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Design of a New Chemoenzymatic Process for Producing Epoxidized Monoalkyl Esters from Used Soybean Cooking Oil and Fusel Oil

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Abstract: The aim of this study was to produce epoxidized monoalkyl esters (EMAE), a valuable class of oleochemicals used in a wide range of products and industries, from used soybean cooking oil (USCO) and fusel oil via a three-step chemoenzymatic process. This process consists of a first enzymatic hydrolysis of USCO to produce free fatty acids (FFA). Here, five microbial lipases with different specificities were tested as biocatalysts. Full hydrolysis of USCO was obtained after a 180 min reaction time under vigorous stirring (1500 rpm) using a non-specific lipase from *Candida rugosa* (CRL). Then, monoalkyl esters (MAE) were produced via the esterification of FFA and fusel oil in a solvent-free system using the lipase Eversa® Transform 2.0 (ET2.0) immobilized via physical adsorption on poly(styrenene-divinylbenzene) (PSty-DVB) beads as a biocatalyst. Different water removal strategies (closed and open reactors in the presence or absence of molecular sieves at 5% m.m^{-1}) on the reaction were evaluated. Maximum FFA conversions of 64.3 ± 2.3% (open reactor after a 30 min reaction time) and 73.5 ± 0.4% (closed reactor after a 45 min reaction time) were observed at 40 °C, using a stoichiometric FFA:fusel oil molar ratio (1:1), without molecular sieves, and 5 mg of immobilized protein per gram of reaction mixture. Under these conditions, maximum FFA conversion was only 30.2 ± 2.7% after a 210 min reaction time in a closed reactor using soluble lipase. Reusability tests showed better retention of the original activity of immobilized ET2.0 (around 82%) after eight successive batches of esterification reactions conducted in an open reactor. Finally, the produced MAE was epoxidized via the Prilezhaev reaction, a classical chemical epoxidation process, using hydrogen peroxide and formic acid as a homogeneous catalyst. The products were characterized by standard methods and identified using proton nuclear magnetic resonance (^{1}H NMR). Maximum unsaturated bond conversions into epoxy groups were at approximately 33%, with the experimental epoxy oxygen content ($OOC_{exp.}$) at 1.75–1.78%, and selectivity (S) at 0.81, using both MAEs produced (open or closed reactors). These results show that this new process is a promising approach for value-added oleochemical production from low-cost and renewable raw materials.

Keywords: epoxidized monoalkyl esters; chemoenzymatic process; waste oil; fusel oil

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1. Introduction

Fossil fuels such as coal, natural gas, and petroleum have been extensively used as raw materials for synthesizing several fuels and chemicals. However, these non-renewable materials have caused a variety of damaging effects to the environment, such as global warming from greenhouse gas (GHG) emissions, land contamination, and air/water pollution [1]. In the last few decades, renewable materials have gained much attention worldwide as promising substitutes for petroleum-based materials, given their environmental benefits

and sustainable properties [1,2]. In fact, many government and environmental organizations have imposed strict regulations that seek to reduce GHG emissions in several countries, by replacing traditional petroleum-based materials with renewable-based materials [2,3]. There are a wide variety of renewable materials for producing biofuels and other valuable products for chemical industries, e.g., different types of agricultural crops, wood, microorganisms, animal manure, human sewage, and agricultural waste [3]. Of these different renewable materials, triacylglycerols (TAGs), such as vegetable and microbial oils, animal fats, waste oils (cooking oils, yellow or brown greases, and sludge or soapstock from refining vegetable oils), and their derivatives (FFAs), have proven to be promising raw materials for producing valuable industrial products [4,5]. Specifically, waste oils, including used cooking oils, have been used as eco-friendly and economic options given their low prices relative to edible vegetable oils, and their use in minimizing environmental problems, given that they can be directly disposed of in natural settings [6,7].

In some instances, the direct use of oleaginous feedstocks, including waste oils, is not recommended, due to the presence of reactive β-hydrogen atoms in the glycerol moiety, and double bonds in unsaturated fatty acids, thus resulting in low thermal and oxidative stabilities [8–11]. In this context, technological advancements have been extensively applied to process them to promote their use in oleochemical industries e.g., chemical modifications to exploit their functional groups [6]. The main chemical modification strategies employed are hydrolysis, esterification, transesterification, hydrogenation, epoxidation and ring-opening reactions [5,9–14]. Interestingly, these different strategies have also been commonly combined to introduce functional groups into their chemical structures to expand their versatility and application in chemical industries [5,11,13,14]. In this field, bio-based epoxidized materials from different oleaginous feedstocks have been widely used as lubricants, plasticizers, acid scavengers, stabilizers, recycled asphalt pavement materials, coatings, and corrosion inhibitors [11,13,15–19]. They can also be transformed into several value-added raw chemical materials for producing biopolymers/bioplastics, including polyols/polyurethanes, estolides, and surfactants via epoxy ring-opening reactions [11,20–25].

Thus, the main objective of this study is to produce and characterize epoxidized monoalkyl esters (EMAE) from USCO and fusel oil via a three-step chemoenzymatic approach. This process first involves a complete hydrolysis of the waste oil to produce FFA, followed by the esterification of the produced FFA with fusel oil, and epoxidation of the produced monoalkyl esters (MAE) in situ, generating performic acid from the reaction of formic acid and hydrogen peroxide. In this study, several lipase preparations with different specificities were used to produce FFA via the hydrolysis of ester bonds of waste oil (USCO), e.g., lipase from *Candida rugosa* (CRL), a non-specific lipase [26]; lipase from *Thermomyces lanuginosus* expressed in *Aspergillus oryzae* (Eversa® Transform 2.0—ET2.0—*sn*-1,3 specific) [27]; *Candida* sp. (CALA—*sn*-2 specific) [28]; *Rhizopus javanicus* (RJL—*sn*-1,3 specific) [29]; and *Aspergillus niger* (ANL—*sn*-1,3 specific) [29]. In this set of experiments, a combination of *sn*-1,3 specific lipase (ET2.0) and a *sn*-2 specific lipase (CALA) was also evaluated. These reactions were conducted in the absence of emulsifier or buffer agents, which require vigorous mechanical stirring to prepare stable water/oil interfaces to improve the catalytic performance of lipases [30]. MAE production from the enzymatic esterification of FFA and fusel oil, the latter being a valuable byproduct from bioethanol production comprising a mixture of ethanol, propanol, isobutanol, and isoamyl alcohol (the main component) [31,32], was performed in an eco-friendly and sustainable process (solvent-free system) [33]. The production of industrial esters via a two-step process (hydrolysis + esterification or "hydroesterification") has been demonstrated to be advantageous, since direct enzymatic transesterification of triacylglycerols requires either long reaction times or high temperatures to obtain high yields [34,35]. Since esterification is a reversible reaction [36–39], water removal strategies were evaluated by performing the reaction in closed or open reactors in the absence or in the presence of molecular sieves to increase ester production. Moreover, previous studies have also demonstrated

that using hydrophobic supports may prevent the formation of an aqueous phase in the biocatalysts' microenvironment that would drastically reduce their catalytic performance and reusability [39,40]. For this purpose, ET2.0, a low-cost commercial lipase preparation with high catalytic activity in non-aqueous media [27,41], was immobilized via physical adsorption on hydrophobic poly(styrene-divinylbenzene) (PSty-DVB) beads, and used as a heterogeneous biocatalyst. This low-cost heterogeneous biocatalyst has also demonstrated high catalytic performance and operational stability (reusability) for producing valuable esters using synthetic or natural starting materials [42–44]. The catalytic performance of this heterogeneous biocatalyst (immobilized lipase) was compared with its soluble form (crude lipase extract). Reusability tests of the biocatalyst after eight successive esterification batch reactions were also conducted in the absence (closed reactor) and presence (open reactor) of water removal strategies. The epoxidation of the produced MAE was performed via Prilezhaev epoxidation (or Prileschajew epoxidation) [11]. This reaction proceeds via the in situ generation of peracids (or peroxyacids) from reactions between carboxylic acids (commonly, formic and acetic acids) and hydrogen peroxide in an aqueous phase, followed by diffusion of peracid molecules to the oil phase to react with the double bonds [11,16,45]. In this study, formic acid, a valuable carboxylic acid from biomass-derived carbohydrates [46], was chosen as a homogeneous catalyst, given its high reactivity relative to acetic acid [15,47,48]. The different products were identified by ^{1}H NMR analysis, and their chemical properties were determined by standard methods. To the best of our knowledge, this is the first study that has investigated the production, via a three-step process, of EMAE from USCO and fusel oil, two renewable and low-cost starting materials commonly found in several countries.

2. Results and Discussion

2.1. FFA Production from Enzymatic Hydrolysis of USCO: Screening the Lipase Sources

In the first step, FFA production from the enzymatic hydrolysis of USCO using a conventional process (mechanical stirring and heating by immersion in a temperature-controlled water bath), in the absence of emulsifier and buffer agents, was investigated. As shown in Figure 1, a linear profile of enzymatic hydrolysis for USCO catalyzed by CRL, a known non-specific lipase, until an almost complete conversion into FFA and glycerol can be observed. As expected, the full hydrolysis reaction was achieved after a 180 min reaction time. On the other hand, hydrolysis reactions catalyzed from lipases from *Aspergillus niger* (ANL) and *Rhizopus javanicus* (RJL), two non-commercial *sn*-1,3-specific lipases, gave a maximum hydrolysis percentage of only 8% after a 360 min reaction time. CALA, a commercial *sn*-2-specific lipase, exhibited maximum hydrolysis at 32% after a 360 min reaction time, thus indicating an almost complete hydrolysis of the ester bonds at the middle position of the glycerol backbone (*sn*-2 position). Thus, a mixture of FFA and diacylglycerols (DAG) are the main products obtained in this process. The hydrolysis percentage is around 45% after a 300 min reaction time, using a *sn*-1,3 specific lipase from *Thermomyces lanuginosus* (Eversa® Transform 2.0—ET2.0) as a biocatalyst. In this case, the maximum hydrolysis percentage was 66.6%, with the expected preferential formation of 2-monoacylglycerol (2-MAG) [49]. Interestingly, the high hydrolysis percentages of refined soybean and waste cooking oils catalyzed by a *sn*-1,3 specific lipase from *Thermomyces lanuginosus* in its immobilized form (Lipozyme TL IM) have been reported on in other studies, albeit under complex experimental arrangements (ultrasound irradiation), and with a process that requires FFA extraction steps using organic solvents [50,51]. Thus, a reaction system using a combination of an *sn*-2 specific lipase (CALA) and an *sn*-1,3 specific lipase (ET2.0) was performed under the same experimental conditions to obtain a complete USCO conversion into FFA using an active and stable low-cost lipase preparation (ET2.0). Previous reports have demonstrated the efficient use of a combination of microbial lipases (enzymatic cocktails) with different specificities, including the combination of *sn*-1,3 specific lipases, as potential biocatalysts for producing FFA mixtures from the hydrolysis of different vegetable oils [52,53]. However, the maximum hydrolysis percentage observed

was 78% after a 660 min reaction time. The objective of this study was to select a lipase preparation or a specific combination that provides full conversion of the waste oil into FFA, the target raw material, for producing esters. Therefore, further tests were conducted using only CRL as a biocatalyst.

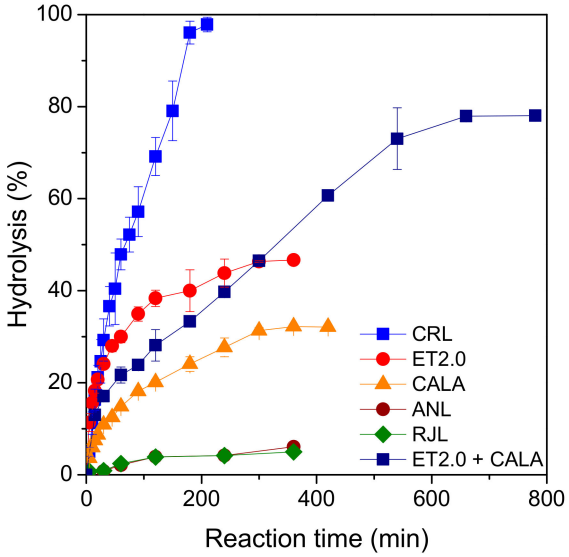

Figure 1. Influence of reaction time on the hydrolysis percentage of USCO catalyzed by microbial lipases with different specificities: CRL (*Candida rugosa*), ET2.0 (*Thermomyces lanuginosus*), CALA (*Candida* sp.), ANL (*Aspergillus niger*), RJL (*Rhizopus javanicus*) and a mixture ET2.0 + CALA. The reactions were conducted in the absence of emulsifier and buffer agents using an oil:water mass ratio of 40%, continuous mechanical stirring (1500 rpm), 40 °C and initial lipase loading of 50 U per gram of reaction mixture. The units of hydrolytic activity used for the combination of specific lipases were 17 U of CALA + 33 U of ET2.0 per gram of reaction mixture.

2.2. Comparative Performance of Soluble or Immobilized Lipase ET2.0 on MAE Production via Esterification in Solvent-Free Systems

The catalytic performance of immobilized lipase ET2.0 in the production of monoalkyl esters (MAE) via the direct esterification of FFA from USCO and fusel oil was investigated and compared with that of its soluble form. In this study, the influence of water removal strategies on the reaction when using molecular sieves and/or esterification reactions conducted in open systems was also evaluated and compared with a reaction system performed in the absence of any water removal strategies (esterification reaction in a closed reactor in the absence of molecular sieves). According to Figure 2, the FFA conversion percentage was only 30.2% after a 210 min reaction time using soluble lipase as biocatalyst, corresponding to a productivity of 0.8 $\mu mol_{FFA} \cdot min^{-1} \cdot mg_{prot}{}^{-1}$ (Table 1). By contrast, immobilized lipase provided better catalytic performance in the reaction, based on FFA conversion percentages and productivity values. In fact, FFA conversion percentages between 64.3 ± 2.3% and 73.5 ± 0.4% were achieved after a 30–45 min reaction time. In this study, the maximum FFA conversion percentage (73.5 ± 0.4%) was achieved after a 45 min reaction time without any water removal strategies (esterification conducted in a closed reactor). A similar FFA conversion percentage (71.8 ± 2.2%) was also observed for the reaction system performed in a closed reactor in the presence of molecular sieves at 5% ($m \cdot m^{-1}$). These reaction systems displayed similar productivity values (9.3 ± 0.1 $\mu mol_{FFA} \cdot min^{-1} \cdot mg_{prot}{}^{-1}$), as shown in Table 1. Interestingly, the esterification reaction conducted in an open reaction (water removal via simple evaporation at atmospheric pressure) exhibited an FFA conversion percentage of 64.3 ± 2.3% after only 30 min, which provided the highest productivity value, at 12.4 $\mu mol_{FFA} . min^{-1} \cdot mg_{prot}{}^{-1}$—almost 16-fold higher than the soluble ET2.0 reaction (see Table 1). These results clearly show better diffusion and starting material molecule access to the active sites of the enzyme adsorbed in its monomeric form in open confor-

mation inside the pores of the PSty-DVB beads [44]. Previous studies have demonstrated improved catalytic performance for ET2.0 immobilized via physical adsorption on several hydrophobic supports, including PSty-DVB beads, to catalyze industrial ester production via esterification reactions [42–44,54,55]. Moreover, esterification reactions conducted in open reactions under moderate experimental conditions (40 °C and atmospheric pressure) were effective in improving ester production (based on FFA consumption productivity—see Table 1). Therefore, subsequent studies (reusability tests) were performed in closed and open reactors without adding molecular sieves.

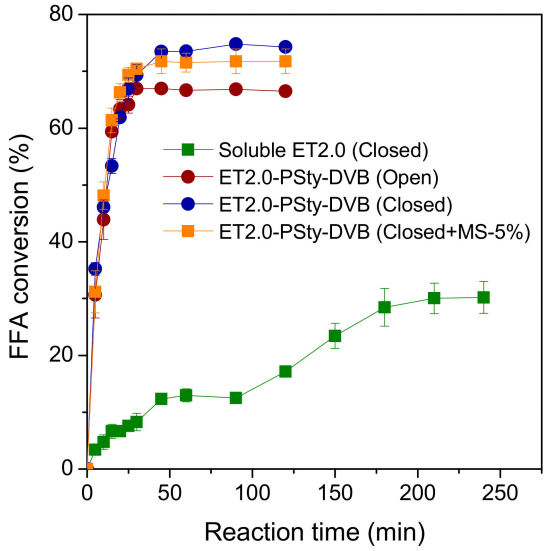

Figure 2. Influence of reaction time on the MAE production in solvent-free systems catalyzed by soluble or immobilized ET2.0 on PSty-DBV beads using different water removal strategies. The reactions were at 40 °C, 240 rpm, stoichiometric FFA:fusel oil molar ratio (1:1), and protein concentration of 5 $mg \cdot g^{-1}$ of reaction mixture.

Table 1. Estimation of productivity for the enzymatic production of monoalkyl esters catalyzed with soluble or immobilized ET2.0 on PSty-DBV beads using different water removal strategies.

Reaction System	Y [a] (%)	t_e [b] (min)	P_{FFA} [c] ($\mu mol_{FFA} \cdot min^{-1} \cdot mg_{prot}{}^{-1}$)
Soluble ET2.0 [d] (Closed reactor)	30.2 ± 2.7	210	0.8
ET2.0-PSty-DVB [e] (Open reactor)	64.3 ± 2.3	30	12.4
ET2.0-PSty-DVB [e] (Closed reactor)	73.5 ± 0.4	45	9.4
ET2.0-PSty-DVB [e] (Closed reactor + MS [f]-5%)	71.8 ± 2.2	45	9.2

[a] FFA conversion percentage at equilibrium; [b] reaction time at equilibrium; [c] Productivity; [d] lipase Eversa® Transform 2.0; [e] lipase Eversa® Transform 2.0 immobilized on PSty-DVB beads; [f] molecular sieves at 5% $m.m^{-1}$.

2.3. Biocatalyst Reusability Studies

Reusability tests were performed for immobilized ET2.0 after eight successive batch esterification reactions, as shown in Figure 3. In this set of experiments, the reaction times corresponded to the required time for achieving a maximum FFA conversion percentage at equilibrium, as reported in Table 1, i.e., 30 min and 45 min conducted in open and closed reactors, respectively. According to the results, a gradual decrease of the catalytic activity of immobilized ET2.0 after successive batch reactions conducted without any water removal strategies (closed reactor in the absence of molecular sieves) can be observed. In

fact, a FFA conversion percentage of 12% (productivity of 1.5 $\mu mol_{FFA}.min^{-1}.mg_{prot}{}^{-1}$), corresponding to a decrease of 84% relative to the original activity (73.5 ± 0.4% in the first batch reaction), was achieved in the eighth batch reaction. These results can be explained by a possible accumulation of produced water molecules on the biocatalyst surface that drastically reduces the partition/diffusion of hydrophobic starting materials such as FFA and isoamyl alcohol from fusel oil to the active sites of the lipase and can shift the reaction equilibrium towards the reverse reaction—ester hydrolysis [36,38,56]. By contrast, esterification reactions performed in an open reactor provided better catalytic activity retention of the heterogeneous biocatalyst after successive batch reactions. In fact, a loss of the original activity of the biocatalyst at around 18% can be observed—from 64.3 ± 2.3% in the first batch to 52.5 ± 0.7% in the eighth batch (productivity of 10.1 $\mu mol_{FFA}.min^{-1}.mg_{prot}{}^{-1}$). These results confirm the requirements for water removal strategies (open reactor) to prevent possible losses in the catalytic performance of the lipase after consecutive batch reactions.

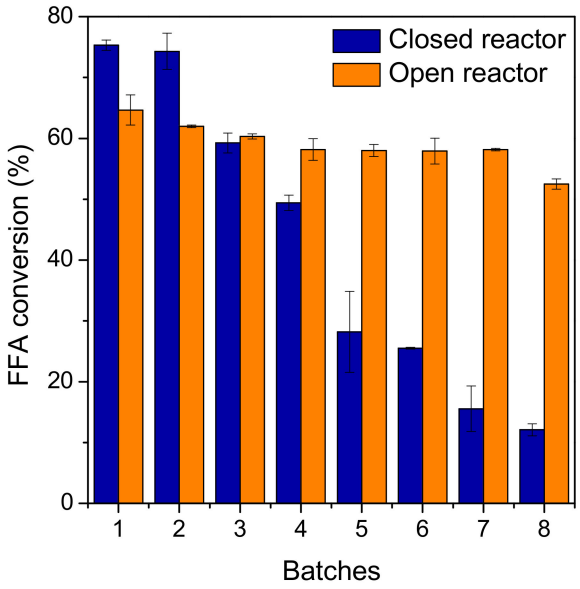

Figure 3. Reusability tests after successive esterification batches of FFA from USCO and fusel oil conducted in closed and open reactor catalyzed by immobilized ET2.0 on PSty-DVB beads. The reactions were at 40 °C, 240 rpm, stoichiometric FFA:fusel oil molar ratio (1:1), and protein concentration of 5 $mg.g^{-1}$ of reaction mixture.

2.4. In Situ Epoxidation of Monoalkyl Esters

In this study, an in-situ epoxidation process was performed under fixed experimental conditions using monoalkyl esters produced via enzymatic esterification in closed or open reactors. The introduction of epoxy oxygen into their chemical structures was determined according to standard methods, and the results are summarized in Table 2. One can observe a similar epoxidation profile for both samples (MAE production in open or closed reactors). The iodine value (IV) for the MAE decreased from 110 g_{I2} per 100 g of esters to 73 g_{I2} per 100 g of esters, corresponding to the conversion of unsaturated bonds into epoxy rings (X) by around 33%. According to Figure S1A,B, the color of the reaction mixtures became light brown, confirming the chemical modification of the produced esters. This partial conversion of double bonds into epoxy groups could be due to the presence of unconverted FFA in the composition, which is corroborated by initial AV values between 5.3 and 5.8 $m_{KOH}.g^{-1}$, reducing the epoxidation process [16]. Under these conditions, the maximum experimental epoxy oxygen content ($OOC_{exp.}$) was 1.75–1.78%. These values correspond to 27% of the theoretical epoxy oxygen content ($OOC_{theor.}$)—around 6.5%. These results also show that this process exhibited high selectivity (above 0.8), suggesting low ring-opening reactions under these conditions, further corroborating previous studies [16,48].

Table 2. Chemical properties of used soybean cooking oil (USCO), monoalkyl esters (MAE) and epoxidized monoalkyl esters (EMAE) produced in this study.

Properties	Units	MAE [a]		EMAE [b]	
		Open Reactor	Closed Reactor	Open Reactor	Closed Reactor
IV [c]	g_{I2} per 100 g	110.2 ± 1.7	109.8 ± 1.1	73.5 ± 0.9	72.6 ± 1.4
X [d]	%	-	-	33.3	33.9
AV [e]	$m_{KOH} \cdot g^{-1}$	5.1 ± 0.3	5.8 ± 0.3	7.1 ± 0.4	7.3 ± 0.2
$OOC_{theor.}$ [f]	%	-	-	6.50	6.47
$OOC_{exp.}$ [g]	%	-	-	1.75 ± 0.01	1.78 ± 0.03
ROC [h]	%	-	-	26.9	27.5
S [i]	Dimensionless	-	-	0.81	0.81

[a] monoalkyl esters; [b] epoxidized monoalkyl esters; [c] iodine value; [d] relative conversion percentage; [e] acid value; [f] maximum theoretical epoxy oxygen content; [g] experimental epoxy oxygen content; [h] relative conversion percentage to oxirane; [i] selectivity.

2.5. H NMR Analysis

The ^{1}H NMR spectra of fusel oil, USCO and their derivatives such as FFA, MAE and EMAE are shown in Figure 4. All the samples presented an intense signal at 1.13–1.17 ppm characteristic of hydrogens of terminal methyl groups (–C$\underline{H}_3$). The signals at 1.49–1.58 ppm of hydrogens of the aliphatic methylene groups (–C$\underline{H}_2$–), and at 2.06–2.11 ppm for allylic hydrogens (–C$\underline{H}_2$–CH=CH–), 2.85–2.95 ppm for bis-allylic hydrogens (–CH=CH–C$\underline{H}_2$–CH=CH–) and at 5.24–5.38 ppm of hydrogens linked to sp^2 carbon—olefinic (–C$\underline{H}$=C$\underline{H}$–) of unsaturated fatty acids are characteristics of USCO and their derivatives (FFA, MAE and EMAE). The signals between 4.10 and 4.40 ppm in the chemical structure of USCO refer to hydrogen atoms of the glycerol moiety that disappeared after hydrolysis step (see FFA spectrum). This confirms the full hydrolysis of USCO (1st step—see Figure 1), in accordance with the titrimetric method. A characteristic peak at 3.85 ppm in the spectrum of fusel oil refers to the methylene hydrogen attached to the hydroxyl group(–C$\underline{H}_2$–OH). The appearance of an intense signal between 4.23 and 4.30 ppm can be observed, one that is characteristic of hydrogen attached to carbon next to the ester group oxygen (–COOC$\underline{H}_2$–), which evidences monoalkyl esters (MAE) production via enzymatic esterification (2nd step), as shown in Figure 2. After epoxidation (3rd step—EMAE production), a decrease in intensity of the characteristic signal of olefinic hydrogens (–C$\underline{H}$=C$\underline{H}$–) at 5.24–5.38 ppm was observed due to partial conversion of unsaturated bonds into epoxy groups. In fact, a slight increase in the intensity of overlapped signals at 1.67 ppm (hydrogen of methylene groups adjacent to epoxy ring: –C$\underline{H}_2$–CHOCH–) and 2.85–3.25 ppm (hydrogens atoms of epoxy groups: –C$\underline{H}$OC$\underline{H}$–) due to introduction of these epoxy groups in the chemical structure of MAE can also be observed. These results confirm the formation of epoxidized monoalkyl esters (EMAE) via a sequential three-step process.

Based on these results, an illustrative scheme of EMAE production by a three-step process was proposed, as shown in Figure 5. This process consists of sequential: (i) enzymatic hydrolysis of USCO, (ii) enzymatic esterification of the produced unsaturated FFA with isoamyl alcohol, the main monoalkyl alcohol present in fusel oil's composition, and (iii) partial epoxidation via conversion of unsaturated bonds in the chemical structure of the produced MAE into epoxy groups.

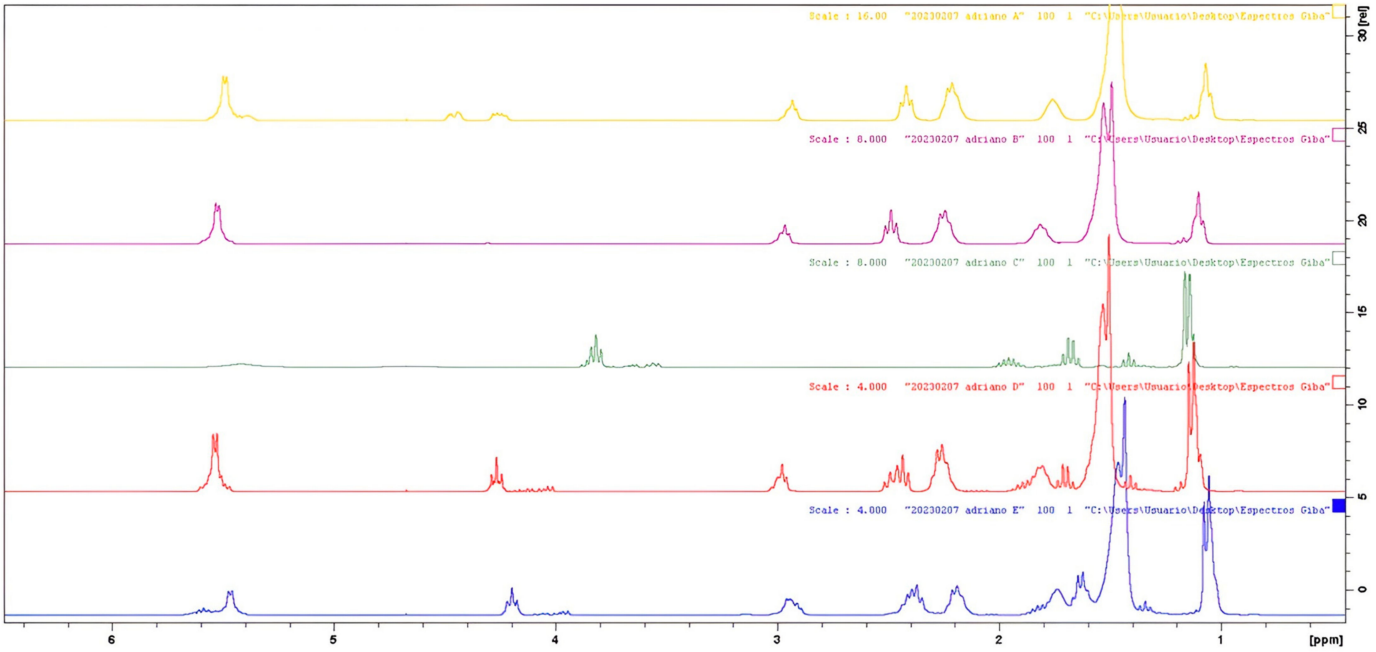

Figure 4. ^{1}H NMR spectra of fusel oil, used soybean cooking oil (USCO) and their derivatives—free fatty acids (FFA), monoalkyl esters (MAE) and epoxidized monoalkyl esters (EMAE).

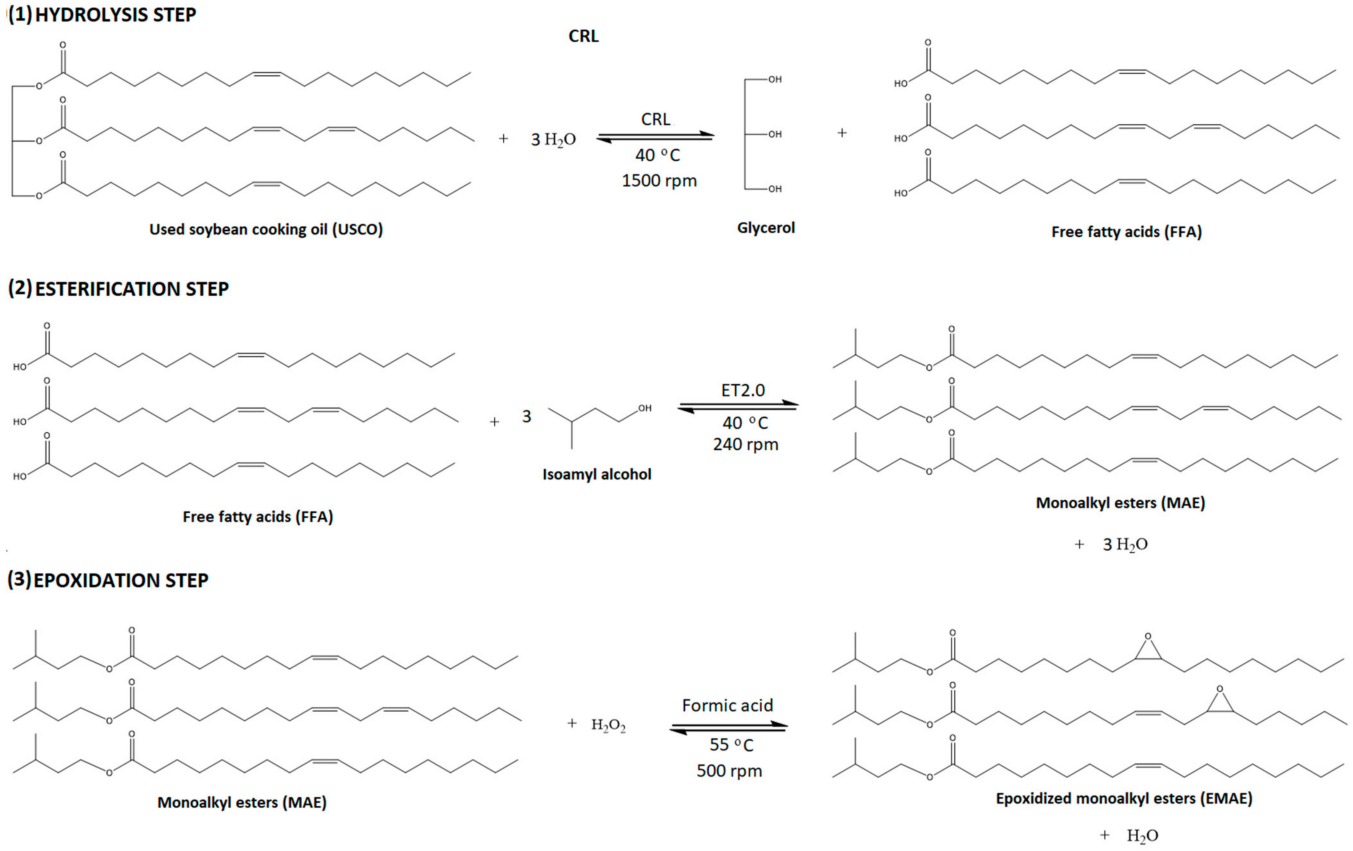

Figure 5. Illustrative scheme of epoxidized monoalkyl esters (EMAE) production from used soybean cooking oil (USCO) and fusel oil via a three-step process.

3. Materials and Methods

3.1. Materials

Commercial lipases from *Thermomyces lanuginosus* expressed in *Aspergillus oryzae* (Eversa® Transform 2.0), *Candida* sp. (CALA) and *Candida rugosa* (CRL) were acquired from Sigma-Aldrich® (St. Louis, MO, USA). Non-commercial lipases from *Aspergillus niger* (ANL) and *Rhizopus javanicus* (RJL) were produced via submerged fermentation [57]. These non-commercial lipases were obtained via sequential precipitation with ammonium sulphate, dialysis in water and lyophilization and used as powder lipase extracts [29,57]. The properties of these lipase preparations are summarized in Table 3. PSty-DVB beads (Diaion® HP-20) were acquired from Supelco® Analytical Products (Bellefonte, PA, USA). The properties of this support were reported in a recent study performed in our lab [44]. Used soybean cooking oil (USCO) was collected, after being used once for French fries preparation, from the restaurant at Federal University of Alfenas (Alfenas, MG, Brazil). Its fatty acid composition (% $m \cdot m^{-1}$) was determined by gas chromatography as follows [58]: 10.7% palmitic, 3.0% stearic, 24.0% oleic, 56.7% linoleic, 5.4% linolenic and 0.1% arachidic acids, with an average FFA molecular mass of 278.6 $g \cdot mol^{-1}$. Fusel oil was acquired from Raízen Tarumã Ltd.a. (Tarumã, SP, Brazil), having the following composition (% $m \cdot m^{-1}$): water (11.6%), ethanol (4.2%), propanol (3.9%), butanol (1.1%), isobutyl alcohol (11.5%) and isoamyl alcohol (67.7%); its average molecular mass was 72.5 $g \cdot mol^{-1}$. Molecular sieve UOP type 3 Å (form of rod, and size of 1/16 in) was purchased from Fluka Analytical (St. Louis, MO, USA). Hydrogen peroxide solution (50% $m \cdot m^{-1}$), formic acid solution (85% $v \cdot v^{-1}$), and Wijs solution (iodine chloride in acetic acid) were acquired from Synth® Ltd. (São Paulo, SP, Brazil). All other reagents and organic solvents were of analytical grade, and acquired from Synth® Ltd.

Table 3. Properties of microbial lipases used in this study.

Properties	Eversa® Transform 2.0	CRL	CALA	RJL	ANL
Source	*Thermomyces lanuginosus*	*Candida rugosa*	*Candida* sp.	*Rhizopus javanicus*	*Aspergillus niger*
Formulation	Liquid	Powder	Liquid	Powder	Powder
Hydrolytic activity [a] ($U \cdot g^{-1}$)	19,856.7	16,156.8	806.4	27,436.5	20,224.9
Protein [b] ($mg \cdot g^{-1}$)	35.8	24.5	16.1	74.9	12.7
Specific activity [c] ($U \cdot mg_{prot}^{-1}$)	554.6	659.5	50.1	366.3	1592.5

[a] determined using olive oil emulsion as substrate—pH 8.0 and 37 °C [59]; [b] determined according to Bradford method [60]; [c] ratio between hydrolytic activity and protein concentration [59].

3.2. Fusel Oil Dehydration

In this study, fusel oil was first dehydrated using anhydrous sodium sulfate, pretreated at 250 °C for 4 h in a muffle furnace. The suspension containing anhydrous Na_2SO_4 (20% $m \cdot v^{-1}$) was added to a closed glass flask immersed in a temperature-controlled thermostatic water-bath under continuous mechanical stirring (240 rpm) at 25 ± 1 °C for 24 h. This procedure was repeated twice before it was used as a starting material in the esterification reaction. The water concentration was reduced from 11.6% $m.m^{-1}$ to ≤2% $m.m^{-1}$, quantified via automatic Titler Karl Fischer (Koheler Model AKF5000—Holtsville, NY, USA).

3.3. Enzymatic Hydrolysis of USCO: Screening the Lipase Preparations

The enzymatic hydrolysis of USCO in a batch system was conducted under fixed experimental conditions, as proposed in a recent study [30]. The reaction systems were prepared by mixing USCO (20 g) and distilled water (30 g) at an oil:water mass ratio of 40% $m.m^{-1}$ in an open polypropylene flask with a 350 mL capacity immersed in a temperature-controlled water bath at 40 ± 1 °C under continuous mechanical stirring

(1500 rpm). To this end, five lipase preparations with different specificities were tested as biocatalysts using a lipase load of 50 units of hydrolytic activity per gram of reaction mixture (50 $U \cdot g^{-1}$), which corresponds to: (i) 3.2 g of CRL powder per Kg of reaction mixture, (ii) 2.5 mL of Eversa® Transform 2.0 per Kg of reaction mixture, (iii) 62.5 mL of CALA per Kg of reaction mixture, (iv) 3.2 g of ANL per Kg of reaction mixture, and (v) 1.82 g of RJL per Kg of reaction mixture. A sixth assay using a combination of commercial lipases Eversa® Transform 2.0 (33 $U \cdot g^{-1}$ that corresponds to 1.7 mL of crude lipase solution per Kg of reaction mixture) and CALA (17 $U \cdot g^{-1}$ that corresponds to 21 mL of crude lipase solution per Kg of reaction mixture), was also performed under the same experimental conditions. Hydrolysis percentage (*HP*) was calculated according to a previous study [61], as shown in Equation (1). The reaction mixtures (100 mL) obtained from the USCO hydrolysis catalyzed by CRL were transferred to a separation funnel (500 mL), followed by the addition of 100 mL of distilled water at 60 °C to separate the aqueous and organic (free fatty acids—FFA) washing phases. The bottom phase (aqueous) was disposed of, and the *FFA* phase was washed three times. Then, anhydrous sodium sulfate (20% $m \cdot v^{-1}$), which was previously dried in a muffle furnace at 250 °C for 4 h, was added to the FFA mixture and kept overnight under static conditions at 25 °C to remove any remaining water traces.

$$HP = \left(\frac{V_{NaOH} \times 10^{-3} \times M_{NaOH} \times M_{FFA}}{m \times \mathrm{f}} \right) \times 100 \quad (1)$$

where V_{NaOH} is the volume of NaOH solution required during titration (mL), M_{NaOH} is the NaOH concentration (40 $mmol \cdot L^{-1}$), MM_{FFA} is the average molecular mass of FFA from USCO (278.6 $g \cdot mol^{-1}$), m is the mass of the sample taken (1 g), and f is the fraction of USCO at start of reaction (f = 0.4).

3.4. Immobilization of Lipase Eversa® Transform 2.0 via Physical Adsorption on PSty-DVB Beads

A quantum of 10 g of PSty-DVB beads were initially soaked in 50 mL of a hydrous ethanol solution (70% $m \cdot m^{-1}$) for 8 h under static conditions at 25 °C, followed by filtration in a Büchner funnel under vacuum, and washing with 200 mL of distilled water [62]. Then, a wet support was added to 190 mL of a lipase solution prepared by mixing 12 mL of liquid lipase formulation (Eversa® Transform 2.0) with 178 mL of a 5 $mmol \cdot L^{-1}$ sodium acetate solution pH 5.0 (0.21 mg protein per mL of solution, which corresponds to an initial protein load of 40 mg of protein per g of support). This suspension was then transferred to a 250 mL closed glass flask and immersed in a temperature-controlled thermostatic water-bath under continuous stirring (200 rpm) at 25 ± 1 °C for 12 h. Finally, the heterogeneous biocatalyst was recovered via filtration in a Büchner funnel under vacuum, washed with an excess of distilled water, and stored at 4 °C for 24 h in a freezer at 4 °C before use. The immobilization process was monitored by measuring the residual protein concentration [60] and hydrolytic activity [42,63] in the supernatant solution at equilibrium. The immobilization yield was calculated as the ratio between the units of hydrolytic activity adsorbed on the support surface and the initial activity [63]. The catalytic activity of the heterogeneous biocatalyst was assayed via the olive oil emulsion hydrolysis method [62]. The maximum adsorbed protein concentration was 31 mg g^{-1} of support, with an immobilization yield of 80%, and a hydrolytic activity at 157.3 ± 12.3 $U \cdot g^{-1}$ of biocatalyst was obtained under the same experimental conditions [44]. One international unit (U) of activity was defined as being the mass of biocatalyst required to release 1 µmol of FFA per min at pH 8.0 (100 $mmol \cdot L^{-1}$ buffer sodium phosphate), 37 °C, and 200 rpm [62].

3.5. General Procedure for the Enzymatic Esterification of FFA and Fusel Oil in a Solvent-Free System

The direct esterification of purified FFA from the enzymatic hydrolysis of USCO and dehydrated fusel oil was conducted in closed or open glass reactors (capacity of 100 mL with a height to diameter ratio of 2) in the absence or presence of molecular sieves (5% mass per mass of reaction mixture—5% $m.m^{-1}$) containing 8 g of starting materials composed

of stoichiometric FFA: fusel oil molar ratio—1:1 (composed of 1.65 g or 23 mmol of fusel oil + 6.35 g or 23 mmol of FFA). The reaction systems were immersed in a temperature-controlled thermostatic water-bath at 40 ± 1 °C under continuous mechanical stirring at 240 rpm containing a fixed biocatalyst concentration of 5 mg of protein per gram of reaction mixture (corresponding to 0.166 g of immobilized lipase or 0.14 g of soluble lipase Eversa® Transform 2.0 per gram of reaction mixture). Samples from the reaction mixtures (50 µL) were periodically withdrawn, diluted in 10 mL of hydrous ethanol solution (92.5% m.m^{-1}), and titrated with a NaOH solution (40 mmol·L^{-1}) using phenolphthalein as an indicator. The FFA conversion percentage (Y—%) was determined as shown in Equation (2) [30,62]. Ester production was performed with two replications, and experimental data are represented as mean ± deviations. In this set of experiments, control assays were prepared with PSty-DVB beads, and no FFA consumption was detected under these experimental conditions, thereby confirming that all of the consumed FFA in the reaction was converted into monoalkyl esters.

$$Y = \left(\frac{FFA_0 - FFA_t}{FFA_0} \right) \times 100 \quad (2)$$

where FFA_0 and FFA_t are the initial and residual FFA concentration after a given reaction time t (mol·L^{-1}), respectively.

Productivity (µmol.min^{-1}·mg$_{prot}$$^{-1}$) was determined according to rearranged equation proposed by [44]—Equation (3):

$$P = \frac{FFA_0 \times Y}{t_e \times m_{prot.}} \quad (3)$$

where FFA_0 is the initial number of moles of FFA in the reaction mixture (23 mmol or 23,000 µmol), Y is average FFA conversion percentage at equilibrium (%), t_e is reaction time at equilibrium (min), and m_{prot} is the protein amount in the reaction mixture (mg).

3.6. Heterogeneous Biocatalyst Reusability Tests

Reusability tests of the heterogeneous biocatalyst prepared in this study (immobilized lipase Eversa® Transform 2.0) were conducted after eight consecutive esterification batches performed in open or closed reactors under fixed experimental conditions, as described above. At the end of each batch of 30 min and 45 min for the reactions performed in open and closed reactors, respectively, the immobilized lipase was recovered via filtration in a Büchner funnel under vacuum, washed with excess cold hexane to remove residual starting materials or products retained on its surface, and was set to dry in a freezer at 4 °C overnight. Afterward, the recovered biocatalyst was re-suspended in a fresh reaction mixture to start new reactions. The acid conversion percentage was determined at the end of each reaction.

3.7. General Procedure for the In Situ Epoxidation of Monoalkyl Esters from USCO and Fusel Oil

In situ epoxidation of the produced MAE was performed according to the methodology proposed by Wang et al. [15]. Initially, 20 g of the mixture of esters produced by esterification (without further separation/purification steps) were introduced in an open polypropylene reactor with a capacity of 350 mL containing 10.4 g of distilled water and immersed in a temperature-controlled water bath at 55 ± 1 °C under continuous mechanical stirring (500 rpm) equipped with a three-blade-helix impeller made of 2 mm thick stainless steel sheets with a diameter of 6 cm. After 15 min of contact, 4.32 g of a formic acid solution at 85% m.m^{-1} were added, followed by a dropwise addition of a 2.5 g H_2O_2 solution at 50% m.m^{-1}. After a 4 h reaction time, the mixture was transferred to a separation funnel (500 mL), followed by the addition of 100 mL of distilled water at 70 °C to wash the aqueous and organic (epoxidized monoalkyl esters) phases. The bottom phase (aqueous) was disposed of, and the organic phase was washed five times. Finally, anhydrous sodium

sulfate (20% m·v^{-1}), previously dried in a muffle furnace at 250 °C for 4 h, was added to the epoxidized esters, and kept overnight under static conditions at 25 °C to remove any traces of water.

3.8. Analytical Methods

3.8.1. Iodine Value (IV)

IV, a parameter that describes the degree of unsaturation in the chemical structure of esters, is defined as the mass of iodine from Wijs solution consumed by 100 g of samples; it was determined according to standard methodology described by American Society for Testing and Materials—ASTM D5554-95 [64]. IV was calculated by the following equation (Equation (4)):

$$IV = \frac{(V_C - V_S) \times 10^{-3} \times \mathrm{C} \times MM_{I2} \times 100}{2 \times \mathrm{m}} \quad (4)$$

where V_C and Vs are the total volume of sodium thiosulfate solution used to titrate the initial (control) and oxidized samples (mL), respectively; C is the molar concentration of sodium thiosulfate solution (0.1 mol·L^{-1}); MM_{I2} is the molecular mass of iodine (253.8 mol·L^{-1}); and m is the mass of the test sample (0.25 g).

3.8.2. Relative Conversion Percentage of Double Bonds

Relative conversion percentage of double bonds or unsaturation degree (X) was determined according to Equation (5) [16,65]:

$$X = \left(\frac{IV_0 - IV_t}{IV_0} \right) \times 100 \quad (5)$$

where IV_0 and IV_t are the iodine values for initial (control) and oxidized (g_{I2} per 100 g) samples, respectively.

3.8.3. Acid Value (AV)

AV, defined as the mass of KOH (in mg) necessary to neutralize 1 g of oil sample, was determined by potentiometric titration according to AOCS Official Method Te 1a-64 [66], as shown in Equation (6):

$$AV = \frac{V \times 10^{-3} \times \mathrm{C} \times MM_{KOH}}{\mathrm{m}} \quad (6)$$

where V is the total volume of sodium hydroxide solution used to titrate the sample (mL); C is the molar concentration of sodium hydroxide solution (0.04 mol·L^{-1}); MM_{KOH} is the molecular mass of potassium hydroxide (56.1 mol·L^{-1}); and m is the mass of the test sample (1 g).

3.8.4. Maximum Theoretical Epoxy Oxygen Content ($OOC_{theor.}$)

$OOC_{theor.}$ was calculated according the following equation (Equation (7)) [15,16]:

$$OOC_{theor.} = \left(\frac{\frac{IV_0 \times MM_O}{2 \times MM_I}}{100 + \frac{IV_0 \times MM_O}{2 \times MM_I}} \right) \times 100 \quad (7)$$

where IV_0 is the initial iodine value of the sample (g_{I2} per 100 g); MM_O is the atomic mass of oxygen (16 u); and MM_I is the is the atomic mass of iodine (126.9 u).

3.8.5. Experimental Epoxy Oxygen Content ($OOC_{exp.}$)

$OOC_{exp.}$ percentage, defined as the mass of oxirane oxygen introduced in 100 g of sample [15], was determined as recommended by Chinese national standard GB/T 1677–2008 (Equation (8)) [67]:

$$OOC_{exp.} = \frac{\left[V_C - \left(V_S - \frac{V_{AV} \times \mathrm{m}}{m_{AV}}\right)\right] \times 10^{-3} \times \mathrm{C} \times MM_O \times 100}{m} \quad (8)$$

where V_C is the volume of NaOH solution used to titrate control sample (mL); Vs is the volume of NaOH solution used to titrate epoxidized esters (mL); V_{AV} is the volume of NaOH solution used to titrate epoxidized esters sample in acid value (AV) determination (mL); m is the mass of test sample (g); m_{AV} is the mass of test sample used in AV determination (g); C is the molar concentration of NaOH solution; and MM_O is the molecular mass of oxirane oxygen (16 $g \cdot mol^{-1}$).

3.8.6. Relative Conversion Percentage to Oxirane (ROC)

This parameter was calculated as follows (Equation (9)) [16,65]:

$$ROC = \left(\frac{OOC_{exp.}}{OOC_{theor.}}\right) \times 100 \quad (9)$$

where $OOC_{exp.}$ is the experimental epoxy oxygen content and $OOC_{theor.}$ is the maximum theoretical epoxy oxygen content.

3.8.7. Selectivity (S)

Selectivity was determined according to following equation (Equation (10)) [15,16]:

$$S = \left(\frac{ROC}{X}\right) \quad (10)$$

where ROC is the relative conversion percentage to oxirane, and X is the relative conversion percentage of double bonds—unsaturation degree (X).

3.9. ^1H NMR Analysis

Supportive identification of chemical structures of starting materials such as fusel oil, USCO and their derivatives (FFA, and non- or oxidized monoalkyl esters—MAE and EMAE, respectively) were given by ^1H nuclear magnetic resonance (NMR) spectroscopy performed on a Bruker AC 300 equipment (Bruker BioSpin, Ettlingen, BW, Germany). The signals of deuterated water (solvent) and trimethylsilane (standard) were suppressed from the spectra.

4. Conclusions

In this study, a three-step process (sequential hydrolysis, esterification, and epoxidation) was proposed to produce epoxidized monoalkyl esters using low-cost and renewable starting materials, e.g., used soybean cooking oil (USCO) and fusel oil. The complete hydrolysis of USCO was achieved after a 3 h reaction time, conducted in the absence of emulsifier and buffer agents, and catalyzed by a non-specific lipase (CRL). The catalytic performance of soluble lipase Eversa® Transform 2.0 was compared with its immobilized form (physical adsorption on a hydrophobic support—Psty-DVB beads). According to the results, the maximum FFA conversion percentage and productivity values were achieved using immobilized lipase, given its better accessibility and partitioning of starting materials to its microenvironment. In this step, different strategies to eliminate water molecules generated during the esterification reaction were also evaluated. According to the results, the maximum FFA conversion percentage was 73.5 ± 0.4%, observed after a 45 min reaction time, and performed in a closed reactor. By contrast, the esterification reaction in an open

reactor showed maximum FFA conversion at 64.3 ± 2.3% after only 30 min. Reusability tests showed the requirement of simple water elimination strategies via evaporation at atmospheric pressure to prevent losses in the catalytic activity of immobilized lipase after successive esterification batch reactions. In situ epoxidation of the produced esters using formic acid as a homogeneous catalyst was confirmed by standard methods and NMR analysis. These results show that the proposed chemoenzymatic process is a promising approach for producing valuable products (MAE and EMAE) for oleochemical industries. Moreover, this study provides new possibilities for investigating the influence of certain parameters on the in situ epoxidation process by using several catalysts, including immobilized lipase Eversa® Transform 2.0, a low-cost and stable enzyme. Further studies should also be conducted using both non-epoxidized (MAE) and epoxidized (EMAE) monoalkyl esters as potential plasticizers in flexible polyvinyl chloride (PVC) film production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/catal13030543/s1, Figure S1: Illustration of the samples of used soybean cooking oil (USCO), monoalkyl esters (MAE) and epoxidized monoalkyl esters (EMAE) for the esterification step (MAE production) conducted in open (A) and closed (B) reactor.

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References

1. Jiang, Z.; Mahmud, A.R.; Maneengam, A.; Nassani, A.A.; Haffar, M.; Cong, P.T. Non linear effect of Biomass, fossil fuels and renewable energy usage on the economic Growth: Managing sustainable development through energy sector. *Fuel* **2022**, *326*, 124943. [CrossRef]
2. Renewable Chemicals | Opportunities & Forecast To 2030. Available online: https://www.psmarketresearch.com/market-analysis/renewable-chemicals-market# (accessed on 7 December 2022).
3. Biomass Explained—U.S. Energy Information Administration (EIA). Available online: https://www.eia.gov/energyexplained/biomass/ (accessed on 7 December 2022).
4. Berenblyum, A.S.; Danyushevsky, V.Y.; Kuznetsov, P.S.; Katsman, E.A.; Shamsiev, R. Catalytic methods for the manufacturing of high-production volume chemicals from vegetable oils and fats (review). *Pet. Chem.* **2016**, *56*, 433–442. [CrossRef]
5. Baena, A.; Orjuela, A.; Rakshit, S.K.; Clark, J.H. Enzymatic hydrolysis of waste fats, oils and greases (FOGs): Status, prospective, and process intensification alternatives. *Chem. Eng. Process.-Process Intensif.* **2022**, *175*, 108930. [CrossRef]
6. Mannu, A.; Garroni, S.; Porras, J.I.; Mele, A. Available Technologies and Materials for Waste Cooking Oil Recycling. *Processes* **2020**, *8*, 366. [CrossRef]
7. 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] [PubMed]

8. Zainal, N.A.; Zulkifli, N.W.M.; Gulzar, M.; Masjuki, H.H. A review on the chemistry, production, and technological potential of bio-based lubricants. *Renew. Sustain. Energy Rev.* **2018**, *82*, 80–102. [CrossRef]
9. Ho, C.K.; McAuley, K.B.; Peppley, B.A. Biolubricants through renewable hydrocarbons: A perspective for new opportunities. *Renew. Sustain. Energy Rev.* **2019**, *113*, 109261. [CrossRef]
10. Bolina, I.C.A.; Gomes, R.A.B.; Mendes, A.A. Biolubricant Production from Several Oleaginous Feedstocks Using Lipases as Catalysts: Current Scenario and Future Perspectives. *BioEnergy Res.* **2021**, *14*, 1039–1057. [CrossRef]
11. Moser, B.R.; Cermak, S.C.; Doll, K.M.; Kenar, J.A.; Sharma, B.K. A review of fatty epoxide ring opening reactions: Chemistry, recent advances, and applications. *J. Am. Oil Chem. Soc.* **2022**, *99*, 801–842. [CrossRef]
12. Ghasemlou, M.; Daver, F.; Ivanova, E.P.; Adhikari, B. Polyurethanes from seed oil-based polyols: A review of synthesis, mechanical and thermal properties. *Ind. Crop. Prod.* **2019**, *142*, 111841. [CrossRef]
13. Belousov, A.S.; Esipovich, A.L.; Kanakov, E.A.; Otopkova, K.V. Recent advances in sustainable production and catalytic transformations of fatty acid methyl esters. *Sustain. Energy Fuels* **2021**, *5*, 4512–4545. [CrossRef]
14. 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.* **2022**, *39*, 25. [CrossRef] [PubMed]
15. Wang, J.; Zhao, X.; Liu, D. Preparation of Epoxidized Fatty Acid Methyl Ester with in situ Auto-Catalyzed Generation of Performic Acid and the Influence of Impurities on Epoxidation. *Waste Biomass Valorization* **2018**, *9*, 1881–1891. [CrossRef]
16. Thuy, N.T.; Lan, P.N. Epoxidation of vietnam rubber seed oil by using peroxophosphatotungstate catalyst complex based on $Na_2WO_4/H_3PO_4/H_2O_2$ with the presence of phase-transfer catalyst. *Mol. Catal.* **2021**, *509*, 111645. [CrossRef]
17. Bai, Y.; Wang, J.; Liu, D.; Zhao, X. Conversion of fatty acid methyl ester to epoxy plasticizer by auto-catalyzed in situ formation of performic acid: Kinetic modeling and application of the model. *J. Clean. Prod.* **2020**, *259*, 120791. [CrossRef]
18. Shi, K.; Ma, F.; Liu, J.; Song, R.-M.; Fu, Z.; Dai, J.-S.; Li, C.; Wen, Y.-L. Development of a new rejuvenator for aged SBS modified asphalt binder. *J. Clean. Prod.* **2022**, *380*, 134986. [CrossRef]
19. Madhusudhana, A.M.; Mohana, K.N.S.; Hegde, M.B.; Nayak, S.R.; Rajitha, K.; Kumar, M.C.S. Functionalized graphene oxide dispersed polyvinyl alcohol-epoxidized linseed oil composite: An eco-friendly and promising anticorrosion coating material. *Colloids Surfaces A Physicochem. Eng. Asp.* **2022**, *650*, 129382. [CrossRef]
20. Gaglieri, C.; Alarcon, R.T.; de Moura, A.; Bannach, G. Vegetable oils as monomeric and polymeric materials: A graphical review. *Curr. Res. Green Sustain. Chem.* **2022**, *5*, 100343. [CrossRef]
21. Ge, Q.-Y.; Dou, Q. "In situ" compatibilization of poly(L-lactic acid)/epoxidized soybean oil bio-blends by reactive additives. *Ind. Crop. Prod.* **2022**, *188*, 115698. [CrossRef]
22. Yang, J.; Xu, S.; Wang, W.; Ran, X.; Ching, Y.C.; Sui, X.; Wei, Y.; Wang, R.; Al-Hada, N.M. Preparation and characterization of bioplastics from silylated cassava starch and epoxidized soybean oils. *Carbohydr. Polym.* **2023**, *300*, 120253. [CrossRef]
23. Armanasco, F.; D'Hers, S.; Chiacchiarelli, L.M. Kinetic and chemorheological modeling of thermosetting polyurethanes obtained from an epoxidized soybean oil polyol crosslinked with glycerin. *J. Appl. Polym. Sci.* **2022**, *139*, e53194. [CrossRef]
24. Chen, Y.; Biresaw, G.; Cermak, S.C.; Isbell, T.A.; Ngo, H.L.; Chen, L.; Durham, A.L. Fatty Acid Estolides: A Review. *J. Am. Oil Chem. Soc.* **2020**, *97*, 231–241. [CrossRef]
25. Polaczek, K.; Kurańska, M.; Prociak, A. Open-cell bio-polyurethane foams based on bio-polyols from used cooking oil. *J. Clean. Prod.* **2022**, *359*, 132107. [CrossRef]
26. De María, P.D.; Sánchez-Montero, J.M.; Sinisterra, J.V.; Alcántara, A.R. Understanding *Candida rugosa* lipases: An overview. *Biotechnol. Adv.* **2006**, *24*, 180–196. [CrossRef] [PubMed]
27. Monteiro, R.R.; Arana-Peña, S.; da Rocha, T.N.; Miranda, L.P.; Berenguer-Murcia, A.; Tardioli, P.W.; dos Santos, J.C.; 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]
28. Monteiro, R.R.; Virgen-Ortiz, J.J.; Berenguer-Murcia, A.; da Rocha, T.N.; dos Santos, J.C.; Alcántara, A.R.; Fernandez-Lafuente, R. Biotechnological relevance of the lipase A from *Candida antarctica*. *Catal. Today* **2021**, *362*, 141–154. [CrossRef]
29. Fregolente, P.B.; Fregolente, L.; Maciel, M.R.; Carvalho, P.O. Screening of microbial lipases and evalutaion of their potential to produce glycerides with high gamma linolenic acid concentration. *Braz. J. Microbiol.* **2009**, *40*, 747–756. [CrossRef]
30. 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. *Enzym. Microb. Technol.* **2022**, *157*, 110019. [CrossRef]
31. Mendoza-Pedroza, J.D.J.; Sánchez-Ramírez, E.; Segovia-Hernández, J.G.; Hernández, S.; Orjuela, A. Recovery of alcohol industry wastes: Revaluation of fusel oil through intensified processes. *Chem. Eng. Process.-Process Intensif.* **2021**, *163*, 108329. [CrossRef]
32. Massa, T.; Raspe, D.; Feiten, M.; Cardozo-Filho, L.; da Silva, C. Fusel Oil: Chemical Composition and an Overview of Its Potential Application. *J. Braz. Chem. Soc.* **2023**, *34*, 153–166. [CrossRef]
33. 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]
34. Gryglewicz, S.; Muszyński, M.; Nowicki, J. Enzymatic synthesis of rapeseed oil-based lubricants. *Ind. Crop. Prod.* **2013**, *45*, 25–29. [CrossRef]
35. Cerón, A.A.; Boas, R.N.V.; Biaggio, F.C.; de Castro, H.F. Synthesis of biolubricant by transesterification of palm kernel oil with simulated fusel oil: Batch and continuous processes. *Biomass Bioenergy* **2018**, *119*, 166–172. [CrossRef]

36. Marty, A.; Dossat, V.; Condoret, J.S. Continuous operation of lipase-catalyzed reactions in nonaqueous solvents: Influence of the production of hydrophilic compound. *Biotechnol. Bioeng.* **1997**, *56*, 232–237. [CrossRef]
37. Colombié, S.; Tweddell, R.; Condoret, J.-S.; Marty, A. Water activity control: A way to improve the efficiency of continuous lipase esterification. *Biotechnol. Bioeng.* **1998**, *60*, 362–368. [CrossRef]
38. Sandoval, G.; Condoret, J.S.; Monsan, P.; Marty, A. Esterification by immobilized lipase in solvent-free media: Kinetic and thermodynamic arguments. *Biotechnol. Bioeng.* **2002**, *78*, 313–320. [CrossRef]
39. Séverac, E.; Galy, O.; Turon, F.; Pantel, C.A.; Condoret, J.-S.; Monsan, P.; Marty, A. Selection of CalB immobilization method to be used in continuous oil transesterification: Analysis of the economical impact. *Enzym. Microb. Technol.* **2011**, *48*, 61–70. [CrossRef]
40. Urrutia, P.; Arrieta, R.; Alvarez, L.; Cardenas, C.; Mesa, M.; Wilson, L. Immobilization of lipases in hydrophobic chitosan for selective hydrolysis of fish oil: The impact of support functionalization on lipase activity, selectivity and stability. *Int. J. Biol. Macromol.* **2018**, *108*, 674–686. [CrossRef]
41. Wancura, J.H.; Fantinel, A.L.; Ugalde, G.A.; Donato, F.F.; de Oliveira, J.V.; Tres, M.V.; Jahn, S.L. Semi-continuous production of biodiesel on pilot scale via enzymatic hydroesterification of waste material: Process and economics considerations. *J. Clean. Prod.* **2021**, *285*, 124838. [CrossRef]
42. Carvalho, W.C.; Luiz, J.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]
43. Júnior, J.G.G.; Mattos, F.R.; Sabi, G.J.; Carvalho, W.C.; Luiz, J.H.; Cren, 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]
44. Júnior, J.M.; Mattos, F.; Costa, G.; Zurlo, A.; Fernandez-Lafuente, R.; Mendes, A. Improved catalytic performance of lipase Eversa® Transform 2.0 via immobilization for the sustainable production of flavor esters—Adsorption process and environmental assessment studies. *Catalysts* **2022**, *12*, 1412. [CrossRef]
45. Cai, J.; Wu, Z.; Gao, N.; Xu, H.; Wang, D.; Zhou, F.; Nie, Y. Novel packed bed reactor designed for Prileschajew epoxidation of fatty acid methyl ester: Intensification of mass/heat transfer. *Chem. Eng. Process.-Process Intensif.* **2022**, *176*, 108960. [CrossRef]
46. Valentini, F.; Kozell, V.; Petrucci, C.; Marrocchi, A.; Gu, Y.; Gelman, D.; Vaccaro, L. Formic acid, a biomass-derived source of energy and hydrogen for biomass upgrading. *Energy Environ. Sci.* **2019**, *12*, 2646–2664. [CrossRef]
47. Petrovic, Z.S.; Zlatanić, A.; Lava, C.C.; Sinadinović-Fišer, S. Epoxidation of soybean oil in toluene with peroxoacetic and peroxoformic acids— kinetics and side reactions. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 293–299. [CrossRef]
48. Jadhav, P.D.; Patwardhan, A.V.; Kulkarni, R.D. Kinetic study of in situ epoxidation of mustard oil. *Mol. Catal.* **2021**, *511*, 111748. [CrossRef]
49. Fernandez-Lafuente, R. Lipase from *Thermomyces lanuginosus*: Uses and prospects as an industrial biocatalyst. *J. Mol. Catal. B Enzym.* **2010**, *62*, 197–212. [CrossRef]
50. Feiten, M.C.; Rosa, C.D.; Treichel, H.; Furigo, A.; Zenevicz, M.C.; de Oliveira, D.; Oliveira, J.V. Batch and fed-batch enzymatic hydrolysis of soybean oil under ultrasound irradiation. *Biocatal. Agric. Biotechnol.* **2014**, *3*, 83–85. [CrossRef]
51. Zenevicz, M.C.P.; Jacques, A.; Furigo, A.F.; Oliveira, J.V.; de Oliveira, D. Enzymatic hydrolysis of soybean and waste cooking oils under ultrasound system. *Ind. Crop. Prod.* **2016**, *80*, 235–241. [CrossRef]
52. Rodrigues, R.C.; Ayub, M.A.Z. Effects of the combined use of *Thermomyces lanuginosus* and *Rhizomucor miehei* lipases for the transesterification and hydrolysis of soybean oil. *Process. Biochem.* **2011**, *46*, 682–688. [CrossRef]
53. Souza, J.E.S.; Monteiro, R.R.C.; Rocha, T.G.; Moreira, K.S.; Cavalcante, F.T.T.; Braz, A.K.D.S.; de Souza, M.C.M.; dos Santos, J.C.S. Sonohydrolysis using an enzymatic cocktail in the preparation of free fatty acid. *3 Biotech* **2020**, *10*, 254. [CrossRef] [PubMed]
54. Choi, S.; Kim, B.H.; No, D.S.; Yoon, S.W.; Lee, M.-W.; Im, D.J.; Kim, I.-H. Lipase-catalyzed synthesis of 2-ethylhexyl palmitate in a solvent free system using step changes in temperature. *Biochem. Eng. J.* **2022**, *177*, 108261. [CrossRef]
55. Facin, B.; Quinto, E.; Valerio, A.; de Oliveira, D.; Oliveira, J.; Fernandez-Lorente, G. Strategies for the immobilization of Eversa® Transform 2.0 lipase and application for phospholipid synthesis. *Catalysts* **2021**, *11*, 1236. [CrossRef]
56. Páez, B.; Medina, A.; Rubio, F.; Moreno, P.; Grima, E. Modeling the effect of free water on enzyme activity in immobilized lipase-catalyzed reactions in organic solvents. *Enzym. Microb. Technol.* **2003**, *33*, 845–853. [CrossRef]
57. Carvalho, P.D.O.; Contesini, F.; Bizaco, R.; Calafatti, S.A.; Macedo, G. Optimization of enantioselective resolution of racemic ibuprofen by native lipase from *Aspergillus niger*. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 713–718. [CrossRef]
58. Santos, K.C.; Cassimiro, D.M.; Avelar, M.H.; Hirata, D.B.; de Castro, H.F.; Fernández-Lafuente, R.; Mendes, A.A. Characterization of the catalytic properties of lipases from plant seeds for the production of concentrated fatty acids from different vegetable oils. *Ind. Crop. Prod.* **2013**, *49*, 462–470. [CrossRef]
59. Alves, M.D.; Aracri, F.M.; Cren, 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]
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. Rooney, D.; Weatherley, L. The effect of reaction conditions upon lipase catalysed hydrolysis of high oleate sunflower oil in a stirred liquid–liquid reactor. *Process. Biochem.* **2001**, *36*, 947–953. [CrossRef]

62. Lage, F.A.; Bassi, J.J.; Corradini, M.C.; Todero, L.M.; Luiz, J.H.; Mendes, A.A. Preparation of a biocatalyst via physical adsorption of lipase from *Thermomyces lanuginosus* on hydrophobic support to catalyze biolubricant synthesis by esterification reaction in a solvent-free system. *Enzym. Microb. Technol.* **2016**, *84*, 56–67. [CrossRef]
63. Boudrant, J.; Woodley, J.M.; Fernandez-Lafuente, R. Parameters necessary to define an immobilized enzyme preparation. *Process. Biochem.* **2020**, *90*, 66–80. [CrossRef]
64. Standard Test Method for Determination of the Iodine Value of Fats and Oils. Available online: https://www.astm.org/d5554-95.html (accessed on 3 December 2022).
65. Suzuki, A.H.; Botelho, B.G.; Oliveira, L.S.; Franca, A.S. Sustainable synthesis of epoxidized waste cooking oil and its application as a plasticizer for polyvinyl chloride films. *Eur. Polym. J.* **2018**, *99*, 142–149. [CrossRef]
66. AOCS Te 1a-64—Acid Value (2017) PDF | PDF | Titration | Chemistry. Available online: https://pt.scribd.com/document/451524050/AOCS-Te-1a-64-Acid-Value-2017-pdf (accessed on 4 December 2022).
67. GB/T 1677-2008: PDF in English. Available online: https://www.chinesestandard.net/PDF.aspx/GBT1677-2008 (accessed on 4 December 2022).