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# Obtaining hydrolysate from macauba oil and its application in the production of methyl esters

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**SUMMARY:** This work aimed to obtain a hydrolyzate rich in free fatty acids (FFA) from the hydrolysis of macauba oil for subsequent esterification and obtaining of methyl esters. To determine the conditions that maximize FFA yield in the hydrolysis step, the effects of buffer solution percentage and catalyst concentration (Lipozyme® RM IM) were determined at 55 ºC and 6 h. From the results, it was verified that both variables evaluated in the experimental range had an influence on the reaction and their increase favored the production of FFA. Additional experiments were carried out to assess the influence of reaction time with a progressive increase up to 8 h. Hydrolyzate with ~92 wt % FFA was obtained and its use in the enzymatic esterification step using Novozym® 435 as catalyst resulted in ~95 % FFA conversion. Regarding the reuse of enzymes at each stage, a ~50 % reduction in FFA yield was found and only 98 % FFA conversion.

***KEYWORDS:*** *Enzymatic catalysis; Esterification; Hydrolysis; Macauba oil; Methyl esters*

**RESUMEN:** ***Obtención de hidrolizado de aceite de macauba y su aplicación en la producción de ésteres metílicos*.** Este trabajo tuvo como objetivo obtener un hidrolizado rico en ácidos grasos libres (AGL) a partir de la hidrólisis del aceite de frutos de macauba, para su posterior esterificación y obtención de ésteres metílicos. Para determinar las condiciones que maximizan el rendimiento de AGL en la etapa de hidrólisis, se determinaron los efectos del porcentaje de solución amortiguadora y la concentración de catalizador (Lipozyme® RM IM) a 55 ºC y 6 h. De los resultados se verificó que ambas variables, en el rango experimental evaluado, tienen influencia en la reacción y su incremento favorece la producción de AGL. Se llevaron a cabo experimentos adicionales para evaluar la influencia del tiempo de reacción, observándose un aumento progresivo hasta las 8 h. Se obtuvo un hidrolizado con ~92 % en peso de FFA y su uso en el paso de esterificación enzimática, usando Novozym® 435 como catalizador, resultó en ~95 % de conversión de FFA. Al investigar la reutilización de enzimas, en cada etapa, se encontró una reducción de ~50 % en el rendimiento de FFA y solo un 98 % en la conversión de FFA.

***PALABRAS CLAVE:*** *Aceite de macauba; Catálisis enzimática; Ésteres metílicos; Esterificación; Hidrólisis*

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## 1. INTRODUCTION

For the past few years, advance research and development on the production of biofuels from renewable sources has been vehemently growing due to the excessive burning of fossil fuels which cause various environmental issues (Bankovicllic *et al.*, 2012). The use of biodiesel has been widely recognized due to its significant contribution to the reduction of greenhouse gas emission, specifically in the transportation sector (Lam *et al.*, 2019).

According to the Brazilian National Petroleum Agency (ANP, 2015), most of the biodiesel produced in Brazil is obtained from soybeans and since this is a crop mainly grown for human consumption, research on the exploitation of other oilseed crops with the potential to produce biodiesel has been reported. The oil from the Macauba fruit (*Acrocomia aculeata*) stands out in this sense, due to the great potential for production, which can be from 1500 to 5000 Kg of oil per hectare (Manfio *et al.*, 2011), higher than the productivity displayed by soybeans, about ~ 576 kg of oil per hectare (Tamagno *et al.*, 2020). The extraction of macauba oil can come from the kernel and the pulp, and the fatty acid composition of the oil extracted from the pulp consists of unsaturated (oleic and linoleic) and saturated (palmitic and stearic) fatty acids (Rosa *et al.*, 2020). The composition of kernel oil is mainly composed of saturated fatty acids (lauric, myristic and palmitic) (Trentini *et al.*, 2018; Rosa *et al.*, 2020) and therefore, because it contains a greater amount of these fatty acids, the use of macauba kernel oil confers the production of biofuels with greater oxidative stability (Trentini *et al.*, 2018). In addition, problems related to the supercooling of biodiesel from this oil were not observed (Menezes *et al.*, 2021).

The application of macauba oil in the synthesis of biodiesel requires its use in crude form (without refining process) in order to reduce raw material costs, which contribute with a high share in production costs. However, crude macauba oil has high acidity, with reports of 70.26% (Silva *et al.*, 2021) and 23% (Raspe *et al.*, 2013) in pulp and kernel oil, respectively. The hydrolysis of triglycerides followed by the esterification of the obtained fatty acids has stood out in obtaining fatty acid esters for substrates with high acidity (Vescovi *et al*., 2016), mainly in the conventional transesterification process with alkaline catalyst, which inevitably generates soap in the presence of these substrates, thus inactivating the catalyst, making separating biodiesel and glycerol expensive and affecting process productivity (Sousa *et al.*, 2010). However, to make this route industrially viable, in addition to the raw material, operational parameters related to the production costs of this biofuel, such as reaction time, energy demand and catalyst performance must be considered (Wancura *et al.*, 2021).

Hydrolysis and esterification reactions have been reported using enzymatic catalysts (Santos *et al.*, 2015; Zhou *et al.*, 2015; Barbosa *et al.*, 2019), mainly due to heterogeneity, the employment of soft conditions (Kabbashi *et al.*, 2015; Nguyen *et al.*, 2017), and the high degree of specificity of the desired substrates, which promotes reaction acceleration and biodegradability, making them less polluting compared to other catalysts and facilitating their reuse (Rodrigues and Ayub, 2011; Vescovi *et al.*, 2016). Although the use of these catalysts still faces problems related to low reaction rates and the need for long periods of time to achieve high yields, their use in the sequential process has stood out, demonstrating its potential to overcome these drawbacks (Wancura *et al.*, 2019).

The use of organic solvents as reaction media for enzymatic reactions provides attractive advantages over traditional systems, such as increased reaction yield over increased substrate solubility, suppression of water-dependent reactions and elimination of microbial contamination (Raspe *et al.*, 2013), besides the influence on catalytic activity and enzyme stability caused by the nature of these solvents. In contrast, other authors report that their effect causes the inactivation of enzymes, high solvent cost, limitations in mass transfer for heterogeneous systems or systems with high viscosity solvents/substrates (Doukyu and Ogino, 2010).

Therefore, the aim of this study was to evaluate the production of esters from the enzymatic hydroesterification of macauba oil in s two-step reaction: oil hydrolysis followed by esterification of the hydrolyzate obtained. The effects of the experimental variables (buffer solution percentage and catalyst concentration) were investigated in the hydrolysis step in order to maximize the free fatty acid (FFA) yield, and to determine the effect of reaction time. The hydrolyzate obtained (with maximum FFA content) was directed to the esterification step. In addition, the reuse of the enzymes used in the hydrolysis and esterification steps was evaluated.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Macauba kernel oil (Cocal Brasil) was used in the reactions, and its chemical composition was previously reported by Raspe *et al.* (2013). Sodium phosphate buffer (Neon), enzyme Lipozyme® *Rhizomucor miehei* (Sigma-Aldrich) and *n*-hexane (Nuclear) were used in the hydrolysis step. In the esterification reactions, the hydrolyzate obtained from the hydrolysis step, methanol (Panreac, 99.9% purity) and enzyme Novozym® 435 (*Candida antarctica* lipase immobilized) were used. Heptane (Nuclear) and ethanol (Anidrol) were used to wash the enzymes in the catalyst reuse tests. . In titration step of the samples, a solution of ethyl ether:ethanol 2:1 (v:v) (Vetec/Nuclear), potassium hydroxide (Nuclear), and phenolphthalein as indicator (Nuclear) were used.

### 2.2. Experimental procedure

The hydrolysis reaction was carried out in a magnetically stirred, jacketed flask (40 mL) connected to a constant temperature bath (Marconi) for temperature monitoring. The reaction was conducted at 55 ºC, with agitation of 400 rpm and the reaction medium was composed of macauba kernel oil, sodium phosphate buffer solution (pH 8.0), *n*-hexane (oil to *n*-hexane mass ratio of 1:1) and Lipozyme® *Rhizomucor miehei* (RM IM) as catalyst (Raspe *et al.*, 2013). The enzyme was maintained at 40 ºC for 1 hour for activation before its addition to the reaction medium. After the reaction time of 6 hours, enzymes were separated by filtration and two phases (oil + solvent and water) were separated by centrifugation and the solvent in the oil phase was dried in an oven to evaporate the excess solvent.

An experimental central composite design (with axial points) was applied to evaluate the effects of process variables on FFA yield using Statistica® 8.0 software (STATSOFT™, Inc.). Buffer solution percentage (A) and catalyst concentration (B) were the variables investigated in the enzymatic hydrolysis, and these factors varied, as shown in Table 1. A total of 11 experiments with different combinations of levels of the variables were performed in duplicate, and the mean values ± standard deviation of the results were reported.

A second-order polynomial model (Barbosa *et al.*, 2019) was adjusted in relation to the responses obtained and the variables investigated. Analysis of variance (ANOVA) was used to evaluate the effects of operational variables and their interactions on the proposed model based on the values of p-value and F, where $p < 0.05$ was used as the threshold of statistical significance.

The effect of reaction time was determined from the conduction of destructive kinetics (in duplicate) in the times of 1, 2, 4, 6, 8 and 10 hours. The reactions were conducted keeping the temperature and buffer solution fixed at 55 ºC and 50 wt% (in relation to the oil mass), respectively, with the evaluation of the addition of lipase in concentrations of 10, 15 and 20 wt% (in relation to the substrate's mass).

The reactions with the macauba oil hydrolyzate were conducted keeping the temperature fixed at 65 ºC and a methanol to FFA molar ratio of 3:1. The reaction conditions were selected based on the work of Cerveró *et al.* (2014). Preliminary tests were conducted with different catalysts (Lipozyme® *Rhizomucor miehei*, Lipozyme® *Thermomyces lanuginosus* and Novozym® 435), which would indicate higher conversions with the use of Novozym® 435 with percentage in the reactions of 10 wt% (relation to substrates mass). These reactions were performed with magnetic stirrers in a batch reactor equipped with condenser and immersed in a temperature-controlled water bath. The hydrolyzate (4 g) was heated until the desired temperature was reached. At this point, methanol and the catalyst (after activation at 40 ºC for 1 hour) were added and esterification began. At the end of each reaction, the catalyst was separated by centrifugation (3000 rpm for 10 minutes), and the alcohol and water were removed from the reaction mixture using a rotary evaporator.

**Table 1**. Actual and coded values of the independent variables, central composite design ($2^2$), for enzymatic hydrolysis of macauba oil.

| | Levels | | | | |
|---|---|---|---|---|---|
| **Factors** | **(-1.41)** | **(-1)** | **(0)** | **(+1)** | **(+1.41)** |
| (A) Buffer (in relation to oil mass) | 21.71 | 10 | 30 | 50 | 78.28 |
| (B) Enzyme (in relation to substrate mass) | 7.92 | 5 | 7.5 | 10 | 22.07 |

### 2.3. Analytical method

The content in free fatty acids (FFA) was determined based on the method Ca 5a-40 (AOCS, 1998), which is based on acid-base titration using an ethanol solution of potassium hydroxide (KOH) previously standardized as the titrant. Each sample was titrated in duplicate and the FFA content was calculated from Equation 1:

$$\text{FFA (wt\%)} = \frac{C \times MM \times v}{(10 \times m)} \quad (1)$$

where C is the concentration of sodium hydroxide (mol $L^{-1}$) used as titrant, MM corresponds to the molar mass of the predominant fatty acids in the sample, v is the volume required for the titration (mL) and m is the mass of sample (g).

The FFA yield of the hydrolysis reactions was calculated from Equation 2:

$$\text{FFA yield (\%)} = \frac{\text{FFA}}{\text{CHI}} \times 100 \quad (2)$$

where FFA corresponds to FFA content produced after the hydrolysis reaction and the CHI content in compounds present in the macauba oil that can be hydrolyzed (considering the initial content of FFA of 23.0 ± 0.4 wt%) reported by Raspe *et al.* (2013).

The macauba oil hydrolysate used was characterized in terms of the free fatty acid and water contents using the official methods recommended by the AOCS (1990): Ca 5a40 and 984.20, respectively. The glycerol content was determined by titration, using the sodium periodate method described by Cocks and Van Rede (1996).

The conversion of the esterification reaction (FFA conversion) was determined according to Equation 3:

$$\text{FFA conversion (\%)} = \frac{\text{FFA}_i - \text{FFA}_f}{\text{FFA}_i} \times 100 \quad (3)$$

where $FFA_i$ is the initial FFA content in the hydrolyzate and $FFA_f$ is the FFA content in the final sample of the reaction medium.

### 2.4. Reuse of lipase

For the reuse assays of the Lipozyme® RM IM and Novozym® 435, batches of hydrolysis and esterification were repeated for 15 cycles of 6 hours and 1 hour, respectively. After each reaction, the biocatalyst was recovered by filtration, washed with heptane and ethanol to remove adsorbed products and dried in oven at 40 ºC for 1 hour, kept in a desiccator for 24 hours and reused in another batch.

## 3. RESULTS

### 3.1. Enzymatic hydrolysis of macauba oil

Table 2 presents the results obtained for the reactions conducted in order to evaluate the effect of

**Table 2**. Central composite design and free fatty acid (FFA) yield obtained from enzymatic hydrolysis of macauba oil carried out at 55 ºC and 6 hours.

| Run | Variables[1] | | FFA yield[2] (%) |
|---|---|---|---|
| | A | B | |
| 1 | 30 (-1) | 10 (-1) | 69.03 ± 0.38 |
| 2 | 30 (-1) | 20 (+1) | 78.09 ± 0.29 |
| 3 | 70 (+1) | 10 (-1) | 71.08 ± 0.82 |
| 4 | 70 (+1) | 20 (+1) | 80.67 ± 0.40 |
| 5 | 21.71 (-1.41) | 15 (0) | 76.67 ± 0.90 |
| 6 | 78.28 (+1.41) | 15 (0) | 80.15 ± 0.48 |
| 7 | 50 (0) | 7.92 (-1.41) | 68.56 ± 0.36 |
| 8 | 50 (0) | 22.07 (+1.41) | 80.17 ± 0.05 |
| 9.1 | 50 (0) | 15 (0) | 77.46 ± 0.05 |
| 9.2 | 50 (0) | 15 (0) | 78.39 ± 0.27 |
| 9.3 | 50 (0) | 15 (0) | 78.49 ± 0.75 |

[1] (A) Buffer solution percentage (in relation to oil mass) and Enzyme concentration (in relation to substrate mass); [2] calculated according FFA content produced after the hydrolysis reaction and CHI content of compounds present in the macauba oil which can be hydrolyzed (23.0 ± 0.4 wt%), mean value (2 replicates) ± standard deviation.

**Table 3.** Analysis of variance (ANOVA) of the quadratic model of free fatty acid (FFA) yield obtained from enzymatic hydrolysis of macauba oil.

| | Sum of squares | Degree of freedom | Mean square | F | $p^1$ |
|---|---|---|---|---|---|
| A (L) | 11.38 | 1 | 11.38 | 35.39 | 0.027 |
| A (Q) | 0.40 | 1 | 0.40 | 1.26 | 0.376 |
| B (L) | 153.71 | 1 | 153.71 | 478.08 | 0.002 |
| B (Q) | 29.66 | 1 | 29.66 | 92.26 | 0.010 |
| A*B | 0.07 | 1 | 0.07 | 0.22 | 0.684 |
| Lack of Fit | 6.19 | 3 | 2.06 | 6.41 | 0.137 |
| Pure Error | 0.64 | 2 | 0.32 | | |
| Total SS | 202.67 | 10 | | | |
| $R^2 = 0.964$ | | | | | |
| $R^2_{adjusted} = 0.949$ | | | | | |

[1] Statistical significance ($p < 0.05$); L - linear effect and Q - quadratic effect.

the process variables for obtaining a hydrolyzate rich in FFA.

The ANOVA of the quadratic model adjusted to the experimental data is presented in Table 3. Significant terms ($p < 0.05$) were obtained, which indicates that the experimental data can adequately describe the model proposed. The F values of 62.53 indicate that the models were significant, since these values were higher than the $F_{critic}$ value (8.89). In addition, the values for $R^2$ and $R^2_{adjusted}$, calculated considering only the significant parameters, showed that the variability of the data (> 90%) is adequately explained by the regression model, which indicates good linearity between the predicted data and the observed data.

Table 3 shows that linear and quadratic terms of all variables were significant for the adjusted model, except for the buffer percentage, which showed influence only on the linear term and the binary interaction which was not significant. From the adjusted model, the linear term of the buffer percentage was the variable that presented a higher F value and a lower p value. The polynomial model for the FFA yield (%) was regressed considering the significant terms as presented in Equation 4:

$$\text{FFA yield (\%)} = 78.11 + 1.19A + 4.38B - 2.29B^2 \quad (4)$$

### *3.1.1. Effect of process variables*

The variables evaluated in the experimental design have a greater influence on the FFA yield (based on the experimental range considered). The greater amount of buffer solution in the reaction medium caused an increase in the interfacial area of the oil-water system, providing a greater number of bonds between the substrates to be catalyzed by the lipase (Zhou *et al.*, 2015). In addition, when the percentage of the buffer solution was increased, there was less variation in the pH of the reaction medium and less aggregation (McClements and Weiss, 2005). In addition, a higher proportion of water changed the balance in favor of the products, improving the reaction rate in each of the hydrolysis steps and accelerating their completion (Wang *et al.*, 2012). This was possibly because lipase, which is a surface-active enzyme, bound with the substrates at the oil–water interface and, with the increased addition of water, the amount of water available for oil to form oil–water droplets increased, thereby increasing the available interfacial area, since the lipase catalyzes the hydrolysis reaction at the interfacial area of emulsion (Nguyen *et al.*, 2017).

Santos *et al.* (2015) observed an increase in the hydrolysis yield from ~35 to 100% FFA with the use of 50 wt% and 90 wt% buffer solution in the reaction, respectively. Zhou *et al.* (2015) evaluated the hydrolysis of unrefined jatropha oil and found that the application of the highest proportion of water (relation to oil mass) (100 wt%) resulted in obtaining ~88 wt% FFA, while using the proportion of 50 wt%, ~75 wt% FFA was obtained. Barbosa *et al.* (2019) reported a ~150% increase in the hydrolysis degree of *Moringa oleifera* Lam oil by varying the oil-to-water mass ratio from 15 to 35 wt%.

The higher catalyst concentration in the reaction medium favored the achievement of higher values for FFA yield, which is the result of increased contact between the substrate and the active sites of the lipase and the cumulative adsorption of the enzyme at the oil-water interface (Santos *et al.*, 2015), leading to increased hydrolysis rates. In general, the reaction rate increases with the greater availability of enzyme in the reaction medium. Zenevicz *et al.* (2016) found a 20% increase in the FFA content obtained from the hydrolysis of soybean oil by increasing the Lipozyme® TL IM percentage from 1 to 10 wt% (based on the total mass of substrates). For the hydrolysis of soybean oil, Corradini *et al.* (2019) obtained 1800 mM and ~500 mM of FFAs using 6 and 2 g of castor seed lipase, respectively.

### *3.1.2. Maximization of FFA yield*

The conditions that maximized the production of FFA from the enzymatic hydrolysis of macauba oil were 50 wt% buffer solution and 20 wt% enzyme, with predicted FFA yields of 80.2%. Verification experiments were conducted (in triplicate) and provided FFA yield of 82.65 ± 0.57%. The predicted experimental values were compared and according to the t-Student test, there was agreement between these values in a significance interval of 0.05, which shows the predictive capacity of the adjusted models.

### *3.1.3. Effect of reaction time*

Considering that for the investigated system the enzyme concentration had a greater influence on the FFA production, the kinetic of the reaction was determined keeping the temperature and buffer solution fixed at 55 ºC and 50 wt% (in relation to the oil mass), respectively, with evaluation of the addition of lipase in concentrations of 10, 15 and 20 wt% (in relation to the substrates mass).

Figure 1 presents the results obtained for the reactions carried out in the interval from 1 to 10 hours and according to this figure it can be seen that the gradual increase in the reaction time resulted in higher FFA yields, with the maximum value of ~88.90% (corresponding to FFA content of 92.08 wt%) obtained using 20 wt% catalyst and after 8 hours of reaction. For the reactions conducted with 15 and 20 wt% of catalyst, there was no increase in yield after 8 hours, indicating that the process equilibrium was reached.

Rodrigues and Ayub (2011) reported yields in the order of 95% FFA after 10 hours of reaction when investigating the hydrolysis of soybean oil using a water-to-soybean oil molar ratio of 3:1, 25 wt% (in relation to mass of oil) of the biocatalyst mixture (combination of 65% *Thermomyces lanuginosus* and 35% *Rhizomucor miehei*) at 30 °C. After 2 hours of

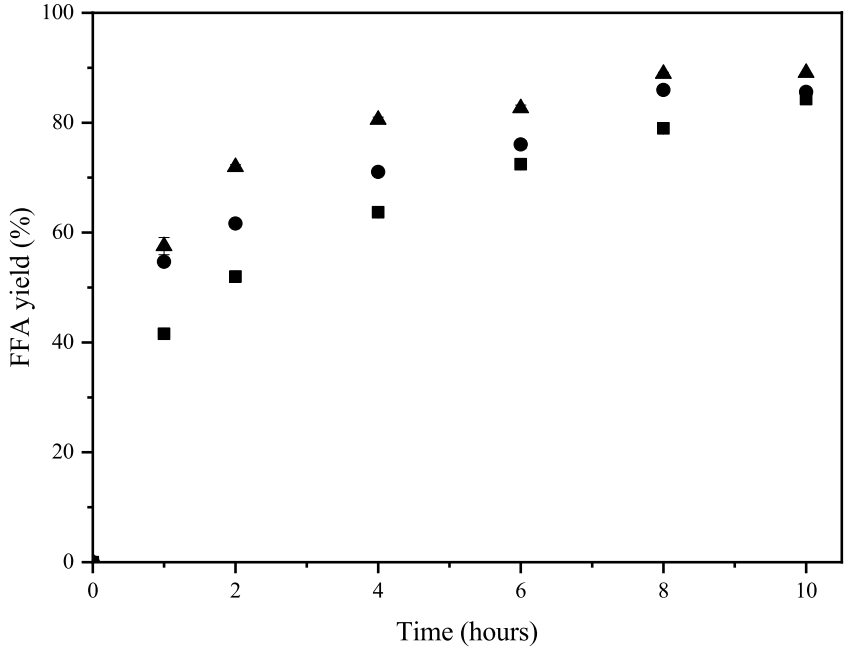


**Figure 1.** Kinetics of the production of free fatty acids (FFA) from enzymatic hydrolysis of macauba oil at 55 ºC, oil-to-*n*-hexane mass ratio of 1:1, 50 wt% of sodium phosphate buffer solution (pH 8.0) (in relation to oil mass) with different percentages of catalyst Lipozyme® RM IM (in relation to substrate mass): ■ 10 wt%; ● 15 wt% and ▲ 20 wt%. Mean value (2 replicates) ± standard deviation.

reaction at 40 °C, with an oil-to-water molar ratio of 1:20 and 10 wt% of Lipozyme® TL IM (in relation to the substrates mass), Zenevicz *et al.* (2016) obtained a maximum yield of 60% FFA in the hydrolysis of soybean oil. Vescovi *et al.* (2016) obtained 100% FFA yield in the hydrolysis of frying oil catalyzed by the immobilized lipase of *Thermomyces lanuginosus*, in a ratio of oil-to-water of 1:4 (v/v) and enzyme/reaction medium of 1:100 (w/v), at 30 °C and 24 h. After 40 hours of hydrolysis catalyzed by Lipozyme® RM IM, Tavares *et al.* (2018) obtained a maximum FFA yield of 74% from crambe oil, under experimental conditions of 2.7 wt% of lipase (in relation to the mass of substrates) and water-to-oil molar ratio of 10:1.

### *3.1.4. Characterization of hydrolyzate*

The macauba oil hydrolyzate collected at 55 ºC, with 50 wt% buffer solution, 20 wt% lipase and 8 hours of reaction showed a free fatty acid content of 92.08 ± 0.28 wt%, water content of 0.865 ± 0.07 wt% and glycerol content of 0.64 ± 0.002 wt%, respectively.

### *3.1.5. Reuse of biocatalyst*

Figure 2 shows the evaluation of the reuse of the enzyme catalyst in reactions conducted at 55 ºC, 50 wt% buffer solution and 20 wt% lipase, evaluated for 15 cycles of 6 hours each. From the data in Figure 2, it can be seen that the efficiency of the lipase declines in the course of its reuse, obtaining ~50% lower yield after 15 cycles compared to cycle 1. The loss in activity observed may be related to the saturation of the active sites of the enzyme during the reaction, since upon reaching its maximum activity, the interfacial effects and obstacles to mass transfer imply a decrease in reaction rates, preventing the enzyme from absorbing more substrate (Corradini *et al.*, 2019). Kabbashi *et al.* (2015) repored that the decrease in product yield may be attributed to desorption of the enzyme from the support and inactivation upon repeated reuse.

Rodrigues and Ayub (2011) verified a drop of ~80% in the hydrolysis yield of soybean oil catalyzed by the mixture of *Thermomyces lanuginosus* and Lipozyme® RM IM after 10 cycles of 10 hours each, relating this behavior to the lack of washing of the catalysts at the end of each process. Vescovi *et al.* (2016) when evaluating the reuse of *Thermomyces lanuginosus* lipase in the hydrolysis of residual cooking oil, determined that enzyme activity decreased during reuse in proportions similar to those reported in this work (~50%), although after only five cycles (10 hours each). According to the authors, this decrease in yield is due to the low pH of

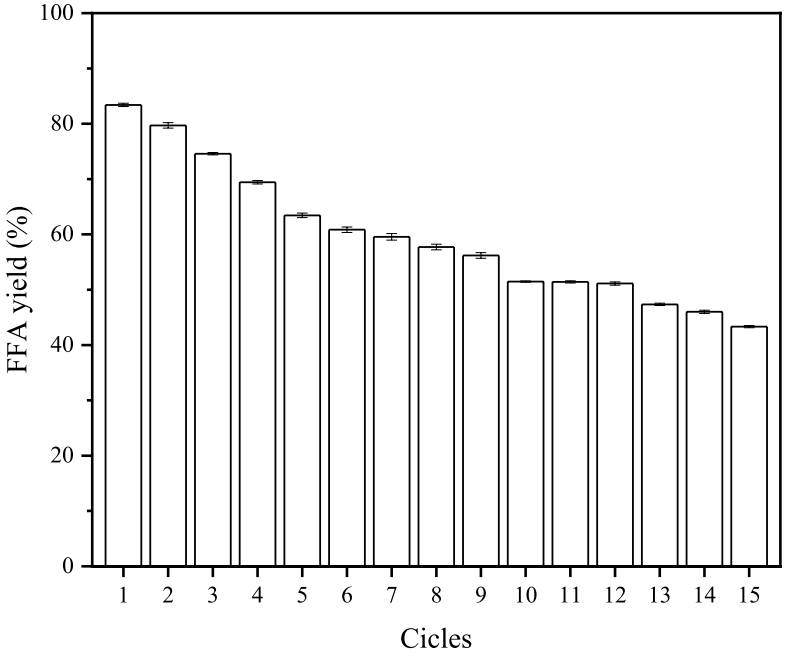


**Figure 2.** Evaluation of free fatty acid (FFA) yield from enzymatic hydrolysis of macauba with Lipozyme® RM IM reuse at 55 ºC, oil-to-*n*-hexane mass ratio of 1:1, 50 wt% of sodium phosphate buffer solution (pH 8.0) (in relation to oil mass) and 20 wt% (in relation to substrate mass) of catalyst and cycle of 6 hours each. Mean value (2 replicates) ± standard deviation.

the reaction medium (around pH 4.6 after 10 hours of hydrolysis), which probably caused enzyme inactivation. Assessing the reuse of Lipozyme® TL IM in the enzymatic hydrolysis of soybean oil, Zenevicz *et al.* (2016) observed that the process maintained the yield at ~60% FFA after 4 cycles (2 hours each).

### 3.2. Reaction carried out with macauba oil hydrolyzate

The esterification step of the FFA in solvent medium (50 wt% of *n*-hexane in relation to the hydrolysate mass) was carried out at different times, as shown in Figure 3. From the data in this figure, it appears that the reaction rate is high in short reaction times (15 min), reaching equilibrium in 60 min with ~95% conversion of FFA.

When investigating the influence of operational conditions on the use of Novozym® 435 in FFA esterification with methanol, Mulalee *et al.* (2015) reported ~ 95% conversion with 5 wt% catalyst (in relation to oleic acid), with a methanol-to-FFA molar ratio of 2:1, at 45 °C after 8 hours. ~97% FFA conversion was obtained by Teixeira *et al.* (2017) in the esterification of FFA of macauba oil, conducted at a methanol-to-FFA molar ratio of 2:1, 5 wt% of Lipozym® 435 (in relation to FFA mass), 30 °C and 60 min. Rosset *et al.* (2019) reported 94.3% conversion in the esterification of soybean oil hydrolyzate with the lipase NS 40116 (enzyme-in-liquid formulation from genetically-modified *Thermomyces lanuginosus* microorganism) at 35 °C, methanol-to-oil molar ratio of 4.5:1 and 12 hours of reaction.

#### *3.2.1. Reuse of biocatalyst*

The reuse of the Novozym® 435 in the esterification reaction was evaluated under the conditions reported in Figure 3 for the reaction time of 1 hour and during 15 cycles, as shown in Figure 4. It was verified from the results that the lipase maintained ~98% of its initial conversion capacity at the end of the evaluated cycles. The maintenance of the catalytic activity of the lipase is related to the low solubility of its support during the reactions (Shin *et al.*, 2020), promoted by operating conditions and the alcohol in the process. Adequate temperature and agitation do not weaken the enzyme support and do not promote its interfacial inactivation (Ortiz *et al.*, 2019). In addition, methanolic esterification promotes less swelling in the reuse of Novozym® 435, causing less loss in activity and degree of catalytic deactivation (Mulalee *et al.*, 2015). At the sqame time, the recovery of the enzyme by washing with heptane may also be responsible for maintaining the stability of Novozym® 435, due to the greater

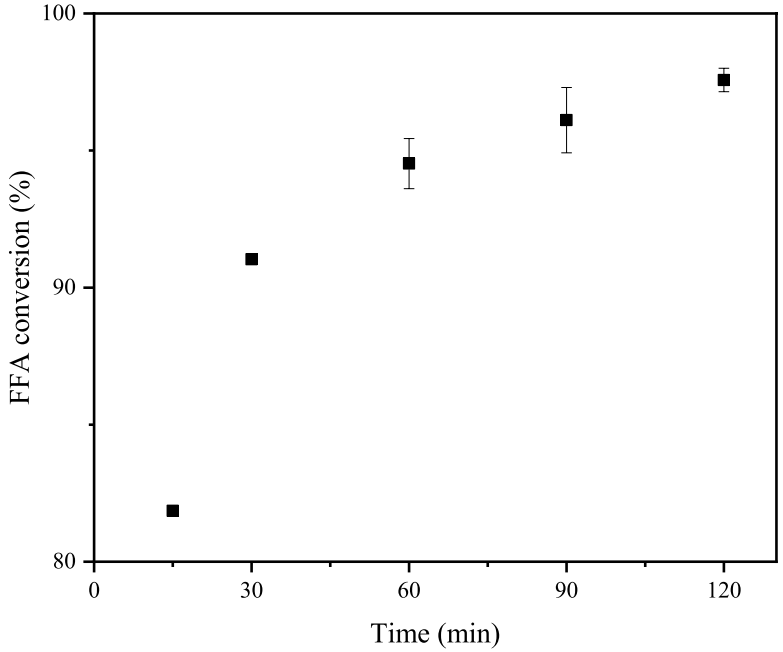


**Figure 3.** Free fatty acid (FFA) conversion from enzymatic esterification of macauba hydrolysate at 65 ºC, methanol-to-free fatty acid of 3:1 and 10 wt% Novozym® 435 (in relation to substrates mass). Mean value (2 replicates) ± standard deviation.

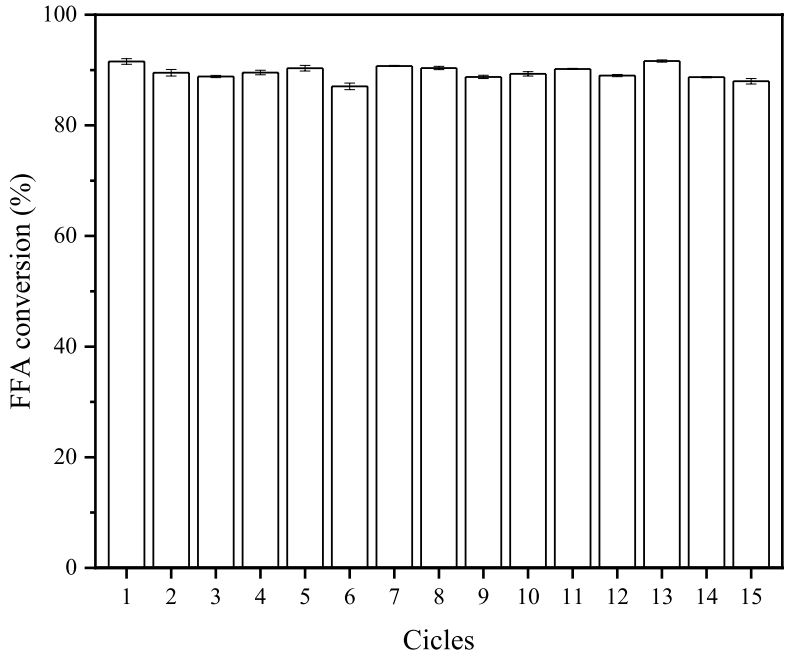


**Figure 4.** Evaluation of free fatty acid (FFA) conversion from enzymatic esterification of macauba hydrolysate with Novozym® 435 reuse at 65 ºC, methanol-to-free fatty acid of 3:1 and 10 wt% (in relation to substrates mass) of catalyst and cycle of 1 hour each. Mean value (2 replicates) ± standard deviation.

removal/dissolution of the constituents linked to the active sites of this enzyme (Chowdhury and Mitra, 2015), restoring the catalyst activity almost completely.

Baek *et al.* (2020) performed the enzymatic synthesis of formate ester through immobilized lipase, and observed that Novozym® 435 could be reused for 10 cycles of 1 hour, keeping the conversions at ~92%. Moreira *et al.* (2020) reported that Novozyme® 435 maintained catalytic activity at the end of 10 consecutive cycles in the enzymatic esterification of babassu FFA in reactions at 48 °C and duration of 4 hours.

## 4. CONCLUSIONS

The present study evaluated the hydroesterification of macauba oil using enzymatic catalysis, with evaluation of the processes of hydrolysis of macauba oil and esterification of the hydrolyzate. Evaluating the effects of the process variables, it was found a yield of ~ 80% in FFA, through positive and significant effects for the percentage of buffer solution and concentration of catalyst in the reaction medium. A hydrolyzate with ~92% FFA was obtained by evaluating the percentage of 20 wt% of catalyst (in relation to oil mass), temperature of 55 ºC, stirring 400 rpm and 50 wt% of buffer solution (in relation to mass of substrates) in the hydrolysis kinetics after 8 hours of reaction. The conversion of the hydrolyzate in the esterification step was evaluated at 65 ºC, methanol to FFA of 3:1 and 10 wt% (in relation to substrates mass) of catalyst, where 95% conversion of the FFA was achieved. In the reuse of the catalysts, the efficiency of the Lipozyme® RM IM lipase decreased ~50% the FFA yield in hydrolysis after 90 hours of the process, while Novozym® 435 maintained ~98% of its initial conversion capacity, at the end of the 15 cycles investigated (15 hours).

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