I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM ·· XV SHEB ·· XV ENZITEC FLORIANÓPOLIS ·· 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
BIOPROCESS ENGINEERING

DEVELOPMENT OF BIOTECHNOLOGICAL AND SUSTAINABLE PROCESSES FOR OBTAINING HIGH-VALUE PRODUCTS FROM MACAUBA (ACROCOMIA ACULEATA) PULP

Norwin S. Rodriguez[1], Guilherme D. Sorita[1], Simone P. Favaro[2], Letícia K. Dos Santos[2,] Marco Di Luccio[1], Alan Ambrosi[1]

(1) Laboratory of Membrane Processes (LABSEM), Department of Chemical and Food Engineering, Federal University of Santa Catarina (UFSC), Trindade, 88040-900 Florianópolis, Santa Catarina, Brazil
(2) Empresa Brasileira de Pesquisa Agropecuária - Embrapa, PqEB, W3 Norte - Asa Norte, Brasília, DF, Brazil.
**Corresponding author: di.luccio@ufsc.br*

ABSTRACT

Aqueous enzymatic extraction (AEE) is an emerging technology that simultaneously extracts oil and other valuable compounds using water as a green solvent. This study explores the potential of AEE as an environmentally friendly extraction method for obtaining oil and other valuable compounds (sugar and phenolic compounds) from macauba pulp, adopting a scale-up and biorefinery approach. The extraction process was evaluated based on oil efficiency (%), and the oil quality was assessed by measuring its acidity. Additionally, the liquid by-product obtained from AEE was characterized by its total solid, phenolic, and sugar content, expanding its potential applications within a biorefinery concept. AEE provided a higher oil efficiency (76.73%) than the control (without enzyme, Aqueous extraction - AE), and the oil presented a low acidity value (4.9 % oleic acid). The liquid by-product from AEE presented a significant amount of phenolic content (0.675mg GAE mL^{-1}) and higher carbohydrate content (AE: 37.03 mg Glu mL^{-1} and AEE: 44.83 mg Glu mL^{-1}). The results obtained in this work showed that AEE is an eco-friendly extraction method to extract oil and other valuable compounds from raw materials with high humidity content.

Keywords: Circular economy, tropical fruits, oil, phenolic compounds, sugar

1 INTRODUCTION

Biotechnology encompasses a range of techniques that utilize microorganisms or enzymes to develop products and processes of important social and economic value. Enzymes and microorganisms are crucial in producing valuable components for food, pharmaceuticals, and agriculture industries, serving as precursors for obtaining primary and secondary metabolites. As biocatalysts, enzymes offer a more efficient and environmentally friendly alternative, facilitating reactions in green media under moderate pH and temperature conditions. These regio- and stereoselective processes often yield higher yields and lower costs than conventional methods (Arroyo, M. et al. 2014). Aqueous enzymatic extraction (AEE) is a sustainable method for obtaining oils, sugar, proteins, phytochemicals, and other valuable compounds from different materials using water as the solvent and specific enzymes to catalyze the degradation of cellular structures. This process, conducted under controlled temperature and pH conditions, maximizes extraction efficiency and produces high-quality products (Arroyo, M. et al 2014). The technique is widely used in the food, pharmaceutical, biofuel, and cosmetics industries because of its efficiency, sustainability, and lower environmental impact than traditional organic solvents.

Macauba (*Acrocomia aculeata*) pulp is a promising source for sustainable oil production for biofuels and food applications. The fruit's pulp boasts a high oil content, predominantly oleic acid, and bioactive compounds like carotenoids. This study explores the AEE for obtaining oil and other valuable compounds (sugar and phenolic compounds) from macauba pulp, adopting a scale-up and biorefinery approach.

2 MATERIAL & METHODS

Materials

Macauba (*Acrocomia aculeata*) fruits were collected at Embrapa Cerrados (Planaltina, DF). The selected fruits (after removing damaged fruits) were washed, pulped, and stored in polyethylene bags at -8 °C until processing. Olimax 101 (Prozyn, São Paulo, Brazil) and Cellic Ctec 3 were used in AEE assays. The chemicals used in the analytical part were HPLC grade.

Methods

Aqueous enzymatic extraction

Scale-up assays were performed to assess the reproducibility of the lab-scale tests of AEE of macauba pulp oil, previously tested by the research group (Favaro, S. et al., 2022 and Sorita, G. et al., 2024). A stainless-steel jacketed reactor (5 L, Kiloclave Type 4, Büchi GmbH, Switzerland) was used to evaluate the AEE on a large scale. The operational parameters (300 rpm at 50 °C) were set according to previous tests, with modifications to adapt to the equipment limitations. The temperature was controlled with a circulating bath (Julabo, Germany). Before the extractions, a uniform mixture of pulp and water (100 °C) was made in 1:2 proportions with the assistance of an electric mixer (RI1364/04, Philips Walita). Two different aqueous extraction treatments were compared: (i) AEE with the use of Olimax 101/Cellic Ctec3 (1:1 w/w) at 5% (by weight of the pulp) and (ii) Aqueous extraction (AE), with the same process conditions but without enzyme assistance (blank). The mixture was placed in the reactor, and the enzymes were added (for AEE). After 2 hours of extraction, the final mixture was heated (90 ± 2 °C for 15 min) for enzymatic inactivation. Then, the reaction medium was placed in separation funnels for solid decantation and submitted to centrifugation (Hitachi, model CR-22GIII) at 7000 rpm for 30 min at 40 °C to separate the oil. After centrifugation, the different phases obtained (oil, liquid, and solid phases) were separated and stored at -8 °C until further analysis. The extractions were performed in triplicate.

Free oil efficiency and acidity index of the oil

The process efficiency was determined according to Equation 1 (based on the oil content previously determined in the pulp before the extraction by Ankom).

$$FOE\ (\%) = \frac{MEO}{MOPA}\ x\ 100 \quad (1)$$

where, FOE: Free oil efficiency (%), MEO: Mass of Extracted Oil, and MOPA: Oil mass pulp determined by Ankom.
The acidity index of the pulp oil was determined with a potentiometric titrator Metrohm (Model Titrando 809, Metrohm, Switzerland) according to the Ca 5a-40 method, expressed as free fatty acid in oleic acid (%).

Liquid by-product characterization

The liquid by-product was characterized by total solids, carbohydrates, phenolic, and protein contents. The former were determined following the American Public Health Association (AWWA-WEF, 1998). Briefly, 0.2 L of the sample (in triplicate) were transferred to a crucible and subjected to drying in an oven at 105 °C for 24 hours. After removal from the oven, they are transferred to a muffle furnace for 12 hours. The dried materials were weighted, and the total solid content was calculated using Equation 2.

$$TSC\ (\%) = \frac{(MCD - MC)}{V}\ x\ 100 \quad (2)$$

where, MCD: crucible mass + dried sample mass after 24h later at 105°C (g), MC: crucible mass (g), V: sample volume (mL).

Total sugars were determined using the sulfuric phenol method, described by Lopez, X. et al; 2017. In a test tube, 29 µL of the diluted sample (1:1000) was mixed, and 29 µL of 5% phenol solution and 142 µL of H_2SO_4 were added. Each sample was shaken in a vortex and left to react for 30 minutes in the absence of light. After that, the absorbance of the samples was read at 490 nm. The results were corroborated with a calibration curve of D+Glucose (Sigma aldrich, powder, 99.5 %) (R^2 = 0.99218). The assay was performed in quintuplicate, and the results were expressed by mg total sugars (Glu) mL^{-1}.

The total phenolic content (TPC) was determined by the Follin- Ciocalteu method (Singleton, V. et al, 1999). Briefly, 600 µL of water, 10 µL of diluted sample (1:1 v/v), and 50µL of Folin reagent were mixed, then 150 µL sodium carbonate (Na_2CO_3) and 190 µL of water were added. The mixture reacted for 2 hours without the presence of light. Absorbances were determined at a wavelength of 760 nm, relating with a gallic acid standard curve (R^2 = 0.9968). The results are expressed EAG mg mL^{-1}.

3 RESULTS & DISCUSSION

Free oil efficiency and acidity

The free oil efficiency and the oil acidity are presented in **Figure 1** and **Figure 2**, respectively.

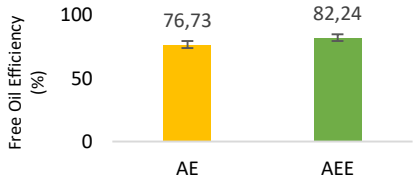

Figure 1. Free oil efficiency extraction of AE and AEE.

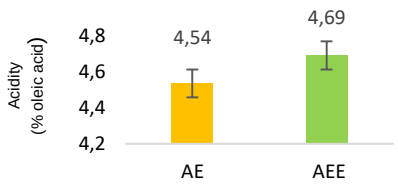

Figure 2. Acidity (%oleic acid) of macauba pulp oil.

As shown in **Figure 1**, the AEE (82.24%) process was more efficient than AE (76.73 %). The use of an enzymatic blend increased (5.51%) the oil efficiency. Macauba plant cell has a complex structure, composed of cellulose, hemicellulose, lignin, and pectin, along with a cellular membrane and layers of proteins and phospholipids surrounding the body oils (Favaro, S. et al; 2022). This composition forms a strong barrier that hinders oil release. As an initial pre-step of AEE, mechanical crushing disrupts the pulp tissue, improving enzyme access to substrates. This interaction is enhanced during malaxation with vigorous shaking at 350 rpm (Sorita, G. et al; 2024). The AE and AEE efficiencies achieved in this work were higher than AEE from others raw materials, cacate (*Oecopetalum mexicanum*) (65%) (Ovando, S. et al; 2018), Safflower (*Carthamus tinctorius* L.), 70.45% (Benkirane, C. et al; 2022) and *Acer truncatum Bunge*, 37.94% (Hu, X. et al; 2022).

Nowadays, macauba pulp oil has been industrially recovered by mechanical pressing (Rivaldi, J. et al., 2022). In this extraction process, the pulp requires a preliminary drying step that demands high energy consumption. Furthermore, after extraction, the efficiency is low (~55%, Sorita, G. et al., 2024), yielding a cake with a high residual oil content (Rivaldi, J. et al., 2022). AEE can be a viable and simpler alternative to innovate the traditional oil extraction process, fitting the ONU goals.

An important parameter to determine the quality of macauba oil is acidity; it allows for measuring the level of rancidity of the oil, that is, it indicates how high the decomposition of triacylglycerides is within the extraction process (Favaro, S. et al; 2022). Based on **Figure 2**, the acidity percentages of the acids obtained (4.54% and 4.69%) are within the range accepted by the Codex Alimentarius for palm oil, as regulations for macauba oil have not yet been established (Pombo, J. et al., 2021). These results suggest that the oil exhibits low triacylglycerides formation, indicating that the AEE and AE processes are suitable for obtaining high-quality oil. Similar results were found by Sorita, G, et al, 2024 (1 – 3.5 % oleic acid) and Faravo S. et al, 2022 (1.5 % oleic acid) in the AEE and AE of macauba pulp oil. Furthermore, the acidity range of macauba pulp oil found in this work were higher than the oil recovery by mechanical pressing (0.75 % oleic acid) and similar or with those obtained by solvent extraction (4.2 % oleic acid Favaro S. et al, 2022). Several factors can contribute to oil's increase in acidity during aqueous extraction, including oxidation, prolonged extraction time, or high temperatures. Controlling these factors during the extraction process can minimize the increase in acidity and ensure the quality of the final product (Favaro, S. et al; 2022).

Characterization of the liquid by-product

The liquid by-product generated after obtaining macauba oil was characterized to determine its potential industrial applications, aligning with the circular economy concept. **Figure 3a** presents the total solid content of AEE and AE extractions. The AEE (5.92) resulted in a slightly higher solid content than the AE (5.27), because the enzymes break the pectic and cellulose to smaller structures, facilitating the solids migration towards the liquid (Ahmadi M. et al; 2013).

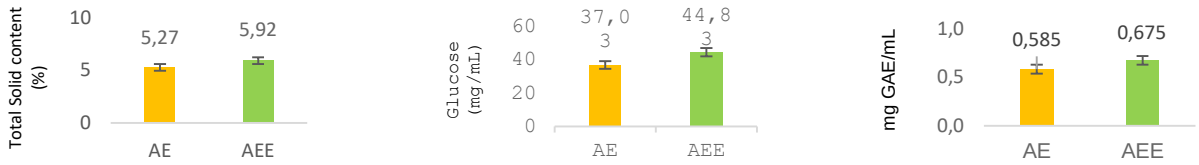

Figure 3. Characteristics of the liquid by-product. (a) Total solids content, (b) Total carbohydrate content, (c) Total phenolic content.

Figure 3b shows the total carbohydrate content of the liquid by-product from AEE and AE. Using cellulases and pectinase enzymes improved the recovery of the carbohydrates (44.83 mg Glu mL^{-1}) compared with the process performed without enzymes (37.03 mg Glu mL^{-1}). The higher total carbohydrate content in the solid by-product shows that this by-product can be used in food industries as prebiotics or to produce second-generation ethanol. However, a more robust characterization is required to select the appropriate use of this fraction, such as identifying low molecular weight carbohydrates and quantifying and identifying xylooligosaccharides and celooligosacarídes.

The liquid by-products of AE and AEE are also a rich source of water-soluble phenolic compounds. These compounds have strong antioxidant capacity and are continually sought after by food researchers aiming to replace chemical ingredients with natural ones to mitigate the side effects of chemical additives in foodstuffs. **Figure 3c** presents the TPC of the liquid by-products (AE and AEE). Consistent with the total carbohydrate content, slightly higher TPC values were achieved for AE (0.585) than AEE (0.675). Those results are also expected since cellulolytic and pectinolytic enzymes can degrade the cellular wall, improving the liberation and recovery of phenolic compounds. Macauba pulp was shown to be a rich source of phenolic compounds.

4 CONCLUSION

This study has effectively established a green and alternative biotechnological method for recovering oil and other valuable compounds from macauba pulp. The AEE process demonstrated high oil efficiency (76.73%) while preserving low acidity level (4.9% oleic acid). AEE also provided a liquid by-product that was rich in phenolic compounds (0.675 mg GAE mL^{-1}) and hydrolyzed carbohydrates (44.83 mg Glu mL^{-1}), suggesting potential applications in various industries. Separating these products through membrane technologies can boost the functionality of this valuable by-product, improving the quality of products and opening up new market avenues for natural and bioactive ingredients.

ACKNOWLEDGEMENTS

The authors thank Embrapa Agroenergia and the EQA/UFSC partner laboratories for collaborating with analysis procedures.

REFERENCES

1 Arroyo, M. Acebal, C. De la Mata, I. 2014. "Biocatálisis y biotecnología". Arbor, 190 (768): a156. doi: http://dx.doi.org/10.3989/arbor.2014.768n4010

2 Sorita, G. Palma, S. De Sousa, D. Da Silva, W. De Oliveira, W. Ambrosi, A. Di Luccio, M. 2024. Aqueous enzymatic extraction of macauba (Acrocomia aculeata) pulp oil: A green and sustainable approach for high-quality oil production. https://www.sciencedirect.com/science/article/pii/S0963996924002308

3 Favaro, S. Smidt, M. Miranda, C. Leal, G. Carvalho, F. Rivaldi, J. 2022. Aqueous extraction to high yield and quality of macauba (Acrocomia aculeata) pulp oil. Applied Food Research, 2(1), 100060. https://doi.org/ 10.1016/j.afres.2022.100060.

4 López, X. Taramuel, A. Arboleda, C. Segura, F. Restrepo, L. 2017. Comparación de métodos que utilizan ácido sulfúrico para la determinación de azúcares totales. Rev. Cubana Quím, 29(2), 2224-5421.

5 Singleton, V. Orthofer, R. Lamuela, R. 1999. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. Methods in Enzymology, 299, 152-178. http://dx.doi.org/10.1016/S0076-6879(99)99017-1

6 Ovando, S. Ovando, G. Borraz, D. Meza, R. 2018. OPTIMIZATION OF THE AQUEOUS ENZYMATIC EXTRACTION OF OIL FROM Oecopetalum mexicanum. https://espacioimasd.unach.mx/index.php/Inicio/article/view/161/530

7 Benkirane,C. Allay, A. Ben Moumen, A. Belhaj, K. Melhaoui, R. Mansouri, F. Abid, M. Serghini, H. Elamrani, A. 2022. Aqueous Enzymatic Extraction of safflower (Carthamus tinctorius L.) seed oil: A preliminary study. 10.1051/e3sconf/202233704010

8 Hu, X. Wang, J. Zhang, W. Zhang, F. Tian, S. Zhou, Z. Ren, Z. 2022. Aqueous enzymatic extraction and quality evaluation of Acer truncatum Bunge seed oil https://onlinelibrary.wiley.com/doi/10.1002/cjce.24623?af=R

9 Rivaldi, J. Smidt, M. Rodas, J. Velázquez, M. 2022 Manual de recolección, almacenamiento y procesamiento de frutos de Mbokaja (Acrocomia aculeata) (4) (PDF) Manual de recolección, almacenamiento y procesamiento de frutos de Mbokaja (Acrocomia aculeata) (researchgate.net)

10 Pombo, J. Marcos C. Barroso, C. Ribeiro, D. Sousa, S. 2021. QUALIDADE FÍSICO-QUÍMICA DOS ÓLEOS BRUTOS DE PALMA E PALMISTE.https://www.gvaa.com.br/revista/index.php/REBAGRO/article/download/8743/10717#:~:text=O%20par%C3%A2metro%20de%20qualidade%20de,Alimentarius%20(FAO%2C%202019).