

Article

Exploiting the Complementary Potential of Rice Bran Oil as a Low-Cost Raw Material for Bioenergy Production

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Abstract: Rice is one of the most consumed cereals in the world. From rice processing, rice bran is obtained, and only a part of this by-product is effectively used. Rice bran oil can be obtained and used as an alternative feedstock for biodiesel production, although few studies exist to support its exploitation. In addition, pretreatment is required to reduce its acidity and allow for its integration in the conventional industrial process. This work evaluated two pretreatment processes aiming to reduce the free fatty acids (FFAs) content of rice bran oil by employing an acid-catalyzed process and a biocatalyzed process. The results allowed us to assess the efficiency and effectiveness of both pretreatments. For that purpose, acid (45, 55 and 65 °C, using H₂SO₄ concentrations of 2 wt.% or 4 wt.% and a methanol:oil molar ratio of 9:1) and enzymatic FFAs conversion (35 °C using a 6:1 methanol:oil molar ratio and 5 wt.% of *Thermomyces lanuginosus*) were evaluated using rice bran oil with an acid value around 47 mg KOH.g⁻¹, and the reaction kinetics were assessed. Acid esterification enabled a 92% acidity reduction (65 °C, 4 wt.% of catalyst) after 8 h, with the final product presenting an acid value of 3.7 mg KOH.g⁻¹ and a biodiesel purity of 42 wt.%. The enzymatic process allowed an acidity reduction of 82%, resulting in a product with an acid value of 7.0 mg KOH.g⁻¹; however, after 24 h, the biodiesel purity was 87 wt.% (almost a two-fold increase compared to that obtained in the homogeneous process), revealing the conversion of both free fatty acids and glycerides. The study of the reaction kinetics of the homogeneous (acid) esterification showed that, for temperatures > 45 °C, the constant rate increased with temperature. A higher constant rate was obtained for the temperature of 55 °C using 4 wt.% of catalyst ($k' = 0.13 \text{ min}^{-1}$). For the heterogeneous (enzymatic) esterification, the constant rate obtained was lower ($k' = 0.028 \text{ min}^{-1}$), as expected. The study revealed the technical viability of the esterification pretreatment of rice bran oil and the important parameters concerning the performance of the pretreatment solutions. Finally, the enzymatic process should be further explored, aiming to develop more ecofriendly processes (water and energy savings) to produce biodiesel from oils with a high acidity (low-cost raw materials).

Keywords: rice bran oil; low-cost feedstock; acid oil; esterification pretreatment; biodiesel



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1. Introduction

Fossil fuels continue to be the most employed resource for energy production despite the environmental problems associated with their exploitation and use [1]. To overcome this problem, environmentally friendly alternatives should be promoted by policymakers, developed at a research level and adopted by the public in general [2]. Since 1880, the global average surface temperature rise is estimated to be 0.85 °C (in 1880–2012), confirming the importance and urgency of global action to minimize climate change [3]. Although there is awareness about the impact of greenhouse gas (GHG) emissions, the consumption of

fossil fuels still continues to increase [4]. The transport sector represents 29% of the total energy consumed in the world and 65% of the global oil consumption [4], contributing with 24% of the CO₂ global emissions. Meanwhile, to face the emissions limits imposed, the use of biofuels has been promoted [1]. Biodiesel is a biofuel produced from renewable sources that is compatible with the requirements of fossil-fuel-based diesel engines, which has a high biodegradability, and thus a low toxicity, and is free of sulfur, representing an eco-friendly alternative to diesel [5–7]. In the biodiesel production chain, most of the costs are associated to the feedstock, representing 80–85% of the total cost of biodiesel production [8,9]. In addition, the use of edible oils for energy purposes is not advisable because this demand increases the prices of such goods, creating competition between the food and biofuels market [7,10].

Rice is the second most consumed cereal in the world, it being only exceeded by wheat. In Portugal, in 2017, the area of rice sown was around 30,000 ha, corresponding to an annual production of around 180 kt [11]. It should also be noted that the national consumption per capita is, on average, 15.3 kg, which is three times more than the European average. By-products such as rice bran might be seen as a non-conventional and low cost feedstock for biodiesel production [12]. This by-product results from the milling process of the rice grain. The processing consists of the removal of the husk (dehulling) and bran layers, finally resulting in the white rice product. The dehulling process generates rice husk equivalent to 20% of the unprocessed paddy rice (rice harvested) [13]. Rice husk is usually used for energy/heat production in the associated industrial process [13]. Bran residue corresponds to 10% of the paddy rice [14].

In the Portuguese context, rice bran is mostly used for animal feed, corresponding to an annual consumption of 8122 tons in 2017 [11], which represents approximately only 44% of the total amount of rice bran produced in Portugal. For this reason, it is of high relevance to study alternatives for the recovery of this by-product, which, from the revised information, does not have an appropriate destiny. The literature reports its high oil content (15–20 wt.%), which is known to be rich in free fatty acids (FFAs), 25 to 50 wt.%, particularly due to the presence of in situ lipases that catalyze the hydrolysis of triglycerides to FFAs [15]. Consequently, such acidic rice bran oil (RBO) must be subjected to pretreatment (first step—esterification reaction), with the objective of reducing its acidity in order to allow its application in the conventional biodiesel production processes (second step—transesterification reaction), where a homogeneous alkaline transesterification reaction requires an oil with an acid value preferably lower than 2 mg KOH.g⁻¹ [16–18]. In the literature, most of the studies available produce bioenergy from RBO using refined oil with a low acidity, highlighting the relevance of studying its direct use.

An esterification reaction (Figure 1) consists in the reaction between FFAs and an alcohol, usually methanol, under the presence of a catalyst (most commonly sulfuric acid) leading to the production of methyl esters (biodiesel) and water.

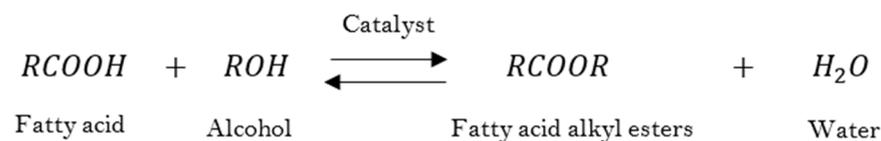


Figure 1. Esterification reaction of free fatty acids.

Esterification pretreatment (to decrease the FFAs content) can be developed using chemical (homogeneous or heterogeneous) or biological (heterogeneous) catalysts. For acid esterification, sulfuric acid is the most used homogeneous catalyst allowing a quick conversion of FFAs into esters [19,20]. The effectiveness of esterification pretreatment for acid oils is directly related with the reaction variables such as the amount of catalyst used, temperature, reaction time and alcohol-to-FFAs molar ratio [21]. The drawbacks of this process are the use of a highly corrosive reagent (mostly sulfuric acid as catalyst) and the need to refine the products and separate/recover the catalyst (homogeneous). In addition,

catalyst removal causes the production of high amounts of wastewater, which requires treatment (environmental and economic costs).

Several studies related to the acid esterification of oils with high FFAs content can be found in the literature [22,23]. In the study developed by Lin et al. [22], the production of biodiesel in two steps started with a pretreatment by acid esterification using a 6:1 methanol:oil molar ratio for 60 min at 50 °C, achieving a reduction in acidity from 40 to 4.8 mg KOH.g⁻¹. Using rice bran oil, in the second stage (alkaline transesterification reaction), it was possible to produce biodiesel with a high purity (around 98.7% of fatty acid methyl esters).

More recently, in a study by Chai et al. [23] that had as its main goal the verification and optimization of the acid esterification of used cooking oil (FFAs content of 5 wt.%), the best conditions obtained were at high a methanol:FFA molar ratio (40:1), catalyzed by 10 wt.% of sulfuric acid in relation to the content of FFAs and at temperatures between 55 and 65 °C. The methanol:FFA molar ratio of 40:1 was very different from that indicated by the National Renewable Energy Laboratory (methanol:FFA molar ratio of 20:1), whose methodology was revised in the mentioned work.

The study of esterification reaction kinetics is of high relevance when aiming to upscale the different processes and to understand the progression of the reaction [1]. According to Kocsisová et al. [24], an esterification reaction is usually described as a second order reaction and, using methanol, the kinetics can be expressed as presented in Equation (1), where v is the reaction rate, k is the rate constant, t is the time and $C(FFA)$ and $C(MeOH)$ are the concentrations of free fatty acids and methanol, respectively.

$$v = -\frac{dC(FFA)}{dt} = k \times C(FFA) \times C(MeOH) \quad (1)$$

A higher reaction rate is possible by increasing the temperature or keeping the reaction far from the equilibrium composition. Due to energy costs, it is, however, advisable to control the reaction temperature and use methanol (alcohol) in excess to ensure that the reaction occurs in the direction of the products [24].

The use of biocatalysts, such as enzymes, might be a good alternative to conventional homogeneous acid esterification, mitigating some of the environmental issues related to wastewater treatment (a heterogeneous process enables easier products separation) and energy demands (mild temperatures are employed) [25,26]. In addition, enzymatic catalysts allow the conversion of low-cost raw materials with a high content of free fatty acids, reducing production costs [27].

In the present work, the potential of acid RBO as a low-cost feedstock for bioenergy production was exploited. For this purpose, the oil was extracted from the by-product and two different catalytic routes were assessed to convert the FFAs present into fatty acid methyl esters, in particular, by enzymatic esterification (where no studies exist) and homogeneous acid esterification in order to contribute to its assimilation by the biodiesel industry.

2. Material and Methods

2.1. Materials

The RBO was extracted from rice bran produced by a Portuguese company from the north of Portugal. The alcohol used in the esterification reaction was methanol (Fischer Scientific $\geq 99\%$). The enzyme used as a catalyst was lipase *Thermomyces lanuginosus* (Lipolase 100 L, activity $\geq 100,000$ U/g), purchased from Sigma-Aldrich. For homogeneous acid esterification, concentrated sulfuric acid (H₂SO₄) was employed (Fischer Chemical). For the oil extraction, petroleum ether (LabChem-Zelienople, PA, USA) was used as a solvent. All the reagents required for analytical procedures were analytical grade.

2.2. Analytical Methods

The oil extracted from rice bran was characterized in terms of its free fatty acids content by volumetric titration (NP EN ISO 660). The oil content was determined in duplicate using

a 100 mL Soxhlet extractor using petroleum ether as a solvent according to NP EN ISO 659 (2002). Oxidation stability was determined by accelerated oxidation using the 873 Biodiesel Rancimat equipment from Metrohm. Kinematic viscosity (40 °C) was measured according to ISO 3104 using capillary viscometers immersed in a thermostatic bath.

The FAME content in the final product was measured by gas chromatography (GC) according to EN 14103. GC analysis was conducted using a Dani Master GC with a DN-WAX capillary column of 30 m, 0.25 mm internal diameter and 0.25 µm film thickness. The following temperature program used was: 120 °C as the starting temperature, rising at 4 °C per minute, up to 220 °C, holding for 10 min. All FAME analyses were performed in duplicate, and the variation was evaluated in terms of the relative percentage difference (RPD) to the mean. In all cases, RPD was less than 1.5%.

2.3. Pretreatment of Rice Bran Oil

Rice bran oil (mostly constituted by C18:1 ≈ 46 wt.%; C18:2 ≈ 39 wt.% and C16:0 ≈ 11 wt.%; 883 g/mol) was submitted to heterogeneous and homogenous catalyzed esterification. Figure 2 shows the schematic procedure adopted for rice bran oil pretreatment.

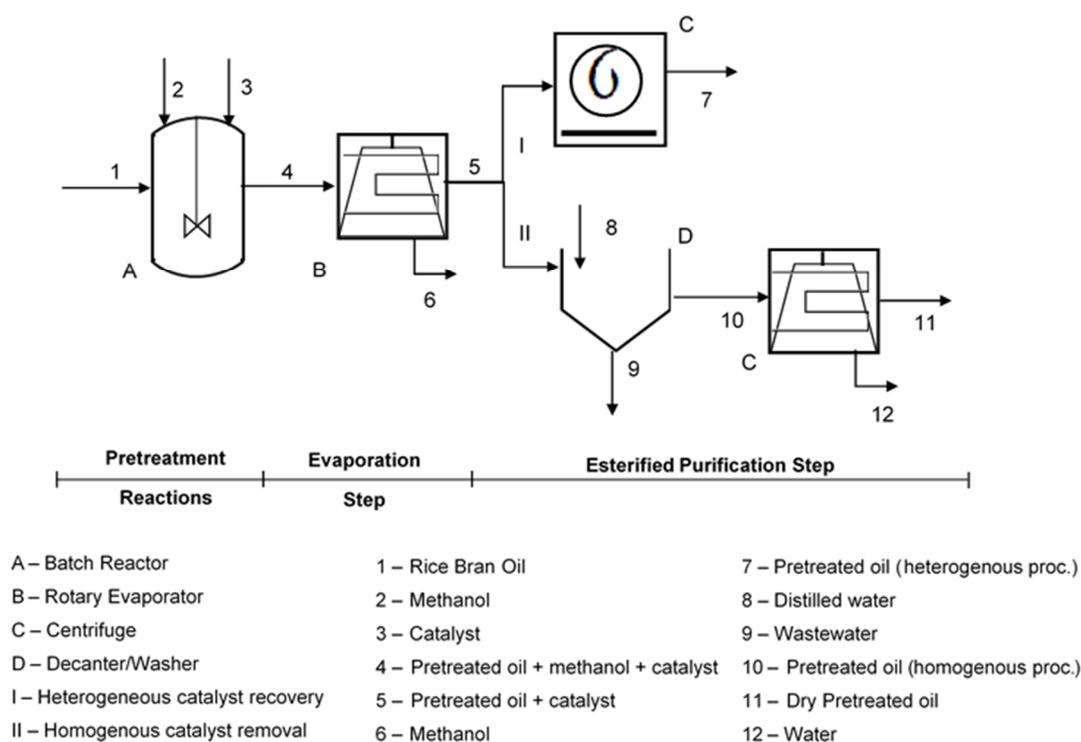


Figure 2. Diagram of RBO pretreatment using both enzymatic esterification and homogeneous acid esterification.

For the homogenous acid esterification reactions, the standard conditions were selected according to the previous studies of the research team [16,28]. Each assay required 15 g of RBO. Three different temperatures of 45 °C, 55 °C and 65 °C and two catalyst amounts of 2 wt.% and 4 wt.% of the oil were evaluated. The methanol:oil molar ratio used was 9:1, this having been reported as effective [16]. Table 1 summarizes the conditions used.

To monitor the progression of the reaction, the acid value of the mixture was measured at 15, 30, 60, 120, 240 and 480 min after the beginning of the reaction. At the end of the esterification reactions (480 min), the excess methanol was removed in a rotary evaporator (T = 65 °C; 200 mbar, 30 min) and the pre-treated oils were washed with 50 vol.% of water to remove the excess catalyst (Figure 1). The acid value of the final product (after purification) and its FAME content were determined. For the condition with the highest FFAs reduction, experiments were performed in duplicate to validate the results.

Table 1. Experimental conditions for homogenous acid esterification.

Assay	Temperature (°C)	Catalyst (wt.%)	Alcohol:Oil Molar Ratio
1	45	2	9:1
2	45	4	9:1
3	55	2	9:1
4	55	4	9:1
5	65	2	9:1
6	65	4	9:1

In the case of heterogenous enzymatic esterification, the RBO was added to a batch reactor placed in an orbital shaking incubator (Agitorb 200IC) where methanol and the enzyme were introduced. The amount of methanol (6:1 methanol:oil molar ratio) was determined taking into account the oil's molecular weight (883 g/mol). The following conditions were also used: stirring at 200 rpm, 24 h, and 35 °C, according to the previous studies of the research team [29] and the recommendations of the catalyst supplier. Methanol addition was performed stepwise after it was verified that a single addition inhibited the enzyme [27,29]; this was conducted as also referred to in the study of Bonet-Ragel et al. [30]. The enzyme concentration selected was 5 wt.% (relative to oil), in agreement with the literature [29,31]. Samples were also collected from the reactor at different time periods (the zero time was considered when the temperature stabilized after catalyst addition), i.e., 15, 30, 60, 120, 240, 480 and 1440 min, to measure the FFAs content. Finally, the excess methanol was recovered in a rotary evaporator (T = 65 °C; 200 mbar, 30 min), and the remaining fraction was centrifuged for 5 min at 3500 rpm with the objective of recovering the enzyme (Figure 2).

The results obtained were used to study the kinetics of the esterification reactions. Considering that the methanol concentration was in excess and thus that it might be considered constant, a pseudo-first-order kinetics model was proposed, considering that $k' = k \times C(\text{MeOH})$, where k' is the constant rate of the pseudo-first-order chemical reaction.

3. Results and Discussion

3.1. Raw Material Properties

The oil content of the rice bran by-product was 18.9 ± 0.5 wt.%. The RBO had a very high acidity of 46.5 ± 0.2 mg KOH.g⁻¹, which was close to the maximum values reported in the literature [15]. As previously mentioned, the high acidity of RBO is mainly related to the presence of lipases and, more specifically, is due to the rupturing of cells during the rice milling, which leads to closer contact between the lipases and the oil, causing its hydrolysis. For this reason, during the storage of rice bran, the quality of the oil decreases. The properties of the RBO are presented in Table 2.

Table 2. Physicochemical properties of rice bran oil.

	Acid Value (mg KOH.g ⁻¹)	Viscosity at 40 °C (mm ² .s ⁻¹)	Oxidation Stability (h)
Result	46.5	29.0	7.6
RPD* (%)	0.3	5	5

* Relative percentage difference to the mean.

From a visual inspection, it was possible to verify that rice bran oil was very viscous at room temperature. This aspect was confirmed by the kinematic viscosity results (29.0 mm².s⁻¹), which were close to the results reported in the literature (32.2 mm².s⁻¹) [32]. When converted into methyl esters, the product's viscosity significantly decreased. A reference value for biodiesel produced from rice bran oil is 6 mm².s⁻¹ [32], which is slightly higher than the limit established for biodiesel quality according to the European standard EN 14214, indicating the need to use it in blends.

In terms of its oxidation stability properties, little information is available in the literature. The value obtained for the RBO's oxidation stability (7.6 h) was higher than that found in another study of rice bran oil with 1 month of storage (3.66 h) [33]. It is expected that the biodiesel produced from the RBO would have an oxidation stability lower than 8 h (EN 14214) due to the characteristics of the oil and the employed process. However, such aspects might be overcome using antioxidants.

3.2. Homogenous Acid Esterification Pretreatment

For a better assessment of the homogenous acid esterification process, the acidity of the oil was monitored at different time intervals (as described in Section 2.3). The results are presented in Figure 3A,B.

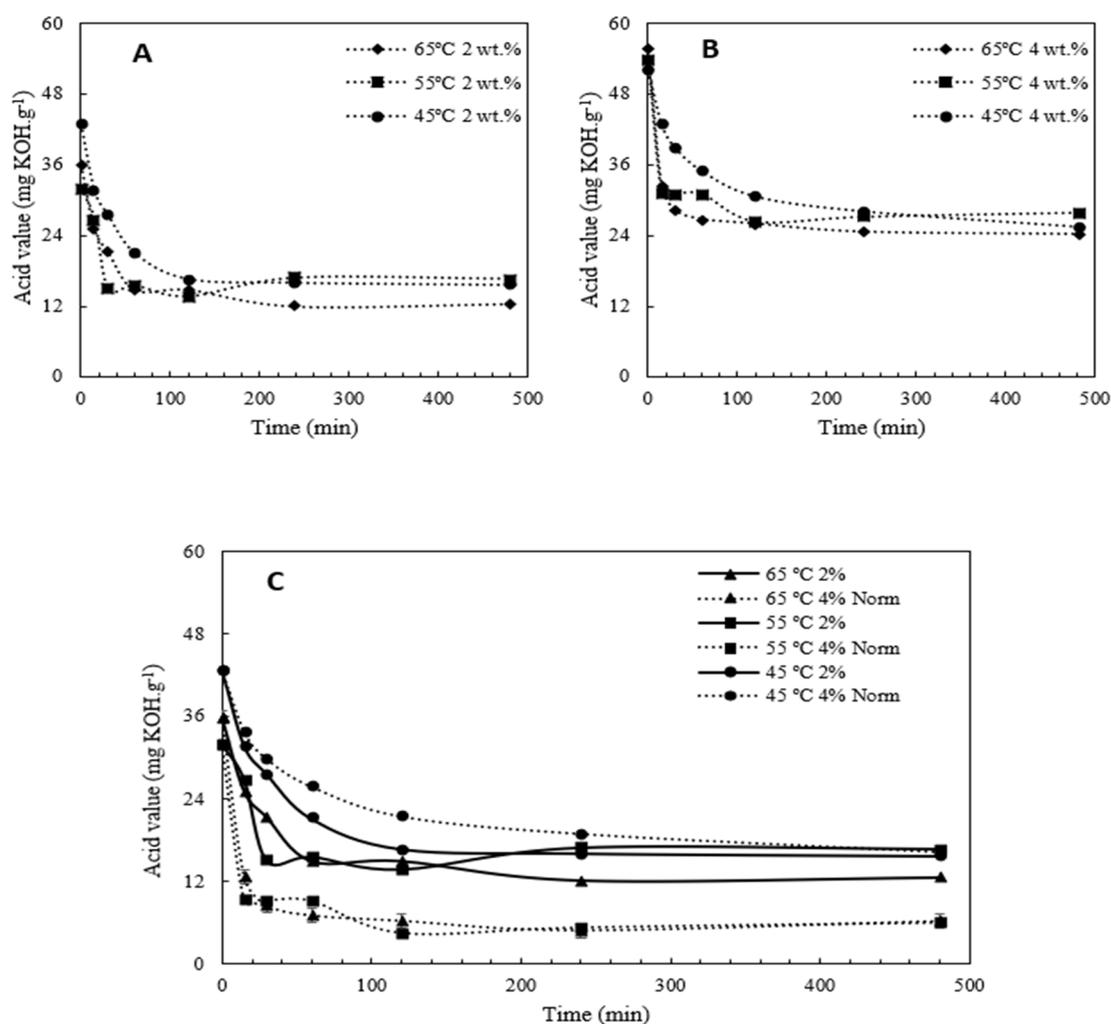


Figure 3. Evolution of the acidity during the homogenous acid esterification of the acid oil for a catalyst amount of 2 wt.% (A) and 4 wt.% (B) at different temperatures; results comparing the use of 2 wt.% catalyst and normalized values in the case of 4 wt.% (C).

The use of different amounts of acid catalyst (2 wt.% or 4 wt.%) led to different initial acid values; thus, direct comparisons were not performed. Consequently, at the end of the acid esterification using a 4 wt.% catalyst concentration (Figure 3B), steady state was reached for a higher acid value than that verified using 2 wt.% (Figure 3A). In agreement, and to support the analysis, Figure 3C shows the acidity evolution for the catalyst concentration of 2 wt.% and for normalized values (4% norm). Normalized values were obtained by discounting the difference between the initial acidity of the products

(from 2 to 4%), which resulted from the excess of mineral acid, to compare the results at the different catalyst concentrations.

According to Figure 3A,B, when increasing the temperature from 45 °C to 55 °C or 65 °C, the rate of the esterification reaction increased significantly during the first two hours (reflected by the higher slope of the curve). After 8 h, similar results were obtained. According to Figure 3C, the results indicated the relevance of increasing the catalyst concentration from 2 to 4 wt.% for the temperatures of 55 °C or 65 °C, whereas at 45 °C, no benefit was evidenced. The results using 55 °C or 65 °C were similar, and this was corroborated by the acid value determined after purification, which was very similar for the products obtained under these conditions (Table 3). In fact, several studies have suggested that the optimal reaction temperature for pretreating acid oil by esterification reaction is in the range of 55 °C to 65 °C [21,23].

Table 3. Characteristics of the homogenous acid esterification final product.

	65 °C, 4 wt.%	65 °C, 2 wt.%	55 °C, 4 wt.%	55 °C, 2 wt.%	45 °C, 4 wt.%	45 °C, 2 wt.%
Acid value, mg KOH.g ⁻¹	3.7	5.4	4.5	7.7	6.8	5.8
Acidity reduction (%)	92	88	90	83	85	88
FAME, wt.%	42	30	35	29	28	24

Among all the experimental conditions studied, the lowest acidities were obtained for the temperatures of 55 °C and 65 °C using a 4 wt.% catalyst concentration (Figure 3C—similar results).

Under the best conditions (higher acidity reduction, 65 °C and 4 wt.% of catalyst), after only 2 h of reaction, the acidity tended to stabilize, showing no relevance in increasing the reaction time. Generally, after 4 h, the acidity of the oil tended to stabilize. A similar trend was obtained in the study developed by Marchetti et al., where acid esterification at different temperatures (35 °C, 45 °C, 55 °C) was assessed using an acid oil with 10.68% acidity (a molar alcohol:acid oil ratio of around 6:1 and a catalyst concentration of 2.26 wt.%). According to the results of that study, the FFAs conversion tended to stabilize after approximately 3 h [34].

Considering that acid esterification requires relatively high temperatures, the time of the reaction is important for reducing process costs. Thus, further studies should be developed regarding the optimization of this parameter.

Equation (2) describes the pseudo-first-order kinetics of the esterification reaction, as previously discussed.

$$v = -\frac{dC(FFA)}{dt} = k' \times C(FFA) \quad (2)$$

After adjusting the results of the present study to a pseudo-first-order reaction, it was verified that the model had a low determination coefficient. As can be observed in Figure 3, this was expected because the acid value did not tend to zero (when the time tended to infinity), which was justified by high acidity of the product and the characteristics of the homogeneous acid esterification reaction. Consequently, the authors propose a modified pseudo-first-order equation, which results in Equation (3), where “a + b” represents the initial FFA concentration and “a” represents the final FFA concentration.

$$C(FFA) = a + be^{-kt} \quad (3)$$

Table 4 shows the values of the different parameters for the modified pseudo-first-order reaction.

Table 4. Parameters obtained for the modified pseudo-first-order reaction in the homogeneous esterification at different temperatures and acid concentrations.

	65 °C, 4 wt.%	65 °C, 2 wt.%	55 °C, 4 wt.%	55 °C, 2 wt.%	45 °C, 4 wt.%	45 °C, 2 wt.%
A	0.388	0.193	0.4249	0.291	0.409	0.2394
B	0.446	0.341	0.383	0.262	0.353	0.396
k' (min ⁻¹)	0.098 ± 0.009	0.037 ± 0.005	0.13 ± 0.05	0.05 ± 0.02	0.021 ± 0.004	0.029 ± 0.002
r^2	0.996	0.987	0.965	0.878	0.978	0.995

As previously verified, the results of the constant reaction rate showed an increase in the reaction kinetics when increasing the temperature and the catalyst concentration and when temperatures higher than 45 °C were used. According to the results, a higher constant rate was obtained for the temperature of 55 °C using 4 wt.% catalyst ($k' = 0.13 \text{ min}^{-1}$). However, considering the error, the final acid value and the acidity reduction (Table 3), the conditions using a temperature of 65 °C and 4 wt.% catalyst led to the best results.

In the study developed by Berrios et al., a pseudo-homogeneous first-order kinetic model was studied for the acid esterification of sunflower oil free fatty acids. For a methanol:oil molar ratio of 20:1 with 5 wt.% catalyst (sulfuric acid) at 60 °C, the constant rate obtained was between 0.010 and 0.020 min⁻¹, lower values than those found in the present study [35]. In the present study, the stirring speed was not considered, although this could have affected the kinetics of the reaction [36]. The authors used the most adequate stirring conditions considering previous studies and the existing laboratory apparatus; however, there is a margin for improvement to optimize this reaction variable.

By analysing in more detail the acid value and the FAME content of the final product obtained at the different experimental conditions (Table 3), different aspects can be detailed. Considering only the esterification reaction, it was expected that lower acid values would correspond to higher FAME contents; however, in some cases it was possible to observe that although the acid value was higher, the FAME content was also higher (e.g., comparing the results at 45 °C using 2 wt.% and at 55 °C using 2 wt.% catalyst). In addition, with lower expression compared to what was verified for the heterogeneous process further described, the results suggested that, apart from the FFAs, mono-, di- and triglycerides were also converted into FAME (24–42 wt.%) by the homogeneous acid transesterification reaction (as further explained), thus affecting these trends.

In the literature, it has been reported that sulfuric acid can catalyze the transesterification reaction in a temperature range from 55 °C to 88 °C [37]. As an example, according to the study developed by Canacki et al. [38], a transesterification reaction performed at 65 °C with 3 wt.% sulfuric acid led to a final FAME content of 88 wt.% after 48 h of reaction. In the study developed by Costa et al. [16], which applied homogenous acid esterification (65 °C, 2.0 wt.% catalyst, 9:1 methanol:acid oil molar ratio) using soapstock acid oil (with an initial acid value of 128.8 mg KOH.g⁻¹), after 7 h, a product with a less than 77% acidity was obtained, presenting a FAME value of 69 wt.%. Considering that esterification and transesterification reactions share reagents and some operational conditions, at the end of the pretreatment process (“esterification”), a FAME content higher than that using only esterification was expected, although much higher reaction times are usually required for relevant conversion through transesterification.

3.3. Heterogenous Enzymatic Esterification Pretreatment

The use of heterogenous catalysts in esterification reactions provides an easier purification of the final product, this being deemed as an eco-friendly alternative process [39]. In fact, to separate the pretreated oil from the catalyst, centrifugation was performed, thus avoiding the production of any wastewater flow. Beyond water savings, the time required for the purification step was also shortened when compared to homogenous esterification.

Based on the literature, it was expected that a 5 wt.% catalyst concentration would be enough to reduce the acidity of the oil to acceptable levels. Although there is the possibility of reusing the enzyme, due to economic constraints the optimization of the enzyme concentration is of high relevance [40], which highlights the relevance of developing future studies in this field. Figure 4 shows the evolution of the acidity reduction at 35 °C (mild temperature) after the addition of methanol in five steps for a 5 wt.% enzyme concentration.

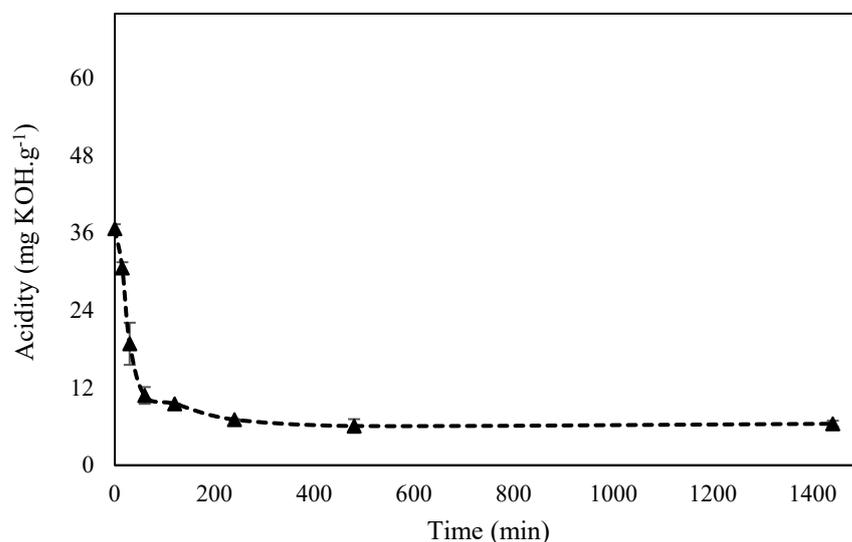


Figure 4. Acidity reduction during heterogenous enzymatic esterification reaction at 35 °C for 6:1 methanol:oil molar ratio with stepwise alcohol addition (5 wt.% catalyst). Errors bars show maximum and minimum values of the experiments in duplicate.

According to Figure 4, at the beginning of the reaction the acidity of the mixture decreased very quickly (almost immediately) to around 36 mg KOH.g⁻¹ (the initial acidity of the oil was 46.5 mg KOH.g⁻¹). However, some of this acidity reduction was due to the addition of methanol (dilution effect). After only 60 min, it was possible to achieve an acidity reduction of 70% (10.8 mg KOH.g⁻¹).

After purification (removal of methanol and enzyme), the rice bran pretreated oil showed an acid value of 7 mg KOH.g⁻¹ (Table 5), which corresponded to a FFAs reduction of 85%.

Table 5. Characteristics of the product obtained after heterogenous esterification of RBO.

	35 °C, 5 wt.%
Acid value, mg KOH.g ⁻¹	7
FAME, wt.%	87

As the initial FFAs content represented only around 23 wt.% of the oil's mass (46.5 mg KOH.g⁻¹ of the acid value for unrefined RBO) that could be converted into FAME, it is possible to conclude that the esterification and transesterification reactions occurred simultaneously and that, in this case, transesterification was extensively promoted. Similar results have been previously found in the literature [29,41].

The first 8 h of reaction corresponded to the period with the highest reduction in acidity (82%). After that, the acidity reduction was very low (only 3%), with the final results after 24 h being similar to those obtained after 8 h.

In the study by Mulalee et al. [31], which evaluated the esterification reaction of acidic palm oil (5 wt.% enzyme, 45 °C, 2:1 ethanol:oil molar ratio, 200 rpm), and in the study by Cruz et al. [29], which evaluated the pretreatment of soapstock acid oil with an FFA content of 65.5 wt.% (4–5 wt.% enzyme, 35 °C, methanol:oil molar ratio 1.5:1, 200 rpm), steady state

was reached after approximately 7 h, with FFAs reductions of 80–90% [29,31]. Finally, the FAME content in the final product was around 68 wt.% [29]. Based on the results of the present study, steady state was achieved after approximately 8 h with an FFAs reduction of 82%. These results are in agreement with those reported in the literature for other types of feedstocks. The differences verified for the FAME content could be related to the different amounts of methanol used (methanol:oil molar ratio 1.5:1 vs. 9:1).

The high conversion of FFAs related to the high FAME content at the end of the process, over a short period of time and using a mild temperature, reveals the importance of enzymatic esterification as an alternative process for bioenergy production using acid RBO.

Regarding cost reduction, Choi et al. [42] showed that it might be possible to perform enzymatic esterification without the addition of enzymes. In that study, a FAME content of 87% could be obtained after 12 days via an in situ lipase-catalyzed reaction with only the addition of methanol [43]. Rice bran is known to contain several types of lipases (phospholipases, glycolipases, esterases), which might improve the economic sustainability of RBO pretreatment aiming at bioenergy production [43]. In agreement, in further studies with RBO, the presence of in situ lipases should be considered.

Equation (4) shows the results for the modified pseudo-first-order kinetic model obtained by enzymatic esterification.

$$C(\text{FFA}) = 0.1 + 0.4693e^{-0.028.t} \quad (4)$$

In this case, the constant rate obtained was $0.028 \pm 0.005 \text{ min}^{-1}$, and a good fitting was found ($r^2 = 0.975$). When the constant rate was compared to the best-established conditions for the homogeneous acid process ($k' = 0.098 \text{ min}^{-1}$), it was clear that the decay in the oil's acidity was faster in the homogeneous process. However, in the end, a higher biodiesel conversion was achieved. V eras et al. [44] studied the kinetics of the enzymatic esterification of FFAs from palm oil (0.805 mol eq. oleic acid; ~26 wt.% considering oleic acid and triolein as reference) using immobilized lipase at higher temperatures of 60 °C (ethanol:oil molar ratio of 5:1 and 1.5% *w/v*), and they obtained a lower constant of 0.015 min^{-1} . The differences between the results were expected due to the reaction conditions employed, namely the type of enzyme, temperature and stirring speed.

4. Conclusions

The results from the present study showed that the high acid value of rice bran oil (47 mg KOH.g^{-1}) could be effectively reduced either by homogenous acid esterification (65 °C or 55 °C, 4 wt.% catalyst during 8 h leading to an acidity reduction between 90–92 wt.%) or enzymatic esterification (35 °C, 5 wt.% catalyst during 24 h leading to an acidity reduction of 85 wt.%).

Homogenous acid esterification was an efficient process to reduce the FFAs content of acid RBO. In terms of the reaction conditions using a homogeneous acid-catalyzed process, an increase in the catalyst concentration from 2 to 4 wt.% for temperatures of 55 °C or 65 °C was beneficial for free fatty acids reduction, whereas at 45 °C no benefits occurred. Temperatures higher than 45 °C improved the reaction rate during the first hours; after 8 h, the differences were small. It was thus concluded that a balance between the temperature and FFAs conversion should be carefully conducted considering economic and environmental factors.

Under all conditions, the results obtained for the homogeneous process showed a very good fit ($r^2 > 0.878$) to a modified pseudo-first-order kinetics model.

Using the biocatalyzed process, better overall results were obtained (higher FAME content in the final product), although the reaction presented a lower constant rate ($k' = 0.028 \text{ min}^{-1}$) when compared with the best conditions of the homogenous acid esterification process ($k' = 0.098 \text{ min}^{-1}$). It was possible to reduce the acidity from around 47 mg KOH.g^{-1} to 11 mg KOH.g^{-1} after only 60 min, showing the final pretreated product a FAME content higher than that obtained using the homogeneous acid catalyst.

At the end of both processes (homogeneous and heterogenous esterification), evidence of transesterification was found. This was very expressive in the case of the biocatalyzed process, in which the FAME content of the product reached 87 wt.%, highlighting the relevance of such a process. This study showed that acid rice bran oil could be used as a raw material for biodiesel production, and that the biocatalyzed conversion of free fatty acids and glycerides might be an interesting alternative process aiming at FAME production without the need for pretreatment. In fact, the easy purification of the final product combined with the use of mild temperatures makes enzymatic catalysis very appealing for bioenergy production. Further studies should also consider in situ lipase-catalyzed processes.

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