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Aqueous-Based Extractions of Macauba (*Acrocomia aculeata*) Pulp Oil: Process Optimization, Scale-up Reproducibility, and Oil Quality

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ABSTRACT: Aqueous extraction processing (AEP) and aqueous enzymatic extraction (AEE) were optimized and scaled up for macauba pulp oil recovery. Initially, the process parameters were evaluated on a laboratory scale. Optimal conditions, enzyme concentration (pectinase, AEP: 0%, AEE: 1% v/v), extraction time (2 h), temperature (50 °C), and stirring rate (350 rpm) were selected for higher oil efficiency. Subsequently, four scale-up trials were conducted: AEP, AEE-pectinase, AEE-cellulase, and AEE-pectinase/cellulase. AEE-pectinase/cellulase system showed the highest oil yield (73%) compared to AEP (51%). The oils had acidity of 2.76−2.93% (oleic acid) and K270 values between 0.16 and 0.19, indicating good oxidative stability. Carotenoid levels varied between 25 and 30 $\mu g\, g^{-1}$, and the fatty acid profile showed elevated amounts of oleic (71%) and linoleic (10%) acids. In conclusion, the scale-up of AEP/AEE proved to be viable, ensuring efficient oil recovery and preserving product quality, highlighting its potential for industrial macauba oil production within the context of sustainable and green food processing industries.

KEYWORDS: *vegetable oil, aqueous enzymatic extraction, pectinase, cellulase, fatty acids*

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1. INTRODUCTION

Macauba is a commercially underexplored Neotropical palm with outstanding food-grade vegetable oil production potential. It provides two distinct oil sources: pulp oil, rich in monounsaturated fatty acids and carotenoids, suitable for the food and biofuels industries, and kernel oil, high in saturated fatty acids, ideal for cosmetics.[1]

Currently, macauba fruits are predominantly harvested from natural populations by smallholder farmers, forming a small-scale processing system. Recent studies estimate that, at planting densities of 450 and 320 plants ha^{-1} in monocropping and integrated systems, each macauba plant produces three to seven bunches per year, corresponding to 320−1080 fruits per plant, which results in estimated yields of 144,000−486,000 and 102,400−345,000 fruits ha^{-1}, respectively.[2,3] Driven by this productive potential, companies such as INOCAS and Acelen Renováveis are developing commercial plantations in Brazil, projected to exceed 50,000 ha by 2030, supplying macauba oil for biofuels, food, and cosmetic applications.[4]

Due to its high tolerance to water-deficits soils, macauba is a strong alternative to major oilseed crops like oil palm, which requires wetter conditions to thrive,[2] contributing to the diversification of the oil crops. Despite those advantages, recovering oil from pulp poses significant industrial challenges. The pulp can contain up to 60% water by mass,[3] making conventional extraction methods (mechanical pressing) unsuitable due to the high energy required for the drying process before extraction. Recently, aqueous extractions (with or without enzyme assistance) have emerged as a green and emerging technology for recovering oil from high-humidity content raw materials.[5−8] This technology can also be applied as an emerging and innovative extraction technique to recover oil from the macauba pulp.

AEP/AEE is an environmentally friendly and safe extraction method, as it occurs in an aqueous medium without generating toxic waste.[8] Its mild temperatures reduce energy consumption compared to solvent extraction, and some enzymes can be reused, enhancing sustainability and cost-effectiveness.[9] However, several factors should be carefully evaluated to improve the oil extraction efficiency and quality and make the process economically viable, including enzyme type, enzyme concentration, solid-to-liquid ratio, degree of seed grinding, stirring speed, extraction temperature, medium pH, and incubation time.[7,10]

The enzyme type is crucial in aqueous enzyme extraction (AEE), affecting cell wall degradation and oil release. Different enzymes target specific components, influencing hydrolysis efficiency and oil quality.[11] After selecting the optimal enzyme, carefully choosing the enzyme concentration is crucial to maximize oil extraction efficiency and minimize costs.[10] While optimal levels enhance yield, excessive concentrations can lead to off-flavors and increased production costs.[10,12] Balancing efficiency and costs is essential for the economic viability of the extraction process.

Enzymes operate best within specific temperature and pH ranges, and variations in those ranges can reduce efficacy,

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leading to incomplete extraction or degradation of the oil and other valuable components.[13] High temperatures can facilitate the oil recovery by decreasing the viscosity of the medium; however, they can compromise fatty acid stability and degrade thermolabile phytochemicals.[6,14] Buffers are often employed to maintain pH levels during enzymatic processes, but their use can increase costs and complicate the treatment of liquid byproducts,[15] particularly at industrial levels. Therefore, the benefits of buffers must be carefully weighed against their environmental and economic implications.

Stirring rate also significantly impacts the oil extraction efficiency and quality. High agitation enhances enzyme−substrate interaction but may cause the incorporation of oxygen to the system, leading to the oxidation and compromising oil quality.[16,17] Low agitation reduces mixing, reducing enzyme−substrate contact and lowering extraction efficiency.[18] Finally, the last but not least important parameter to optimize is extraction time. While prolonged AEE enhances yield, excessive duration can increase oil acidity, degrade compounds, and reduce profitability.[7] Balancing the extraction time ensures quality and economic viability.

Up to now, a previous study by our research group evaluated the enzymatic activity of different commercial enzyme pools for AEE of macauba pulp oil on the laboratory scale. Among the enzymes tested, pectinase and cellulase/hemicellulase pools demonstrated the most promising results, yielding high oil recovery rates. This previous work guided the selection of the most promising enzymes for further optimization in AEE, as it focused on enzyme types but did not evaluate the influence of key process parameters on the extraction efficiency. In this study, extraction was performed under extreme conditions, such as prolonged extraction times and high enzyme concentrations, which are economically unfeasible for industrial application. Despite these conditions, the extracted oil exhibited good quality, demonstrating the viability of the aqueous-based extraction technique as an alternative to conventional mechanical pressing.

Based on the most promising enzymatic pools identified by Sorita et al.[17] and considering the importance of process parameters in aqueous-based extraction processes, particularly regarding scale-up feasibility, the present study aimed to (i) evaluate, at laboratory scale, the effect of key process parameters (enzyme concentration, extraction time, temperature, and stirring rate) on AEE of macauba pulp oil using a pectinase pool, and, in parallel, assess the same parameters (excluding enzyme concentration) for AEP to identify the optimal conditions for a high-efficiency process; and (ii) scale up both optimized AEP and AEE to assess the feasibility of increasing the process scale, evaluate reproducibility, and identify potential challenges for industrial application. To support this assessment, the oils obtained from the scale-up trials were characterized in terms of process efficiency, oil quality (acidity, moisture content, oxidative stability as measured by K232 and K270 indices, and total carotenoid content), and identity parameters (fatty acid profile and iodine value).

2. MATERIALS AND METHODS

2.1. Materials. Macauba (*Acrocomia aculeata*) fruits (50 kg, approximately) were carefully selected and collected from the experimental area of Embrapa Cerrados (Planaltina, DF, Brazil; 15°36′09.6″S 47°43′34.5″W). The selected fruits were washed, pulped, and stored in polyethylene bags (at −8 °C) until aqueous extraction.

Preliminary nutritional characterization of macauba fruits was performed before AEP/AEE extractions (in triplicate) based on AOAC methodology.[19] Pulp moisture was determined in an oven at 105 °C. Oil content of pulp and residual solids was measured using an XT15 extractor (ANKOM Technologies). Protein content was determined via CHNS/O elemental analysis (PerkinElmer PE2400) using a conversion factor of 6.25. Crude fiber and ash were analyzed by acid/alkali digestion (Ankom 200, AOCS Ba 6a-05) and thermogravimetric analysis (TGA 801, LECO), respectively. The nutritional composition (on a wet basis) of the fruits was: moisture 40.58% ± 0.01, oil 32.83% ± 0.70, protein 2.85% ± 0.07, carbohydrate 10.60% ± 0.38, ash 1.58% ± 0.04, and fiber 11.55% ± 1.03.

The enzymes used in this study were (i) Cellic CTec3 - Novozymes - Araucaria-PR, Brazil (224 U mL^{-1}) composed of cellulase and hemicellulase complex, and (ii) Olimax 101 - Prozyn − São Paulo-SP, Brazil (993.106 U mL^{-1} g^{-1} min^{-1}), a pectinase enzyme pool. The chemicals used in this study were analytical grade or HPLC grade. Deionized water and citrate buffer were the solvents in the extractions.

2.2. Methods. *2.2.1. Laboratory-Scale Assays. 2.2.1.1. AEP/AEE Extraction Procedure.* AEP/AEE was conducted following the method described by Sorita et al.[17] In summary, 30 g (12.2 g dry weight) of fresh macauba pulp was homogenized with an aqueous solution (water or 0.1 mol L^{-1} citrate buffer) at a mass ratio of 1:1 pulp/liquid, using a food blender (Mixer Phillips 1336). Enzymes were then added at concentrations ranging, according to the following sections, from 0.5 to 6.3% (enzyme mass/homogenate mass), while the AEP was conducted without enzyme. Based on the highest process efficiency results and the good oil quality from our previous study,[17] Olimax 101 was selected to optimize the AEE process parameters (extraction time, enzyme concentration, temperature, and stirring rate).

The stirring rate was controlled using an IKA Overhead Stirrer (RW20, IKA, Germany) apparatus in a temperature-controlled reactor (Alpha RA8, Lauda, Germany). After the extraction, the enzyme inactivation was performed by placing the reaction medium in a water bath (Solab, model SL1506) at 90 °C for 10 min. Following enzyme inactivation, the samples were centrifuged (RC6 plus, Thermo Scientific) at 12,500 rpm (18,725*g*) for 10 min to separate the four phases: free oil, cream emulsion, liquid, and solid fractions. Each fraction was carefully separated, and the process efficiency was calculated according to eq 1. The total oil content (theoretical mass) of the raw material was quantified using petroleum ether with a fat extractor (XT15, ANKOM Technologies, Macedon, NY, AOCS, Am 5−04 method).

$$\text{efficiency}\ (\%) = (m_{\text{freeoil}}/m_{\text{oilmacaubapulp}}) \times 100 \quad (1)$$

where m_{freeoil} is the mass of free oil obtained after extraction (g), and $m_{\text{oilmacaubapulp}}$ is the theoretical mass of oil (g).

2.2.1.2. Evaluating the Extraction Time by Kinetics Studies. The first parameter evaluated in the AEP/AEE process was extraction time, utilizing a destructive kinetic mode. The extraction time was investigated through three kinetic assays with varying enzyme concentrations: (i) 0% (control) using water, (ii) 1%, and (iii) 6.3%.[17] Assay (i) employed water as the solvent, while assays (ii) and (iii) used a 0.1 mol/L citrate buffer (pH 5.5).

The extractions were conducted in batch mode, following the protocols outlined in Section 2.2.1.1. The temperature (50 °C) and stirring rate (350 rpm) were chosen based on our prior study.[17] For both AEP and AEE, the kinetic assays were carried out over 8 h. All assays were performed in triplicate, and the oil efficiency (%) was calculated according to eq 1.

Three models were fitted to the oil extraction efficiency (%) versus time (hours) data to evaluate the kinetic parameters: (i) first-order, (ii) pseudo-second order, and (iii) the Elovich model. These models were applied to provide insights into various aspects of the extraction kinetics, facilitating a comprehensive analysis of the process.

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Nonlinear regressions were performed using OriginLab 2022 software. The equations and evaluated parameters for each model are provided in the Supporting Information, eqs S1−S3 for (i), (ii), and (iii), respectively.

2.2.1.3. The Enzyme Concentration Evaluation. The enzyme concentration was optimized to minimize excessive use and to reduce process costs. For this, AEP/AEE were conducted by varying Olimax 101 concentrations from 0.25 to 1.25% (considering the total mass of the reactive medium, pulp + water or buffer), with the following fixed parameters: 350 rpm, 50 °C for 2 h (determined by the kinetics assays) using citrate buffer (0.1 mol L^{-1}) or water, with the extraction procedure described at Section 2.2.1.1. As previously reported, although using a buffer can enhance the extraction process by stabilizing enzyme activity, it also increases the cost and complicates wastewater treatment. Therefore, enzyme activity was evaluated in both buffer and water to assess its effectiveness under different conditions while aiming to minimize industrial costs.

2.2.1.4. Effect of Temperature and Stirring Rate on Process Efficiency of AEP and AEE at Laboratory Scale. The experimental design for AEP followed a 2^2 full-factorial design (FFD), while AEE was evaluated by using a central composite rotational design (CCRD). Both considered temperature (°C) and stirring rate (rpm) as independent variables, with levels defined in Table 1, and oil efficiency as the response variable.

Table 1. Experimental Values and Coded Levels of the Independent Variables (Temperature and Stirring Rate) Used in the 2^2 Full-factorial Design (AEP) and CCRD Design (AEE, with Pectinase Assistance) of Macauba Pulp Oil

AEP - FFD design			
	levels		
variable	−1	0	+1
A − temperature (°C)	30	50	70
B − rotation (rpm)	125	275	425

AEE - CCRD design					
	levels				
variable	−1.41	−1	0	+1	+1.41
A − temperature (°C)	36	40	50	60	64
B − rotation (rpm)	209	250	350	450	491

This selection of different models was driven by the specific requirements of each process: AEE requires a broader range of conditions to optimize enzymatic activity. It was hypothesized that enzymatic extraction at lower temperatures could yield better efficiencies than nonenzymatic extraction at higher temperatures.

The choice of experimental design variable values considered the preliminary tests, equipment limitations, and conditions that would allow the preservation of oil quality. For temperature, a maximum limit of 60 °C was defined to minimize the oxidation of unsaturated fatty acids in the lipid fraction, which negatively influences the oil quality.[6] The temperature range was also considered to be the optimal activity of Olimax 101, which is between 40 and 60 °C (product data sheet), with an optimal temperature of 55 °C.

For AEP, the extraction procedures were performed according to Section 2.2.1.1 using deionized water as solvent (1:1 w/w) for 4 h (determined according to kinetics results). AEP experiments were performed in duplicate with three replicates at a central point.

AEE experiment optimization employs a commercial pectinase pool Olimax 101 (1% w/w) using 0.1 mol L^{-1} citrate buffer as a solvent for 2 h (defined in kinetic assay). The enzymatic reaction time was initiated with the addition of the enzyme and stopped by inactivating the enzyme (as described in Section 2.2.1.1). Duplicate of each point was carried out. Tables 2 and 3 show each variable's coded and actual values for AEP and AEE, respectively. The generated design for AEP contains 7 experiments, with 4 factorial (in replicates) and 3 central points, while the AEE design contains 11 experiments with 3 central, 4 factorials, and 4-star points. The response (dependent variable) was the oil efficiency (%), calculated according to eq 1. For both designs, the model accuracy was evaluated using the regression coefficient (R^2), the *p*-value of the model, and the lack of fit.

2.2.2. Scaling Up AEP and AEE of Macauba Pulp Oil. 2.2.2.1. Extraction Procedure. Scale-up assays were conducted to evaluate the reproducibility of AEP and AEE laboratory-scale tests for macauba pulp oil extraction. Extractions were conducted in a 5 L stainless-steel jacketed reactor (Kiloclave Type 4, Büchi GmbH, Switzerland) equipped with an anchor-type stirrer and a temperature sensor. Aqueous extractions were performed using 2000 g of pulp with water as the solvent instead of a buffer to simplify the process and avoid the introduction of additional chemicals, aligning with a greener and more sustainable approach. The optimal operational parameters were defined in Section 2.2.1.4: a solid/liquid ratio of 1:1 (w/w), stirring at 350 rpm, and a temperature of 50 °C. For AEE, the enzyme concentration was set according to Section 3.2 (1%). Temperature was regulated using a circulating bath (Julabo, Germany), and the extraction time was fixed at 2 h based on kinetic assays.

Although kinetic studies indicated that 4 h was optimal for AEP, shorter extraction times were selected for scale-up trials to compare AEP and AEE directly. Based on previous findings highlighting the high efficiency of pectinase (Olimax 101) and cellulase/hemicellulase (Cellic CTec3) enzyme pools,[17] their combination was also tested to assess its potential for improving oil yield and quality. Consequently, four aqueous extraction treatments were conducted and compared: (i) AEE with Olimax 101 (AEE-pectinase), (ii) AEE with Cellic CTec3 (AEE-cellulase), (iii) AEE with a 1:1 (w/w) combination of Olimax 101 and Cellic CTec3 (AEE-pectinase/cellulase), and (iv) without enzyme assistance (AEP).

After 2 h of extraction, the final mixture was heated to 90 ± 2 °C for 10 min to inactivate the enzymes. The reaction medium was then transferred to separation funnels for solid decantation and subjected to centrifugation (Thermo Scientific, RC6 Plus) at 8000 rpm (7871*g*) for 10 min to separate the oil. Extractions were performed in triplicate, and process efficiency was determined using eq 1.

2.2.2.2. Characterization of Macauba Pulp Oil. Oil Humidity: Moisture content was determined according to the drying loss method described by AOAC.[19] Oil samples were heated in a drying oven at 105 °C until a constant weight was achieved. Moisture was estimated based on the difference between the initial and final sample weights, attributed to water evaporation.

Free Fatty Acids and Conjugated Dienes and Trienes: The extracted oils were chemically characterized to evaluate their quality based on acidity, and molar absorptivity (K232 and K270). The acidity, expressed as an oleic acid percentage (%) of the extracted oils, was determined using a potentiometric titrator (Titrando 809, Metrohm, Switzerland) following the Ca 5a-40 method.[19] Additionally, the specific extinction coefficients (molar absorptivity) at 232 nm (K232) and 270 nm (K270) were measured as indicators of primary and secondary oxidation, respectively, using the Ca 6a-40 method.[19] This assay was conducted with a spectrophotometer using iso-octane-grade UV/HPLC as the solvent.

Total Carotenoid Content (TCC): TCC[20] was measured by diluting oil samples in 10 mL of petroleum ether, and absorbance was recorded at 450 nm using a spectrophotometer (Shimadzu, Japan). The results were expressed as $\mu g\ g^{-1}$.

Fatty Acid Profile and Iodine Index: The fatty acid profile was analyzed following the Ce 1−62 method, which involved converting the oil samples into fatty acid methyl esters (FAME).[19] The detection of FAME was performed by using gas chromatography coupled to a mass spectrometry detector (MDGC/GCMS-2010, Shimadzu, Japan), utilizing a capillary column (OmegaWax, 15 m × 0.10 mm × 10 μm, Supelco). The initial column temperature was set at 120 °C and subsequently increased at 25 °C/min until reaching 260 °C, where it was maintained for 5 min. The injector temperature was set to 250 °C, with a split ratio of 200:1. The flow rates for hydrogen, synthetic air, and helium were 40, 400, and 30 mL min^{-1}, respectively,

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Table 2. Experimental Design Matrix and Recovery Efficiency of Macauba Pulp Oil Obtained in Aqueous Extraction Processing (AEP) at Laboratory Scale (Independent Variables: Temperature and Stirring rate; Dependent Variable: Oil Efficiency)[a]

Run	Variables (Coded level)		Oil extraction efficiency (%)	Visual inspection of the result after centrifugation
	X1 Temperature (°C)	X2 Stirring rate (rpm)		
1	30 (-1)	125 (-1)	19.38 ± 4.19	
2	70 (+1)	125 (-1)	44.85 ± 0.45	
3	30 (-1)	425 (+1)	4.49 ± 0.51	
4	70 (+1)	425 (+1)	46.19 ± 2.34	
5	50 (0)	275 (0)	33.29	
6	50 (0)	275 (0)	32.43	
7	50 (0)	275 (0)	38.55	

[a]Experiments for runs 1−4 were conducted in duplicate, and results are expressed as mean ± standard deviation. The central point was performed in triplicate, and individual values are presented.

and a 1 μL volume was injected. Fatty acids were identified by comparing relative retention times to those of a FAME standard (C13:0, Sigma-Aldrich, 99.89% purity). Quantification was done by normalizing the peak areas of the FAMEs against the total peak area in the GC Solution software. Results (%) are reported as mean values, along with their standard deviations. The iodine index (g I_2 100 g^{-1}) was calculated based on the fatty acid profile, following the protocols outlined by AOAC.[19]

Nutritional Quality: The nutritional quality of macauba oils was evaluated according to Chen & Liu,[21] using two indexes: atherogenicity index (AI, eq 2) and thrombogenicity index (TI, eq 3).

$$\mathrm{AI} = (\mathrm{C12{:}\,0} + (4 \times \mathrm{C14{:}\,0}) + \mathrm{C16{:}\,0}) / (\Sigma\,\mathrm{MUFA} + \Sigma\,\mathrm{MUFA} + \Sigma\,\omega 6 + \Sigma\,\omega 3) \quad (2)$$

$$\mathrm{TI} = (\mathrm{C14{:}\,0} + \mathrm{C16{:}\,0} + \mathrm{C18{:}\,0}) / [(0.5 \times \Sigma\,\mathrm{MUFA}) + (0.5 \times \Sigma\,\omega 6) + (3 \times \Sigma\,\omega 3) + (\Sigma\,\omega 3 / \Sigma\,\omega 6)] \quad (3)$$

C12:0, C14:0, C16:0, *and* C18:0: the mass percentage of lauric, myristic, palmitic, and stearic acids, respectively; MUFA: monounsaturated fatty acids; $\omega 6$ and $\omega 3$: relative percentage mass of C18:2 (linoleic) and C18:3 (linolenic) acids, respectively.

2.3. Statistical Analysis. All tests were performed in triplicate, and the results are expressed as means ± standard deviations. The experimental design results (FFD and CCRD) were analyzed (ANOVA) with a confidence level of 95% applying the Response Surface Methodology (RSM). Tukey test was conducted to find significant differences between treatments ($p < 0.05$). All statistical analyses were performed with the Software Statistica (Statsoft Inc.).

3. Results and Discussion. *3.1. Selection of the Extraction Time.* Kinetic studies were performed to identify the optimal processing time for the AEP and AEE procedures. Based on the overall extraction curve, the mass transfer mechanisms were assessed using first-order, pseudo-second order, and Elovich's models. Model performance was evaluated by using the coefficients of determination (R^2), root-mean-square error (RMSE), and residual mean square (RMS). Higher R^2 and lower RMSE values indicate a better fit to the experimental data.[22] The fitted parameters for the models are detailed in Table S1 (Supporting Information), and Figure 1 and illustrate the fitted kinetics curves.

Kinetic studies offer valuable insights into extraction mechanisms. As indicated in Table S1, all models exhibited fitted R^2 values greater than 0.9, suggesting a good fit for explaining the oil release mechanism of macauba pulp by AEP and AEE. The first-order model was determined to be the most suitable for AEP (kinetic (i)), as it showed the highest R^2 and the lowest RMSE and RMS values among the models tested. For the lab-scale aqueous extraction without enzyme addition, a reaction time of 4 h (Figure 1A) was necessary to achieve stable oil recovery efficiency (65.2%), with no significant difference observed compared to efficiencies at 6 h (66.1%) and 8 h (66.67%), as confirmed by the ANOVA Tukey test.

Regarding assays performed with 1% (kinetic (ii)) and 6.3% (kinetic (iii)) of pectinase (Figure 1B,C, respectively), the data was better fitted by the Elovich model, with determination coefficients (R^2) of 0.99 and 0.98, respectively. The kinetics curves (i) and (iii) showed a high initial adsorption rate and progressively reduced oil efficiency until the end of the process. In assay (ii), performed with 1% of Olimax 101, the oil extraction efficiency in the first 13 min was 41.26%, while for assay (iii), with a higher enzyme concentration (6.3%), it was 53.77%. The higher enzyme concentration leads to a higher reaction velocity in the first 30 min.

The experimental data in Figure 1 show that the extraction processes occurred in two stages. The first stage is characterized by a rapid extraction rate, observed at 4, 3, and 2.5 h for assays (i), (ii), and (iii), respectively. In this stage, the macauba oil extraction efficiency increases fast, corroborating the high values of q_e (first- and pseudo-second-order models) and A (Elovich's model) parameters (Table S1, Supporting Information). Pectin constitutes the middle lamella, and its function is to bind the cells together, while it is also part of the primary cell wall matrix. Degradation of the pectin may significantly increase the oil release by reducing the barrier effect, reported as one of the dominant resistances for mass transfer.[23]

In the first stage, the mechanical treatment (stirring rate) in kinetics ((i), without enzyme addition, AEP), probably fragments the external structures surrounding the oil, facilitating the solubilization of the small polar compounds by the water, as well as the oil droplet coalescence and separation. For assays (ii) and (iii), the pectin

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Table 3. Experimental Design Matrix and Recovery Efficiency of Macauba Pulp Oil Obtained in Aqueous Enzymatic Extraction (AEE) at Laboratory Scale (Independent Variables: Temperature and Stirring Rate; Dependent Variable: Oil Efficiency)[a]

Run	Temperature (°C)	Stirring rate (rpm)	Oil extraction efficiency (%)	Visual inspection of the result after centrifugation
1	36 (-1.41)	350 (0)	75.35[a] ± 0.57	
2	64 (+1.41)	350 (0)	60.30[b] ± 2.59	
3	50 (0)	350 (0)	66.45[ab] ± 6.40	
4	50 (0)	350 (0)	62.88 [ab] ± 0.80	
5	50 (0)	350 (0)	65.65 [ab] ± 6.14	
6	50 (0)	209 (-1.41)	74.09[ab] ± 0.92	
7	50 (0)	491 (+1.41)	72.41[ab] ± 0.46	
8	40 (-1)	450 (+1)	68.29[ab] ± 7.47	
9	60 (+1)	450 (+1)	72.83[ab] ± 3.55	
10	40 (-1)	250 (-1)	64.29[ab] ± 2.55	
11	60 (+1)	250 (-1)	70.80[ab] ± 1.02	

[a]Legend: Means (n = 2) followed by the different letters in the columns indicate a significant statistical difference by Tukey's test ($p \leq 0.05$)

structures are hydrolyzed by the pectinase pool, which weakens (together with the mechanical treatment) the oil-pectin-water emulsion. The destabilization of those systems facilitates the solubilization of the hydrolyzed pectin by the water, breaking the emulsion and consequently releasing the oil. In addition, the quick breakdown of the most superficial pectin molecules facilitates the penetration of enzymes (with the assistance of mechanical stirring rate) into the most internal structures of the plant structure, increasing the efficiency of the process[24] in a short extraction time. This effect was not observed at assay (i), in which at least 4 h was necessary to achieve an efficiency higher than 50%.

The second stage tends to be slow in all reactions. For assay (i), the mechanical action after 4 h was not able to break the more internal pulp structures, decreasing the extraction rate. For enzymatic kinetics (AEE, assays (ii) and (iii)), after 3 and 2.5 h, the enzymatic substrate (pectin) is depleted, and the remaining oil is probably emulsified by other compounds (such as proteins and carbohydrates). The kinetics mechanism, explained by first- and pseudo-second-order and Elovich models has also led to a good fitting of the aqueous enzymatic extraction kinetics from other vegetable raw materials, such as passion fruit seed oil[25] and *Mentha* leaf oil.[26]

The extraction time was set considering the low oil extraction efficiency variation and that long extraction times can decrease the oil quality due to the extraction of other unwanted compounds during this period.[18] Few variations in efficiency were observed from 4 and 2 h for AEP and AEE up to the end of the reaction. Then, those incubation times were established for the following laboratory-scale experiments.

3.2. Optimization of Enzyme Concentration. Once the extraction time was defined (2 h, Section 3.1), AEE were conducted using pectinase (Olimax 101) at concentrations ranging from 0 to 1.25% to study the effect of enzyme concentration (% w/w, regarding pulp + solvent) and the type of solvent (buffer or water) on the extraction efficiency (%) of macauba pulp oil. The results are shown in Figure 2.

For the extraction performed with water (Figure 2), the extraction efficiency increased with an increase in enzyme concentration, achieving an efficiency value of 58.59% using 1.25% of the enzyme. It is important to point out that a low-efficiency increase (9.3%) was observed with the increase of the enzyme concentration from 0.5 to 1.25%. Besides, no significant differences in oil recovery efficiency were observed in the extractions performed with water and buffer for 0.25 and 0.5% enzyme concentrations.

The extraction using citrate buffer 0.1 mol L^{-1} (Figure 2) did not present the same behavior as the extraction performed with water. The maximum efficiency (68.55%) of oil recovery was observed at 1.25% (statistically equal to 1 and 0.75%). Silva et al.[27] reported a

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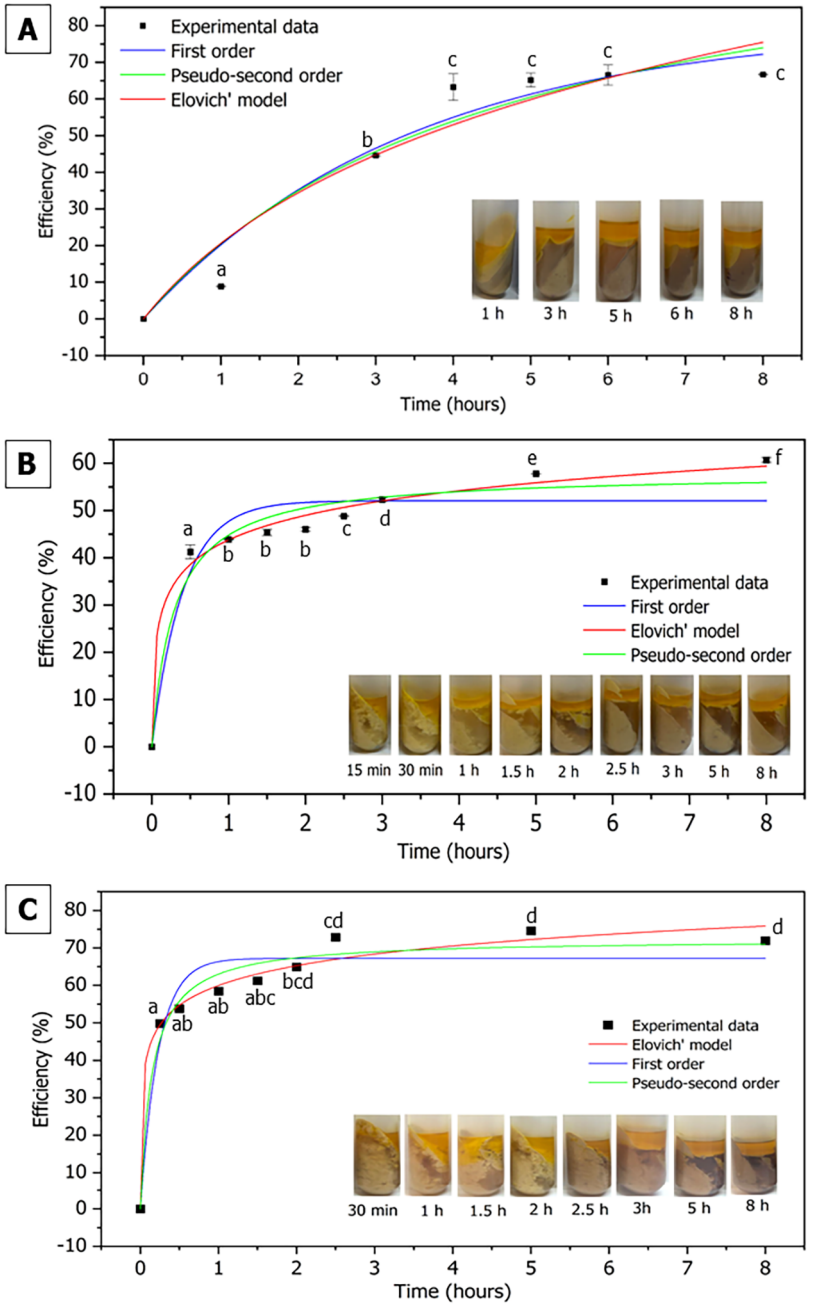


Figure 1. Extraction kinetics curves (First- and pseudo−second−order and the Elovich model) for (A) kinetic (i) − AEP, without enzyme, 1:1 w/v solid/liquid ratio, at 50 °C 350 rpm, (B) kinetic (ii) − AEE using pectinase (Olimax 101) at 1% (w/w), 1:1 w/v solid/liquid ratio, at 50 °C 350 rpm, and (C) kinetic (iii) − AEE using pectinase (Olimax) at 6.3%. (w/w), 1:1 w/v solid/liquid ratio, at 50 °C 350 rpm.

similar efficiency value of 67.8% in aqueous enzymatic oil extraction from tucumã (*Mauritia Flexuosa*) pulp using pectinase (1.32% w/v) at 45 °C for 2 h. Given the compositional and structural similarities between tucumã and macauba fruits, comparable extraction efficiencies can be expected under optimized enzymatic conditions, particularly with respect to enzyme concentration.

In summary, considering the industrial process applicability, the high enzyme costs, and the low oil efficiency rate increase after the enzyme concentration of 1% for extractions using water and buffer, these concentrations were considered optimal for subsequent assays.

3.3. Effect of Temperature and Stirring Rate on AEP and AEE Process Efficiency. 3.3.1. AEP: Full-Factorial Design (FFD). To efficiently extract oil from macauba pulp by an aqueous medium (without enzymes), a full-factorial design (FFD) combined with response surface methodology was applied. The effects of temperature and stirring rate on oil efficiency (%), and the figure of each run (after centrifugation steps) are shown in Table 2.

The analysis of variance (ANOVA) for the experimental results of the FFD is shown in Table S2 (Supporting Information). A good fit was achieved, considering the coefficient of determination ($R^2 > 0.9$) and the adjusted coefficient of determination (R^2-adj = 0.85), respectively, indicating that the empirical model explained at least 93% of the data for the oil efficiency (%) of macauba pulp. A lack-of-fit test was used to support the adequacy and how well the model fitted the data, and results showed that the model could adequately fit the experimental data (p-value >0.05).

The first-order model equation developed by FFD for oil extraction efficiency is presented in eq S4 (Supporting Information). The regression coefficients of the model allowed for the determination of the influence of each independent variable on the responses. A higher

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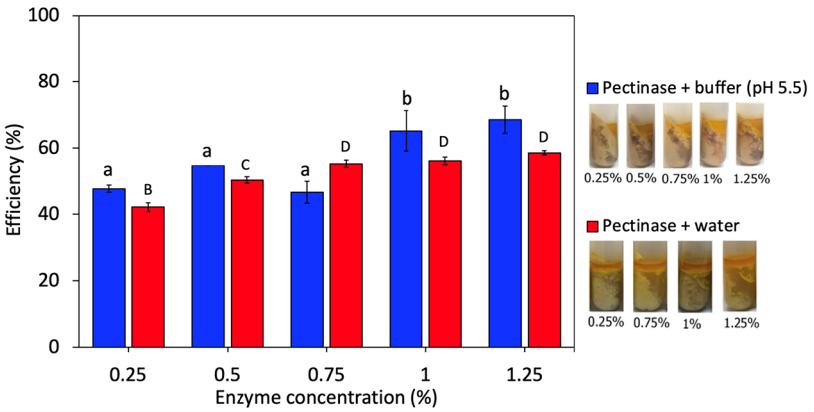


Figure 2. Effect of pectinase (Olimax 101) concentration (0.25−1.25%) on AEE oil extraction efficiency (%) using water and buffer (citrate buffer 0.1 mol L^{-1}, pH 5.5). Legend: Different lowercase and uppercase letters indicate a significant statistical difference for the effect of enzyme concentration test using citrate buffer and water, respectively, by Tukey's test ($p \leq 0.05$).

absolute coefficient value implies a greater effect on the independent variable, and the sign indicates if this contribution increases (+) or decreases (−) the response.[28] Furthermore, the temperature ($X1$) and its interaction with stirring rate ($X1*X2$) influenced the response positively, and the stirring rate ($X2$) had a negative effect. Wei et al.[14] also observed no significant effect of stirring rate (rpm) on *Cinnamomum camphora* seed oil yield extracted using ultrasound-assisted aqueous enzymatic extraction (UAAEE).

Figure S1 (Supporting Information) shows the correlation between the predicted and observed efficiency (%) value of macauba pulp oil obtained at different temperatures and stirring rate conditions. The observation of Figure S1 (Supporting Information) corroborates the good fitting of the model, where the values predicted from the model were in satisfactory agreement with those obtained from the real data. Based on the statistical tests, the model was accepted as suitable for representing the correlation between the oil extraction efficiencies and the temperature and stirring rate.

The highest (46.19%) and the lowest (4.49%) oil recovery efficiency were obtained at 70 °C/425 rpm and 30 °C/425 rpm, respectively. Figure 3A,B show that temperature has the most important effect on extraction efficiency, agreeing with several AEP and AEE studies, such as almonds,[29] tiger nuts,[30] pine nuts,[31] and tucumã pulp.[6]

In summary, increasing the temperature from −1 (30 °C) to +1 (70 °C) improves the oil extraction efficiency. Generally, increasing the process temperature could reduce the surface tension and viscosity of the extraction medium, promoting the diffusion of the solution into the matrix and, consequently, improving the extraction efficiency.[30] However, high process temperatures are not preferred in many aspects, such as process economy and product quality. The exposure of macauba pulp to higher temperatures (>60 °C) may cause the degradation of phytochemicals (such as carotenoids and phytosterols) and promote hydrolytic reactions of the triacylglycerides, increasing the oil acidity, which may compromise the oil quality.[32] Those reactions are not desired in food industries since they confer a rancid smell and off-odor to the oil, while in biofuels, increased acidity can impair conversion efficiency and raise processing costs.

As reported previously, the model showed that the stirring rate did not significantly affect the oil extraction efficiency. However, the stirring rate should be cautiously analyzed since it can influence oil extraction through several physical mechanisms depending on its intensity. A higher stirring rate can increase oil release by rupturing intact macauba pulp cells, creating the shear and turbulence, and rupturing the solid matrices that entrap oil within the disrupted cells (after the grinding treatment). This effect increases the contact of the small oil droplets that exit more efficiently out of the cellular matrix into the bulk fluid and facilitates the coalescence.[11] However, it can also introduce oxygen, leading to oxidation, which can compromise oil quality.[16] Thus, for this study, a moderate stirring rate is preferred.

After calculating the response desirability profile (employing the Statistica 13.0 software), the optimal conditions for AEP were determined as 70 °C and 350 rpm, with a model predicting 48.79% of process efficiency. Thus, considering the optimum conditions predicted by the mathematical model, the industrial costs, technological relevance of macauba pulp oil, and considering that high process temperature can promote negative quality on macauba pulp oil, a temperature of 50 °C and a stirring rate of 350 rpm at a solid/liquid ratio of 1:1 (m/v) were set for the scale-up trials.

3.3.2. AEE: Central Composite Rotational Design (CCRD). As previously reported, a central composite design (CCRD) was applied to evaluate the effect of temperature (°C) and stirring rate (rpm) on oil efficiency from macauba pulp by AEE. For this reason, the temperature and stirring rate ranges selected were 36−64 °C and 209−491 rpm, respectively. The effects of the temperature and stirring rate on the oil efficiency (%) are shown in Table 3.

The analysis of variance (ANOVA) for the experimental results of the CCRD is shown in Table S3 (Supporting Information). The ANOVA analysis reveals that the quadratic term of the stirring rate is the only significant factor in oil efficiency (p = 0.01). Linear and quadratic terms of temperature, the linear term of stirring rate, and the interaction between those parameters are not statistically significant ($p > 0.05$). Those results suggest that within the selected range, the enzyme activity is relatively stable and not highly sensitive to changes in temperature and linear stirring rate.

The model explains only 28% of the variability (R^2 = 0.28) and has a low adjusted R^2 (0.06), suggesting a poor fit. Moreover, the significant lack of fit (p = 0.01) indicates that the model does not adequately describe the relationship among the variables. Given the lack of significant impact of temperature and linear stirring rate on oil efficiency, it can be inferred that lower values of these parameters might achieve efficiencies similar to those of higher values within the tested range. This result benefits process optimization, suggesting that lower agitation rates and temperatures can be used without compromising oil extraction efficiency.

Thus, the Tukey test was applied to Table 3 data to evaluate the differences between the treatments, and it was possible to observe that only runs 1 and 2 presented, statistically, oil efficiency differently. The only differences between those runs were temperature, run 1 (36 °C) and run 2 (64 °C), the first run at level −1.41, and the second at level +1.41. Temperatures above 60 °C probably denature the pectinase pool, decreasing the oil efficiency.

Comparing the AEP (Section 3.3.1) and AEE models, the presence of the enzyme provides several advantages despite a less well-fitting model. The enzyme allows for stable activity across various temperatures and stirring rates, indicating flexibility in operating conditions without a large loss of efficiency. This consistency makes

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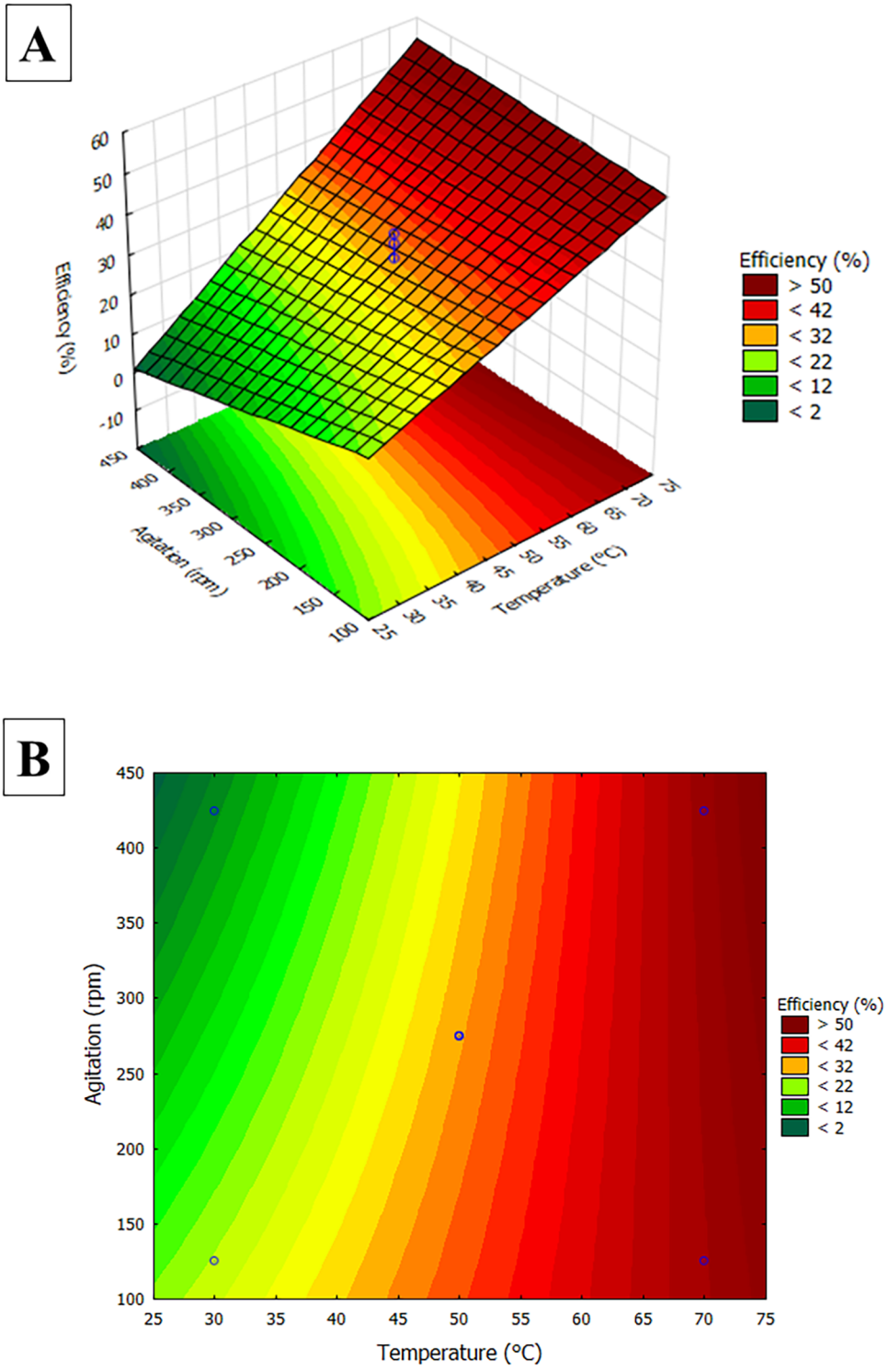


Figure 3. Surface response (A) and contour (B) plots of the efficiency of the AEP of macauba pulp oil.

the process feasible with lower temperatures and stirring rates, leading to significant operational savings.

Thus, considering the oil recovery efficiency (%) presented in Table 3 and analyzing the results individually and based on Tukey's mean comparison test, the temperature was set at 50 °C (best results from runs 6 and 7), and the stirring rate was set at 350 rpm (the best result of run 1, setting 50 °C). These parameter values were also chosen to match those chosen for AEP (section 3.3.1), facilitating a direct comparison between AEP and AEE on the scale-up trial.

3.4. Reproducibility of AEP/AEE in Scaling-Up Trials. 3.4.1. Process Efficiency. After the investigation of the main parameters that influence the oil recovery efficiency and quality by AEP and AEE of macauba pulp oil on a laboratory scale, scale-up trials were conducted to identify the feasibility and the potential problems that might occur on an industrial commercial scale. The results for oil recovery efficiency (%) and chemical characterization (oil humidity, acidity, TCC, K232, and K270) are shown in Table 4.

The scaling up of AEP and AEE applied for macauba pulp oil recovery presented process efficiencies ranging from 51 to 73%, respectively. All AEE methods exhibited statistically similar efficiencies, indicating that adding Olimax, Cellic CTec3, or both enzymes to the extraction process did not lead to a statistically

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Table 4. Oil Recovery Efficiency (%) and Chemical Characterization (Oil Humidity, Acidity, TCC, K232, and K270) of AEP and AEE Performed in a 5 L Reactor[b]

Efficiency and Chemical properties	Extraction Method					
	AEP	AEE-pectinase	AEE-cellulase	AEE-pectinase/cellulase	[a]SE	[a]MP
Efficiency (%)	$51.00^{c} \pm 0.05$	$67.00^{b} \pm 0.02$	$71.00^{b} \pm 0.02$	$73.00^{b} \pm 0.06$	$96.82^{a} \pm 1.00$	$63.55^{c} \pm 6.64$
Humidity (%)	0.31 ± 0.17	0.21 ± 0.07	0.26 ± 0.04	0.21 ± 0.01		
Acidity (oleic acid, %)	$2.76^{a} \pm 0.01$	$2.93^{a} \pm 0.37$	$2.14^{b} \pm 0.12$	$2.46^{ab} \pm 0.02$	$0.36^{c} \pm 0.01$	$0.59^{c} \pm 0.25$
K 232	$4.98^{a} \pm 0.50$	$2.48^{b} \pm 0.10$	$2.53^{b} \pm 0.12$	$2.65^{b} \pm 0.07$	$1.23^{c} \pm 0.18$	$1.66^{c} \pm 0.07$
K 270	$0.16^{a} \pm 0.01$	$0.17^{a} \pm 0.10$	$0.19^{a} \pm 0.01$	$0.18^{a} \pm 0.01$	$0.11^{b} \pm 0.03$	$0.11^{b} \pm 0.01$
TCC ($\mu g\ g^{-1}$)	$28.82^{c} \pm 0.82$	$30.16^{c} \pm 0.78$	$27.58^{c} \pm 0.34$	$25.02^{c} \pm 0.23$	$71.98^{a} \pm 1.73$	$51.33^{b} \pm 6.37$

[a]Data extracted from Sorita et al. .[17] Means followed by the different letters in the same line indicate a significant statistical difference by Tukey's test ($p \leq 0.05$). [b]Legend: AEP: aqueous extraction processing (without enzyme), AEE-pectinase: AEE performed with Olimax 101, AEE-cellulase: AEE performed with Cellic CTec3, AEE-pectinase/cellulase: AEE was performed with Olimax 101 and Cellic CTec3 (1:1 w/w). Values are expressed as mean ± standard deviation.

significant difference in the oil extraction efficiency. Moreover, using water instead of a buffer solution did not affect the process efficiency, highlighting a key advantage: eliminating the need for buffering agents simplifies the process, reduces costs, and minimizes potential environmental impacts. These efficiency values were comparable or higher than those observed in Section 3.3.2 (Table 3, experiments 3, 4, and 5), demonstrating that the process efficiencies obtained at the laboratory scale were successfully reproduced in the pilot-scale trials. All experiments from the AEE scale up showed efficiencies statistically higher than mechanical pressing in the study by Sorita et al.[17] (63.55%), showing a good performance and an important green alternative to conventional extraction methods.

Another positive result is that the efficiencies obtained from the AEP were slightly higher than the optimum conditions predicted by the FFD (Section 3.3.1) at 70 °C and 350 rpm. Those differences may be related to the extractor's equipment used in the different tests. The reactor used in the scale-up has better sealing than the reactor used in the laboratory scale and may have prevented the solvent from escaping; furthermore, the scale-up reactor presented baffles around the reactor, which probably improved the stirring rate and the cell wall breaking, facilitating oil release.

It is evident that there is a lack of scaling-up studies of AEP and AEE in the literature. For instance, Favaro et al.[9] performed AEE of macauba pulp oil (0.2 kg of wet pulp at a solid−liquid ratio of 1:2.5 w/w at 50 °C) using cellulase pool (Cellic Ctec3) with water as solvent in a pilot scale. The authors achieved an efficiency of 85.7% (calculated based on the oil remaining at the solid residue), and only a small fraction of oil was recovered in the free form. Otherwise, it is important to note that in this work a significant amount of oil recovered from the aqueous extractions was obtained in free form. Those differences can also be attributed to the maturation stages of the fruits. However, enhancement is still necessary to improve oil efficiencies and to increase competitiveness with well-established methods. A study by Dias et al.[15] scaled up the AEE for simultaneous oil and protein recovery from almond flour. In contrast to this work, the authors chose a different strategy. First, AEP was performed (0.7 kg of almond flour, a solid−liquid ratio of 1:10 w/v, 50 °C, and pH 9), generating three products (liquid, skim-protein-rich, and cream-oil rich fractions). Then, the cream was demulsified using enzymatic (pH 8.0, 50 °C, 0.8% w/w of the enzyme) and chemical demulsification methods (pH 5.0 and 55 °C). The processes achieved slightly lower efficiencies than the present work: 65.77 and 65.53%, respectively. Furthermore, the enzymatic treatment reduced 29% of the extraction time and provided a greater concentration of unsaturated fatty acids (C18:2 and C18:3). However, it is crucial to note that Dias et al.[15] used a solid−liquid ratio of 1:10, resulting in significant water usage (10 L of solvent per kg of pulp). In contrast, the present study employed a much lower solid/liquid ratio of 1:1, enhancing resource efficiency and minimizing the environmental impact related to water treatment.

3.4.2. Chemical Properties. Evaluating the chemical properties of macauba oil is key to ensuring product quality, process efficiency, and regulatory compliance in scale-up studies. The chemical properties of the oils obtained by aqueous extraction and conventional methods are also presented in Table 4.

Acidity is an important index to evaluate the rancidity and oxidation of oil samples. The acid value shows the free fatty acid contents in oils, and a higher acid value always means a more severe oxidation of oils.[32] The aqueous extractions resulted in the following acidity values: AEP (2.76%), AEE-pectinase (2.93%), AEE-cellulase (2.14%), and AEE-pectinase/cellulase (2.46%). All values were within the acceptable low-grade acidity range (<5%) for palm oil according to Codex Alimentarius (1999), as no standard exists for macauba pulp oil.

The extinction coefficients K232 and K270 are useful parameters to evaluate the oil's oxidative status. The highest K232 value was observed on AEP (4.98), followed by AEE treatments (AEE-pectinase: 2.48, AEE-cellulase: 2.53, and PS-AEE-pectinase/cellulase: 2.65) oils. Favaro et al.[9] found a lower value (2.9) for K232 and a higher value (0.33) for K270 (0.33) for macauba pulp oil obtained in AEE on a pilot scale using cellulase enzyme (Cellic CTec3).

Although the oils obtained at pilot scale were classified as having acceptable quality, their acidity (2.14−2.93%) and K232 values (2.48−4.98) were notably higher than those reported in our previous study for solvent- (0.36%) and mechanically extracted (0.59%) macauba oils.[17]

Although the acidity and molar absorptivity values observed in the oils extracted by AEP/AEE in this study are higher than those reported for conventional methods in previous studies, this difference cannot be attributed solely to processing conditions. Oil acidity is significantly influenced by intrinsic factors of the raw material, such as the degree of ripeness and postharvest storage time of the fruit prior to processing.[3] Previous studies have shown that the harvest season, physiological state, and storage conditions of the fruit strongly affect endogenous lipolytic activity, resulting in variations in free fatty acid content.[33] In this case, the fruits used by Sorita et al.[17] originated from a different batch than those used in the present study, which, in parts, may explain the discrepancy in acidity and molar absorptivity values. Therefore, direct comparison of acidity values between studies should be approached with caution, considering the variability in fruit lots and raw material conditions.

The TCC ranged from 25.02 to 30.16 $\mu g\ g^{-1}$; these values are lower than those reported by Tilahun et al.[34] (83.44 $\mu g\ g^{-1}$), and the differences may be related to the processing conditions and the genetic diversity within this species.

In summary, although differences in process parameters (such as extraction time and enzyme concentration) and extraction apparatus may influence the chemical characteristics of the macauba pulp oil obtained by AEP and AEE, the measured values remain within the quality limits established by international standards, suggesting that the oil meets acceptable quality criteria even on larger scales. This consistency indicates that scaling up the AEE process does not adversely affect the oil quality, suggesting that the process is robust and scalable without compromising the product's chemical properties

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Table 5. Fatty acid (FA) Composition, Iodine Index, and Nutritional Properties of AEP and AEE Oils from Macauba Pulp Obtained in the Scale-Up Trials[a,b]

	extraction method			
fatty acid (%)	AEP	AEE-pectinase	AEE-cellulase	AEE-pectinase/cellulase
lauric, C12:0	0.06[a,b] ± 0.01	0.03[b] ± 0.01	0.08[a] ± 0.01	0.07[a] ± 0.01
myristic, C14:0	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
palmitic, C16:0	11.40[b] ± 0.02	11.69[a] ± 0.08	11.66[a] ± 0.12	11.54[a,b] ± 0.03
palmitoleic, C16:1	1.51[b] ± 0.01	1.57[a] ± 0.02	1.58[a] ± 0.02	1.52[b] ± 0.01
stearic, C18:0	1.23 ± 0.01	1.23 ± 0.01	1.19 ± 0.06	1.25 ± 0.01
oleic, C18:1 (ω9, n9c)	71.68[a] ± 0.04	70.82[b] ± 0.19	71.07[b] ± 0.20	71.46[a] ± 0.09
oleic, C18:1 isomer	2.91[a,b] ± 0.02	3.16[a] ± 0.25	3.01[a,b] ± 0.03	2.83[b] ± 0.02
linoleic, C18:2 (ω6, n6cis)	9.95[b] ± 0.01	10.22[a] ± 0.06	10.16[a] ± 0.06	10.04[b] ± 0.03
linoleic, C18:2 (ω6, n6trans)	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.01
linolenic, C18:3 (ω3, n3)	0.77 ± 0.01	0.82 ± 0.01	0.80 ± 0.03	0.81 ± 0.01
eicosenoic, C20:1 (ω11, n9)	0.16 ± 0.01	0.17 ± 0.03	0.16 ± 0.01	0.16 ± 0.01
heneicosanoic, C21:0	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.03	0.13 ± 0.01
SFA	12.92 ± 0.03	13.16 ± 0.03	13.16 ± 0.17	13.11 ± 0.04
MUFA	76.27[a] ± 0.02	75.72[b] ± 0.02	75.82[b] ± 0.15	75.97[b] ± 0.06
PUFA	10.81[c] ± 0.01	11.12[a] ± 0.05	11.02[a,b] ± 0.07	10.92[b,c] ± 0.03
PUFA/SFA	0.84 ± 0.01	0.84 ± 0.01	0.84 ± 0.01	0.83 ± 0.01
MUFA/UFA	0.88[a] ± 0.01	0.87[b] ± 0.01	0.87[a,b] ± 0.01	0.87[a,b] ± 0.01
II (g I_2 100 g^{-1})	86.91 ± 0.02	87.00 ± 0.03	86.91 ± 0.21	86.87 ± 0.03
AI	0.14[b] ± 0.01	0.14[a,b] ± 0.01	0.14[a] ± 0.01	0.16[a] ± 0.01
TI	0.28 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0.28 ± 0.01

[a]Legend: SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, UFA = unsaturated fatty acids (MUFA + PUFA), II = iodine index, AI = atherogenicity Index, TI = thrombogenicity index, AEP = aqueous extraction processing (without enzyme) in a pilot scale, AEE-pectinase = AEE performed with Olimax 101 in a pilot scale, AEE-cellulase = AEE performed with Cellic CTec3 in a pilot scale, AEE-pectinase/cellulase = AEE performed with Olimax 101 and Cellic CTec3 (1:1 w/w) in a pilot scale. [b]Values expressed as mean ± standard deviation, means followed by the different letters in the same line indicate a significant statistical difference by Tukey's test ($p \leq 0.05$).

(at this scale-up stage). This reliability is crucial for industrial applications, showing that larger-scale operations can replicate the results achieved in the laboratory, leading to predictable and controlled outcomes.

Currently, there is no standardized harvesting method for macauba, and the development of such a method should consider the ripening stage of the fruits. This is crucial as fruit maturity directly influences extraction efficiency and oil quality. Implementing better control over fruit selection and harvesting conditions could help improve these parameters, reduce quality losses, and support the establishment of a more consistent and efficient extraction process.

Additionally, no significant differences in efficiency and oil quality were observed when using pectinase, cellulase/hemicellulase, or both enzymes in AEE, implying that the choice of enzyme can be flexible, allowing selection based on other factors such as cost, availability, and ease of use, without compromising process efficiency or the quality of the final product. Nevertheless, further research is required to enhance the proposed green extraction technique and make it more economically feasible for industrial manufacturing.

3.4.3. Identity Properties (Fatty Acid Profile and Iodine Index) and Nutritional Index. The fatty acid profile of the extracted macauba pulp oils under AEP and AEE scale-up tests was analyzed, and the statistical difference between the extraction protocols was tested ($p \leq 0.05$). The results are presented in Table 5.

Monounsaturated fatty acids (MUFA) constitute the most abundant fraction observed in the extracted oils, primarily palmitoleic acid (C16:1), oleic acid (C18:1), and eicosenoic acid (C20:1). Oleic acid (C18:1, ω9) was the predominant fatty acid, ranging from 73.98% (AEE-pectinase) to 74.59% (AEP). Interestingly, using a blend of pectinase and cellulase enzymes showed statistically equal values to AEP, showing that for this specific fatty acid, the use of this enzyme blend did not affect the oil's oleic acid composition, compared with the process without enzyme.

The saturated fatty acids (SFA) ranged from 12.92 to 13.16% (AEP, AEE-pectinase, and AEE-cellulase, respectively). Palmitic acid (C16:0), stearic acid (C18:0), myristic acid (C14:0), heneicosanoic acid (C21:0), and lauric acid (C12:0) were the saturated fatty acids identified in macauba pulp oils.

Two polyunsaturated fatty acids (PUFA), linoleic acid (C18:2, ω6, n6cis, and n6trans) and linolenic acid (C18:3, ω3), were observed in the oils. Similar values of PUFA were observed for AEE-pectinase (11.12%) and AEE-cellulase (11.02%), and slightly lower values were achieved for AEP (10.81%) and AEE-pectinase/cellulase (10.92%). Among the PUFAs, linoleic acid, C18:2 (ω6, n6cis) was the most predominant (AEP: 9.95, AEE-pectinase: 10.22, AEE-cellulase: 10.16, and AEE-pectinase/cellulase: 10.04).

Identity Index (II) measures the unsaturated content or double bonds that can react with iodine. It is defined as the percentage of iodine absorbed by the oil. It is a valuable metric for assessing the degree of unsaturation in macauba pulp oil fatty acids.[6] The values for II across different scale-up aqueous extraction treatments of macauba pulp oil were ~87 g of I_2 100 g^{-1}, reflecting the consistency in the degree of unsaturation in their fatty acid profiles.

Nutritional indexes, particularly AI and TI, may be used to evaluate the potential impacts of macauba pulp fatty acid composition on cardiovascular health. The results for the nutritional quality (PUFA/SFA, MUFA/UFA, AI, and TI) of fatty acids from macauba pulp oils are presented in Table 5.

According to Table 5, the PUFA/SFA ratio of the extracted oils was closer to 0.84, while MUFA/UFA presented values closer to 0.87. These values are considered good, reflecting a beneficial profile for cardiovascular health due to the predominance of unsaturated fats over saturated fats in the oils.[35]

The atherogenicity index (AI) indicates the correlation between the sum of SFAs and the sum of UFAs. Only SFAs with chain lengths of 12, 14, or 16 carbon atoms are pro-atherogenic, as they promote lipid adhesion to circulatory and immune system cells.[36] The thrombogenicity index (TI) defines the thrombogenic potential of fatty acids, reflecting their propensity to form blood clots.[36] It delineates the contribution of various fatty acids, elucidating the association between prothrombogenic FAs (C12:0, C14:0, and C16:0) and antithrombogenic fatty acids (MUFAs and the ω3 and

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ω6 families). AI values ranged from 0.14 to 0.16, while TI remained around 0.28. These values were lower than those reported by Silva et al.[37] for macauba kernel oil (AI: 3.14, TI: 1.21), likely due to differences in lipid composition influenced by the fatty acid profile of both oils. Macauba pulp oil contains a higher proportion of monounsaturated fatty acids, while the kernel oil is richer in saturated fats, particularly C16:0.

In summary, this study demonstrated that the aqueous extraction scale-up was highly reproducible, achieving oil quality and efficiency comparable to laboratory-scale results. However, to fully realize its potential, further research is essential to scale up the process and improve its economic feasibility for industrial use. Furthermore, further studies should also investigate the functional and technological properties of liquid and solid coproducts, unlocking their potential for alternative uses. Additionally, the scale-up strategy presented herein may serve as a basis for the engineering design of equipment adapted to aqueous extraction processes on an industrial scale. This approach could drive the circular economy in macauba pulp oil production, offering sustainable solutions for the industry.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.5c00881.

Kinetic modeling and analysis of variance (ANOVA) applied to AEP and AEE of macauba pulp oil (PDF)

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G.D.S.: conceptualization; investigation; data curation; formal analysis; methodology; writing—original draft. S.P.F.: conceptualization; data curation; funding acquisition; investigation; methodology; project administration; resources; supervision; writing—review and editing. L.K.d.S.: formal analysis; methodology. I.P.M.: Formal analysis; methodology. W.P.d.S.: formal analysis; methodology. R.G.: writing—review and editing. A.A.: conceptualization; data curation; funding acquisition; writing—review and editing; supervision; resources; project administration; investigation. M.D.L.: conceptualization; data curation; funding acquisition; investigation; project administration; resources; supervision; writing—review and editing.

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