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BIOPROCESS ENGINEERING

Development of biotechnological and sustainable processes for obtaining high-value products from macauba (*Acrocomia aculeata*) pulp

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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 (Embrapa. 2004). Enzymes and microorganisms are crucial in producing valuable components for food, pharmaceuticals, and agriculture industries. These biological agents can serve as precursors for obtaining primary metabolites, including vitamins, nucleotides, amino acids, and secondary metabolites (Kordi, M. et al., 2022). 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). Moreover, integrating environmental technologies to extract oil and other valuable compounds is an important strategy to contribute to the circular economy development in oil industries (Gao, Y. et al., 2024).

Aqueous enzymatic extraction (AEE), an emerging biotechnological process, is a sustainable method for obtaining oils, sugar, proteins, phytochemicals, and other valuable compounds from different materials. This innovative method applies water as the solvent and specific enzymes to catalyze the degradation of cellular structures (Amahdi, M. et al., 2013). In AEE, the raw material is first ground to facilitate the and suspended in water, followed by the addition of enzymes such as proteases, cellulases, or pectinases, which facilitate the release of target components. This process is conducted under controlled temperature and pH conditions, maximizing extraction efficiency, and producing 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. It has recently emerged as an eco-friendly oil extraction method. Many advantages are achieved compared to conventional oil extraction: the raw material is processed without a drying pre-treatment, avoiding high energy spending (Arroyo, M. et al., 2014 and Sorita, G. et al., 2024). Also, two rich by-products are formed, a liquid and solid fraction, with compositions varying according to the specific characteristics of the raw material and the enzymes applied in the process. The liquid fractions are rich in hydrolyzed molecules, such as cello oligosaccharides, pectic compounds, amino acids, or short molecules of proteins. In contrast, the solid fraction can be a valuable source of fibers and other non-hydrolyzed compounds (Gao, Y. et al., 2024).

Both fractions produced in AEE, the liquid and solid, can be utilized as value-added products to create new formulations or to increase the value of existing ones. For instance, the hydrolyzed sugar in the liquid by-product can be used as prebiotics in food formulations or as substrates for other biotechnological processes, such as second-generation ethanol production (Soleum. 2024).

Proteins, another valuable component, can be used to produce vegan-based products or enhance the nutritional content of existing products (Soleum. 2024). These applications highlight the versatility and potential of this biotechnological process, making it a green and environmentally friendly approach.

In this context, macauba (*Acrocomia aculeata*) pulp is identified as a promising oleaginous source for AEE due to its high humidity content. Indigenous to Tropical and Subtropical America, this palm species holds significant potential for sustainable oil production for biofuels and food applications. Its fruits are characterized as globe-shaped with rigid skin, with color ranging from yellow to brown depending on ripeness (Soleum, 2024). The pulp of these fruits boasts a high oil content, with oleic acid being the predominant component, along with carotenoids. Its bioactive composition qualifies it as a reducer of LDL and a promoter of HDL in the body (Aoqu, M. 2012). Per cultivated hectare, the macauba palm produces about 9 tons of oil compared to the African palm, which only produces 3.8 tons per hectare (Soleum. 2024). Due to this, international companies are highly investing in producing those fruits in Brazil for biofuels, showing the high economic importance of this culture (Soleum. 2024). Given the substantial investment in this crop, developing green and sustainable processes for the simultaneous recovery of oil and other valuable compounds is paramount and merits significant research efforts.

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.

2 MATERIAL & METHODS

Materials

Macauba (*Acrocomia aculeata*) fruits from the experimental area of Embrapa Cerrados, Planaltina/DF. The selected fruits (after removing damaged fruits) were washed, pulped, and stored in polyethylene bags (at -8 °C) until AEE processing. Olimax 101 (Prozyn – São Paulo-SP, Brazil), a pectinase enzyme pool, 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 in this study 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) equipped with an anchor-type stirrer and a temperature sensor was used to evaluate the AEE on a larger scale. The operational parameters (300 rpm at 50 °C) were set according to previous tests (Favaro, S. et al., 2022 and Sorita, G. et al., 2024), 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% (concerning the 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 chemical properties 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).

Equation 1:

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

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.

To measure total solids were determined following the methodology described by American Public Health Association; American Water Works Association- Water Environment Federation (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.

Equation 2:

$$\mathrm{TSC}\ (\%) = \frac{(MCD - MC)}{\mathrm{V}}\ \mathrm{X}\ 100$$

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 and Masuko, T. et al; 2005 and Suzanne, N. 2017. In a test tube, 29µL of the diluted sample (1:1000) was mixed, and 29µL of 5% phenol solution and 142µL 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 taken at 490nm. 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 according to Singleton, V. et al; 1999 and Kuhnen, S. et al; 2011 protocols. Briefly, 600µL of water, 10µL of diluted sample (1:1 v/v), and 50µL of Folin reagent were mixed, then 150µL sodium carbonate (Na_2CO_3) and 190L µL of water were added. The mixture was reacted for 2 hours without the presence of light. Subsequently, readings were carried out in a spectrophotometer at a wavelength of 760nm (in triplicate). The sample absorbances were corroborated with a gallic acid standard curve (R^2 = 0,9968). The results were expressed EAG mg mL^{-1}.

3 RESULTS & DISCUSSION

Free oil efficiency and acidity

The free oil efficiency in extracting oil from macauba pulp by AE and AEE is presented in **Figure 1**.

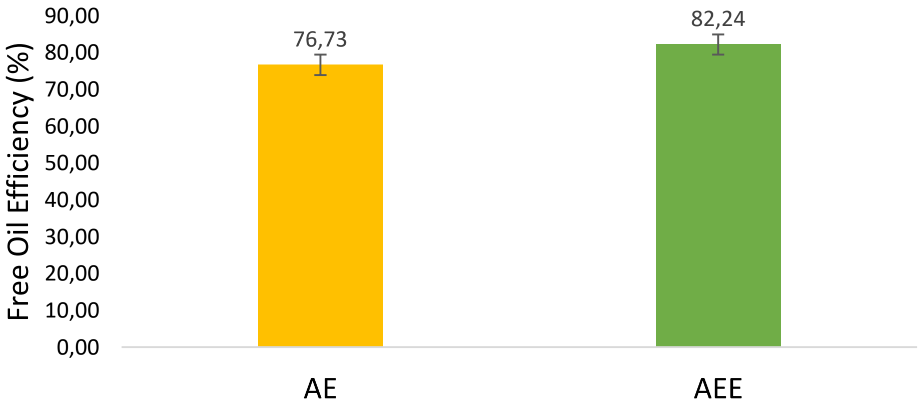

Figure 1. Free oil efficiency extraction of AE and AEE.

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; Oliveira, I. et al; 2017). The results from oil acidity are presented in **Figure 2.**

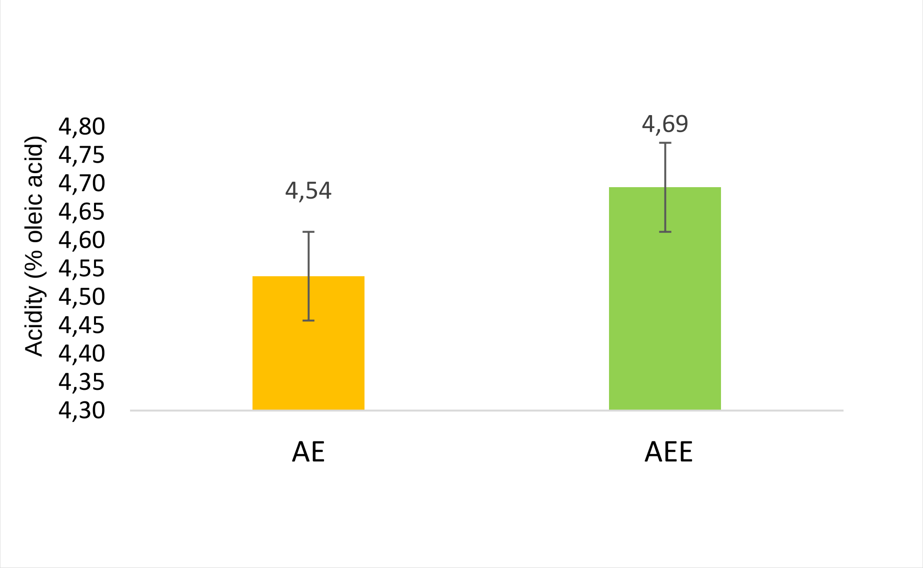

Figure 2. Acidity (%oleic acid) of macauba pulp oil.

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 founded 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 founded 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 liquid by-product

The liquid by-product generated after obtaining macauba oil was characterized to determine its potential industrial applications. Understanding its composition and providing valuable information about its nutritional content can lead to applications in the food industry for human consumption, as well as in pharmaceuticals and other biotechnological processes. This approach aligns with the AEE process and the circular economy concept. The aqueous residue obtained was subjected to characterization assays to identify and quantify the composition of compounds that may present commercial interest and propose interesting insights to further separation and concentration processes, such as membrane processes.

Figure 3 presents the total solid content of AEE and AE extractions. As expected, AEE (5,92) presented a slightly higher solid content than AE (5,27). This was expected since, the enzymes break the pectic and cellulose structures, then the smaller structures, the solids migrate towards the liquid (Ahmadi M. et al; 2013) then an increase in their concentration occurs.

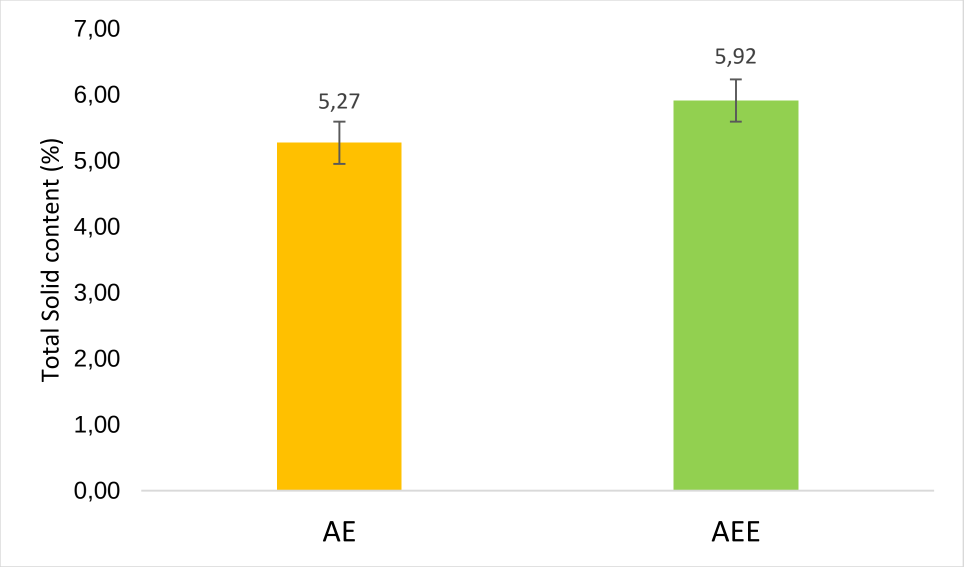

Figure 3. Percentage of total solids in each extraction sample analyzed

Figure 4 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}).

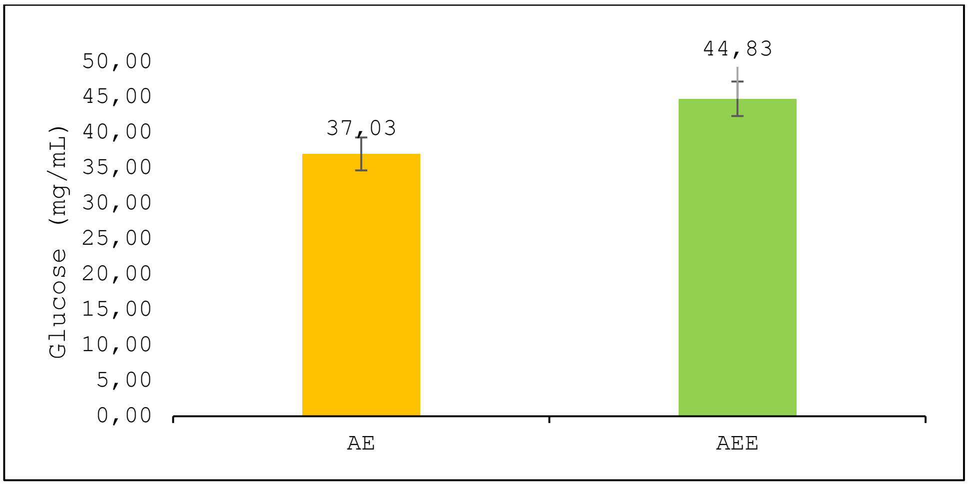

Figure 4. Total carbohydrate content of liquid by-product

The higher total carbohydrate content in the solid by-product shows that this by-product can be used in food industries as prebiotics (Bamigbade, G. et al; 2022) or for the production of second-generation ethanol (Soleum, 2024). 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 celooligosacarídeos.

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. Numerous efforts have been made to identify natural antioxidants from plant sources (Ghandahari, A. et al; 2018), primarily due to safety concerns and the toxicity of synthetic antioxidants

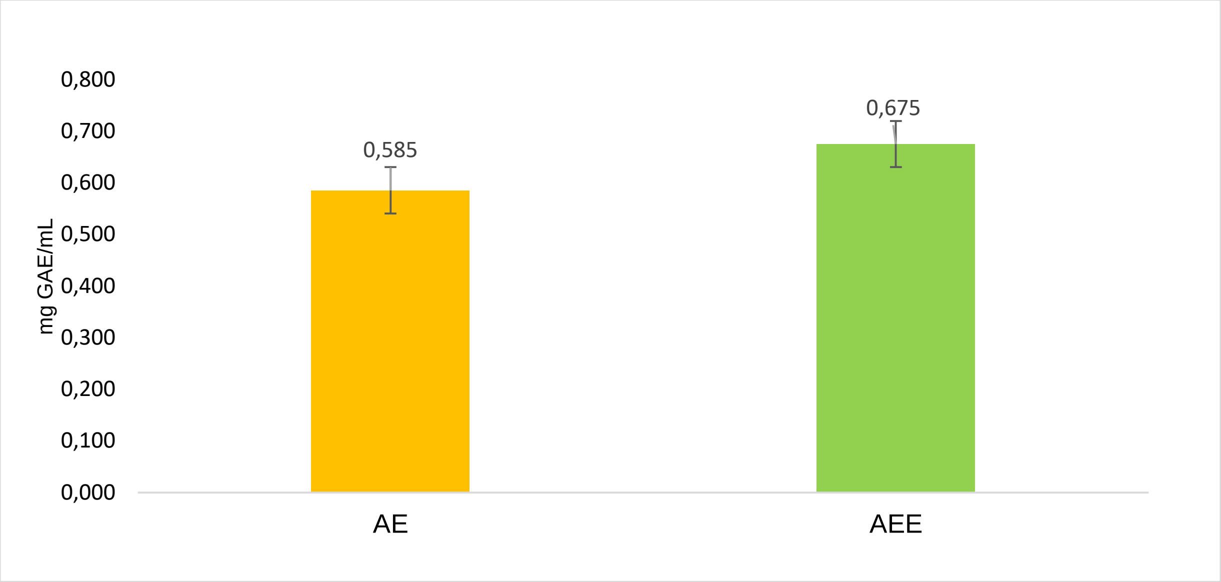

Figure 5. Total phenolic content (mg GAE mL^{-1}) of the liquid by-product of AE and AEE.

Figure 5 presents the TPC of the liquid by-products (AE and AEE). Consistent with the total carbohydrate content, slightly higher TPC values were achieved for AEE (0,585) than AE (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 (Cascaes, A. et al; 2021)

Macauba pulp was shown to be a rich source of phenolic compounds. For instance, (Dos Santos, J. et al; 2021) identified the phenolic composition of macauba pulp and showed the high predominance of conjugated phenolics in the pulp. Flavanols were the main class in the conjugated (86.73%). The authors identified catechin, epicatechin gallate, epigallocatechin gallate, and procyanidin B2 as the main flavonol compounds. Also, the same study showed the antioxidant potential of the phenolic fraction of macauba pulp (DPPH: 109.73 µg TEAC 100 g^{-1}, and ORAC: 104.47 µg TEAC 100 g^{-1}).

These compounds exhibit antioxidant, anti-inflammatory, antimicrobial, anticancer, and neuroprotective properties. They have been studied extensively for their potential therapeutic applications in various diseases and health conditions, including cardiovascular diseases, cancer, diabetes, and neurodegenerative disorders (Cascaes, A. et al; 2021). The recovery and separation of these compounds through membrane processes into aqueous by-products of macauba pulp oil is an environmentally friendly and ecological practice. This approach minimizes waste and presents an opportunity to add value to oil industries. By recovering flavonoids and other bioactive compounds from aqueous streams, these industries can enhance their product offerings with high-value ingredients that can be used in food, pharmaceuticals, and cosmetic formulations. Despite the good TPC results, a more robust characterization is required to identify and link the phenolic compounds with the respective biological activity, such as HPLC-MS/MS.

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, achieving a remarkable rate of 76.73% while preserving oil quality with a low acidity level (4.9% oleic acid). AEE also provided a rich liquid by-product that was rich in phenolic compounds (0.675mg GAE mL^{-1}) and hydrolyzed carbohydrates (44.83 mg Glu mL^{-1}), suggesting potential applications in various industries, such as food, pharmaceuticals, cosmetics, and nutraceuticals for their antioxidant properties and other bioactive functionalities. Separating those products through membrane technologies can boost the functionality of this valuable by-product, improving the quality of end products and opening up new market avenues for natural and bioactive ingredients.

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