, and perfumes, thus exploring its potential beyond the culinary domain [2].

While Brazil remains the principal producer of açai, Colombia has witnessed a surge in its production due to its wild growth within the Amazon rainforest and the biogeographical Chocó region. This growth has provided economic opportunities in areas that would otherwise be more vulnerable [3, 4].

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Despite these benefits, açai pulp constitutes merely 15% of the fruit's weight, with the seeds representing the majority of the volume, it is estimated that acai pulp production generates over 1 million tons of waste. Improper disposal of açai seeds as waste in open landfills has led to environmental damage and public health concerns [5]. The acai seed is described as a lignocellulosic material composed of a fibrous layer and an endosperm that covers the embryo, whose polysaccharide-rich content serves as a promising renewable source for the production of enzymes, biofuels, or thermal energy, as well as highly valued chemical compounds in the industry [6].

The Acai seed has been used as a renewable energy source and to create various products in support of a circular economy. This approach promotes environmental conservation. A study found that acai seeds can be used to produce biofuels such as biogas and ethanol [7]. Other investigation showed that utilizing acai seed-based biogas production could provide over 40% of the energy needs of an acai processing facility, resulting in a significant reduction of its carbon footprint [8]. The seeds have been utilized for manufacturing activated carbon, which has facilitated soil enhancement [9], and for producing biocomposite materials like synthetic wood [10]. Additionally, their ashes have been tested as a partial substitute for Portland cement in construction mortars [11]. The production of mannose, polyphenols, and electricity through one-step oxalic acid hydrolysis in biorefinery setups was found to be the most optimal option in regards to economic and environmental benefits [12].

The research focused on Brazilian açai seeds has identified hemicellulose, primarily composed of linear mannan chains (> 50%), as a prominent component [13], rendering these seeds a valuable source for mannan-based products such as mannan oligosaccharides (MOS) or mannose-derived sugars and prebiotics. Common sources of mannan or its derivatives, such as mannan oligosaccharides, are agricultural, agroforestry, or agri-food residues like palm kernel cake (PKC) or softwoods. However, these sources often require robust purification processes due to their lower mannan content (< 35%) [12].

Mannooligosaccharides (MOS) derived from various plant sources offer a spectrum of health benefits with applications spanning pharmaceutical and animal industries. The balance of intestinal microorganisms profoundly affects host health, and MOS contributes to this balance by enhancing beneficial microbes and increasing short-chain fatty acid production [14]. The reduction of pathogenic bacteria through pH modulation and membrane alterations underscores the potential of MOS to enhance overall well-being. The emerging role of plant-derived MOS as prebiotic agents holds significant promise for gut health.

The production of mannan oligosaccharides involves the partial hydrolysis of raw materials, achieved through

acid/base additions, enzymatic systems, or environmentally friendly processes like steam explosion, with the latter two being more environmentally sustainable. The characterization of waste is important to identify potential processes and applications for them as raw materials. Furthermore, despite extensive characterization efforts directed at Amazonian açai seeds, there is a paucity of reported information regarding the structure and composition of seeds from regions distinct from the Amazon, such as the biogeographical Chocó rainforest. Enhanced understanding of this primary resource and the proposition of novel valorization alternatives offer a potential avenue for enhancing the well-being of communities, as exemplified by small-scale biorefineries centered on the adequate utilization of tropical forest fruits [15]. Thus, it becomes pivotal to explore potential relationships and differences in the seed composition of açai from already characterized regions.

In this sense, this study aims to characterize the physico-chemical and structural properties of açai seeds and present preliminary outcomes on the production of mannan oligosaccharide-rich substrates through enzymatic hydrolysis of açai seeds sourced from the Colombian Pacific region.

2 Methodology

2.1 Raw material

The seeds were obtained from Refrescos del Litoral Ltda., a company located in Quibdó, Chocó-Colombia. They arrived in resealable bags and were washed several times with distilled water to eliminate any remaining fruit particles and external fiber layer. Following this, the seeds were kept in sub-zero temperatures (below 0 °C) in the dark for no more than 2 weeks before undergoing analysis and enzymatic treatment.

2.2 Chemical and structural characterization

2.2.1 Compositional analysis

The seeds were bisected and subjected to a drying process at a temperature of 70 °C for a duration of 24 h. Subsequently, the dried seeds were ground using a 0.5-mm mesh knife mill (Model TE-650/1 Tecnal, SP, Brazil) to obtain particles with sizes below 0.4 mm, ensuring uniformity in particle size distribution. The resulting dry powder samples were stored in resealable bags under dry and light-free conditions.

The moisture content of the samples was determined gravimetrically through vacuum drying, in accordance with the AOAC 926.12 standard and their extractive content was determined using water–ethanol solvents, following the NREL/TP-510–42619 method [16]. The

raw material devoid of extractives was used to quantify holocellulose and cellulose, in accordance with ASTM standard D-1104 and NREL/TP-510–42620 [17], respectively. Hemicellulose content was indirectly measured as the difference between holocellulose and cellulose. The quantification of insoluble lignin followed the NREL/TP-510–42618 standard [18]. Protein content was determined using the Kjeldahl method [19]. Ash content was obtained in accordance with the NREL/TP-510–42622 standard [18], while fat content was determined using n-hexane via reflux percolation in a Soxhlet apparatus [20].

2.2.2 X-ray diffraction analysis

Crystalline nature of dehydrated and milled açai seeds was analyzed through X-ray diffraction (XRD). The X-ray diffraction spectra were acquired using a Rigaku Miniflex II diffractometer with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) operating at 15 mA. The measurements were taken within a 2θ range of 5° to 50° , employing a scan speed of $1^\circ/\text{min}$ and a step size of 0.2° .

The crystallinity index (CI) was calculated using two distinct methods [6]. The first method, as described in Eq. 1, calculates the CI as the difference between the maximum intensity of the crystalline peak (I_c) and the minimum intensity of the amorphous peak (I_{am}), normalized by the maximum intensity of the crystalline peak:

$$CI = (I_c - I_{am}) \times 100 / I_c \quad (1)$$

The second method, as described in Eq. 2, calculates the CI as the ratio of the crystalline area of peaks to the total peak area:

$$CI = \text{crystalline area} \times 100 / (\text{crystalline area} + \text{amorphous area}) \quad (2)$$

In this calculation, the OriginPro 8.1 software was utilized to determine the crystalline index, relying on the proportion of the crystalline area of the peaks to the entire peak area.

2.2.3 FTIR spectroscopy

The dehydrated and milled açai seeds spectrum was measured using a NICOLET i5 FTIR spectrometer with a DTGS detector, in a KBr pellet (5:95 ratio sample:KBr) at 20°C and 62% humidity, scanning from 450 to 4000 cm^{-1} with a spectral resolution of 4 cm^{-1} . The FTIR spectra were analyzed by examining the positions of the spectral

bands to identify the main functional groups. This identification was based on comparisons with literature sources.

2.3 Enzymatic hydrolysis

The previously stored samples were ground and passed through an 80-mesh sieve ($180 \mu\text{m}$) to achieve a homogeneous suspension in the enzymatic solutions. The powdered seed underwent an enzymatic hydrolysis test using Rohalase®GMP enzyme (1303 U/g enzyme for locust bean substrate), which primarily contains mannanase at 50°C with an enzyme loading of 17.5 U/g substrate (dry seed) for a period of 1 to 24 h. The hydrolysis test was repeated three times. The operating conditions for enzymatic hydrolysis were determined by aligning the enzyme technical data sheet recommendations with the findings from preliminary experiments conducted in this study.

The responses were analyzed for reducing sugars (RS) using the 3,5-dinitrosalicylic acid (DNS) method and mannan-oligosaccharides (MOS) content using high-performance liquid chromatography. Aliquots (2 mL) were extracted every 4 h after the initial hour of the reaction and then centrifuged at 10°C for 5 min at 13500 rpm , and the analysis was performed on the supernatant.

2.3.1 Quantification of reducing sugars

The analysis of reducing sugars (RS) was conducted using the DNS method as reported by Wood et al. (2012) [21], with some modifications. Briefly, $40 \mu\text{L}$ of the sample was mixed with $1960 \mu\text{L}$ of the DNS reagent and then heated at 100°C for 5 min. Subsequently, the mixture was cooled in water at 20°C for 5 min. Finally, 1 mL of distilled water was added, and the solution was transferred to a cuvette and left to stand for 3 min before measuring the absorbance at 540 nm . The absorbance values were compared with a calibration curve ranging from 5 to 30 gr/L , using D-mannose as the standard.

2.3.2 Quantification of MOS content

To determine the mannoooligosaccharide content in the enzymatic hydrolysates, high-performance liquid chromatography (HPLC) analysis was performed using a Shimadzu Lachrom Elite L-2490/EZChrom Elite system equipped with a refractive index detector. A Eurokat Ca column ($10 \mu\text{m}$, $300 \times 4 \text{ mm}$) with water as the eluent (0.15 mL/min) was used for separation. The column and detector temperatures were set at 65°C and 35°C , respectively.

Each mannoooligosaccharide was calibrated using mannobiose (M2), mannatriose (M3), mannotetraose (M4), and mannopentaose (M5) standards (Megazyme, Ireland). The

mannooligosaccharide results are presented as the summation of M2 to M5.

2.3.3 Hydrolysis yield

The calculation of the yield for all MOS (M2–M5), and the hydrolysis ratio were performed with based by Li et al. (2018) [22] with modifications employing the following equations:

$$\text{MOS yield} = \text{total mass of M2} - \text{M5} / 100\text{g dry basis seed} \quad (3)$$

$$\% \text{Hydrolysis ratio} = \text{total mass of M2} - \text{M5} \times 100\% / \text{total mannan}^* \quad (4)$$

The determination of the mannan content* for this calculation was established based on an averaged value derived from literature sources [13, 23], which was then adjusted by the determined hemicellulose content in the açai seeds in this study. Thus, the mannan content is $0.1894 \times \text{dry basis seed gr.}$

2.4 Statistical analysis

All statistical analyses were carried out by computer software (SPSS version 16.0, Chicago, IL, USA) using one-way analysis of variance (ANOVA). The differences between mean values were considered significant at $p < 0.05$. The graphs were performed using Microsoft Excel 2020.3. Data are reported as mean $\pm$ SD of three independent experiments (each experiment was conducted in triplicate).

3 Results and discussion

3.1 Chemical and structural characterization

Acquiring information on the seed's chemical composition is essential in developing the most suitable processing methods. Data published in the literature about the composition of açai seeds indicates significant variation in the proportions of their components. Table 1 compares the results of characterizing açai seeds in this study with those reported by other authors. Before analysis, the seeds in each of the three cases were dehydrated using hot air and subsequently ground, following a similar method as utilized in this study. The açai fruits in Table 1 were harvested from various regions of the Brazilian Amazon during both winter and summer seasons, which accounts for the significant variations in reported contents. Nevertheless, the Brazilian açai seeds are predominately composed of lignocellulosic biomass, primarily cellulose (34–53%), hemicellulose (6–18%), lignin (3–25%), and protein (4–7%) [6]. The seeds studied in this research have cellulose content lower than the reported

Table 1 Composition of açai seed powder

Component	This work*	Buratto et al. (2021) [24]	Barros et al., 2021 [25]
Cellulose	11.58 ± 1.66	8.5	45.49
Hemicellulose	37.88 ± 2.19	48.1	21.08
Lignin	14.12 ± 3.69	16.4	24.36
Extractives (water)	4.09 ± 1.00	13.1	4.48
Extractives (ethanol)	11.76 ± 3.46	0.9	
Fats	2.64 ± 0.85	3.5	-
Moisture	7.55 ± 0.05	-	7.17
Protein	3.67 ± 0.05	9.3	-
Ash	1.57 ± 0.06	0.96	1.01

* $n = 3 \pm$ standard deviation

ranges and higher hemicellulose content, consistent with findings from Buratto et al. (2021) [24].

Sugars and oligosaccharides found in hemicellulose can be released from plant cell walls through various methods, including acid, thermal, enzymatic hydrolysis, or microbial fermentation. Dilute inorganic acids that can hydrolyze hemicelluloses containing xylan are not easily hydrolyzed when they contain mannan. Releasing mannan oligosaccharides and free mannose from mannan-rich residues could be done by mannan-degrading enzymes. This process allows for milder conditions that are less damaging to the environment [13].

The hemicellulose content discovered in this research contradicts prior studies on Açai seed composition that have identified cellulose as the primary polysaccharide [26, 27]. Nevertheless, the high concentration of mannan, as indicated in several studies, is consistent with the hemicellulose content found in our research. De Lima et al. (2021) reported a significant amount of carbohydrates in the cellular wall of the açai seed endosperm, showing a total carbohydrate content of 76%, with mannose identified as the primary constituent, and a lignin content of 11%. Monteiro et al. (2019) reported similar findings with $52.46 \pm 1.51\%$ mannan content [13].

The high levels of water and ethanol extractives in açai seeds are consistent with their high concentration of polymeric procyanidins. Both the aqueous and ethanolic extracts of açai seeds are rich in polymeric procyanidins, which have strong antioxidant properties [28].

Compared to the papers reviewed in Table 1, the material analyzed has a lower lignin content. Typically, when processing lignocellulosic biomass for biofuel production, it is convenient to pre-treat the raw material to separate the structural bonds between lignin and carbohydrates, including cellulose and hemicellulose. This facilitates access to the conversion of these materials for optimal total reducing

sugar production, thereby improving the efficiency of biofuel production. The objective of this research is to hydrolyze the mannan of the seed, reducing the production of monomeric sugars and increasing the production of mono-oligosaccharides to achieve prebiotic properties of the hydrolysate. In addition, the ash content reported in Table 1 could inhibit β -mannanase, which is responsible for converting seed mannan to simple sugars and oligosaccharides. To prevent this in lignocellulosic materials, a pretreatment consisting of washing combined with steam explosion is usually used to remove a good portion of the lignin and soluble ash cations. Since acai seeds have a lower lignin and ash content than other lignocellulosic sources, it is worth investigating whether the lignin and ash concentrations are sufficiently low to eliminate the need for pretreatment, which would result in economic and environmental benefits in seed processing.

3.2 X-ray diffraction analysis

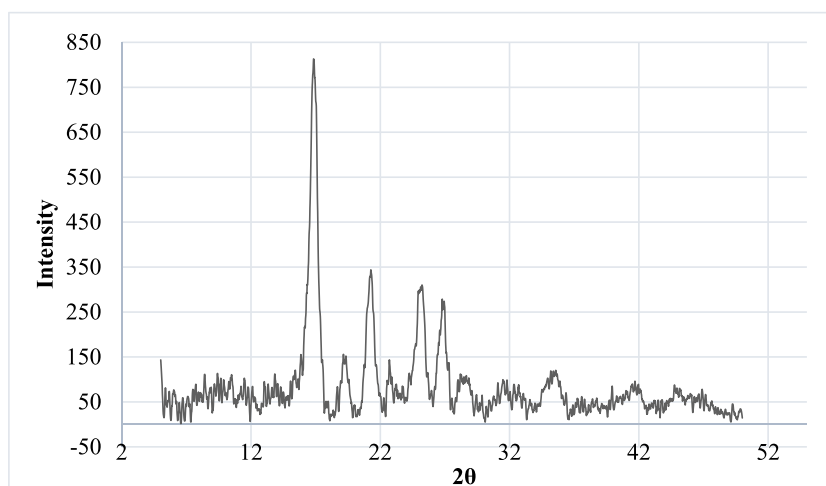
XRD analyses were conducted to confirm the presence of cellulose and mannans in the ground açai seed samples. The natural crystalline forms of cellulose comprise cellulose Ia and Ib, collectively known as cellulose I [29]. The diffraction pattern of cellulose I is commonly represented by two distinct 2θ peaks at approximately 16.0° and 22.0° , respectively [30]. The latter peak, attributed to the 200-cellulose crystal plane, tends to be more intense than the former- which is due to the 110 cellulose crystal plane.

The ground and dried samples of açai seeds display a first peak at a 2θ angle of 16.8° (Fig. 1), which is consistent with the presence of cellulose in the material [31]. A low-intensity second peak (19.4°) is associated with hexagonally packed structures [32] and a moderate-intensity peak of the 200 cellulose crystal plane (2θ : 21.4°) is observed. Finally, the peaks at 2θ : 25° and 26.8° are characteristic of type I

mannan, which comprises linear chains of β ,1–4-linked mannose units [33]. This confirms that cellulose is not the primary polysaccharide found in acai seeds and is consistent with the chemical characterization, which indicates that hemicelluloses have a higher concentration than cellulose. De Lima et al. (2021) [6] reported the absence of detectable cellulose peaks in the acai seed. Their diffractograms revealed the presence of mannan in the material, which was also noted in similar findings reported by Monteiro et al. (2019) [13].

In this study, the crystallinity of acai seed material was estimated to be 77.89% using Eq. 1 and 60.79% using Eq. 2. These levels of crystallinity are comparable to those reported by the authors referred in above paragraph. The crystallinity index (CI) is a quantitative measure that describes the amount of crystalline material present in a biopolymer. It has been applied to various biopolymers, including cellulose, chitosan, and glucomannan [34, 35]. In enzymatic hydrolysis, the digestibility of the material by the enzyme is influenced by its degree of crystallinity (CI). The CI is related to the accessibility of the enzyme to the microfibrils of the biopolymer in the cell walls of the lignocellulosic material. As such, CI is a factor that would can affect the rate of hydrolysis in the reaction [36]. According to reports, the significant level of crystallinity obstructs the enzyme that generates mannose from reaching the polymer, thereby restricting the production of monosaccharides in the product [37]. Apparently, the enzyme's access restrictions to areas of high substrate crystallinity primarily affect the β -mannosidase in the enzyme cocktail, responsible for mannose production. Based on the hydrolysis tests conducted in this study, the β -mannanase present in the enzymatic cocktail appears to be capable of partially hydrolyzing the substrate despite its high degree of crystallinity. The results suggest that the β -mannanase does not face any significant challenges in this regard.

Fig. 1 X-ray diffraction pattern of açai seeds



3.3 FTIR analysis

The spectra carried out using FTIR spectroscopy allows for the study of the chemical structure of açai seeds by identifying the functional groups of the analyzed samples. The results are plotted in Fig. 2 that presents the characteristic signals related to lignocellulosic materials. The band at 3380 cm^{-1} corresponds to the O–H stretching vibrations of cellulose and hemicellulose in the material, with the C–H stretching vibrations (2920 cm^{-1}) closely linked to it [38]. The band at 1635 cm^{-1} represents the characteristic vibration of flexing hydroxyl groups of water absorbed in the material. On the other hand, functional groups associated with lignin are also observed.

The band at 1465 cm^{-1} corresponds to C–H bending vibrations, while the band at 1350 cm^{-1} corresponds to deformation and stretching of methyl (CH₃) groups in aromatic rings. Additionally, the C–O stretching at 1261 cm^{-1} (guaiacyl ring) of lignin and the deformation planes of aromatic rings' C–H (1102 cm^{-1}) can be observed [39]. Furthermore, the vibration at 1036 cm^{-1} corresponds to the C–O bending related to cellulose presence, and vibrations at $815\text{--}872\text{ cm}^{-1}$ correspond to the glucosidic bond vibrations in the material, with the latter specifically related to β -D-mannopyranose. Furthermore, the intensities of the peaks were compared to FTIR results for mannan in the literature, which typically manifest at 3320 cm^{-1} (OH vibrations), 2934 cm^{-1} (CH₂ vibrations), 1635 cm^{-1} (CO vibrations), and 1022 cm^{-1} (COC vibrations) [40]. Interestingly, values closely resembling these were also discerned in the spectrum reported within this study. The prevalence of hydroxyl groups suggests a greater concentration of cellulose and hemicellulose in the material. Although lignin was detected by the vibrations of the aromatic rings, its content is not significantly higher than that of cellulose and hemicellulose in

the functional groups of the spectrum. This outcome is connected to the physicochemical characterization. The arrangement of these chemical groups in this context determines the ease with which these materials can break down into smaller sugars during hydrolysis processes. The low detected content and intensity of lignin suggest that the material is not strongly safeguarded by lignin, indicating that the hydrolysis process should not be significantly impacted. However, possible contamination with this material during the purification process should be taken into account in subsequent steps.

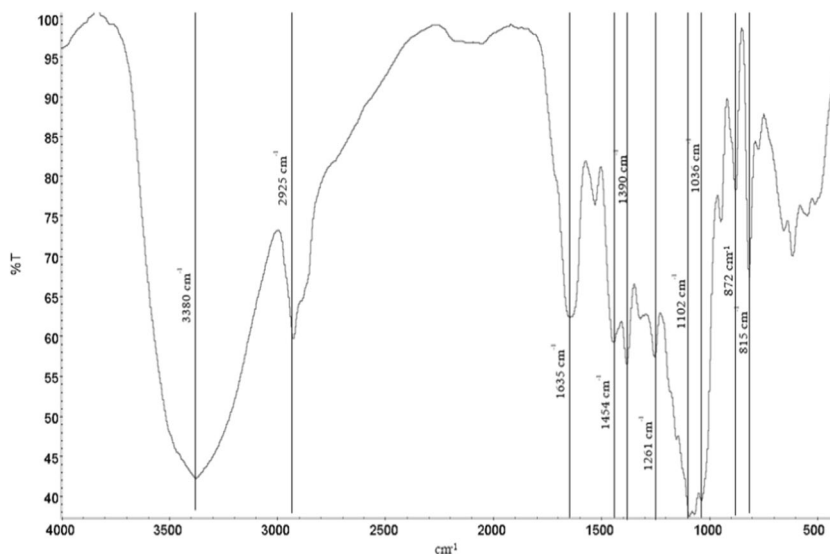
In this context, this study also managed to detect characterizations previously reported in the literature, linked to the presence of mannans as a primary component of the açai seed. This finding further validates its potential utility as a source for the extraction of mannoooligosaccharides (MOS) and various other types of sugars typical in lignocellulosic materials.

3.4 Enzymatic hydrolysis

Most methods for determining carbohydrase activity involve analyzing the reducing sugars formed due to the enzymatic cleavage of glycosidic bonds in the substrate. Although the DNS assay does not provide stoichiometric data for oligosaccharides, typically yielding higher values than the actual number of hemiacetal reducing groups, it is widely used for measuring carbohydrase activities, such as amylases, β -mannanases, pectinases, and xyloglucanases [41].

In the hydrolysis system with 100 g/L of açai seed powder, the enzyme activity in the solution was 1.75 U/mL . The time course of enzymatic hydrolysis of mannan in açai seed was monitored by quantifying the products of the reaction, namely, reducing sugars and mannoooligosaccharides (M2–M5). The concentration of reducing sugars (RS) reflected the rate of hydrolysis of açai seed powder. By measuring

Fig. 2 FTIR spectra of açai seed



the RS in the reaction media at each time point (Fig. 3), we observed that the concentration of reducing sugars generated by hydrolysis remained within the range of 10.5–11 g/L, with no significant differences observed in reducing sugar formation from the tenth hour onwards ($p > 0.05$). In contrast, the concentrations of MOS continued to increase until the nineteenth hour, after which no significant differences in the total MOS concentration were evident ($p > 0.05$). The differences between the quantification of MOS content and reducing sugars lie in the variety of oligosaccharides of various lengths produced in the hydrolysate, resulting in sugars with different reducing potential [42]. Additionally, there is an observable difference of almost 2 g/L between the concentration of reducing sugars and the MOS content. Monomeric sugars exhibit higher reducing power. The release of glucose and mannose due to the presence of cellulases and mannosidases in the enzymatic cocktail might also be affecting the content of reducing sugars.

MOS are non-digestible oligosaccharides with a low degree of polymerization (DP) of 2 to 20 monomers linked

by O-glycosidic bonds [43]. Prebiotic anticancer and antioxidant activities have been reported for oligosaccharides with a DP of 2 (M2) to 6 (M6) [44]. This is the main reason for our interest in focusing exclusively on M2 to M5 mannose oligosaccharides.

The hydrolysis of açai seed produced the four different types of manno oligosaccharides monitored: di-, tri-, tetra-, and penta-saccharides from the first hour of the reaction. Figure 4 shows the temporal distribution of the generation of these oligomers, indicating an increase in manno biose (M2) production primarily and a decrease in the other three types of MOS (M3–M5). Consequently, it can be proposed that the accumulation of M2 at an extended incubation time may be attributed to the further hydrolysis of the oligosaccharides from M3 to M5. This observation explains the relatively low increase in MOS content between the first and last hour of monitoring, where the MOS concentration increases by approximately 13%.

The concentration of MOS obtained was compared with those observed in other studies using enzymatic hydrolysis

Fig. 3 Monitoring sugars and MOS production from açai seed hydrolysis

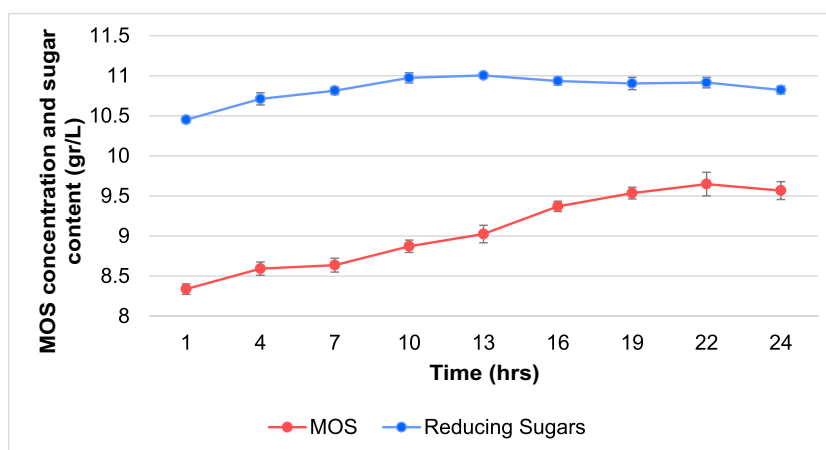
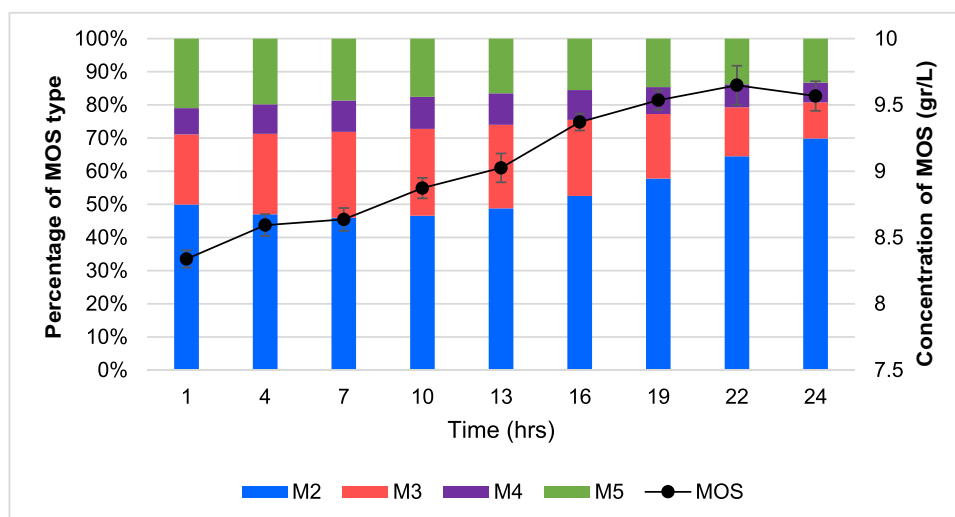


Fig. 4 Temporal distribution of MOS types from enzymatic açai seed hydrolysis



to obtain MOS from agro-industrial residues. In the case of copra meal, a concentration of 4.6 g of MOS per liter of hydrolysate was reported by Ghosh et al. (2015) [45] while spent coffee grounds showed a concentration of 25 g of MOS per liter of hydrolysate, according to Chauhan et al. (2014) [46]. The value obtained from the açai seed hydrolysate falls within the range of 8–10 g of MOS per liter of hydrolysate reported for materials suitable for MOS production. This comparison indicates that the enzymatic hydrolysis of acai seeds offers a potential source of MOS with a concentration appropriate for further applications.

Additionally, a comparable study explored the production of oligosaccharides and low molecular weight polysaccharides from konjac flour using β -mannanase, with varying substrate concentrations ranging from 50 to 200 g/L, pH 5.5, and 20 U/mL of β -mannanase in the reaction media. In that study, the concentration of reducing sugars was maintained in the range of 9–13 g/L when the Konjac flour concentration was 200 g/L, and the average degree of polymerization of the released sugars was 9.78 during the 1- to 2-h reaction time. Notably, when konjac flour was added at a concentration of 50 g/L, the hydrolysis process rapidly completed in a short period, resulting in a lower average degree of polymerization of the polysaccharide. The authors concluded that achieving high concentrations of konjac oligosaccharides and reducing monosaccharides during the production process is possible with a high substrate concentration, which reduces free water within the reaction system, effectively controlling the degree of polymerization and yielding high-quality konjac oligosaccharides [47]. In this current study, we employed similar substrate concentrations (100 g/L), and it was observed that the oligosaccharides with the highest molecular weight among those evaluated were obtained within the first hour of reaction. This observation suggests that further tests could be conducted within a narrower range of reaction time (between 0 and 2 h), while maintaining the other conditions of the reaction system, to verify if superior results can be achieved in this shorter time frame, resulting in potential time and resource savings.

Finally, concerning the % hydrolysis and oligosaccharide production yield, results of $10.79 \pm 0.05\%$ and 51.39 ± 0.11 g M2-M5/g mannan were achieved, respectively. This outcome was compared with findings from Li et al. (2018) [22], who subjected PKC to steam explosion pretreatment to obtain MOS. Their reported values for % hydrolysis ranged between 10.5 and 19.7%, and the oligosaccharide yields of M2-M4 ranged from 24.1 to 45.7 g/g mannan. Remarkably, these values closely resembled those obtained in this study. However, it is worth noting that the reported values from the comparative study appear higher due to the exclusion of M5 oligomers from their quantification.

4 Future prospects

Research on mannoooligosaccharides (MOS) derived from acai seeds should prioritize optimizing enzymatic extraction and validating it against alternative hydrolysis methods. Additionally, analytical validation of obtained products, technoeconomic and environmental analysis of production, and exploration of bioactive characteristics are crucial.

Validation of alternate hydrolysis methods could improve the efficiency and sustainability of MOS production. Analytical validation, as well as techno-economic and environmental assessments, would ensure their applicability and feasibility. Investigating their prebiotic, antioxidant, and anti-inflammatory properties presents new perspectives on gastrointestinal health and overall well-being, promoting their use in functional foods and pharmaceuticals. Together, these interdisciplinary avenues will facilitate the complete utilization of MOS's potential as healthful and beneficial agents in diverse applications within the human and animal food industry.

Currently, MOS's functional food additive status in food applications is restricted to laboratory-scale trials and necessitates expansion. In addition, greater emphasis should be placed on efficiently implementing downstream and upstream processing methods that are necessary for a seamless and economical process.

5 Conclusions

The acai seeds studied from the Colombian Pacific region have lower cellulose and lignin contents, as well as higher hemicellulose contents, compared to the ranges reported for acai seeds grown in Brazil. These characteristics indicate that they can be utilized in enzymatic hydrolysis processes to obtain sugars or mannoooligosaccharides without the necessity of customary pretreatments. This will result in both economic and environmental benefits during the seed processing. This finding further validates its potential utility as a source for the extraction of MOS and various other types of sugars typical in lignocellulosic materials.

The enzymatic hydrolysis of açai seed was found to be a method for producing a mixture of mannoooligosaccharides, including di-, tri-, tetra-, and penta-saccharides. Notably, there was a predominant accumulation of mannobiose (M2) during the hydrolysis process. Comparing the MOS concentration with other studies, the açai seed hydrolysate fell within the range of MOS extracted from other materials. These results underscore the potential of açai seed as a sustainable and valuable source of

mannooligosaccharides, offering promising prospects for their application as prebiotics and functional food additives. Further exploration of these compounds from açai seed could lead to significant advancements in various industries seeking natural and eco-friendly alternatives.

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Author contribution Sarha Lucia Murillo Franco: conceptualization, formal analysis, experimental work, visualization, investigation, writing—original draft. Juan David Galvis Nieto: methodology, experimental work, writing and editing. Carlos Eduardo Orrego Alzate: conceptualization, resources, supervision, project administration, writing—review and editing.

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Declarations

Competing interests The authors declare no competing interests.

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References

- Laurindo LF et al (2023) Açai (*Euterpe oleracea* Mart.) in health and disease: a critical review. *Nutrients* 15(4):989. <https://doi.org/10.3390/nu15040989>
- Barbosa JR, de Carvalho Junior RN (2022) Food sustainability trends - how to value the açai production chain for the development of food inputs from its main bioactive ingredients? *Trends Food Sci Technol* 124:86–95. <https://doi.org/10.1016/j.tifs.2022.04.005>
- Portafolio, “El Açai: opción para deforestación en la Amazonia,” Portafolio.co. [Online]. Available: <https://www.portafolio.co/internacional/acai-opcion-deforestacion-amazonia-62086>. Accessed 8 May 2023
- “Fondo Acción | La Palma murrapo: una especie no maderable con potencial en el Darién.” [Online]. Available: <https://fondoaccion.org/2021/02/04/la-palma-murrapo-una-especie-no-maderable-con-potencial-en-el-darien/>. Accessed 8 May 2023
- Sato MK et al (2020) Biochar as a sustainable alternative to açai waste disposal in Amazon, Brazil. *Process Saf Environ Prot* 139:36–46. <https://doi.org/10.1016/j.psep.2020.04.001>
- De Lima ACP et al (2021) Physicochemical characterization of residual biomass (seed and fiber) from açai (*Euterpe oleracea*) processing and assessment of the potential for energy production and bioproducts. *Biomass Conv Bioref* 11(3):925–935. <https://doi.org/10.1007/s13399-019-00551-w>
- Maciel-Silva FW, Mussatto SI, Forster-Carneiro T (2019) Integration of subcritical water pretreatment and anaerobic digestion technologies for valorization of açai processing industries residues. *J Clean Prod* 228:1131–1142. <https://doi.org/10.1016/j.jclepro.2019.04.362>
- Sganzerla WG, Ampese LC, Parisoto TAC, Forster-Carneiro T (2023) Process intensification for the recovery of methane-rich biogas from dry anaerobic digestion of açai seeds. *Biomass Conv Bioref* 13(9):8101–8114. <https://doi.org/10.1007/s13399-021-01698-1>
- Sato MK, de Lima HV, Costa AN, Rodrigues S, Pedroso AJS, de Freitas Maia CMB (2019) Biochar from Acai agroindustry waste: study of pyrolysis conditions. *Waste Manag* 96:158–167. <https://doi.org/10.1016/j.wasman.2019.07.022>
- de Melo Barbosa A, Rebelo VSM, Martorano LG, Giacon VM (2019) Caracterização de partículas de açai visando seu potencial uso na construção civil. *Matéria (Rio J.)* 24(3):e12435. <https://doi.org/10.1590/s1517-707620190003.0750>
- Rocha JHA et al (2022) Circular bioeconomy in the amazon rainforest: evaluation of açai seed ash as a regional solution for partial cement replacement. *Sustainability* 14(21):14436. <https://doi.org/10.3390/su142114436>
- Jorge FTA, da Silva AS, Brigagão GV (2022) Açai waste valorization via mannose and polyphenols production: techno-economic and environmental assessment. *Biomass Conv Bioref*. <https://doi.org/10.1007/s13399-022-02681-0>
- Monteiro AF, Miguez IS, Silva JPRB, da Silva AS (2019) High concentration and yield production of mannose from açai (*Euterpe oleracea* Mart.) seeds via mannanase-catalyzed hydrolysis. *Sci Rep* 9(1):1. <https://doi.org/10.1038/s41598-019-47401-3>
- Ayimbila F, Keawsompong S (2022) Characteristics and bioactive properties of agro-waste and yeast derived manno-oligosaccharides. *Biocatal Agric Biotechnol* 45:102522. <https://doi.org/10.1016/j.bcab.2022.102522>
- Poveda-Giraldo JA, Piedrahita-Rodríguez S, Aristizabal NS, Salas-Moreno M, Alzate CAC (2022) Prefeasibility analysis of small-scale biorefineries: the annatto and açai case to improve the incomes of rural communities. *Biomass Conv Bioref*. <https://doi.org/10.1007/s13399-022-03479-w>
- Sluiter A (2008) Determination of extractives in biomass: laboratory analytical procedure (LAP); Issue Date 7/17/2005. Technical Report. Available: <https://www.nrel.gov/docs/gen/fy08/42619.pdf>. Accessed 6 Nov 2023
- Hames B, Ruiz R, Scarlata C, Sluiter A, Sluiter J, Templeton D (2008) Preparation of samples for compositional analysis: laboratory analytical procedure (LAP); Issue Date 08/08/2008. Technical Report. Available: <https://www.nrel.gov/docs/gen/fy08/42620.pdf>. Accessed 6 Nov 2023
- Sluiter AD et al (2004) Determination of structural carbohydrates and lignin in biomass. [Online]. Available: <https://www.semanticscholar.org/paper/Determination-of-Structural-Carbohydrates-and-in-Sluiter-Hames/e8b210c00c005b0a2fd7aab65b2e96b4189b8e98>. Accessed 7 Aug 2023
- Lim PY (1987) Protein determination by Kjeldahl method. <http://aquaticcommons.org/id/eprint/26807>. [Online]. Available: <https://aquadocs.org/handle/1834/40994>. Accessed 11 Sep 2022
- Rivas B, Torrado A, Torre P, Converti A, Domínguez JM (2008) Submerged citric acid fermentation on orange peel autohydrolyzate. *J Agric Food Chem* 56(7):2380–2387. <https://doi.org/10.1021/jf073388r>
- Wood IP, Elliston A, Ryden P, Bancroft I, Roberts IN, Waldron KW (2012) Rapid quantification of reducing sugars in biomass hydrolysates: improving the speed and precision of the

- dinitrosalicylic acid assay. *Biomass Bioenerg* 44:117–121. <https://doi.org/10.1016/j.biombioe.2012.05.003>
22. Li Y, Yi P, Liu J, Yan Q, Jiang Z (2018) High-level expression of an engineered β -mannanase (mRmMan5A) in *Pichia pastoris* for manno-oligosaccharide production using steam explosion pretreated palm kernel cake. *Biores Technol* 256:30–37. <https://doi.org/10.1016/j.biortech.2018.01.138>
 23. Rambo MKD, Schmidt FL, Ferreira MMC (2015) Analysis of the lignocellulosic components of biomass residues for biorefinery opportunities. *Talanta* 144:696–703. <https://doi.org/10.1016/j.talanta.2015.06.045>
 24. Buratto RT, Cocero MJ, Martín Á (2021) Characterization of industrial açai pulp residues and valorization by microwave-assisted extraction. *Chem Eng Process- Process Intensif* 160:108269. <https://doi.org/10.1016/j.cep.2020.108269>
 25. de Sá Barros S et al (2021) Sementes de açai (*Euterpe precatoria* Mart) como uma nova fonte alternativa de celulose: extração e caracterização. *RSD* 10(7):e31110716661. <https://doi.org/10.33448/rsd-v10i7.16661>
 26. Oliveira J, Martins L, Komesu A, Filho RM (2015) Evaluation of alkaline delignification (naoh) of acai seeds (*euterpe oleracea*) treated with h₂so₄ dilute and effect on enzymatic hydrolysis. *Chem Eng Trans* 43:499–504. <https://doi.org/10.3303/CET1543084>
 27. Wycoff W et al (2015) Chemical and nutritional analysis of seeds from purple and white açai (*Euterpe oleracea* Mart.). *J Food Compos Anal* 41:181–187. <https://doi.org/10.1016/j.jfca.2015.01.021>
 28. Melo PS, de Oliveira Rodrigues Arrivetti L, de Alencar SM, Skibsted LH (2016) Antioxidative and prooxidative effects in food lipids and synergism with α -tocopherol of açai seed extracts and grape rachis extracts. *Food Chem* 213:440–449. <https://doi.org/10.1016/j.foodchem.2016.06.101>
 29. Atalla RH, VanderHart DL (1984) Native cellulose: a composite of two distinct crystalline forms. *Science* 223(4633):283–285. <https://doi.org/10.1126/science.223.4633.283>
 30. French AD, Cintrón MS (2013) Cellulose polymorphy, crystallite size, and the Segal Crystallinity Index. *Cellulose* 20(1):583–588. <https://doi.org/10.1007/s10570-012-9833-y>
 31. Filson PB, Dawson-Andoh BE, Schwegler-Berry D (2009) Enzymatic-mediated production of cellulose nanocrystals from recycled pulp. *Green Chem* 11(11):1808. <https://doi.org/10.1039/b915746h>
 32. Scudino S, Donnadieu P, Surreddi KB, Nikolowski K, Stoica M, Eckert J (2009) Microstructure and mechanical properties of Laves phase-reinforced Fe–Zr–Cr alloys. *Intermetallics* 17(7):532–539. <https://doi.org/10.1016/j.intermet.2009.01.007>
 33. Grimaud F et al (2019) In vitro synthesis and crystallization of β -1,4-mannan. *Biomacromol* 20(2):846–853. <https://doi.org/10.1021/acs.biomac.8b01457>
 34. Orrego CE, Valencia JS (2009) Preparation and characterization of chitosan membranes by using a combined freeze gelation and mild crosslinking method. *Bioprocess Biosyst Eng* 32(2):197–206. <https://doi.org/10.1007/s00449-008-0237-1>
 35. Tatirat O, Charoenrein S, Kerr WL (2012) Physicochemical properties of extrusion-modified konjac glucomannan. *Carbohydr Polym* 87(2):1545–1551. <https://doi.org/10.1016/j.carbpol.2011.09.052>
 36. Park S, Baker JO, Himmel ME, Parilla PA, Johnson DK (2010) Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. *Biotechnol Biofuels* 3(1):10. <https://doi.org/10.1186/1754-6834-3-10>
 37. Hägglund P (2002) Mannan-hydrolysis by hemicellulases: enzyme-polysaccharide interaction of a modular beta-mannanase. Doctoral Thesis (compilation), Dr Henrik Stålbrand, Getingevägen 60, Box 124, 221 00 LUND, Sweden. Available: <https://lup.lub.lu.se/search/publication/f48e132a-9725-45e9-9eb7-28d585e87014>. Accessed 6 Nov 2023
 38. Avanthi A, Banerjee R (2016) A strategic laccase mediated lignin degradation of lignocellulosic feedstocks for ethanol production. *Ind Crops Prod* 92:174–185. <https://doi.org/10.1016/j.indcrop.2016.08.009>
 39. Mvondo RRN, Meukam P, Jeong J, Meneses DDS, Nkeng EG (2017) Influence of water content on the mechanical and chemical properties of tropical wood species. *Results Phys* 7:2096–2103. <https://doi.org/10.1016/j.rinp.2017.06.025>
 40. Rabyk M et al (2018) Mannan-based conjugates as a multimodal imaging platform for lymph nodes. *J Mater Chem B* 6. <https://doi.org/10.1039/C7TB02888A>
 41. Gusakov AV, Kondratyeva EG, Sinitsyn AP (2011) Comparison of two methods for assaying reducing sugars in the determination of carbohydrase activities. *Int J Anal Chem* 2011:e283658. <https://doi.org/10.1155/2011/283658>
 42. Arnlund Bååth J, Martínez-Abad A, Berglund J, Larsbrink J, Vilaplana F, Olsson L (2018) Mannanase hydrolysis of spruce galactoglucomannan focusing on the influence of acetylation on enzymatic mannan degradation. *Biotechnol Biofuels* 11(1):114. <https://doi.org/10.1186/s13068-018-1115-y>
 43. Roberfroid M, Slavin J (2000) Nondigestible oligosaccharides. *Crit Rev Food Sci Nutr* 40(6):461–480. <https://doi.org/10.1080/10408690091189239>
 44. Jana UK, Suryawanshi RK, Prajapati BP, Kango N (2021) Prebiotic manno-oligosaccharides: synthesis, characterization and bioactive properties. *Food Chem* 342:128328. <https://doi.org/10.1016/j.foodchem.2020.128328>
 45. Ghosh A, Verma AK, Tingirikari JR, Shukla R, Goyal A (2015) Recovery and purification of oligosaccharides from copra meal by recombinant endo- β -mannanase and deciphering molecular mechanism involved and its role as potent therapeutic agent. *Mol Biotechnol* 57(2):111–127. <https://doi.org/10.1007/s12033-014-9807-4>
 46. Chauhan PS, Sharma P, Puri N, Gupta N (2014) A process for reduction in viscosity of coffee extract by enzymatic hydrolysis of mannan. *Bioprocess Biosyst Eng* 37(7):1459–1467. <https://doi.org/10.1007/s00449-013-1118-9>
 47. Tang X, Zhu X, Yang Y, Qi Z, Mu Y, Huang Z (2020) Research article product composition analysis and process research of oligosaccharides produced from enzymatic hydrolysis of high-concentration konjac flour. *ACS Omega* 5(5):2480–2487. <https://doi.org/10.1021/acsomega.9b04218>

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