

Article

Biocatalytic Production of Solketal Esters from Used Oil Utilizing Treated Macauba Epicarp Particles as Lipase Immobilization Support: A Dual Valorization of Wastes for Sustainable Chemistry

José Miguel Júnior ^{1,2} , João V. B. Dimas ² , Milson S. Barbosa ³, Raphael A. B. Gomes ⁴, Ana K. F. Carvalho ⁵ , Cleide M. F. Soares ^{6,7} , Roberto Fernandez-Lafuente ^{8,*}  and Adriano A. Mendes ^{1,2,*} 

- ¹ Graduate Program in Biotechnology, Federal University of Alfenas, Alfenas 37130-001, MG, Brazil; jose.junior@sou.unifal-mg.edu.br
 - ² Institute of Chemistry, Federal University of Alfenas, Alfenas 37130-001, MG, Brazil; joao.dimas@sou.unifal-mg.edu.br
 - ³ Federal Institute of Education, Science and Technology of Paraíba—Campus Cajazeiras, Cajazeiras 58900-000, PB, Brazil; barbosamilson@hotmail.com
 - ⁴ School of Nutrition, Federal University of Ouro Preto, Ouro Preto 35400-000, MG, Brazil; raphabgomes@gmail.com
 - ⁵ Department of Basic and Environmental Sciences, Engineering School of Lorena, University of São Paulo, Lorena 12602-810, SP, Brazil; anacarvalho@usp.br
 - ⁶ Graduate Program in Process Engineering, Tiradentes University, Aracaju 49032-490, SE, Brazil; cleide.mara@souunit.com.br
 - ⁷ Institute of Technology and Research, Aracaju 49032-490, SE, Brazil
 - ⁸ Departamento de Biotálisis, Instituto de Catálisis y Petroleoquímica—Consejo Superior de Investigaciones Científicas (ICP-CSIC), Campus UAM-CSIC, 28049 Madrid, Spain
- * Correspondence: rfl@icp.csic.es (R.F.-L.); adriano.mendes@unifal-mg.edu.br (A.A.M.)



Citation: Miguel Júnior, J.; Dimas, J.V.B.; Barbosa, M.S.; Gomes, R.A.B.; Carvalho, A.K.F.; Soares, C.M.F.; Fernandez-Lafuente, R.; Mendes, A.A. Biocatalytic Production of Solketal Esters from Used Oil Utilizing Treated Macauba Epicarp Particles as Lipase Immobilization Support: A Dual Valorization of Wastes for Sustainable Chemistry. *Catalysts* **2024**, *14*, 693. <https://doi.org/10.3390/catal14100693>

Academic Editor: Evangelos Topakas

Received: 18 September 2024

Revised: 1 October 2024

Accepted: 4 October 2024

Published: 5 October 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: This study describes the production of solketal esters from used soybean cooking oil (USCO) via enzymatic hydroesterification. This process consists of the complete hydrolysis of USCO into free fatty acids (FFAs) catalyzed by crude lipase extract from *Candida rugosa* (CRL). The resulting FFAs were recovered and utilized as the raw material for an esterification reaction with solketal, which was achieved via an open reaction. For this purpose, lipase Eversa[®] Transform 2.0 (ET2.0) was immobilized via physical adsorption on treated epicarp particles from *Acrocomia aculeata* (macauba), a lignocellulosic residue. A protein loading of $25.2 \pm 1.3 \text{ mg g}^{-1}$ with a support and immobilization yield of $64.8 \pm 2.5\%$ was achieved using an initial protein loading of 40 mg g^{-1} of support. The influence of certain parameters on the esterification reaction was evaluated using a central composite rotatable design (CCRD). Under optimal conditions, a FFAs conversion of $72.5 \pm 0.8\%$ was obtained after 150 min of reaction at 46°C using a biocatalyst concentration of 20% wt. and a FFAs–solketal molar ratio of 1:1.6. The biocatalyst retained 70% of its original activity after ten esterification batches. This paper shows the conversion of two agro-industrial waste into valuable materials (enzyme immobilization support and solketal esters).

Keywords: solketal esters; hydroesterification; lipase; agro-industrial wastes; valorization

1. Introduction

Solketal esters, a valuable class of chemical compounds extensively used as dietary supplements, antimicrobial agents, oxygenated fuel additives, and plasticizing agents, are traditionally produced by esterification and/or transesterification reactions catalyzed by acid or basic classical chemical catalysts [1–6]. These reaction systems require the utilization of high temperatures to achieve high levels of conversion/high yields [1–4,7]. Moreover, complex reaction schemes utilizing hazardous organic solvents or ionic liquids

to improve the miscibility of the raw materials are required [1,3,6–8]. Therefore, the utilization of lipases as catalysts is of great interest due to their advantages compared to traditional chemical routes; for example, they require less extreme experimental conditions, reducing energy demand, and have high specificity and selectivity, reducing the formation of undesirable byproducts, minimizing the generation of wastes, and simplifying the purification steps [9,10].

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are a class of enzymes that catalyzes the hydrolysis of triacylglycerols to glycerol and free fatty acids (FFAs) [11,12]. They have been utilized as biocatalysts in the production of solketal esters, as lyophilized crude extracts [13,14], as lyophilized mold mycelia [15–17], or in immobilized forms using different supports [8,18–24]. These studies have also been preferentially conducted in solvent systems using hazardous classical organic solvents [13,16,18,22,23]. In a previous study performed in our group, a saturated solketal ester was produced via enzymatic esterification using palmitic acid as the raw material [22]. Under optimal conditions, a maximum acid conversion of 83% was achieved after 150 min in a heptane medium. The use of solvent-free reaction systems in the enzymatic production of several chemicals, including solketal esters, has attracted attention due to its potential for use in environmentally friendly processes of industrial interest. The use of these systems can avoid the use of any additional organic solvent, although these systems have their own drawbacks [9].

In this context, the objective of the present study was to develop a sustainable means of producing solketal esters from used soybean cooking oil (USCO) via a two-step enzymatic process (hydroesterification), as shown in Figure 1. This process has been extensively utilized in ester production [25,26], since the direct transesterification of triacylglycerols with various alcohols, including solketal, requires high temperatures or long reaction times to obtain high ester yields because the high viscosity of the reaction medium increases the mass transfer limitations [2,20,27,28]. The first step comprises the complete hydrolysis of USCO, utilizing a non-specific lipase from *Candida rugosa* (CRL) as a biocatalyst [29]. After eliminating the glycerin (which can become a competitor for the target reaction), the resulting FFAs were esterified with solketal in open reactors in a reaction catalyzed by lipase Eversa[®] Transform 2.0 (ET2.0), a low-cost means of lipase preparation with high catalytic activity that is reusable in esterification reactions [30,31]. In this reaction, ET2.0 was immobilized via physical adsorption [32,33] on treated *Acrocomia aculeata* (macauba) epicarp particles (Figure S1). This oleaginous plant has been considered a highly promising oil-producer. This oil may be useful in the production of biodiesel and biokerosene for aviation [34,35]. However, its processing generates large amounts of wastes. Therefore, this study intends to transform this agro-industrial waste, which is currently mainly disposed of in nature, into a valuable lipase immobilization support. Although the utilization of lignocellulosic wastes as enzyme support materials has been extensively studied [36–38], the use of this renewable support to immobilize enzymes has not been reported in the literature to date. However, these materials require pre-treatment to ensure the development of appropriate particle features, such as a specific size, surface, and pore diameter, to provide stable and active biocatalysts with a high enzyme-retention capacity [36].

This study also aims to present a promising strategy to mitigate the deleterious and hazardous effects of the improper disposal of waste cooking oils to the environment, as this can damage plants, animals and marine life [39]. Therefore, the production of valuable chemicals using recycling waste cooking oils can offer several benefits, such as economic, social, environmental, and waste management benefits. Moreover, this study also intends to expand the potential application of Eversa[®] Transform 2.0 (ET2.0) in industrial processes.

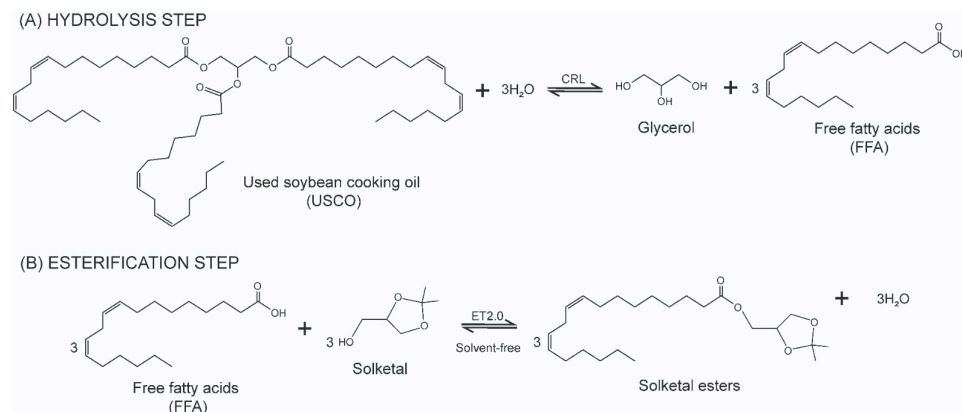


Figure 1. Illustrative scheme of the enzymatic production of solketal esters from USCO via the hydroesterification process.

2. Results and Discussion

2.1. Macauba Epicarp Particles Characterization

In this study, preliminary tests were conducted using untreated macauba epicarp particles as a support material to immobilize ET2.0. However, a very small amount of adsorbed enzyme, or immobilized protein loading— $\text{IP} \leq 5 \text{ mg}_{\text{protein}} \text{ g}^{-1}$, was used as a support, using a high level of lipase loading (40 mg g^{-1} of support) was obtained due to the release of components (extractives) of this material in the immobilization supernatant, resulting in strong turbidity (a milky appearance). Therefore, the use of macauba epicarp particles as a lipase immobilization support requires efficient pre-treatment technologies to remove extractives, hemicellulose, and/or lignin fractions to provide a material with better features [36,37]. A series of pre-treatment methods was proposed based on recent reports, such as acid pre-treatment and solvent extraction processes [40–42]. In this study, the influence of pre-treatment techniques on the properties of macauba epicarp particles was evaluated by FT-IR, XRD, and SEM analyses and compared with those of their untreated form.

An FT-IR analysis was conducted to identify changes in functional groups induced by the treatment of macauba epicarp particles within $3600\text{--}500 \text{ cm}^{-1}$. According to Figure 2, both untreated and treated samples exhibited a broad band at 3329 cm^{-1} related to O–H stretching vibrations, which corresponds to the presence of alcoholic and phenolic hydroxyl groups in their structures.

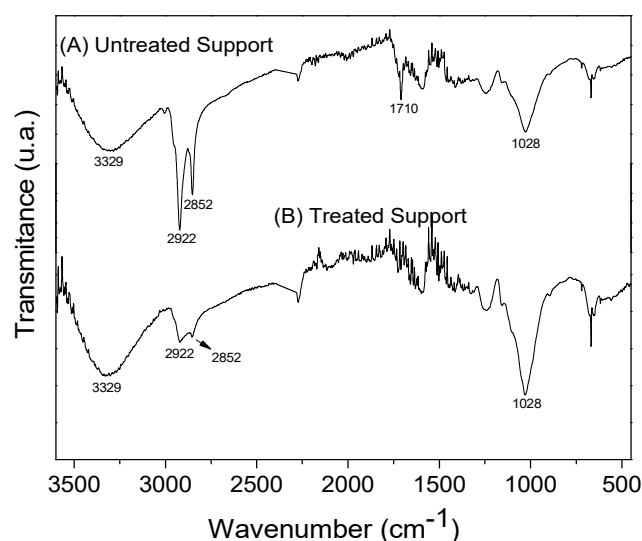


Figure 2. FT-IR spectra of untreated (A) and treated (B) macauba epicarp particles.

On the other hand, a sharp decrease in the intensity of the band between 2922 cm^{-1} and 2852 cm^{-1} was observed after the treatment, assigned to the stretching vibrations of C–H bonds of methyl and methylene groups, in addition to the disappearance of the band associated with the stretching vibrations of the C=O bond in carbonyl groups of 1710 cm^{-1} . This disappearance of this characteristic band suggests a possible cleavage in acetyl and uronic esters, and/or ester bonds of ferulic and *p*-coumaric acids in the structure of the lignocellulosic materials [43]. These results indicate the partial removal of hemicellulose and lignin fractions during the treatment process [44]. Moreover, an increase in the intensity of the characteristic band at 1028 cm^{-1} related to C–OH stretching vibrations was also observed, which suggests an increase in the percentage of cellulose content in the chemical composition of the treated support material [44,45]. These results confirm that changes occurred in the chemical composition of macauba epicarp after the treatment processes.

The X-ray diffraction patterns for untreated and treated macauba epicarp particles are illustrated in Figure 3. A typical sharp peak in the untreated support material at 2θ values between 16 and 25° , which is characteristic of crystalline cellulose, was observed. After the treatment process, an increase in the intensity of this peak, with the concomitant appearance of a peak at a 2θ value of around 34° , was detected. This X-ray diffractogram reveals the loss of amorphous components such as lignin, hemicellulose, and extractive fractions after the sequential treatment process [44,46], which corroborates the results of the FT–IR analysis.

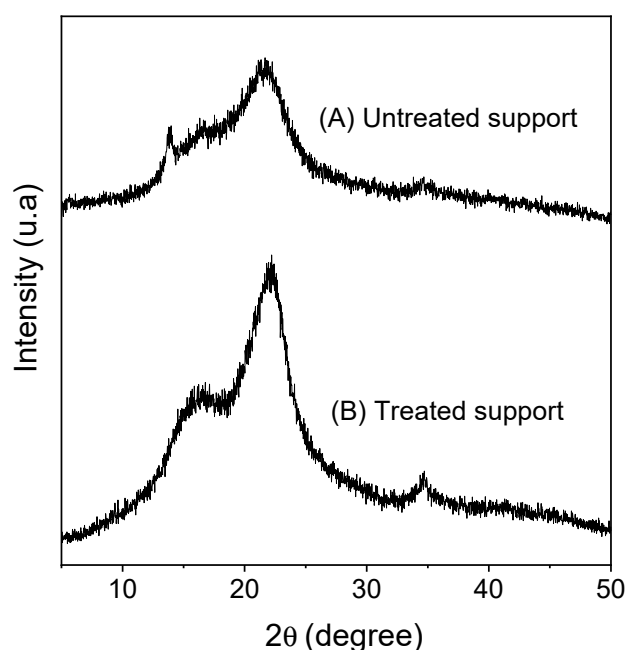


Figure 3. X-ray patterns of untreated (A) and treated (B) macauba epicarp particles.

An SEM analysis was performed in order to investigate the changes in the morphology of the macauba epicarp particles after the treatment process (Figure 4). Untreated macauba epicarp particles have an irregular structure (Figure 4A) and a smooth and highly ordered surface structure. Due to this non-porous structure (Figure 4B), a low capacity to adsorb ET2.0 may be expected. After the treatment process, the material presented a porous structure that resulted in the exposure of its internal surface (Figure 4C). These images show that the rigid structure of the native material was disarranged due to the cleavage and/or partial removal of hemicellulose and/or lignin fractions and extractives, in accordance with FT–IR and XRD analyses. Moreover, the mass balance shown in Figure S2 also reveals a mass loss of around 54% (from $19.1 \pm 0.7\text{ g}$ to $8.88 \pm 0.4\text{ g}$) after the combined treatment processes, which resulted in the formation of a porous structure. This can improve its specific area and, in this way, could perhaps improve its enzyme adsorption capacity.

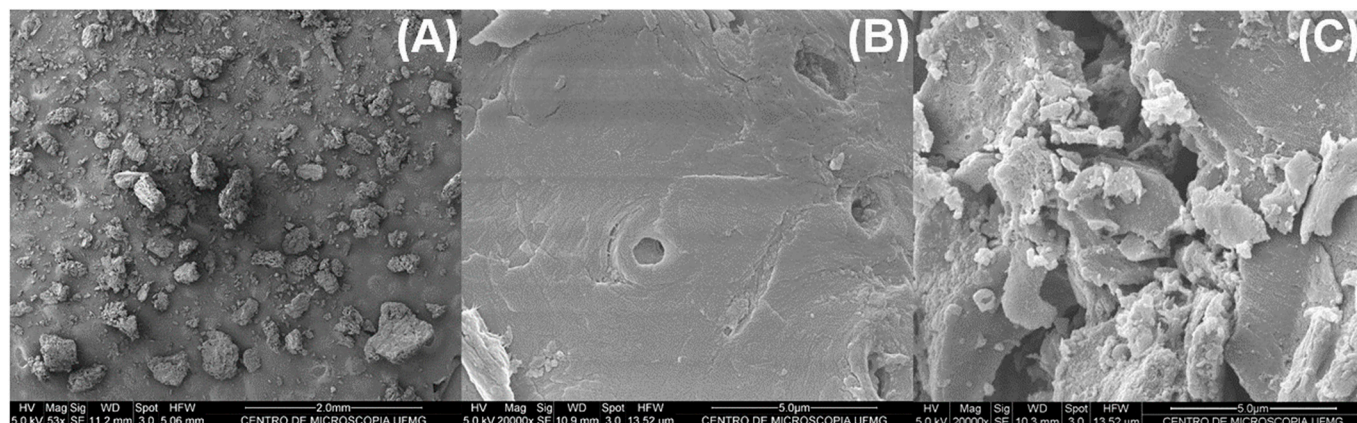


Figure 4. SEM images of untreated macauba epicarp particles at a magnification of $53\times$ (A) and $20,000\times$ (B), and treated particles at magnification of $20,000\times$ (C).

2.2. Characterization of the Heterogeneous Biocatalyst Prepared via Physical Adsorption

The support material that was produced was used in the physical adsorption of ET2.0 and its properties were compared with those of its soluble form (liquid lipase extract). In this study, the immobilization process was performed at pH 5.0 [47]. Under this condition, the maximum hydrophobic interaction between ET2.0 and support surface via interfacial activation is expected, since the isoelectric point of this lipase is around 4.4 [48]. Initially, the stability of the soluble ET2.0 was evaluated under the immobilization conditions (5 mmol L^{-1} buffer sodium acetate, pH 5.0, at $25\text{ }^{\circ}\text{C}$ and 15 h). Under these conditions, the lipase fully retained its original hydrolytic activity. This shows that all units of hydrolytic activity disappeared in the supernatant of the immobilization suspension due to the physical adsorption of the enzyme on the support surface. According to our results, $25.2 \pm 1.3\text{ mg protein g}^{-1}$ of support was immobilized (almost five times higher than that obtained using the untreated support material), and this corresponds to 62.5% of the initial protein. This value was very similar to the immobilization yield that was obtained (YI value of $64.8 \pm 2.5\%$) based on the units of hydrolytic activity that disappeared in the supernatant during the immobilization suspension, thus showing that the ET2.0 formulation has a very low amount of contaminant proteins [49]. This demonstrates that the capacity of treated macauba epicarp particles to immobilize lipase was very similar to or even higher than that of other supports, such as the silica–alumina supports Siral 20 and Siral 40 [50], functionalized rice husk silica [51], metal–organic frameworks [52], and commercial Purolite® supports [53]. The resulting immobilized lipase exhibited a hydrolytic activity of $356.6 \pm 23.5\text{ U g}^{-1}$ when using olive oil as a biocatalyst, and a specific activity of $14.1\text{ U mg}_{\text{protein}}^{-1}$. This value corresponds to only 2.5% of the original specific activity of the liquid ET2.0 extract ($560\text{ U mg}_{\text{protein}}^{-1}$). This could be explained by diffusion problems caused by the olive oil emulsion penetrating the support pores where the enzyme molecules are located [30]. In this study, the resulting biocatalyst (immobilized ET2.0) was then utilized in the optimized production of solketal esters in a solvent-free system. Under such conditions, where the medium is just the reaction reagent and is in a homogenous solution, the raw materials (FFAs and solketal) have better access to the biocatalyst particle and, thus, a better catalytic performance is expected during the esterification reaction.

2.3. Optimization of the Enzymatic Production of Solketal Esters via Esterification Reaction

The effect of three relevant parameters (FFAs–solketal molar ratio, reaction temperature, and biocatalyst concentration) on ester production following the esterification of FFAs was evaluated using a full CCRD approach. The levels of each independent variable, as well as fixed parameters (initial mass of raw materials, mechanical stirring intensity, and reaction time), were chosen based on previous studies [22,30,31] and preliminary tests. Esterification is a thermodynamically controlled process [9]. Therefore, the reactions

were conducted in an open reactor allowing for simple water removal via evaporation at atmospheric pressure. Moreover, a molar excess of solketal was utilized, which is more easily separated from the reaction mixture at the end of the process (purification steps).

The coded and actual values of these parameters and their response (experimental and predicted FFAs conversion percentage) are shown in Table 1. According to our results, the experimental FFAs conversion percentage at 40 min of reaction varied from $6.2 \pm 3.5\%$ (Run #1) to $55.7 \pm 2.4\%$ (Run #14). This large difference between the minimum and maximum response variable values clearly shows the importance of the variables proposed in this study in the optimization process. It is possible to observe that both the experimental and predicted FFAs percentage values were very similar, showing the high level of accuracy obtained by the proposed polynomial model (see Figure S3).

Table 1. CCRD experimental design for the enzymatic production of solketal esters via esterification conducted using solvent-free systems.

Runs	Independent Variables' Coded (Actual) FFAs Conversion (%)			FFAs Conversion (%) ^a
	FFAs–Solketal Molar Ratio	Temperature (°C)	Biocatalyst Concentration (% wt.)	
1	−1 (1:1.6)	−1 (46)	−1 (8)	6.2 ± 3.5
2	+1 (1:3.4)	−1 (46)	−1 (8)	30.2 ± 2.8
3	−1 (1:1.6)	+1 (64)	−1 (8)	23.4 ± 2.2
4	+1 (1:3.4)	+1 (64)	−1 (8)	24.6 ± 1.7
5	−1 (1:1.6)	−1 (46)	+1 (17)	44.5 ± 0.5
6	+1 (1:3.4)	−1 (46)	+1 (17)	40.5 ± 2.1
7	−1 (1:1.6)	+1 (64)	+1 (17)	43.0 ± 0.3
8	+1 (1:3.4)	+1 (64)	+1 (17)	30.1 ± 3.8
9	−1.68 (1:1)	0 (55)	0 (12.5)	21.7 ± 1.4
10	+1.68 (1:4)	0 (55)	0 (12.5)	22.2 ± 2.2
11	0 (1:2.5)	−1.68 (40)	0 (12.5)	28.9 ± 0.1
12	0 (1:2.5)	+1.68 (70)	0 (12.5)	39.8 ± 2.1
13	0 (1:2.5)	0 (55)	−1.68 (5)	19.3 ± 0.9
14	0 (1:2.5)	0 (55)	+1.68 (20)	55.7 ± 2.4
15	0 (1:2.5)	0 (55)	0 (12.5)	35.7 ± 1.3
16	0 (1:2.5)	0 (55)	0 (12.5)	30.2 ± 1.3
17	0 (1:2.5)	0 (55)	0 (12.5)	31.5 ± 0.3
18	0 (1:2.5)	0 (55)	0 (12.5)	33.0 ± 2.2

^a Experimental values obtained after 40 min of reaction.

The significance of each individual parameter and their interactions on the response variable (FFAs conversion percentage), as determined by Student's *t*-test, is shown in Table 2. The analysis of variance (ANOVA) shows that the mean, quadratic term of the FFAs–solketal molar ratio (x_1^2), the linear term of the biocatalyst concentration (x_3), and the interactions ($x_1.x_2$, $x_1.x_3$, and $x_2.x_3$) were highly significant variables at the 95% confidence level (*p*-values < 0.05). On the other hand, the non-significant parameters (*p*-values > 0.05) were the linear terms of the FFAs–solketal molar ratio (x_1), the linear and quadratic terms of the reaction temperature (x_2 and x_2^2), and the quadratic term of biocatalyst concentration (x_3^2); these were excluded from the model.

Table 2. Regression coefficients, standard errors, *p*-values, and analysis of variance (ANOVA) for the developed model of the enzymatic production of solketal esters.

Parameters	Regression Coefficients	Standard Errors	<i>p</i> -Values	
Mean	32.64	1.68	0.0000	
x ₁	0.66	0.91	0.4897 *	
x ₁ ²	−3.96	0.95	0.0031	
x ₂	1.32	0.91	0.1875 *	
x ₂ ²	0.43	0.95	0.6605 *	
x ₃	9.88	0.91	0.0000	
x ₃ ²	1.53	0.95	0.1457 *	
x ₁ .x ₂	−3.96	1.19	0.0106	
x ₁ .x ₃	−5.27	1.19	0.0022	
x ₂ .x ₃	−2.92	1.19	0.0400	
ANOVA				
Source of Variation	Sum of Squares	Degree of Freedom	Mean Square	<i>F</i> -Test
Regression	2058.6732	9	228.74	20.11
Residual	90.9850	8	11.37	
<i>Lack of fit</i>	74.0784	5	14.82	2.63
<i>Pure error</i>	16.9066	3	5.64	
Total	2149.6582	17		

$$R^2 = 0.9577; F_{0.05,9,8} = 3.39$$

x_1 , x_2 , and x_3 represent the FFAs–solketal molar ratio, reaction temperature, and biocatalyst concentration, respectively. * Non-significant parameters at 95% confidence level.

The significant terms were utilized to obtain the second-order polynomial model used to describe the reaction, as shown by Equation (1):

$$Y(\%) = 32.64 - 4.30x_1^2 + 9.88x_3 - 3.96x_1 \cdot x_2 - 5.27x_1 \cdot x_3 - 2.92x_2 \cdot x_3 \quad (1)$$

where x_1 , x_2 , and x_3 represent the FFAs–solketal molar ratio, reaction temperature, and biocatalyst concentration, respectively.

The coefficient of determination, or correlation (R^2), was utilized to show the adequacy of the proposed model. According to Table 2, a R^2 value of 0.9577 was obtained. The adequacy of the model was also confirmed by Fisher's *F*-test (see Table 2).

The calculated *F*-value (20.11) was greater than the tabulated *F*-value at the 5% significance level (3.39), which clearly shows its significance. On the other hand, the calculated *F*-value for lack of fit (2.63) was not significant (*p*-value > 0.05). These results show that the second-order polynomial model can be utilized to predict and determine the optimal conditions for the production of solketal esters via esterification reaction using the proposed biocatalyst, as shown in Figure 5A,B.

The 3D response surface and contour plots of the effect of the independent variables of bio-catalyst concentration and FFAs–solketal molar ratio on the reaction are shown in Figure 5A. As expected, a gradual increase in the FFAs conversion percentage can be observed with an increase in the biocatalyst concentration from the lowest (5% wt.—coded value of −1.68) to the highest (20% wt.—coded value of +1.68) levels. In fact, the highest coefficient and highest positive value in Equation (1) were obtained for the linear term of biocatalyst concentration (+9.88 x_3), demonstrating its significant effect on the esterification reaction. These results show that the maximum FFAs conversion percentage can be achieved by utilizing a slight excess of solketal in the reaction mixture, i.e., an FFAs–solketal molar ratio of 1:1.6 (coded value of −1). The mutual interaction between these variables was significant at the 95% confidence level (−5.27 $x_1 \cdot x_3$ —see Equation (1)). Therefore, the maximum FFAs conversion percentage can be obtained at the highest biocatalyst concentration level—20% *w/w* of reaction mixture and an FFAs–solketal molar ratio of 1:1.6.

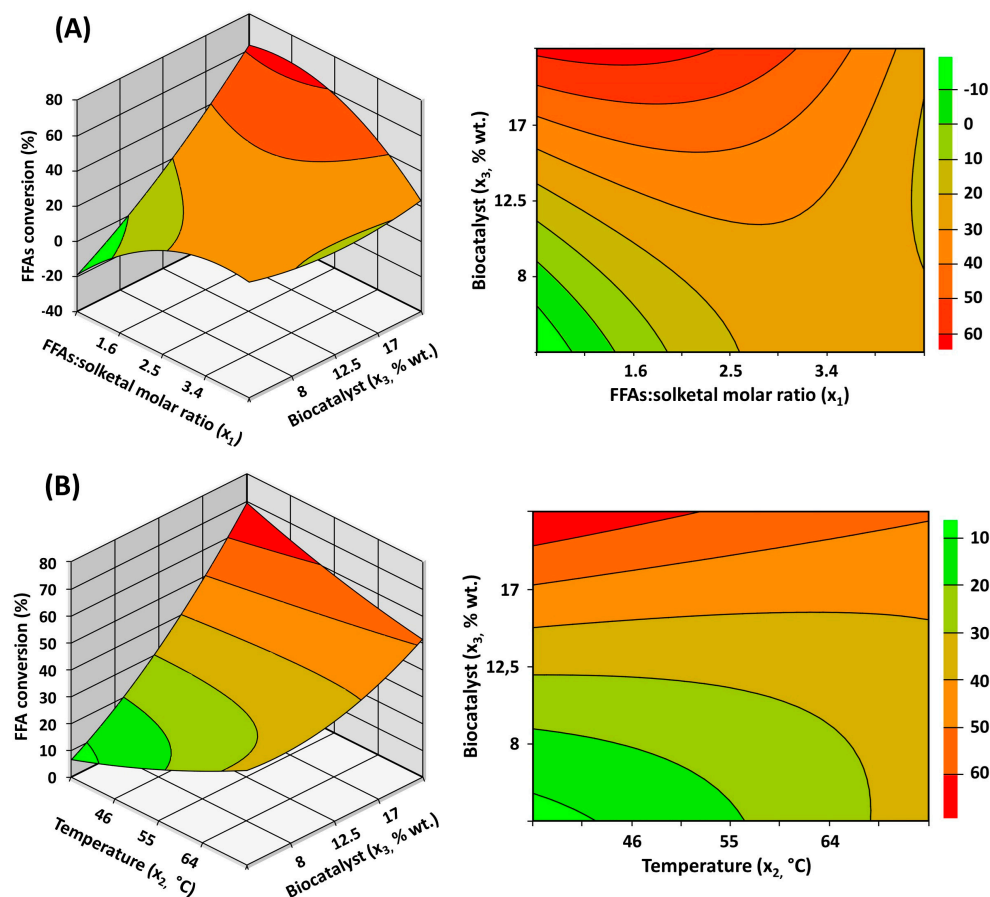


Figure 5. Three-dimensional response surface and contour plots for the enzymatic production of solketal esters. Effect of the FFAs–solketal molar ratio and biocatalyst concentration (A), and reaction temperature and biocatalyst concentration (B), on the FFAs conversion percentage.

The effects of the reaction temperature and biocatalyst concentration are illustrated in Figure 5B. The three-dimensional response surface and contour plots analyses show that the maximum FFAs conversion percentage can be observed at the highest biocatalyst concentration (20% *w/w*; coded value of +1.68) and reaction temperature of 46 °C (coded value of −1). The interaction between these two individual parameters was also significant at the 95% confidence level, with a negative coefficient value ($-2.92x_2 \cdot x_3$); see Equation (1). This means that a positive effect on FFAs conversion can also be achieved by using the highest biocatalyst concentration (20% *w/w*) and a reaction temperature of 46 °C.

The validation of the second-order polynomial model was performed by conducting the reaction under optimal experimental conditions: a FFAs–solketal molar ratio of 1:1.6, reaction temperature of 46 °C, and biocatalyst concentration of 20% *w/w*. Under these conditions, the maximum FFAs conversion percentage value of $50.8 \pm 1.0\%$ was achieved, which was similar to the predicted value ($Y = 54.7\%$). Therefore, subsequent tests were conducted under such conditions.

2.4. Reaction Course

The reaction course was studied under the optimal experimental conditions established by CCRD (FFAs–solketal molar ratio of 1:1.6, reaction temperature of 46 °C, and biocatalyst concentration of 20% *w/w* of reaction mixture). In this set of experiments, soluble ET2.0 was not used as a biocatalyst due to its lower stability/activity than its immobilized form in non-aqueous media and strong aggregation that may result in increased mass transfer limitations [30,31]. According to Figure 6, a linear profile of ester production was observed during the first 50 min of the reaction, showing the satisfactory

diffusivity of the raw material molecules (FFAs and solketal) to the internal biocatalyst microenvironment. After this period, a decrease in the reaction rate could be observed due to a possible increase in the viscosity of the reaction mixture, thus restricting raw materials' access to the biocatalyst's surface. Moreover, the decrease in the concentration of substrates and the increase in concentration of the product can slow the reaction rate and, additionally, the product may be inhibited as it accumulates. The highest FFAs conversion percentage (around $72.5 \pm 0.8\%$) was achieved after 150 min of reaction. These results show that an appropriate immobilization technique was capable of improving the enzyme performance in this process, in accordance with previous reports [30,31,49,54–56].

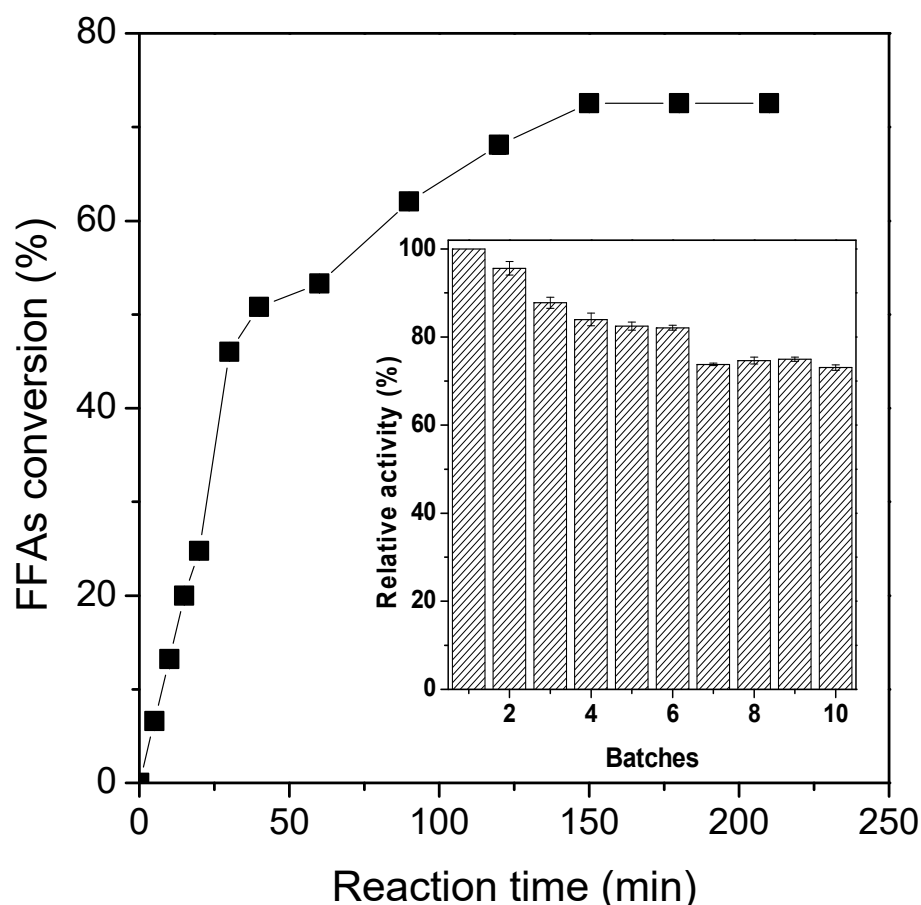


Figure 6. Reaction course of the esterification reaction catalyzed by immobilized ET2.0. The operational stability tests after successive esterification batches in a solvent-free system are shown. The reactions were conducted at 46 °C using an FFAs–solketal molar ratio of 1:1.6 and biocatalyst concentration of 20% *w/w* of reaction mixture.

2.5. Operational Stability Tests of the Immobilized Biocatalyst

The operational stability of the immobilized ET2.0 after ten successive esterification batches under optimal experimental conditions was analyzed. According to Figure 6 (inset), the immobilized ET2.0 led to a steady and progressive decrease in the activity until the seventh batch. This value remained unaltered until the 10th batch (FFAs conversion values around of 51% after 150 min of reaction). This value corresponds to 70% of its original activity (FFAs conversion of $72.5 \pm 0.8\%$ at the first batch). This decrease in catalytic performance could be due to thermal inactivation [54], the desorption of some lipase molecules from the support surface during the washing steps, and/or the possible accumulation of unconverted raw materials and/or products in the support, which could alter the microenvironment of the enzyme [57]. These results clearly demonstrate the satisfactory stability of ET2.0 after successive batch reactions under moderate conditions.

2.6. Identification of the Reaction Products via FT-IR and NMR Analysis

The FT-IR spectra of the raw materials (solketal and FFAs) and products (solketal esters) are shown in Figure 7. The spectrum of FFAs shows intense bands at 2922 cm^{-1} and 1710 cm^{-1} , attributed to the stretching of C-H overlapping with O-H bonds and the stretching vibrational band of the carbonyl group (-C=O) from the carboxylic moiety. The solketal spectrum presents an intense band at 3429 cm^{-1} , attributed to the axial deformation of -O-H bonds. The bands between 2991 and 2881 cm^{-1} correspond to the vibrations in the symmetrical and asymmetrical stretching of -C-H methyl and methylene groups. Moreover, the characteristic band between 1051 and 1157 cm^{-1} corresponds to the asymmetric vibrations of the -C-O-C-O-C- bond present in its structure. The spectrum of the solketal esters mixture shows an intense band at 1740 cm^{-1} , characteristic of the carbonyl group of the ester that forms after esterification, and the bands at 1221 and 1160 cm^{-1} correspond to the stretching vibrational of the -C(=O)O- group.

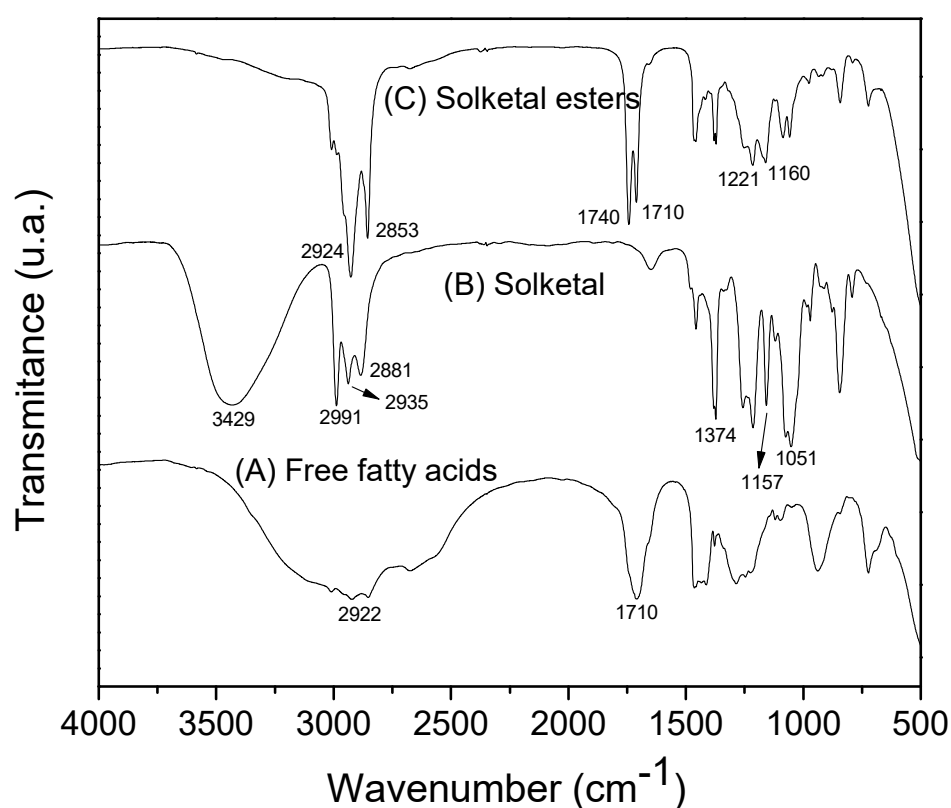


Figure 7. FT-IR spectra of free fatty acids from USCO (A), solketal (B), and solketal esters (C).

The production of solketal esters was also confirmed by NMR analysis. The ^1H NMR spectrum (Figure 8A) showed specific signals of a two-hydrogen triplet at 2.32 ppm for the methylene group bound to the carbonyl group from the ester moiety $[\text{-CH}_2(\text{C=O})\text{OCH}_2\text{-}]$, and a two-hydrogen triplet at 4.13 ppm for the methylene group attached to the oxygen atom $[\text{-CH}_2(\text{C=O})\text{OCH}_2\text{-}]$. The ^{13}C NMR spectrum (Figure 8B) shows an intense signal at 172.51 ppm that corresponds to the carbonyl group of the ester $[\text{-CH}_2(\text{C=O})\text{OCH}_2\text{-}]$. These spectroscopy analyses confirmed the production of solketal esters.

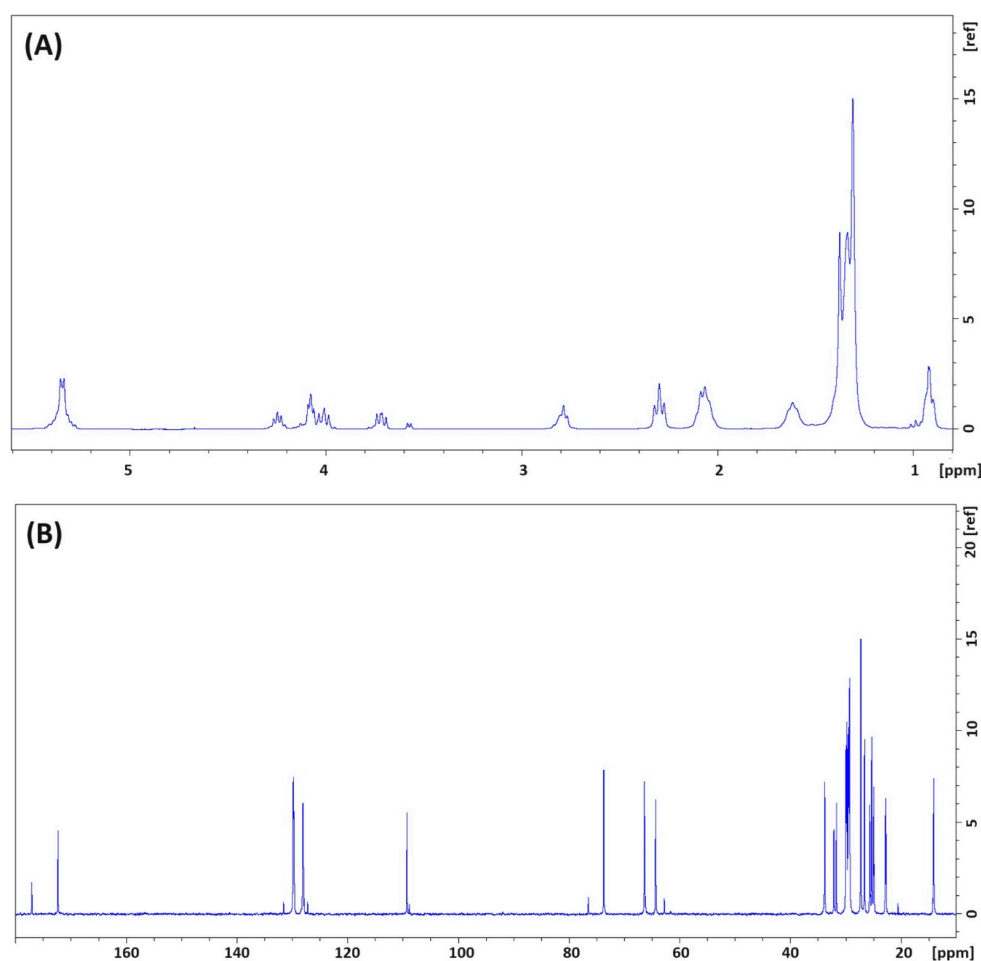


Figure 8. ^1H (A) and ^{13}C (B) NMR spectra of solketal esters.

2.7. Comparing with Previous Reports

The enzymatic production of solketal esters using different raw materials (synthetic monoesters, oils and fats, and their derivatives—FFA) has been reported in previous reports, as shown in Table S1. In general, these reaction systems require large amounts of solvent and long reaction times to achieve a high yield or high conversion values. Solketal esters from soybean oil [17] and methyl ricinoleate [20] were produced in solvent-free systems. However, reusability tests after successive batch reactions were not reported in these studies (see Table S1). This activity of this new heterogeneous biocatalyst demonstrated high retention after successive esterification batches were performed in a solvent-free system. This study shows that the immobilization of ET2.0 on epicarp macauba particles may lead to the highest catalytic performance and simpler downstream processes for the separation/purification of the esters. This new process can be considered an attractive option for the sustainable production of solketal esters due to its high catalytic activity and reusability.

3. Materials and Methods

3.1. Materials

Macauba fruits were manually harvested from the Cerrado biome at the Federal University of São João del-Rei, Campus Sete Lagoas—latitude: $19^\circ 28' 32.18''$ S; longitude: $44^\circ 11' 44.808''$ W (Sete Lagoas, MG, Brazil). Lipase Eversa[®] Transform 2.0 (ET2.0), a lipase derived from the lipase from *Thermomyces lanuginosus*, expressed in *Aspergillus oryzae*, (liquid extract, a hydrolytic activity of $19,856.7$ units mL^{-1} , and a protein concentration of 33 mg mL^{-1}), a lipase from *Candida rugosa* (CRL) (powder extract, a hydrolytic activity of

16,156.8 units g^{-1} , and a protein content of 24.5 mg g^{-1}), and solketal were acquired from Sigma-Aldrich Co. (St. Louis, MO, USA). USCO was collected after being used once for the preparation of french fries in a restaurant at the Federal University of Alfenas (Alfenas, MG, Brazil). Its fatty acid composition and properties were described in a recent study [51]. All other chemicals were of analytical grade and were acquired from Synth[®] (São Paulo, SP, Brazil).

3.2. Preparation of Macauba Epicarp Particles

Macauba fruits were first washed with distilled water to remove impurities and dried for 15 h in an oven at 60 °C to facilitate epicarp removal. Then, the epicarp was separated and crushed in a knife-mill, and the particles with a size of 75 to 120 μm were selected using vibratory sieves. Subsequently, 30 g of the material was refluxed with 400 mL of 3.5% *w/w* HCl solution at 120 °C for 2 h [58]. This experimental procedure was conducted twice. The treated particles were collected through filtration in a Buchner funnel under vacuum using Whatman n° 41 filter paper, washed with deionized water and dried for 15 h in an oven at 60 °C before use. Then, the support material was transferred to a 500 mL closed flask with 200 mL 70% *w/w* ethanol solution and immersed in a temperature-controlled water-bath under continuous orbital stirring (200 rpm) at 25 ± 1 °C for 15 h. This procedure was also performed twice. Finally, the treated material was collected by filtration in a Buchner funnel under vacuum using a Whatman n° 41 filter paper and dried for 15 h in an oven at 60 °C before use. The process flow chart for the preparation of treated macauba epicarp particles (material support) and the average mass loss after each step of the proposed process is illustrated in Figure S2.

3.3. Support Characterization

The morphology of untreated and treated macauba epicarp particles was determined via scanning electron microscopy (SEM) using Quanta 200 FEI equipment under a vacuum operating at 5 kV. These materials were also analyzed via Fourier transform infrared (FT-IR) spectrometry using a Nicolet iS50 FT-IR Thermo Scientific spectrophotometer (Madison, WI, USA). The samples were scanned between 3600 and 500 cm^{-1} at a spectral resolution of 4 cm^{-1} . X-ray diffraction (XRD) data were collected on Empyrean Panalytical diffractometer using $\text{CuK}\alpha$ radiation ($\lambda = 1.5418 \text{ \AA}$) at the 2θ range of 5°–50°.

3.4. General Immobilization Procedure of ET2.0 for Treated Macauba Epicarp Particles

The immobilization of ET2.0 on treated macauba epicarp particles was conducted according to the methodology reported in previous studies [47,59]. This process consisted of the preparation of a suspension by adding 10 g of the support to 190 mL of a lipase solution prepared with 12.1 mL of crude lipase solution and 178 mL of 5 mmol L^{-1} sodium acetate solution pH 5.0 (initial protein loading of 40 mg g^{-1} of support). The resulting suspensions were added to 300 mL closed flasks and immersed in a temperature-controlled water-bath under continuous orbital stirring (200 rpm) at 25 ± 1 °C for 15 h. The immobilized biocatalyst was recovered via filtration in a Buchner funnel under vacuum using Whatman n° 41 filter paper and thoroughly washed with distilled water to remove unbounded lipase molecules. The adsorption process was monitored by measuring the residual protein concentration [60], and hydrolytic activity units [47] in the supernatant solution. The heterogeneous biocatalyst was stored at 4 °C for 24 h prior to use. The hydrolytic activity of ET2.0 was assessed according to the olive oil emulsion hydrolysis method [47,59]. One international unit (U) of activity was defined as being the biocatalyst mass necessary to produce 1 μmol of FFAs per min at pH 8.0 (100 mmol L^{-1} buffer sodium phosphate), 37 °C, and 200 rpm.

Immobilization yield percentage (IY) was calculated as shown in Equation (2) [61].

$$IY = \left(\frac{EA_0 - EA_f}{EA_0} \right) \times 100 \quad (2)$$

where EA_0 and EA_f are the initial and residual enzymatic activity in the supernatant immobilization (U mL^{-1}), respectively.

Immobilized protein loading (IP — mg g^{-1} of support) at equilibrium was calculated as shown in Equation (3):

$$IP = \frac{V_{enz} \times (C_0 - C_f)}{m} \quad (3)$$

where V_{enz} is the volume of the enzyme solution (mL); C_0 and C_f are, respectively, the initial and residual protein concentration in the immobilization supernatant (mg mL^{-1}); and m is the mass of support (g).

The specific activity (SA — $\text{U mg}_{\text{protein}}^{-1}$) was calculated as shown in Equation (4):

$$SA = \frac{HA}{IP} \quad (4)$$

where HA is the hydrolytic activity of the biocatalyst (U g^{-1} for soluble ET2.0 and U g^{-1} for immobilized lipase) and IP is the immobilized protein loading (mg g^{-1} of support).

3.5. Enzymatic Hydroesterification of USCO

The production of FFAs via the hydrolysis of USCO catalyzed by a non-specific lipase (lyophilized CRL extract) in the absence of an emulsifier and buffer agents was conducted according to the methodology described in previous reports [30,31,51]. The reaction system was prepared by mixing 20 g of waste oil and 30 g of distilled water in a closed reactor (350 mL polypropylene flask) immersed in a temperature-controlled water-bath under continuous mechanical stirring (1500 rpm) at $40 \pm 1^\circ\text{C}$. The reaction was started by adding 50 units of hydrolytic activity of CRL per g of reaction mixture, corresponding to 3.2 g powder CRL extract per Kg of reaction mixture. Under these experimental conditions, a hydrolysis percentage of above 98% was achieved after 3 h of reaction [51]. The resulting FFAs were then recovered in a separation funnel, followed by washing with distilled water at 60°C , and dehydrated by adding anhydrous sodium sulfate for use as a raw material in the esterification reaction.

The esterification of FFAs and solketal was performed in 100 mL open glass bottles (height to diameter ratio of 2) containing 6 g of raw materials and appropriate amounts of the immobilized lipase. The reaction mixtures were immersed in a temperature-controlled water-bath under fixed continuous orbital stirring (240 rpm). At regular intervals, 50 μL aliquots were taken from the reaction mixture and suspended in 10 mL, 95% w/w ethanol solution. The resulting solution was strongly stirred and titrated with 30 mmol L^{-1} NaOH solution using phenolphthalein as indicator to calculate FFAs conversion percentage (Y), as shown in Equation (5) [51,59]. In this set of experiments, all reactions were performed with two replicates and the experimental values are represented as mean $\pm$ s.d. Control assays were performed using only macauba epicarp particles and no FFAs consumption was detected under such conditions.

$$Y(\%) = \left(\frac{FFAs_0 - FFAs_t}{FFAs_0} \right) \times 100 \quad (5)$$

where $FFAs_0$ is the initial concentration (mol.L^{-1}) and $FFAs_t$ is the residual concentration after a certain time t (mol.L^{-1}).

The scheme of the sequential enzymatic hydrolysis of trilinolein, the most abundant triacylglycerol present in soybean oil [62,63], and the esterification of the resulting FFAs with solketal in a solvent-free system is shown in Figure 1.

3.6. CCRD Optimization of Solketal Esters' Production via Esterification Reaction

In this set of experiments, a full CCRD with three independent parameters, such as FFAs–solketal molar ratio (from 1:1 to 1:4), reaction temperature (from 40 to 70°C), and

biocatalyst concentration (from 5 to 20% w/w), was proposed to optimize the enzymatic production of solketal esters. The levels of each independent variable were determined based on previous studies performed by our research group [22,30,31]. The statistical approach consisted of eighteen runs—eight factorial points, six axial points, and four center points. The reactions were performed in a random order to reduce possible systematic errors using a fixed initial mass of raw materials (6 g), continuous orbital stirring (240 rpm), and a fixed reaction time (40 min). The dependent variable (response) was FFAs conversion percentage. The experimental data were analyzed at a 95% confidence level utilizing the software Protimiza Experimental Design (<https://experimental-design.protimiza.com.br>, accessed on 4 October 2024) to obtain the 3D response surface and contour plots. The relation between coded independent variables and their interactions and response was described using a quadratic polynomial equation (Equation (6)). In this study, the empirical model was developed considering only the statistically significant parameters (p -value < 0.05).

$$Y(\%) = a_0 + \sum_{i=1}^n a_i x_i + \sum_{i=1}^n a_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^n a_{ij} x_i x_j + e \quad (6)$$

where Y is the predicted FFAs conversion percentage; n is the number of independent variables (3); x_i and x_j are coded independent variables; a_0 , a_i , a_{ii} , and a_{ij} are the regression coefficients of intercept, linear, quadratic, and interaction terms, respectively; i and j are the index numbers of each independent variable; and e is random error.

The effects of the main variables and their interactions were estimated using variance analysis (ANOVA). The adequacy of the quadratic model was evaluated via the coefficient of determination (R^2), probability (p -value), lack of fit, and Fischer's " F " test. Three-dimensional response surface and contour plots were used to determine the optimal experimental conditions. The experimental model was validated via the production of solketal esters production under the conditions predicted by the quadratic model. The experimental results were compared with the predicted values using Equation (6).

3.7. Effect of Reaction Time on Enzymatic Solketal Esters' Production

The effect of the reaction time on solketal esters production was conducted under the optimal conditions obtained from the analysis of the 3D response surface and contour plots (an FFAs–solketal molar ratio of 1:1.6 (6 g of reaction mixture that corresponds to 3.4 g of FFAs and 2.6 g of solketal), 46 °C, and a biocatalyst concentration of 20% w/w , corresponding to an immobilized protein concentration of 5.05 mg g^{-1} of the reaction mixture).

3.8. Operational Stability Tests of the Immobilized ET2.0

These tests were conducted under optimal experimental conditions (an FFAs–solketal molar ratio of 1:1.6 (6 g of reaction mixture that corresponds to 3.4 g of FFAs and 2.6 g of solketal), 46 °C, and a biocatalyst concentration of 20% w/w) after 150 min of reaction (time required to achieve maximum FFAs conversion percentage). At the end of each esterification batch, the heterogeneous biocatalyst was recovered from the reaction mixture via filtration in a Buchner funnel under vacuum and washed with cold hexane to remove any compounds retained on its surface. The biocatalyst was stored overnight at 4 °C under static conditions. Finally, the recovered biocatalyst was resuspended in a fresh reaction mixture to start a new esterification batch.

3.9. Separation of the Esters

Firstly, the heterogeneous biocatalyst was recovered from the reaction mixture via filtration in a Buchner funnel under vacuum. The resulting reaction mixture was then transferred to a 500 mL separation funnel, followed by the addition of 200 mL distilled water at 60 °C. The bottom phase (washing water) was eliminated and the upper phase was

washed three times following this protocol. Finally, 20% *w/w* anhydrous sodium sulfate, dried in a muffle furnace at 250 °C for 24 h, was added to the esters and kept overnight under static conditions at 25 °C to remove water traces.

3.10. Identification via FT-IR and NMR Analyses

The FT-IR spectra for raw materials (FFAs and solketal) and product (solketal esters) were recorded on a Spectrum GX FT-IR spectrometer (Perkin Elmer, Shelton, CT, USA). These spectra were acquired after 32 scans between 4000 and 400 cm^{-1} with a spectral resolution of 4 cm^{-1} using KBr as a matrix. The ^1H (300 MHz) and ^{13}C (75 MHz) nuclear magnetic resonance (NMR) spectra of solketal esters were recorded on Bruker AC 300 equipment (Bruker BioSpin, Billerica, MA, USA). The signals of deuterated water (solvent) and tetramethylsilane (internal standard) were suppressed from the spectra.

4. Conclusions

In this study, a new and sustainable process was developed for the production of a valuable class of chemical compounds (solketal esters) from used soybean cooking oil. This two-step process is called hydroesterification. For this purpose, a new and active heterogeneous biocatalyst was prepared via immobilization through the physical adsorption of a low-cost commercial lipase (ET2.0) on an agro-industrial support material (macauba epicarp particles). This study demonstrated that this material required some further treatment steps to allow for its use in lipase immobilization and in the production of a heterogeneous biocatalyst with a high enzyme-loading capacity and successful catalytic performance. This could be achieved via the formation of a porous structure, which was confirmed by the mass balance that was obtained in the treatment process (mass loss of around of 54%), and by FT-IR, XRD, and SEM analyses. This generation of porosity allowed for the adsorption of ET2.0 molecules in its internal surface. This study clearly showed that the resulting immobilized lipase was more active and stable during esterification reactions conducted under moderate conditions (46 °C). Under these conditions, a linear profile of solketal esters' production was observed, thus demonstrating that raw materials molecules (FFAs and solketal) could access the internal biocatalyst microenvironment at a satisfactory rate. This is a relevant aspect of the proposed process, since it could allow for the production of an important class of chemicals to be achieved with a low energy demand. The development of an eco-friendly process utilizing waste cooking oil from food processing (raw material) and lignocellulosic residue (enzyme support material) can be considered a promising approach for oleochemical industries. Therefore, further studies will be conducted on the application of the resulting solketal esters as bio-based plasticizers for poly(vinyl chloride) (PVC) films. Moreover, the study findings provide new possibilities for further studies regarding the application of the generated glycerol in the hydrolysis step in the production of solketal.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/catal14100693/s1>, Figure S1: Representation of macauba palm tree (A); palm tree with fruit bunches (B), fruits (C), and treated macauba epicarp particles (D).; Figure S2. Process flow chart for the preparation of the support from macauba epicarp particles.; Figure S3. The relationship between experimental and predicted FFAs conversion percentage values for the enzymatic production of solketal esters from USCO.; Table S1. Literature survey regarding the enzymatic production of solketal esters in batch systems.

Author Contributions: Conceptualization, A.A.M. and R.F.-L.; methodology, J.M.J., J.V.B.D., M.S.B., R.A.B.G., A.K.F.C. and C.M.F.S.; software, M.S.B., C.M.F.S. and A.A.M.; validation, J.M.J., J.V.B.D., M.S.B. and C.M.F.S.; formal analysis, R.F.-L. and A.A.M.; investigation, J.M.J., J.V.B.D., M.S.B., R.A.B.G., A.K.F.C. and C.M.F.S.; resources, J.M.J. and J.V.B.D.; data curation, J.M.J., J.V.B.D., M.S.B., R.A.B.G., A.K.F.C. and C.M.F.S.; writing—original draft preparation, J.M.J., M.S.B. and C.M.F.S.; writing—review and editing, R.F.-L. and A.A.M.; visualization, J.M.J., J.V.B.D., M.S.B., R.A.B.G., A.K.F.C. and C.M.F.S.; supervision, A.A.M.; project administration, R.F.-L. and A.A.M.; funding

acquisition, R.F.-L. and A.A.M. All authors have read and agreed to the published version of the manuscript.

Funding: The present study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)—Brazil—Finance Code 001. The authors are also thankful for the financial support of Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)—Brazil (Process APQ-01691-21) and Ministerio de Ciencia e Innovación, and Agencia Estatal de Investigación (Spanish Government) (PID2022-136535OB-I00). A.A.M. thanks the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research fellowship (PQ-2 CA BI, Grant 306253/2023-2). J.M.J. and J.V.B.D. thank the FAPEMIG and CNPq Foundation for the student fellowships, respectively.

Data Availability Statement: All data generated or analyzed during this study are included in this published article and its Supplementary Information Files.

Acknowledgments: The authors thank the UFMG Microscopy Center (<http://www.microscopia.ufmg.br>, accessed on 4 October 2024) for providing the equipment and technical support for the SEM analysis and the Laboratory of Crystallography (Institute of Chemistry—UNIFAL/MG) for the XRD analysis. We also thank Ángel Berenguer-Murcia (Universidad de Alicante, Alicante, Spain) for the kind suggestions during the writing of this paper.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

References

- Kenar, J.A.; Knothe, G. 1,2-Isopropylidene Glycerol Carbonate: Preparation, Characterization, and Hydrolysis. *J. Am. Oil Chem. Soc.* **2008**, *85*, 365–372. [\[CrossRef\]](#)
- Mariam, N.M.D.N.S.; Hoong, S.S.; Arniza, M.Z.; Adnan, S.; Ismail, T.N.M.T.; Yeong, S.K.; Yusop, M.R. Optimisation of Reaction Parameters for the Synthesis of Solketal Levulinate as Potential Additive. *J. Oil Palm Res.* **2022**, *34*, 524–534. [\[CrossRef\]](#)
- Neamtu, C.; Stepan, E.; Plesu, V.; Bozga, G.; Tuluc, A. Synthesis and Characterization of New Solketal Alkylesters Usable as Diesel Biobased Fuel Additives. *Rev. Chim.* **2019**, *70*, 1167–1172. [\[CrossRef\]](#)
- Yang, J.; Ma, W.J.; Li, N.; Zhou, J.H.; Sun, H.Z. Synthesis of Tung Oil Monoglyceride. *Adv. Mater. Res.* **2014**, *941–944*, 1021–1025. [\[CrossRef\]](#)
- Perosa, A.; Moraschini, A.; Selva, M.; Noè, M. Synthesis of the Fatty Esters of Solketal and Glycerol-Formal: Biobased Specialty Chemicals. *Molecules* **2016**, *21*, 170. [\[CrossRef\]](#)
- Sultanova, R.M.; Khusnutdinova, N.S.; Borisova, Y.G.; Raskildina, G.Z.; Meshcheryakova, S.A.; Samorodov, A.V.; Zlotsky, S.S. Selective Synthesis of Some Carboxylic Acids Esters. *Russ. J. Gen. Chem.* **2023**, *93*, 1–7. [\[CrossRef\]](#)
- Barbosa, S.L.; Lima, P.C.; dos Santos, W.T.P.; Klein, S.I.; Clososki, G.C.; Caires, F.J. Oxygenated Biofuels: Synthesis of Fatty Acid Solketal Esters with a Mixture of Sulfonated Silica and (Bu₄N)(BF₄) Catalyst. *Catal. Commun.* **2019**, *120*, 76–79. [\[CrossRef\]](#)
- Lozano, P.; Gomez, C.; Nicolas, A.; Polo, R.; Nieto, S.; Bernal, J.M.; García-Verdugo, E.; Luis, S.V. Clean Enzymatic Preparation of Oxygenated Biofuels from Vegetable and Waste Cooking Oils by Using Spongellike Ionic Liquids Technology. *ACS Sustain. Chem. Eng.* **2016**, *4*, 6125–6132. [\[CrossRef\]](#)
- Sousa, R.R.; Silva, A.S.; Fernandez-Lafuente, R.; Ferreira-Leitão, V.S. Solvent-Free Esterifications Mediated by Immobilized Lipases: A Review from Thermodynamic and Kinetic Perspectives. *Catal. Sci. Technol.* **2021**, *11*, 5696–5711. [\[CrossRef\]](#)
- Stergiou, P.Y.; Foukis, A.; Filippou, M.; Koukouritaki, M.; Parapouli, M.; Theodorou, L.G.; Hatziloukas, E.; Afendra, A.; Pandey, A.; Papamichael, E.M. Advances in Lipase-Catalyzed Esterification Reactions. *Biotechnol. Adv.* **2013**, *31*, 1846–1859. [\[CrossRef\]](#)
- Verger, R. “Interfacial Activation” of Lipases: Facts and Artifacts. *Trends Biotechnol.* **1997**, *15*, 32–38. [\[CrossRef\]](#)
- Schmid, R.D.; Verger, R. Lipases: Interfacial Enzymes with Attractive Applications. *Angew. Chemie Int. Ed.* **1998**, *37*, 1608–1633. [\[CrossRef\]](#)
- Mine, Y.; Zhang, L.; Fukunaga, K.; Sugimura, Y. Enhancement of Enzyme Activity and Enantioselectivity by Cyclopentyl Methyl Ether in the Transesterification Catalyzed by *Pseudomonas cepacia* Lipase Co-Lyophilized with Cyclodextrins. *Biotechnol. Lett.* **2005**, *27*, 383–388. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zniszczol, A.; Walczak, K. Lipases Aided Esterification of (2,2-Dimethyl-1,3-Dioxolan-4-Yl)Methanol. *Lett. Org. Chem.* **2014**, *11*, 6–12. [\[CrossRef\]](#)
- Da Silva Junior, V.A.; Shigueyuki Kanda, L.R.; Zandoná-Filho, A.; Corazza, M.L.; Sutile De Lima, C. Effect of Supercritical Carbon Dioxide over the Esterification of Levulinic Acid with Ethanol Using Montmorillonite K10 as Catalyst. *J. CO₂ Util.* **2020**, *39*, 101158. [\[CrossRef\]](#)
- Romano, D.; Ferrario, V.; Molinari, F.; Gardossi, L.; Montero, J.M.S.; Torre, P.; Converti, A. Kinetic Resolution of (R, S)-1,2-O-Isopropylidene-glycerol by Esterification with Dry Mycelia of Moulds. *J. Mol. Catal. B Enzym.* **2006**, *41*, 71–74. [\[CrossRef\]](#)
- Torregrosa, R.; Yara-Varón, E.; Balcells, M.; Torres, M.; Canela-Garayoa, R. Entirely Solvent-Free Biocatalytic Synthesis of Solketal Fatty Esters from Soybean Seeds. *Comptes Rendus Chim.* **2016**, *19*, 749–753. [\[CrossRef\]](#)

18. Boncel, S.; Zniszczoł, A.; Szymańska, K.; Mrowiec-Białoń, J.; Jarzebski, A.; Walczak, K.Z. Alkaline Lipase from *Pseudomonas Fluorescens* Non-Covalently Immobilised on Pristine versus Oxidised Multi-Wall Carbon Nanotubes as Efficient and Recyclable Catalytic Systems in the Synthesis of Solketal Esters. *Enzyme Microb. Technol.* **2013**, *53*, 263–270. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Itabaiana, I.; Gonçalves, K.M.; Zoumpanioti, M.; Leal, I.C.R.; Miranda, L.S.M.E.; Xenakis, A.; De Souza, R.O.M.A. Microemulsion-Based Organogels as an Efficient Support for Lipase-Catalyzed Reactions under Continuous-Flow Conditions. *Org. Process Res. Dev.* **2014**, *18*, 1372–1376. [\[CrossRef\]](#)
20. Johnny, J.; Jatla, A.; Eruva, V.K.; Misra, S.; Kaki, S.S. Synthesis, Characterization and Evaluation of 1-Monoacylglycerols of Unsaturated Fatty Acids as Potential Bioactive Lipids. *Grasas Aceites* **2019**, *70*, e325. [\[CrossRef\]](#)
21. Junior, I.I.; Flores, M.C.; Sutili, F.K.; Leite, S.G.F.; Leandro, L.S.; Leal, I.C.R.; De Souza, R.O.M.A. Fatty Acids Residue from Palm Oil Refining Process as Feedstock for Lipase Catalyzed Monoacylglycerol Production under Batch and Continuous Flow Conditions. *J. Mol. Catal. B Enzym.* **2012**, *77*, 53–58. [\[CrossRef\]](#)
22. Mendoza-Ortiz, P.A.; Gama, R.S.; Gómez, O.C.; Luiz, J.H.H.; Fernandez-Lafuente, R.; Cren, E.C.; Mendes, A.A. Sustainable Enzymatic Synthesis of a Solketal Ester—Process Optimization and Evaluation of Its Antimicrobial Activity. *Catalysts* **2020**, *10*, 218. [\[CrossRef\]](#)
23. Rocha, K.S.C.; Queiroz, M.S.R.; Gomes, B.S.; Dallago, R.; de Souza, R.O.M.A.; Guimarães, D.O.; Itabaiana, I.; Leal, I.C.R. Lipases of Endophytic Fungi *Stemphylium lycopersici* and *Sordaria* Sp.: Application in the Synthesis of Solketal Derived Monoacylglycerols. *Enzyme Microb. Technol.* **2020**, *142*, 109664. [\[CrossRef\]](#)
24. Villa, R.; Alvarez, E.; Nieto, S.; Donaire, A.; Garcia-Verdugo, E.; Luis, S.V.; Lozano, P. Chemo-Enzymatic Production of Omega-3 Monoacylglycerides Using Sponge-like Ionic Liquids and Supercritical Carbon Dioxide. *Green Chem.* **2020**, *22*, 5701–5710. [\[CrossRef\]](#)
25. Mendes, A.A.; Soares, C.M.F.; Tardioli, P.W. Recent Advances and Future Prospects for Biolubricant Base Stocks Production Using Lipases as Environmentally Friendly Catalysts: A Mini-Review. *World J. Microbiol. Biotechnol.* **2023**, *39*, 25. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Pourzolfaghar, H.; Abnisa, F.; Daud, W.M.A.W.; Aroua, M.K. A Review of the Enzymatic Hydroesterification Process for Biodiesel Production. *Renew. Sustain. Energy Rev.* **2016**, *61*, 245–257. [\[CrossRef\]](#)
27. Gryglewicz, S.; Muszyński, M.; Nowicki, J. Enzymatic Synthesis of Rapeseed Oil-Based Lubricants. *Ind. Crops Prod.* **2013**, *45*, 25–29. [\[CrossRef\]](#)
28. Sankaranarayanan, S.; Jindapon, W.; Ngamcharussrivichai, C. Valorization of Biodiesel Plant-Derived Products via Preparation of Solketal Fatty Esters over Calcium-Rich Natural Materials Derived Oxides. *J. Taiwan Inst. Chem. Eng.* **2017**, *81*, 57–64. [\[CrossRef\]](#)
29. Domínguez De María, P.; Sánchez-Montero, J.M.; Sinisterra, J.V.; Alcántara, A.R. Understanding *Candida rugosa* Lipases: An Overview. *Biotechnol. Adv.* **2006**, *24*, 180–196. [\[CrossRef\]](#)
30. Carvalho, W.C.A.; Luiz, J.H.H.; Fernandez-Lafuente, R.; Hirata, D.B.; Mendes, A.A. Eco-Friendly Production of Trimethylolpropane Triesters from Refined and Used Soybean Cooking Oils Using an Immobilized Low-Cost Lipase (Eversa® Transform 2.0) as Heterogeneous Catalyst. *Biomass Bioenergy* **2021**, *155*, 106302. [\[CrossRef\]](#)
31. Guedes Júnior, J.G.E.; Mattos, F.R.; Sabi, G.J.; Carvalho, W.C.A.; Luiz, J.H.H.; Cren, É.C.; Fernandez-Lafuente, R.; Mendes, A.A. Design of a Sustainable Process for Enzymatic Production of Ethylene Glycol Diesters via Hydroesterification of Used Soybean Cooking Oil. *J. Environ. Chem. Eng.* **2022**, *10*, 107062. [\[CrossRef\]](#)
32. Bolivar, J.M.; Woodley, J.M.; Fernandez-Lafuente, R. Is Enzyme Immobilization a Mature Discipline? Some Critical Considerations to Capitalize on the Benefits of Immobilization. *Chem. Soc. Rev.* **2022**, *51*, 6251–6290. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Rodrigues, R.C.; Virgen-Ortiz, J.J.; dos Santos, J.C.S.; Berenguer-Murcia, Á.; Alcántara, A.R.; Barbosa, O.; Ortiz, C.; Fernandez-Lafuente, R. Immobilization of Lipases on Hydrophobic Supports: Immobilization Mechanism, Advantages, Problems, and Solutions. *Biotechnol. Adv.* **2019**, *37*, 746–770. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Lopes, D.D.C.; Steidle Neto, A.J.; Mendes, A.A.; Pereira, D.T.V. Economic Feasibility of Biodiesel Production from Macauba in Brazil. *Energy Econ.* **2013**, *40*, 819–824. [\[CrossRef\]](#)
35. Silva, L.N.; Fortes, I.C.P.; De Sousa, F.P.; Pasa, V.M.D. Biokerosene and Green Diesel from Macauba Oils via Catalytic Deoxygenation over Pd/C. *Fuel* **2016**, *164*, 329–338. [\[CrossRef\]](#)
36. Bijoy, G.; Rajeev, R.; Benny, L.; Jose, S.; Varghese, A. Enzyme Immobilization on Biomass-Derived Carbon Materials as a Sustainable Approach towards Environmental Applications. *Chemosphere* **2022**, *307*, 135759. [\[CrossRef\]](#)
37. Girelli, A.M.; Chiappini, V. Renewable, Sustainable, and Natural Lignocellulosic Carriers for Lipase Immobilization: A Review. *J. Biotechnol.* **2023**, *365*, 29–47. [\[CrossRef\]](#)
38. Girelli, A.M.; Astolfi, M.L.; Scuto, F.R. Agro-Industrial Wastes as Potential Carriers for Enzyme Immobilization: A Review. *Chemosphere* **2020**, *244*, 125368. [\[CrossRef\]](#)
39. Foo, W.H.; Chia, W.Y.; Tang, D.Y.Y.; Koay, S.S.N.; Lim, S.S.; Chew, K.W. The Conundrum of Waste Cooking Oil: Transforming Hazard into Energy. *J. Hazard. Mater.* **2021**, *417*, 126129. [\[CrossRef\]](#)
40. Mankar, A.R.; Pandey, A.; Modak, A.; Pant, K.K. Pretreatment of Lignocellulosic Biomass: A Review on Recent Advances. *Bioresour. Technol.* **2021**, *334*, 125235. [\[CrossRef\]](#)
41. Parchami, M.; Agnihotri, S.; Taherzadeh, M.J. Aqueous Ethanol Organosolv Process for the Valorization of Brewer's Spent Grain (BSG). *Bioresour. Technol.* **2022**, *362*, 127764. [\[CrossRef\]](#)

42. Wu, D.; Wei, Z.; Mohamed, T.A.; Zheng, G.; Qu, F.; Wang, F.; Zhao, Y.; Song, C. Lignocellulose Biomass Bioconversion during Composting: Mechanism of Action of Lignocellulase, Pretreatment Methods and Future Perspectives. *Chemosphere* **2022**, *286*, 131635. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Chawla, S.K.; Goyal, D. Optimization of Pretreatment of Wheat Straw Using Response Surface Methodology for Production of Lactic Acid Using *Bacillus sonorensis* Strain DGS15. *Bioenergy Res.* **2023**, *16*, 967–978. [\[CrossRef\]](#)
44. Du, S.K.; Su, X.; Yang, W.; Wang, Y.; Kuang, M.; Ma, L.; Fang, D.; Zhou, D. Enzymatic Saccharification of High Pressure Assist-Alkali Pretreated Cotton Stalk and Structural Characterization. *Carbohydr. Polym.* **2016**, *140*, 279–286. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Qureshi, A.S.; Ji, X.; Khushk, I.; Mirjatt, A.N.; Tunio, A.A.; Huang, Y. Ionic Liquid and Diluted Sulfuric Acid Combinatorial Pretreatment for Efficient Sugarcane Bagasse Conversion to L-Lactic Acid. *Ind. Crops Prod.* **2023**, *204*, 117272. [\[CrossRef\]](#)
46. Kim, D.Y.; Lee, B.M.; Koo, D.H.; Kang, P.H.; Jeun, J.P. Preparation of Nanocellulose from a Kenaf Core Using E-Beam Irradiation and Acid Hydrolysis. *Cellulose* **2016**, *23*, 3039–3049. [\[CrossRef\]](#)
47. Alves, M.D.; Aracri, F.M.; Cren, É.C.; Mendes, A.A. Isotherm, Kinetic, Mechanism and Thermodynamic Studies of Adsorption of a Microbial Lipase on a Mesoporous and Hydrophobic Resin. *Chem. Eng. J.* **2017**, *311*, 1–12. [\[CrossRef\]](#)
48. Monteiro, R.R.C.; Arana-Peña, S.; da Rocha, T.N.; Miranda, L.P.; Berenguer-Murcia, Á.; Tardioli, P.W.; dos Santos, J.C.S.; Fernandez-Lafuente, R. Liquid Lipase Preparations Designed for Industrial Production of Biodiesel. Is It Really an Optimal Solution? *Renew. Energy* **2021**, *164*, 1566–1587. [\[CrossRef\]](#)
49. Martínez-Sánchez, J.A.; Arana-Peña, S.; Carballares, D.; Yates, M.; Otero, C.; Fernandez-Lafuente, R. Immobilized Biocatalysts of Eversa® Transform 2.0 and Lipase from *Thermomyces lanuginosus*: Comparison of Some Properties and Performance in Biodiesel Production. *Catalysts* **2020**, *10*, 738. [\[CrossRef\]](#)
50. Pedro, K.C.N.R.; da Silva, J.V.V.; Cipolatti, E.P.; Manoel, E.A.; Campisano, I.S.P.; Henriques, C.A.; Langone, M.A.P. Adsorption of Lipases on Porous Silica-Based Materials for Esterification in a Solvent-Free System. *3 Biotech* **2023**, *13*, 380. [\[CrossRef\]](#)
51. Sabi, G.J.; Gama, R.S.; Fernandez-Lafuente, R.; Cancino-Bernardi, J.; Mendes, A.A. Decyl Esters Production from Soybean-Based Oils Catalyzed by Lipase Immobilized on Differently Functionalized Rice Husk Silica and Their Characterization as Potential Biolubricants. *Enzyme Microb. Technol.* **2022**, *157*, 110019. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Shomal, R.; Du, W.; Al-Zuhair, S. Immobilization of Lipase on Metal-Organic Frameworks for Biodiesel Production. *J. Environ. Chem. Eng.* **2022**, *10*, 107265. [\[CrossRef\]](#)
53. Tacias-Pascacio, V.G.; Peirce, S.; Torrestiana-Sanchez, B.; Yates, M.; Rosales-Quintero, A.; Virgen-Ortiz, J.J.; Fernandez-Lafuente, R. Evaluation of Different Commercial Hydrophobic Supports for the Immobilization of Lipases: Tuning Their Stability, Activity and Specificity. *RSC Adv.* **2016**, *6*, 100281–100294. [\[CrossRef\]](#)
54. Badgujar, V.C.; Badgujar, K.C.; Yeole, P.M.; Bhanage, B.M. Investigation of Effect of Ultrasound on Immobilized *C. rugosa* Lipase: Synthesis of Biomass Based Furfuryl Derivative and Green Metrics Evaluation Study. *Enzyme Microb. Technol.* **2021**, *144*, 109738. [\[CrossRef\]](#)
55. Morales, A.H.; Hero, J.S.; Ledesma, A.E.; Martínez, M.A.; Navarro, M.C.; Gómez, M.I.; Romero, C.M. Tuning Surface Interactions on MgFe₂O₄ Nanoparticles to Induce Interfacial Hyperactivation in *Candida rugosa* Lipase Immobilization. *Int. J. Biol. Macromol.* **2023**, *253*, 126615. [\[CrossRef\]](#)
56. Morales, A.H.; Hero, J.S.; Ledesma, A.E.; Perez, H.A.; Navarro, M.C.; Gómez, M.I.; Romero, C.M. Interfacial Hyperactivation of *Candida rugosa* Lipase onto Ca₂Fe₂O₅ Nanoparticles: pH and Ionic Strength Fine-Tuning to Modulate Protein-Support Interactions. *Langmuir* **2023**, *39*, 12004–12019. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Badgujar, K.C.; Bhanage, B.M. The Green Metric Evaluation and Synthesis of Diesel-Blend Compounds from Biomass Derived Levulinic Acid in Supercritical Carbon Dioxide. *Biomass Bioenergy* **2016**, *84*, 12–21. [\[CrossRef\]](#)
58. Zhang, S.; Gao, H.; Li, J.; Huang, Y.; Alsaedi, A.; Hayat, T.; Xu, X.; Wang, X. Rice Husks as a Sustainable Silica Source for Hierarchical Flower-like Metal Silicate Architectures Assembled into Ultrathin Nanosheets for Adsorption and Catalysis. *J. Hazard. Mater.* **2017**, *321*, 92–102. [\[CrossRef\]](#)
59. Machado, N.B.; Miguez, J.P.; Bolina, I.C.A.; Salviano, A.B.; Gomes, R.A.B.; Tavano, O.L.; Luiz, J.H.H.; Tardioli, P.W.; Cren, É.C.; Mendes, A.A. Preparation, Functionalization and Characterization of Rice Husk Silica for Lipase Immobilization via Adsorption. *Enzyme Microb. Technol.* **2019**, *128*, 9–21. [\[CrossRef\]](#)
60. Bradford, M.M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254. [\[CrossRef\]](#)
61. Boudrant, J.; Woodley, J.M.; Fernandez-Lafuente, R. Parameters Necessary to Define an Immobilized Enzyme Preparation. *Process Biochem.* **2020**, *90*, 66–80. [\[CrossRef\]](#)
62. Li, M.; Butka, E.; Wang, X. Comprehensive Quantification of Triacylglycerols in Soybean Seeds by Electrospray Ionization Mass Spectrometry with Multiple Neutral Loss Scans. *Sci. Rep.* **2014**, *4*, 6581. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Park, Y.W.; Jeong, M.K.; Park, C.; Lee, J. Distribution of Triacylglycerols and Fatty Acids in Soybean Oil with Thermal Oxidation and Methylene Blue Photosensitization. *J. Am. Oil Chem. Soc.* **2011**, *88*, 373–380. [\[CrossRef\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.