Açaí seeds as a source of prebiotics (mannan oligosaccharides)

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Introduction: The açaí (*Euterpe oleracea*) is recognized as an exotic fruit with high antioxidant power (Marcason, 2009). Brazil is the main producer of this material, however, in Colombia there are several regions where it is beginning to be used on a larger scale, since the açaí palm grows wild in those places, its use does not affect wild systems, and also facilitates the development economy of vulnerable regions in the country (García-Cardona *et al.*, 2022). However, the seed and fiber content of the açaí are about 85% or more of the total dry weight, being the pulp the rest of the fruit. Açaí fruits are generally consumed fresh and the seeds are discarded as waste in open landfills, which generates environmental damage and problems of health (Sato *et al.*, 2020). There are studies that show that the main component of the açaí seed corresponds to mannan (> 50%), a crystalline polysaccharide with a high percentage of substitution (Monteiro et al., 2019). Currently, the sources used to obtain substrates rich in mannan sugars usually come from palm kernel cake or soft wood, systems that usually require robust machines for purification since they have mannan percentages below 38% (Jorge, da Silva and Brigagão, 2022). This work aims to present a preliminary study of the seeds characterization and the process of obtaining a substrate rich in mannan oligosaccharides from açaí seeds of the Colombian Pacific by enzymatic hydrolysis.

Methodology: Açaí seed samples were dried and ground (particle size<0.4 mm). Physicochemical characterization of seed powder was done as follows: Holocellulose and cellulose were determined using the ASTM (D-1104) NREL/TP-510-42620 standards (Hames *et al.*, 2008). With the sample free of extractives, hemicellulose was quantified indirectly from the difference with holocellulose. Soluble lignin content was determined with TAPPI T222. Also, the structure of the material was determined with XRD using a Rigaku Miniflex II diffractometer with a Cu Kα radiation angle of 15 mA in the 2θ range of 2° to 80° with a scanning speed of 1°/min and a step angle of 0.2. °.

The seed powder enzymatic hydrolysis tests were carried out using the enzyme Rohalase®GMP (enzymatic preparation containing mainly mannanase) in a relation enzyme-substrate ratio of 0.675% after three reaction times (3,13 and 23 hr) and three pH levels (1.5, 6.5 and 11.5) at 50°C. The sugar analysis was carried out using a monomer type reducing sugar (RS) analysis based on the DNS method with modifications (Wood *et al.*, 2012). It was also determined the total content of soluble sugars (TSS), that corresponds to the monomers and oligomers content in the hydrolyzed sample, using the sulfuric phenol method (Nielsen, 2010). For both methods, D-mannose was used as standard. The yield of the hydrolysis was calculated as the amount of TSS per the initial raw material concentration, both in gr/L. The difference between TSS-RS was taken an indicator of the mannan polysaccharides content (Luo *et al.*, 2022) in the sample of the hydrolyzed material under the reported conditions.

Results: The average concentrations of cellulose, hemicellulose and lignin in the seed were 12.53 ± 1.66, 40.98 ± 2.19 and 15.28 ± 3.69 %, respectively. These results that can be compared with those of Buratto *et al.*, (2021) that reported 8.5 ± 0.10, 48.1 ± 0.45. and 16.4±1.7%, respectively. The higher hemicellulose content shows the prevalence of mannan chains. The presence of mannan chains was also confirmed by the XRD analysis, Figure 1 presents a representative diffractogram of the açaí seed powder showing the typical peaks of synthetic mannan chain systems of type α 1-4 mannan, indicating the presence of these compounds to a greater extent in the seeds.(Grimaud *et al.*, 2019)

Figure 1. XRD diffractogram of açaí seeds from the Colombian Pacific region

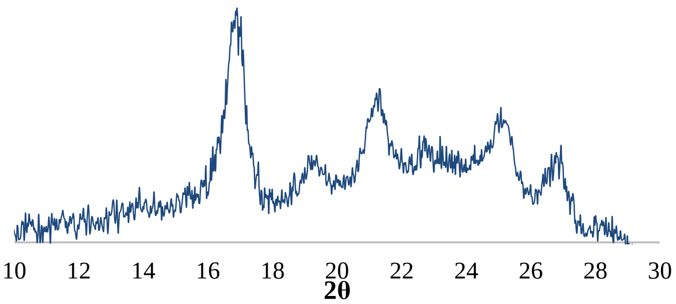

Table 1 shows the preliminary results of yield, estimated index of oligosaccharides (IPC) and reducing sugars (RS) at the end of some of the hydrolysis tests. For the extreme values of pH (1.5 and 11.5) mannose was mainly released and practically no oligosaccharides were present. In pH 6.5 tests, as the reaction time elapses, the increase in IPC indicated an increasing concentration of oligosaccharides.

Table 1. Preliminary results of the hydrolysis of açaí seeds

pH Buffer	Time	Carbohydrate Content		
	Hours	Yield=TSS/ Sample weight (%)	IPC*=TSS – RS [gr/L]	RS ** [gr/L]
6.5	23	39.4±0.27	3.18±0.46	8.64±
6.5	13	27.8±0.42	1.62±0.29	6.73±
6.5	3	13.6±0.035	0.25±0.03	3.82±
1.5	13	23.6±0.93	0.029±0.00	7.38±
11.5	13	35.7±0.45	0.047±0.00	11.16±

** Estimated index of polysaccharide content´(IPC) ** Reducing sugar content*

Conclusions: The results of the characterization of the acai seed from the Colombian Pacific showed a high content of hemicellulose and specific characteristics of crystallinity of links of linear mannan chains, so this raw material can be considered as an alternative source for obtaining high amounts of mannose polysaccharides and monosaccharides. At extreme pH levels, it is observed that the preferential reaction to obtain mannose, with a higher yield of total sugar content, while at intermediate pH, with the passage of time, an increase is observed both in mannose content and in that of soluble polysaccharides, which allows us to assume that non-extreme pH conditions may favor the production of prebiotic material.

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