
Açaí seeds as a source of prebiotics (mannan-oligosaccharides)

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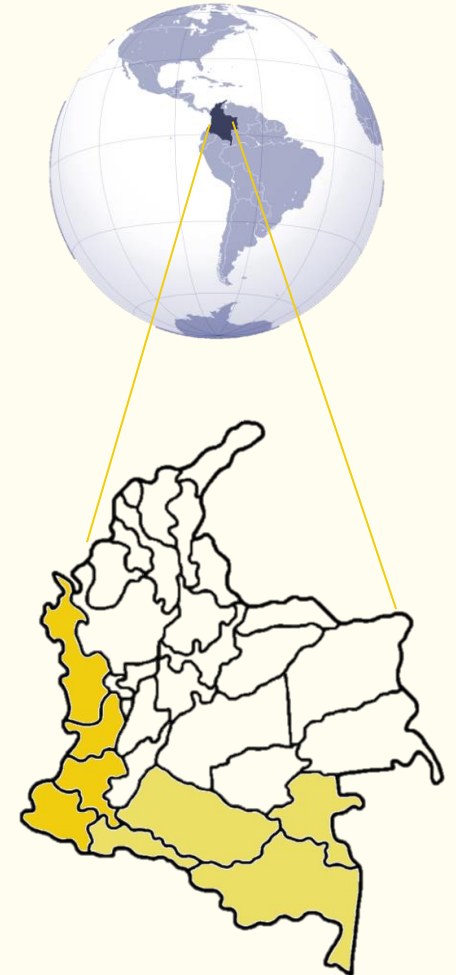
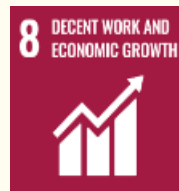
Introduction



The açaí (*Euterpe oleracea*) is recognized as an exotic fruit with high antioxidant power.



Colombia is taking advantage of the areas where the açaí palm grows naturally to facilitate its extensive utilization.



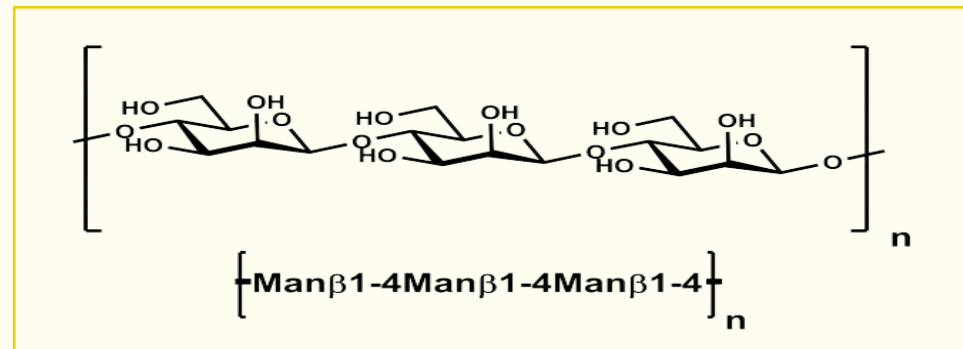
Introduction



The seed and fiber content of the açai are about 85% or more of the total dry weight [1].



There are studies that show that the main component of the açai seed corresponds to mannan (> 50%), a crystalline polysaccharide [2].



[1] Sato, M.K. *et al.* (2020) 'Biochar as a sustainable alternative to açai waste disposal in Amazon, Brazil', *Process Safety and Environmental Protection*, 139, pp. 36–46. Available at: <https://doi.org/10.1016/j.psep.2020.04.001>.

[2] Monteiro, A.F., Miguez, I.S., Silva, J.P.R.B. *et al.* High concentration and yield production of mannose from açai (*Euterpe oleracea* Mart.) seeds via mannanase-catalyzed hydrolysis. *Sci Rep* 9, 10939 (2019). <https://doi.org/10.1038/s41598-019-47401-3>

The sources used to obtain substrates rich in mannan sugars are systems that usually require robust machines for their purification, since they present mannan percentages below 40% [1].

Table 1. Mannan content in agroindustrial waste

Raw material	%Mannan	Reference
Palm Kernel Cake (PKC)	35.2	(Sathitkowitchai et al., 2022) [2]
Açaí Seed	53.8	(Rambo et al., 2015) [3]
Spent Coffee Ground (SCG)	39.3	(Jooste et al., 2013) [4]
Pine wood	15.79	(Ståhl et al., 2018)



- [1] Jorge, F.T.A., da Silva, A.S. and Brigagão, G.V. (2022) 'Açaí waste valorization via mannose and polyphenols production: techno-economic and environmental assessment. <https://doi.org/10.1007/s13399-022-02681-0>.
- [2] Sathitkowitchai, W., Ayimbila, F., Nitisinprasert, S., & Keawsompong, S. (2022). Selection of pretreatment method and mannanase enzyme to improve the functionality of palm kernel cake. <https://doi.org/10.1016/j.jbiosc.2022.06.016>
- [3] Rambo, M. K. D., Schmidt, F. L., & Ferreira, M. M. C. (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>.
- [4] Jooste, T., García-Aparicio, M. P., Brienzo, M., van Zyl, W. H., & Görgens, J. F. (2013). Enzymatic Hydrolysis of Spent Coffee Ground. <https://doi.org/10.1007/s12010-013-0134-1>
- [5] Ståhl, M., Nieminen, K., & Sixta, H. (2018). Hydrothermolysis of pine wood. *Biomass and Bioenergy*, 109, 100–113. <https://doi.org/10.1016/j.biombioe.2017.12.006>

Presentation Objective

This is a preliminary study on the characterization of açai seeds and the process of enzymatic hydrolysis to obtain a mannan sugar-rich substrate from the Colombian Pacific.

Methodology

Determination of lignocellulosic content, crystallinity and the presence of specific functional groups in açai seeds

Structure Characterization

Table 2. International standard techniques for chemical characterization

Component	Technique
Cellulose	NREL / TP-510-42620
Holocellulose	ASTM standard (D-1104)
Hemicellulose	By difference
Lignin	TAPPI T222

X-ray diffraction



15 A, 30 mV, 2θ range from 2° to 80° with speed of sweep of 1°/min and a step angle of 0.2°.

Infrared spectroscopy



KBr pellet (95:5), Range 400-4000 cm⁻¹, Resolution 4 cm⁻¹.

Methodology

Pretreatment



Açaí seed samples were dried (70°C) and ground (particle size < 0.4 mm).



Conditions of enzymatic hydrolysis

The seed powder was mixed with the enzyme cocktail (enzymatic preparation containing mainly mannanase) in a specific enzyme-substrate ratio of 0.675% at 50°C and 350 rpm.

Sugar Quantification

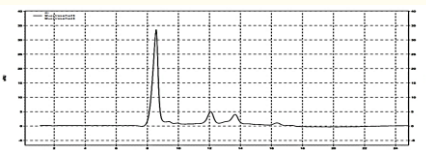


Total soluble sugars (TSS)
(Phenol-Sulfuric Method)



Reducing sugar content (RS)
(DNS method)

Estimated index of polysaccharide content (IPC) = TSS - RS



HPLC Analysis

Table 2. Conditions of enzymatic hydrolysis of açaí seeds

pH	Time
6.5	23
6.5	13
6.5	3
1.5	13
11.5	13

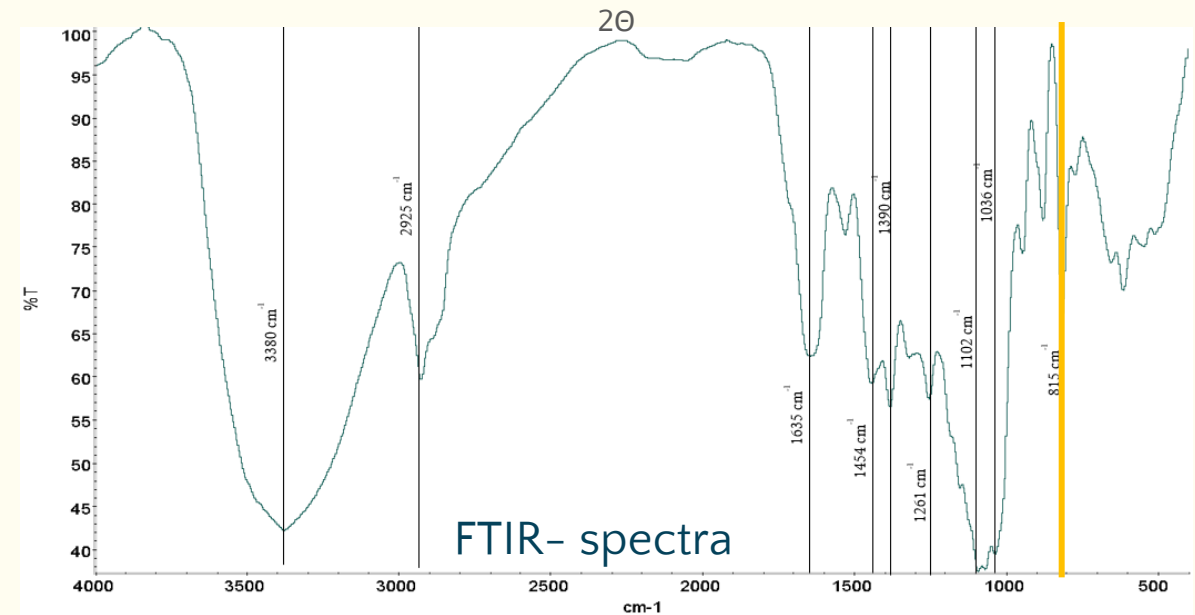
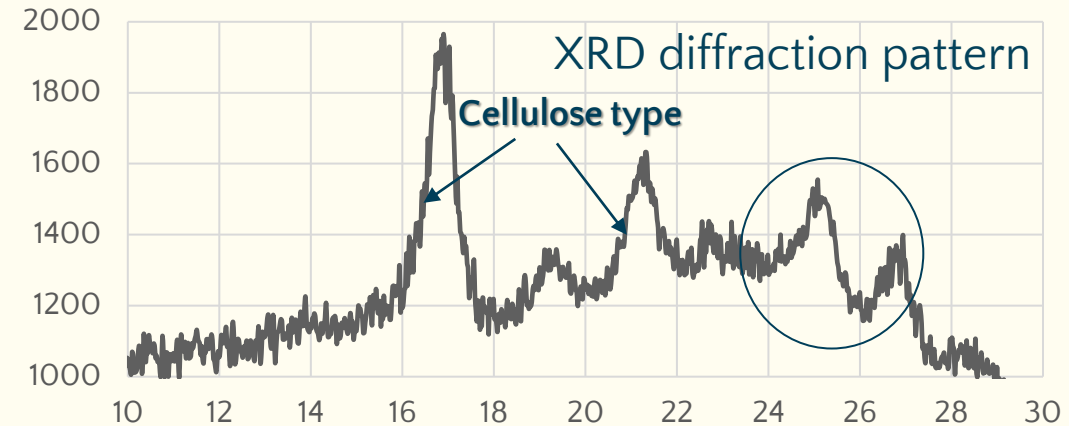


Results

The results show a high hemicellulose content, high crystallinity and spectral intensity typical of mannan-rich products.

Table 3. Chemical characterization of açai seeds

Component	%Composition	
	This Work	Buratto, R.T. <i>et al.</i> (2021)
Cellulose	16.30±1.66	8.5±0.10
Holocellulose	69.61±0.64	-
Hemicellulose	53.30±2.19	48.1±0.45
Lignin	19.87±3.69	16.4±1.7



[1] Buratto, R.T. *et al.* (2021) 'Formulation of açai (*E. oleracea* Mart.) Pulp and seeds extracts by co-precipitation in Supercritical Antisolvent (SAS) technology', *The Journal of Supercritical Fluids*, 169, p. 105090. Available at: <https://doi.org/10.1016/j.supflu.2020.105090>.

For the extreme values of pH (1.5 and 11.5) mannose was mainly released and practically no polysaccharides were present. In pH 6.5 tests, as the reaction time elapses, the increase in IPC indicated an increasing concentration of oligo and polysaccharides.

Table 4. Preliminary results of the hydrolysis of açai seeds

pH	Time (Hours)	Carbohydrate Content	
		IPC* = TSS – RS [gr/L]	RS ** [gr/L]
6.5	23	3.18±0.46	8.64±0.27
6.5	13	1.62±0.29	6.73±0.05
6.5	3	0.25±0.03	3.82±0.05
1.5	13	0.029±0.012	7.38±0.02
11.5	13	0.047±0.017	11.16±1.96

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

Table 5. Comparative Studies

pH	Time (Hr)	Raw Material	Carbohydrate Content	Ref.
			[gr product/L]	
6.0	5	Locust Bean	0.52	[1]
5.5	3	Spend Coffee Ground	1.20	[2]

[1] Kumar Suryawanshi, R., & Kango, N. (2021). Production of manooligosaccharides from various mannans and evaluation of their prebiotic potential. Food Chemistry, 334, 127428. <https://doi.org/10.1016/j.foodchem.2020.127428>

[2] Wongsiridetchai, C., Chiangkham, W., Khlahiran, N., Sawangwan, T., Wongwathanarat, P., Charoenrat, T., & Chantorn, S. (2018). Alkaline pretreatment of spent coffee grounds for oligosaccharides production by mannanase from Bacillus sp. GA2(1). Agriculture and Natural Resources, 52(3), 222–227. <https://doi.org/10.1016/j.anres.2018.09.012>

Conclusion

- Açai seeds can be considered as an alternative source for obtaining high amounts of mannose polysaccharides, oligosaccharides and monosaccharides.
- At 1.5 and 11.5 pH levels (13 h), it is observed that the preferential reaction to obtain mannose, with a higher yield of total sugar content.
- At intermediate pH, when increase the reaction time also exist an increase in mannose content and in the soluble polysaccharides (including mannan-oligosaccharides).

Future Work

1. Optimization for oligosaccharide production
 2. Validation of prebiotic activity for the product obtained
 3. Heterogeneous enzymatic catalyst
 4. Technoeconomical and enviromental assesment
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THANKS

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