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Optimizing the enzymatic production of biolubricants by the Taguchi method: Esterification of the free fatty acids from castor oil with 2-ethyl-1-hexanol catalyzed by Eversa Transform 2.0

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ABSTRACT

The solvent-free esterification of the free fatty acids (FFAs) obtained by the hydrolysis of castor oil (a non-edible vegetable oil) with 2-ethyl-1-hexanol (a branched fatty alcohol) was catalyzed by different free lipases. Eversa Transform 2.0 (ETL) features surpassed most commercial lipases. Some process parameters were optimized by the Taguchi method (L16′). As a result, a conversion over 95% of the FFAs of castor oil into esters with lubricants properties was achieved under optimized reaction conditions (15 wt% of biocatalyst content, 1:4 molar ratio (FFAs/alcohol), 30 °C, 180 rpm, 96 h). The substrates molar ratio had the highest influence on the dependent variable (conversion at 24 h). FFAs/2-ethyl-1-hexanol esters were characterized regarding the physicochemical and tribological properties. Interestingly, the modification of the FFAs with 2-ethyl-1-hexanol by ETL increased the oxidative stability of the FFAs feedstock from 0.18 h to 16.83 h. The biolubricants presented a lower friction coefficient than the reference commercial mineral lubricant (0.052 ± 0.07 against 0.078 ± 0.04). Under these conditions, ETL catalyzed the oligomerization of ricinoleic acid (a hydroxyl fatty acid) into estolides, reaching a conversion of 25.15% of the initial FFAs (for the first time).

1. Introduction

Vegetable oils and animal fats have been employed as lubricating materials since the ancient Egyptian civilization [1]; however, as the petroleum industry developed, vegetable oils and animal fats were substituted by mineral oils [2]. Lubricants especially control friction and wear losses in interacting surfaces in most industries (e.g., transportation, manufacturing and energy), being mineral oils the most utilized lubricants due to their low price, availability and high performance [2,3]. Regardless of the convenient features of mineral-based lubricants, they are obtained from non-renewable resources and are toxic to the environment due to leakage/spillage and/or improper disposal [4–6]. The growing concerns about the depletion of petroleum reserves and the difficulties in avoiding or even mitigating the environmental problems of mineral lubricants have been boosting the use of non-toxic and biodegradable feedstocks (e.g., vegetable oils and animal fats) to produce bio-based lubricants (formally, biolubricants) [4,6,7]. Vegetable oils may be employed as a bio-based lubricating material due to their excellent lubricity, high viscosity index and flash point. However, they must be further modified to enhance, for example, their oxidative stability [7,8].

The transesterification of vegetable oils or the esterification of the corresponding FFAs are the most common strategies for producing high-performance biolubricants [6,9,10]. Acid catalysts have been mainly used to perform the esterification of FFAs with fatty alcohols or polyols to avoid soap formation that occurs using alkaline catalysts [11]. Nevertheless, the chemical production of biolubricants is performed at high temperature and, as such, it is high energy-demanding. Moreover, the acid catalysts may lead to equipment corrosion and further effluent post-treatment complications in the downstream. In this context, the esterification of FFAs catalyzed by enzymes (e.g., lipases, EC.3.1.1.3) may be a suitable alternative to the chemical esterification of FFAs to

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produce high-performance biolubricants [6,12,13].

The physiological function of lipases is to hydrolyze triacylglycerols (e.g., from vegetable oils) into FFAs and glycerol; however, at low water activity, lipases may catalyze the esterification of FFAs [6,12,13]. Therefore, lipases are the most employed biocatalyst in the esterification of FFAs with suitable alcohols to produce high-performance biolubricants [6,12,13]. The lipase Eversa Transform 2.0 (ETL) is an industrially genetically engineered enzyme derived from the lipase from *Thermomyces lanuginosus* (TLL), being produced by submerged fermentation of a genetically modified *Aspergillus oryzae* strain [14]. ETL was primarily designed to produce biodiesel using feedstocks with any FFAs content – i.e., from low acidity to high acidity oils, such as residual oils – in a one-batch process (transesterification of acylglycerols/esterification of FFAs) [14]. ETL is a low-cost liquid lipase preparation, accounting for approximately 5% of the final expenses in biodiesel production [15]. Compared to biodiesel, biolubricant is a high added-value product; thus, ETL may be even more suitable to produce it. However, the acyl acceptors to produce biodiesel are small chain alcohols, whereas fatty alcohols and polyols are employed to produce biolubricants; that way, due to enzyme specificity, ETL may not be so efficient for this application. This enzyme is offered in the market to be used in free form to catalyze the production of biodiesel, saving the costs of immobilization and also losing the advantages that the immobilization may offer [14]. In the production of biolubricants, few reports evaluated the performance of free ETL in the esterification of FFAs with fatty alcohols and polyols [16,17]. This is the primary objective of the current paper, even though the use of immobilized Eversa can alter the results, this first evaluation intends to study the behavior of the free enzyme as suggested by the supplier [14]. Very likely, results can be improved using immobilized forms of Eversa [18–20], as immobilization can improve enzyme stability, selectivity and specificity [21–31].

Regarding feedstocks, biolubricants may be produced from edible and non-edible oils [32,33]. Non-edible vegetable oils may be a better option due to food security and the possibility of plant cultivation under harsh environments; in addition, non-edible vegetable oils are usually cheaper than edible vegetable oils [7,8]. Among the non-edible vegetable oils, castor oil is a promising feedstock to produce biolubricants due to its composition [34–40]. Castor oil is mainly composed of ricinoleic acid (up to 90%), which is a hydroxy fatty acid [35,38]. Castor oil has a higher viscosity and a lower viscosity index than other vegetable oils [35]. The selection of vegetable oil should be evaluated, and the rational design of high-performance biolubricants should also consider the selection of a suitable alcohol [6]. Substituting the glycerol backbone of vegetable oils by a more suitable alcohol (e.g., branched fatty alcohols or polyols) may result in a biolubricant with enhanced oxidative stability [10]. For instance, due to its potential to improve the physico-chemical properties of castor oil, 2-ethyl-1-hexanol is a branched fatty alcohol widely employed in the production of biolubricants by chemical catalysts [41–47]; however, its potential has not been exploited in the enzymatic modification of castor oil to produce biolubricants yet.

Apart from the selection of the proper biocatalyst and feedstocks (vegetable oil and alcohol), the process parameters (e.g., biocatalyst content, substrates molar ratio (FFAs/alcohol), reaction temperature, stirring rate and reaction time) must be carefully evaluated to optimize the production of biolubricants as much as possible [48–50]. In the majority of recent literature, the optimization of process parameters has been mainly performed by univariate design, in which the influence of one independent variable is evaluated at a time for its effect on the dependent variable [51]. Nevertheless, depending on the number of process parameters and levels evaluated, it may be very time, cost and labor-intensive [52]. The Taguchi methods are powerful statistical designs for product development and industrial engineering [52]. Nevertheless, in partial fractional statistical designs, the Taguchi methods provide enough information to accurately and reliably optimize process parameters with the minimum number of experiments [52,53]. As other robust design protocols, the Taguchi methods evaluate controllable and uncontrollable process parameters by the signal to noise (S/N) ratio and, thus, aims to mitigate noise effects and to reduce variation in order to lead towards the development of cost-effective processes [52,53].

As a reaction system, we have decided to utilize a solvent-free system. This system has the advantage of saving the use of any organic solvent in the reaction, increasing the concentration of the substrates and, that way, the volume productivity [54–58]. However, it is a complex system. Changing the ratio of substrates, the properties of the reaction media are changed (polarity, viscosity, etc.); that way, the change on the enzyme performance may be derived from several causes. These media properties change when the reaction proceeds, which can cause unexpected effects on the reaction courses [58].

Herein, the esterification of the FFAs from castor oil with 2-ethyl-1-hexanol in a solvent free media catalyzed by free ETL was evaluated regarding the process parameters (biocatalyst content, substrates molar ratio (FFAs/alcohol), reaction temperature, stirring rate and reaction time) by the Taguchi method. First, to check if really this enzyme may be a good option as biocatalyst of this reaction, it was compared with some of the most popular commercial lipases: lipases A and B from *Candida antarctica* (CALA and CALB) [59–62], lipase from *Rhizomucor miehei* (RML) [63,64] and lipase from *Thermomyces lanuginosus* (TLL) [65]. The product was further characterized regarding its physico-chemical and tribological properties. To the best of the authors' knowledge, it is the first report in the scientific literature to optimize the esterification of the FFAs from castor oil with 2-ethyl-1-hexanol catalyzed by free ETL.

2. Materials and methods

2.1. Materials

ETL (protein concentration of 27.50 mg/mL in the commercial preparation), CALA (protein concentration of 7.7 mg/mL in the commercial preparation), CALB (protein concentration of 7.9 mg/mL in the commercial preparation), RML (protein concentration of 3.2 mg/mL in the commercial preparation) and TLL (protein concentration of 21 mg/mL in the commercial preparation) were kindly donated by Novozymes Spain (Alcobendas, Spain). FFAs from castor oil were purchased from Azevedo Indústria e Comércio de Óleos LTDA (São Paulo, Brazil). According to the supplier, castor oil is composed of ricinoleic acid (82–90 wt%), linoleic acid (2–8 wt%), oleic acid (2–7 wt%), stearic acid (2 wt%) and palmitic acid (2 wt%). 2-ethyl-1-hexanol was purchased from Dinâmica (São Paulo, Brazil). All other used chemicals were of analytical grade. The chrome steel alloy balls (hardness of 64 HRC, diameter of 12.7 mm and initial surface roughness of 0.015 μm) used in the tribology tests were purchased from CYH Rolamentos e Esferas (São Paulo, Brazil). A commercial mineral lubricant (with a flow behavior of 20 W-50) was purchased from Elf (São Paulo, Brazil). The software Statistica® (Statsoft South America, São Caetano do Sul, Brazil) was used to create and analyze the design of experiments by the Taguchi method.

2.2. Methods

All experiments were performed at least in triplicate, and the results are presented as the average of these values, with a standard deviation typically below 5%.

2.2.1. Evaluation of different biocatalysts as catalysts of the production of biolubricants

Prior to experimental design, five liquid lipase preparations (ETL, CALA, CALB, RML and TLL) were evaluated under the same reaction conditions (lowest level of the experimental design: 30 °C, 120 rpm, 1:1 molar ratio (FFAs/alcohol) and 5 wt% of biocatalyst content) regarding the esterification of the FFAs from castor oil and 2-ethyl-1-hexanol for 0.5–8 h. The ester productivity of the five liquid lipase preparations was calculated as the ratio of the consumed FFAs (mmol) per space time (h)

and protein mass (g).

2.2.2. Experimental design

A design of experiments by the Taguchi method with a standard orthogonal array L16′ ("L" represents the Latin square and "16″ represents the number of experiments) was employed to screen five factors at four levels each in the esterification of the FFAs from castor oil with 2-ethyl-1-hexanol catalyzed by ETL, as depicted in Table 1. The signal/noise (S/N) ratios for the conversion of each assay of the L16′ orthogonal array were calculated by a "larger-is-better" function, as the main goal is to maximize the response (conversion). The S/N ratios for each assay were calculated by Eq. 1.

$$\frac{S}{N} = -10\log\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_i^2}\right) \quad (1)$$

In which, y is the conversion to the corresponding essay, i is the number of replicates and n is the number of responses for the combination of factor levels in any specific parametric combination according to Table 1. The predicted S/N ratio under optimal conditions to achieve the highest conversion was estimated by Eq. 2.

$$\frac{S}{N_{predicted}} = \frac{\overline{S}}{N} + \sum_{j=1}^{n}\left(\frac{s}{N_j} + \frac{\overline{s}}{N}\right) \quad (2)$$

In which, $\overline{S}/N$ is the arithmetic mean of all S/N ratios, S/N_j is the S/N ratio at the optimal point for each factor and n is the number of factors significantly affecting the process.

2.2.3. Biolubricant production

To produce 2-ethylhexyl esters (biolubricant), the FFAs from castor oils (5 g), 2-ethyl-1-hexanol (1:1–1:4 molar ratio, FFAs/2-ethyl-1-hexanol) and ETL (aqueous buffer solution, without any further treatment) (5–20 wt% of the FFAs) were incubated in an open vessel (50 mL, 42 mm diameter and 60 mm height) in an orbital shaker (120–180 rpm) at 30–60 °C for 4–24 h, as depicted in Table 1. After the specific reaction time for each assay, ETL was removed from the reaction medium by centrifugation (10,000 rpm and 5 min), as it forms a semi-solid phase in contact with 2-ethyl-1-hexanol; then, the unreacted 2-ethyl-1-hexanol and the produced water was separated from the product by Kugelrohr reduced pressure distillation (70 °C and 1 h). Finally, the Acidity Index (AI) of the product was determined using 1 g aliquots (free of ETL and water) diluted in 10 mL of ethyl alcohol and 3 drops phenolphthalein and titrated with sodium hydroxide (0.1 M) [66,67]. The AI was calculated by Eq. 3.

$$AI(mgNaoH/g) = MW_{NaOH} \times M_{NaOH} \times f \times \frac{V_{NaOH}}{m} \quad (3)$$

In which, MW_{NaOH} (g/mol) is the molecular weight of NaOH; M_{NaOH} (mol/L) is the molarity of the NaOH solution; f is the correction factor determined by NaOH standardization; V_{NaOH} (mL) is the volume of NaOH spent on the titration; and, m (g) is the mass of the sample to be analyzed. The conversion of the FFAs from castor oil and 2-ethyl-1-hexanol into 2-ethylhexyl esters was calculated by Eq. 4, considering the acidity at time zero (AI_0) and time t (AI_t) [66,67].

$$Conversion(\%) = \frac{AI_0 - AI_t}{AI_0} \quad (4)$$

Table 1
Independent factors and the corresponding levels to screen the esterification of the free fatty acids (FFAs) from castor oil with 2-ethyl-1-hexanol catalyzed by Eversa Transform 2.0.

	Temperature (°C)	Stirring (rpm)	Time (h)	Molar Ratio (FFAs/Alcohol)	Biocatalyst (wt%)
Level 1 (L1)	30	120	4	1:1	5
Level 2 (L2)	40	140	8	1:2	10
Level 3 (L3)	50	160	16	1:3	15
Level 4 (L4)	60	180	24	1:4	20

2.2.4. Estolide production

To evaluate the potential of ETL to produce estolides of the FFAs from castor oil, a reaction under the same optimal conditions to produce the 2-ethylhexyl esters by ETL was performed, but without using 2-ethyl-1-hexanol. The conversion of the FFAs from castor oils into estolides was evaluated as in Section 2.2.2.

2.2.5. Feedstock and product characterization

The feedstock (FFAs from castor oil) and the products (2-ethylhexyl esters and estolides) were characterized regarding kinematic viscosity at 40 °C and 100 °C in a viscometer (Anton Paar, SVM 3000, Graz, Austria), according to ASTM D445 [68]. The Viscosity Index (VI) and Iodine Value (IV) were determined according to the ASTM D2270 and D5554, respectively [69,70]. The oxidative stability was determined by the Rancimat method in a Professional Biodiesel Rancimat (Methohm, 893, Herizau, Switzerland), according to the EN 14112 [71].

The composition of the feedstock and products was determined by Fourier-Transform Infrared spectroscopy with Attenuated Total Reflection (FTIR-ATR, for short) in the scanning range of 400–4000 cm^{-1} and 32 scans at a resolution of 4 cm^{-1} in a spectrophotometer (Shimadzu, IRTracer-100, Kyoto, Japan).

The feedstock and products were further characterized regarding tribology in a four-ball tester coupled to a rheometer (DHR-3, TA Instruments, New Castle, USA). First, the ball was cleaned with acetone and dried under ambient temperature. The four-ball test was conducted: one ball rotated at constant sliding speed (0.46 m/s), temperature (75 °C) and loading force (55 N) against three fixed balls submerged in the evaluated sample (FFAs from castor oils, biolubricant, estolides and commercial lubricant) during 1 h. Finally, the wear morphology and wear scar diameter were determined in optical microscopy (Zeiss, Oberkochen, Germany) [47].

3. Results and discussion

3.1. Biocatalyst screening

Four commercial preparations of free lipases (CALA, CALB, RML and TLL) were compared to ETL in the esterification of the FFAs from castor oil with 2-ethyl-1-hexanol to check if the use of this new enzyme was competitive with some of the most popular enzyme commercial. As depicted in Fig. 1, CALA, CALB and RML exhibited a similar performance: an increase in the conversion over time, but with a minimal increase in conversion after 4 h of reaction. ETL and TLL exhibited an increase in conversion over time, but with a more significant increase in conversion after 4 h of reaction. ETL exhibited a very adequate performance, similar to the performance found for the parent lipase (TLL) [14]. Table 2 shows that the activities of TLL and ETL were higher than the activities of the other 3 enzymes, and very similar each other. That way, the optimization of this reaction using the new enzyme, even designed for the utilization of short-chain alcohols, presents itself as a reasonable idea, being cheaper than TLL, the parent enzyme [14].

3.2. Experimental design

To study the esterification of FFAs from castor oil with 2-ethyl-1-hexanol catalyzed by ETL as biocatalyst, an experimental design by the Taguchi method with a standard orthogonal array L16′ was performed as detailed in Section 2.2.1. Table 3 depicts the combination of the five

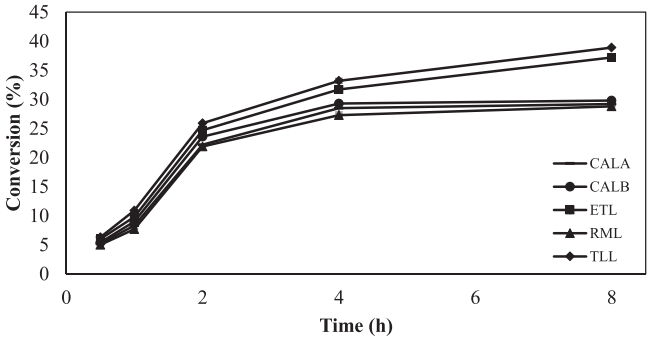

Fig. 1. Biocatalyst screening for the esterification of the free fatty acids from castor oil with 2-ethyl-1-hexanol. Reaction condition: 30 °C, 120 rpm, 1:1, 5 wt % of biocatalyst content and 0.5–8 h. (CALA: lipase A from *Candida antarctica*; CALB: lipase B *Candida antarctica*; ETL: Eversa Transform 2.0; RML: lipase from *Rhizomucor miehei*; TLL: lipase from *Thermomyces lanuginosus)*. The experiments were carried out in triplicate for each group. $p < 0.05$, one-way ANOVA test. Further details are provided in Section 2.2.

Table 2
Biocatalyst productivity for the esterification of the free fatty acids from castor oil with 2-ethyl-1-hexanol. Reaction condition: 30 °C, 120 rpm, 1:1, 5 wt% of biocatalyst content and 8 h. (CALA: lipase A from Candida antarctica; CALB: lipase B Candida antarctica; ETL: Eversa Transform 2.0; RML: lipase from Rhizomucor miehei; TLL: lipase from Thermomyces lanuginosus). The experiments were carried out in triplicate for each group. $p < 0.05$, one-way ANOVA test. Further details are provided in Section 2.2.

Biocatalyst	Productivity $\mu molg^{-1}h^{-1}$
CALA	2445.5 ± 6.00
CALB	2495.7 ± 5.56
ETL	3115.5 ± 6.00
RML	2412.0 ± 16.67
TLL	3257.9 ± 10.67

independent variables (reaction temperature, stirring rate, reaction time, substrates molar ratio and biocatalyst content) at four levels each with conversion as dependent variable and S/N ratio, which was calculated by the "bigger-the-better" function (Eq. 1). The maximum speed of our facilities limits the maximum stirring rate, and 20% of biocatalyst content was the maximum amount of enzyme considered as the limit to have the possibility of handling the reaction mixture. It should be considered that the enzyme is in an aqueous buffer; that way, adding more ETL means adding more water to the system. A substrate molar ratio 1:4 is also the limit to have an adequate industrial process; further increasing the alcohol content would dilute the acid and reduce the overall productivity of the process, even if the alcohol is recovered and reused. The use of conversion as dependent variable at a maximum of 24 h enables us to see the reaction rate as at that reaction time the equilibrium has not been reached in any of the reactions, but it is easy to make longer reaction cycles if desired (as in fact, we have performed).

As depicted in Table 3, the highest conversion of the FFAs from castor oil and 2-ethyl-1-hexanol into 2-ethylhexyl esters was experimentally achieved at the superior level (L4) for all independent variables, except for reaction temperature (L1). As such, the highest conversion (74.19%) was achieved at run 4 (30 °C, 180 rpm, 24 h, 1:4 molar ratio (acid/alcohol) and 20 wt% of biocatalyst content).

Nevertheless, Eq. 2 predicted that the highest S/N ratio (38.05) and, thus, the highest conversion (75.46%) would be achieved at 50 °C (L3), 180 rpm (L4), 24 h (L4), 1:4 (FFAs/alcohol) and 15 wt% of biocatalyst content, as depicted in Fig. 2. Then, an experiment was run under these theoretical optimal reaction conditions to validate the predicted value of the dependent variable, resulting in a conversion of over 74%. Even though the highest conversion (74.46%) was achieved under the optimal theoretical reaction conditions, a very similar conversion (74.19%) was achieved at run 4, but at room temperature (30 °C).

The reaction conditions of run 4 are less energy-demanding and, thus, these sub-optimal conditions were selected as selected conditions and the following analyses were performed under these conditions. Overall, the performance of ETL observed in this study is similar to the one previously reported for the esterification of fatty acids with similar carbon chain length (> C18) with fatty and/or branched alcohols [16,72,73].

In a similar solvent-free approach, Triveli et al. employed ETL as biocatalyst in the esterification of oleic acid with different alcohols (oleyl alcohol, octanol, hexanol, 3,7-dimethyloctan-1-ol and 2-ethyl-1-hexanol) [72]. Even though ETL was primarily designed to produce biodiesel using short-length carbon chain alcohols (e.g., methyl and ethyl alcohol) [14], it was reported that the performance of esterification of ETL increased as the carbon chain length increased [72]. Strictly, a conversion of 53% was reached for the esterification of oleic acid with 2-ethyl-1-hexanol under optimized reaction conditions (30 °C, 200 rpm, 18 h, 1:1 (acid/alcohol), 1 wt% of biocatalyst content) [72]. Similarly, ETL was employed as biocatalyst in the solvent-free esterification of oleic acid with octanol, reaching a conversion of less than 30% at 45 °C, 200 rpm, 5 h, 1:4 molar ratio (acid/alcohol) and 5 wt% of biocatalyst content [16]. In another approach, ETL was immobilized onto a hybrid

Table 3
L16′ orthogonal array to screen the esterification of the free fatty acids (FFAs) from castor oil with 2-ethyl-1-hexanol by Eversa Transform 2.0. Further details are provided in Section 2.2.

Run	Temperature (°C)	Stirring (rpm)	Time (h)	Molar Ratio (FFAs/Alcohol)	Biocatalyst (wt%)	Conversion (%)	S/N Ratio
1	30	120	4	1:1	5	29.7 ± 1.2	29.4
2	30	140	8	1:2	10	47.7 ± 1.3	33.6
3	30	160	16	1:3	15	65.8 ± 1.9	36.4
4	30	180	24	1:4	20	74.2 ± 1.5	37.4
5	40	120	8	1:3	20	57.4 ± 1.4	35.2
6	40	140	4	1:4	15	63.9 ± 1.5	36.1
7	40	160	24	1:1	10	37.8 ± 1.3	31.6
8	40	180	16	1:2	5	49.1 ± 1.4	33.8
9	50	120	16	1:4	10	64.7 ± 2.0	36.2
10	50	140	24	1:3	5	58.3 ± 1.4	35.3
11	50	160	4	1:2	20	48.8 ± 1.4	33.8
12	50	180	8	1:1	15	43.0 ± 1.3	32.7
13	60	120	24	1:2	15	49.7 ± 1.4	33.9
14	60	140	16	1:1	20	36.8 ± 1.3	31.3
15	60	160	8	1:4	5	63.6 ± 1.5	36.1
16	60	180	4	1:3	10	57.8 ± 1.4	35.2

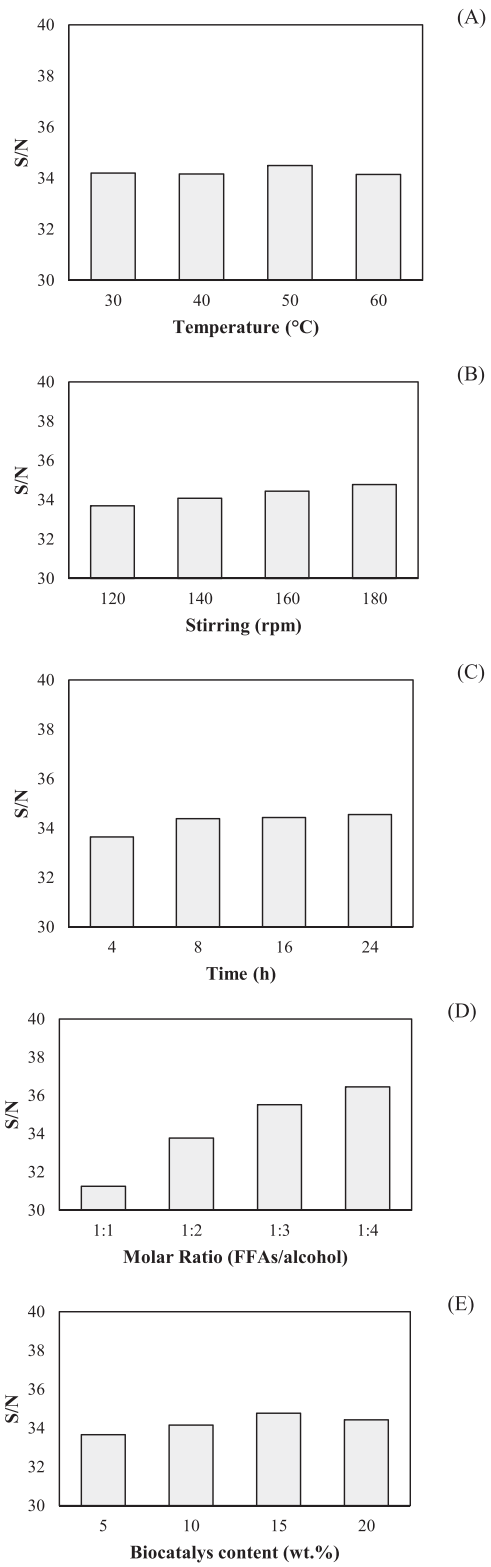

Fig. 2. Signal/Noise (S/N) ratios plots for (A) reaction temperature (°C), (B) stirring rate (rpm), (C) reaction time (h), (D) substrates molar ratio (free fatty acids/alcohol) and (E) biocatalyst content (wt%) obtained from Eq. 1. Further details are provided in Section 2.2.

support of chitosan and agarose activated by glutaraldehyde; then, the immobilized ETL was employed as biocatalyst in the solvent-free esterification of oleic acid with 2-ethyl-1-hexanol, reaching a conversion of approximately 40% [73]. That way, our results are very competitive and surpass the literature results.

In Table 4, the ranking of the independent variables is based on the delta values (the difference of the S/N ratio values between the highest and the lowest levels). Among the independent variables evaluated, the substrate molar ratio (FFAs/2-ethyl-1-hexanol) is the one that most influenced the conversion (dependent variable). At a significance level of 10% ($\alpha = 0.1$), the molar substrate ratio was the independent variable with the lowest *p*-value and, thus, the highest statistical significance, as depicted in Table 5. In solvent-free esterification of monocarboxylic acids, the alcohol may be used beyond the stoichiometric ratio, thereby being used as reactant and a solvent. In the production of biolubricants, as the esterification proceeds, the viscosity of the medium may increase, so that the excess alcohol may decrease medium viscosity and, thus, favor mass transfer. Furthermore, the acid usually exerts adverse effects on enzyme properties (due to its ionic and amphipathic character). Therefore, the excess of alcohol may promote some positive effects in a solvent-free system: reducing the concentration of acid, facilitating a high conversion of acid, and acting as a solvent that can reduce the impact of the biolubricant progressive concentration during the reaction in the physical features of the reaction media.

As depicted in Table 4 and Table 5, the biocatalyst content, stirring rate and reaction time presented a lower influence (similar delta and *p*-value) on the conversion, compared to the substrates molar ratio. However, the highest S/N ratios for all these independent variables were reached at the highest level (L4), except for the biocatalyst content.

Considering that the viscosity of the reaction medium increases as the esterification proceeds toward the production of the biolubricant, a faster stirring in the reaction medium may favor the mixing of the substrates. The maximum conversion observed using 15% biocatalyst may be derived from the increase in the water content when further increasing the biocatalyst content (the enzyme is in aqueous buffer). However, some failures in properly dispersing the enzyme particles (in medium with low water content, the enzymes will be precipitated) cannot be discarded. In any case, the obtained values were much better than the previously reported ones.

The reaction temperature was the independent variable with the lowest influence on the conversion. The optimal temperature of ETL is 50 °C; however, it is stable at 60 °C even after 24 h of incubation [74]. In addition, as reaction temperature increases, the viscosity of the reaction medium decreases, thereby mitigating mass transfer resistance. However, at 60 ºC, it is clear that there is a decrease in the conversion, and this should be associated with some distortion of the enzyme at this temperature; as it has been reported, stability in thermal inactivation experiments may be not fully coupled to operational stability and optimal reaction temperature [75].

Even though the highest S/N ratio is reached at a high level (L3), a similar S/N ratio and, thus, a similar conversion is reached at the lowest level (L1). Therefore, even though ETL has the highest conversion at 24 h at 50 °C (L3) and an increase in reaction temperature may facilitate fluidity of the medium, the production of the biolubricant was proposed to be performed at 30 °C (L1), reducing the energy consumption of the process, as yields were virtually identical.

Overall, the highest S/N ratio was reached at the highest level of stirring rate, reaction time and substrate molar ratio; however, even

Table 4
S/N ratios for reaction temperature (°C), stirring rate (rpm), reaction time (h), substrates molar ratio (free fatty acids/alcohol) and biocatalyst content (wt%) at each level. Further details are provided in Section 2.2.

Level	Temperature	Stirring	Time	Molar Ratio	Biocatalyst
1	34.2	33.7	33.6	31.2	33.7
2	34.2	34.1	34.4	33.8	34.1
3	34.5	34.4	34.4	35.5	34.8
4	34.1	34.8	34.5	36.4	34.4
Delta	0.3	1.1	0.9	5.2	1.1
Ranking	**5**	**3**	**4**	**1**	**2**

Table 5
ANOVA results for the L16′ orthogonal array (α = 0.1). {} indicates that the variable (reaction temperature) has no (or negligible) effect. Further details are provided in Section 2.2.

	dF	SS	MS	*F*-value	*p*-value
Temperature	{3}	{0.33}	-	-	-
Stirring	3	2.65	0.88	8.12	0.06
Time	3	2.03	0.68	6.21	0.08
Molar Ratio	3	62.92	20.97	193.03	0.00
Biocatalyst	3	2.60	0.87	7.99	0.06
Residual	3	0.33	0.11	-	-
Total	15	70.53	-	-	-

under these conditions, it was not possible to achieve the full conversion of the FFAs from castor oil and 2-ethyl-1-hexanol into biolubricant. As explained above, only reaction time could be prolonged from our point of view. Therefore, a set of reactions was performed under the optimal conditions (30 °C, 180 rpm, 1:4 molar ratio (acid/alcohols) and 15% of biocatalyst content), but evaluating reaction times up to 96 h, as depicted in Fig. 3. The conversion increased as the time increased, reaching 95.70% of conversion after 96 h of reaction. These yields were interesting, but the reaction rate was somehow slow for industrial application. The immobilization of the enzyme could be a simple solution for this problem. For example, ETL immobilized via physical adsorption (interfacial activation) onto hydrophobic support (polystyrene-divinylbenzene) presented an improved behavior in the production of biolubricants, also enabling a simple enzyme recovery [76]. Similarly, magnetic cross-linked aggregates of ETL were compared to free ETL in the production of biolubricant [77]. As a result, a conversion of approximately 90% was achieved for the immobilized ETL, whereas a conversion of approximately 34% was achieved for the free ETL after 72 h [77]. In this sense, a proper immobilization protocol may enhance ETL performance, reaching higher conversions even after shorter reaction times. That way, the immobilization of ETL seems to be a simple solution to improve enzyme performance in these reactions significantly.

3.3. Estolide production

Hydroxyl fatty acids (e.g., ricinoleic acid) may be oligomerized to produce estolides by the esterification of the hydroxy moiety of one hydroxy fatty acid molecule with the carboxyl moiety of another fatty acid molecule [78]. In this sense, to evaluate the performance of ETL in the oligomerization of ricinoleic acid, which is the major constituent of castor oil, into estolides, a reaction without 2-ethyl-1-hexanol was performed under optimal conditions (30 °C, 180 rpm, 96 h and 15 wt% of biocatalyst content). In this case, the solvent-free system means that FFAs are the medium only initial components, which may be deleterious for the enzymes performance. As depicted in Fig. 4, a FFA consumption suggests that ETL can perform this reaction even under this drastic condition. It should be considered that lipase-catalyzed oligomerization of ricinoleic acid into estolides is not an easy task. For instance, the production of estolides resulting from the oligomerization of ricinoleic acid cannot be catalyzed by Novozym® 435 (a commercial immobilized lipase B from *Candida antarctica* biocatalyst) or Lipozym® RM-IM (a commercial immobilized lipase from *Rhizomucor miehei* biocatalyst) [38]. The conversion of the FFAs from castor oil into estolides catalyzed by ETL increased over reaction time, reaching a moderate 25.12% of conversion after 96 h. Therefore, ETL is suitable to produce estolides even under these drastic conditions; however, other reaction settings should be further evaluated to reach higher conversion increasing the enzyme activity (e.g., evaluating different organic solvents). Furthermore, it cannot be discarded that some of the esters produced in the presence of alcohols can be estolides, even though the 4-fold excess of 2-ethyl-1-hexanol should reduce this possibility, it is not possible to discard the presence of some traces of these components in the final product.

Due to the intrinsic interest of these estolides, further research should be performed to optimize this reaction (including medium and biocatalysts optimization). We just pointed out the possibility of using ETL as an alternative enzyme to the already reported lyophilized lipase from *Candida rugosa* (Lipomod 34 MDP) to catalyze this interesting reaction [38].

3.4. Product characterization

Table 6 depicts the physicochemical properties of the feedstock (FFAs from castor oils) and the biolubricant – 95.70% of conversion into esters after centrifugation to remove ETL and reduced pressure distillation to remove non-reacted and remaining alcohol and produced water – and estolides – 25.15% of conversion into oligomeric esters after centrifugation to remove ETL and reduced pressure distillation the produced water. For both, biolubricant and estolides, the non-reacted FFAs were not removed from the reaction medium prior to characterization.

Lubricants may protect the material from corrosion and/or oxidation [79]. On the other hand, vegetable oils may confer poor corrosion and/or oxidation protection; indeed, highly acidic vegetable oils or the corresponding FFAs will extend the material ability to prevent corrosion and/or oxidation. The transesterification/esterification of highly acid vegetable oils or the corresponding FFAs may reduce the acidity index, thus reducing the risks of corrosion [7,8]. Herein, the esterification of the FFAs from castor oil with 2-ethyl-1-hexanol reduced the acidity index of the feedstock approximately 117-fold (from 91.13 mg NaOH/g to 0.78 mg NaOH/g). Regarding the production of estolides, the oligomerization of ricinoleic acid reduced the acidity index of the feedstock approximately 1.3-fold (from 91.13 mg NaOH/g to 68.38 mg NaOH/g).

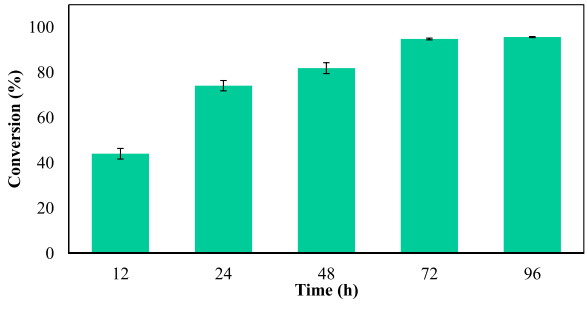

Fig. 3. Conversion of the free fatty acids (FFAs) from castor oil and 2-ethyl-1-hexanol into biolubricant over time. Reaction conditions: 30 °C, 180 rpm, 12–96 h, 1:4 molar ratio (FFAs/alcohol) and 15 wt% of biocatalyst content. The experiments were carried out in triplicate for each group. $p < 0.05$, one-way ANOVA test. Further details are provided in Section 2.2.

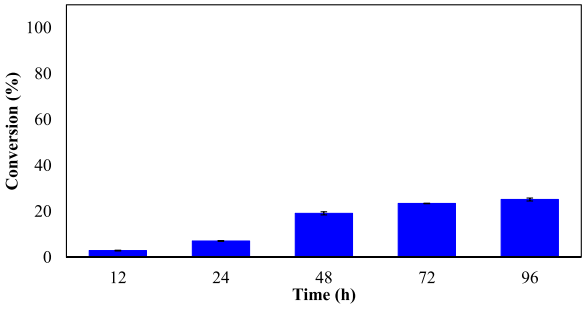

Fig. 4. Conversion of the free fatty acids from castor oil into estolide over time. Reaction conditions: 30 °C, 180 rpm, 12–96 h and 15 wt% of biocatalyst content. The experiments were carried out in triplicate for each group. $p < 0.05$, one-way ANOVA test. Further details are provided in Section 2.2.

Table 6
Physiochemical properties of the feedstock (free fatty acids from castor oil), the reactions mixture obtained under optimal conditions using 2-ethyl-1-hexanol (biolubricant) and in its absence (estolide). The experiments were carried out in triplicate for each group. $P < 0.05$, one-way ANOVA test. Further details are provided in Section 2.2.

	Feedstock	Biolubricant	Estolide
Acidity Index (mg NaOH/g)	91.13 ± 0.25	0.78 ± 0.00	68.38 ± 0.61
Iodine Index (gI_2/100 g)	86.30 ± 0.56	32.80 ± 0.24	66.00 ± 0.03
Oxidative Stability (h)	0.18	16.83	16.11
Viscosity @ 40 °C (mm^2/s)	139.31	29.73	246.60
Viscosity @ 100 °C (mm^2/s)	13.46	5.24	25.51
Viscosity Index	90.31	107.17	128.99

Therefore, to avoid corrosion, further oligomerization of ricinoleic acid or its esterification with alcohol must be performed to use estolides as a biolubricant or additive, which is out of the scope of this study. Beyond the reduction on the acidity index, the biological modification of the FFAs from castor oil with ETL was able to reduce the iodine number of the feedstock from 86.30 gI_2/100 g to 32.80 gI_2/100 g (2-ethylhexyl esters) and 66.00 gI_2/100 g (estolide). The iodine number measures the degree of unsaturation of the feedstock and products (i.e., the higher the iodine number, the higher the amount of double bonds, and, thus, tendency to oxidation) [80]. Usually, epoxidation followed by oxirane-ring opening has been employed to decrease the degree of unsaturation of biolubricants [81,82]. This study did not employ such a strategy; however, as stated so far, while esterifying the FFAs from castor oil with 2-ethyl-1-hexanol, ETL may simultaneously oligomerize ricinoleic acid into estolides. Estolides may be produced by the esterification of the hydroxy moiety of one fatty acid with the carboxyl moiety of another fatty acid; in addition, estolides may be produced by the reaction between the carboxylic moiety of one fatty acid with the double bonds of another fatty acid, thereby resulting in the reduction of unsaturation degree of the biolubricant [78]. Overall, the biological modification of the FFAs of castor oil with 2-ethyl-1-hexanol catalyzed by ETL was able to enhance the oxidative stability of the feedstock approximately 94-fold (from 0.18 h to 16.83 h), probably due to the production of estolides since the stability of the estolide was very similar to one of the 2-ethylhexyl ricinoleate.

In terms of the viscosity index, the esterification of the FFAs from castor oil with 2-ethyl-1-hexanol resulted in a biolubricant (2-ethylhexyl esters) with a higher viscosity index (107.17) compared to the feedstock (90.31). In a similar study, the FFAs from castor oil were esterified with 2-ethyl-1-hexanol catalyzed by p-toluenesulfonic acid, resulting in a biolubricant with a viscosity index of 89.2 [47]. Therefore, the modification of the FFAs from castor oil with 2-ethyl-1-hexanol may enhance the viscosity index of the biolubricant (i.e., high viscosity index infers that the viscosity of the lubricant will be slightly affected by temperature changes [83]). According to the American Petroleum Institute (API), lubricant base stocks with a viscosity index of 80 to 120 are categorized into groups I and II [84]. Lubricants from group I are usually used as engine oils, hydraulic oils, heat transfer oils, while lubricants from group II are used as engine oils, automatic transmission fluid and turbine oils [85].

Beyond the physico-chemical characterization, the feedstock (FFAs from castor oil) and the products (2-ethylhexyl esters and estolides) were characterized by FTIR-ATR to determine the functional groups, as depicted in Fig. 5. Accordingly, there is a sharp peak for the FFAs from castor oil, biolubricant and estolide around 1750 cm^{-1}, related to the carbonyl groups (C═O). In details, the FFAs from castor oil present a peak at 1750 cm^{-1}, which is related to the C═O stretch of a carboxylic acid, whereas the biolubricant presents a peak at 1750 cm^{-1}, which is related to the C═O stretch of an ester linkage [47]. Overall, it suggests the esterification of the FFAs from castor oil with 2-ethyl-1-hexanol; however, as expected, the spectra of the FFAs from castor oil and estolide are very similar as both are carboxylic esters.

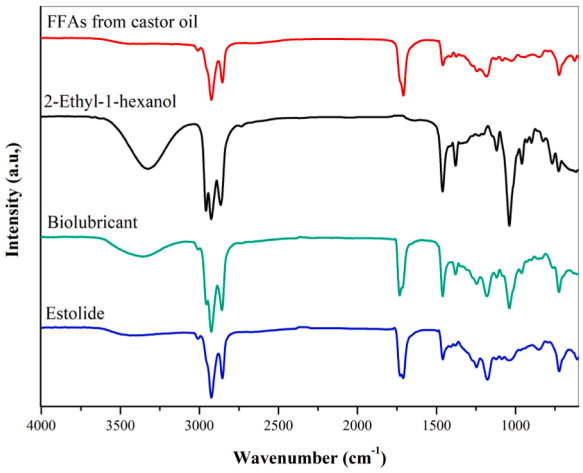

Fig. 5. Fourier transform infrared-attenuated total reflectance spectra of the feedstock (free fatty acids from castor oil and 2-ethyl-1-hexanol) and products (biolubricant and estolide).

For both 2-ethyl-1-hexanol and biolubricant, there is a rounded peak in the region around 3400–3200 cm^{-1}, which is related to the hydroxyl groups (O-H) of the alcohol. The rounded peak in the region around 3400–3200 cm^{-1} in the biolubricant may be attributed to un-reacted and distilled 2-ethyl-1-hexanol. Amines also have N-H stretches in the region 3400–3200 cm^{-1}; however, N-H stretches tend to be sharper and may present more than one peak depending on the number of N-H bonds. Therefore, it may suggest that there is no residue of biocatalyst in the products (biolubricant and estolide).

The tribological tests are a powerful tool to evaluate the friction, wear, and lubrication of interacting surfaces [3]; however, they are not widely employed in the characterization of biolubricants produced by enzymatic biocatalysts. For instance, natural palm stearin was transesterified with methanol by immobilized lipase B from *Candida antartica* (CALB, for short) [86]. Then, the produced biolubricant was compared to a commercial mineral lubricant (SAE 40) regarding the tribological performance (FC: friction coefficient, WSD: wear scan diameter) [86]. As a result, the biolubricant presented a slightly lower FC than the commercial lubricant; the bio-based lubricant produced considerably larger WSD than the mineral one [86]. Later on, the same research group evaluated the tribological properties of a biolubricant produced from the epoxidation of palm stearin methyl ester catalyzed by immobilized CALB [49]. Compared to a commercial mineral lubricant (SAE 40), the produced biolubricant presented a lower FC and larger WSD than the commercial lubricant [49].

Herein, the FC and WSD of the biolubricant were compared with the ones of a commercial mineral lubricant (20 W-50). The tribology tests of the estolide were not performed due to its high acidity index (68.38 mg NaOH/g), which may lead to equipment corrosion and make its use unfeasible as a biolubricant base. Again, further improvements in reaction engineering must be performed to enhance the production of estolides and, thus, reduce its acidity index; however, it is out of the scope of this communication. Table 7 depicts the average FC and WSD of the biolubricant (2-ethylhexyl esters) and commercial mineral lubricant (20 W-50). Regarding the anti-friction properties, the lubricating

Table 7
Average friction coefficient (FC) and wear scan diameter (WSD) of the biolubricant (2-ethylhexyl esters) and lubricant (20 W-50). The experiments were carried out in triplicate for each group. $P < 0.05$, one-way ANOVA test.

	FC	WSD (μm)
Biolubricant	0.052 ± 0.07	209.72 ± 3.01
Lubricant	0.078 ± 0.04	140.36 ± 1.36

material reduces friction by forming a stable film on the metal surface [87]. Therefore, the lower the FC, the higher the resistance to friction of the lubricant film is. Accordingly, the FC for the biolubricant was lower than that for the lubricant. The biolubricant (ester) is a polar substance, whereas the lubricant (hydrocarbon) is a nonpolar substance. Therefore, the polar moieties of the biolubricant are adsorbed on the metal surface, forming a film and, thus, preventing direct metal-to-metal contact; meanwhile, the nonpolar moieties of the lubricant remain away from the metal surface [87].

Nevertheless, the WSD of the biolubricant was higher than that of the lubricant, as depicted in Table 7. Castor oil comprises up to 95% of unsaturated fatty acids (ricinoleic acid, linoleic acid and oleic acid). The enzymatic modification of the FFAs of castor oil reduced the iodine number up to 2.6-fold (Table 6); however, it remains highly unsaturated. As such, the biolubricant is still very susceptible to oxidation and degradation, thereby reducing the metal surface protection against damage [49,86]. The epoxidation followed by oxirane-ring opening is one of the strategies to reduce the unsaturation content of biolubricants [6]; however, it is out of the scope of this study.

Nevertheless, the wear morphology (Fig. 6) of the surfaces lubricated with the biolubricant is smoother due to the better surface protection against damage provided by the ester layer adsorbed on the metal surface; however, as the tribology test continues and the biolubricant is oxidized by the air, such protection is reduced and, thus, eventually, the metal surface is damaged. As a result, the biolubricant presents a higher WSD than the lubricant. As depicted in Fig. 6, even though the lubricant presented a lower WSD, it resulted in higher damage as the metal surface presented deeper grooves and several scratches and material detachments.

4. Conclusion

ETL have probed to exhibit better properties for the esterification of the FFAs (mainly ricinoleic acid) from castor oil with 2-ethyl-1-hexanol that the other assayed commercial free lipases, except the “mother” enzyme TLL, that is very similar. After optimizing the reaction conditions by the Taguchi method, and prolonging the reaction time to 96 h, a conversion of 95.7% into biolubricant was achieved at 30 °C, thereby reducing energy consumption compared to other publications and with yields comparable to the best described in literature. The produced biolubricant exhibited lower friction coefficient and smoother material damage than a commercial biolubricant. Nevertheless, the reaction was

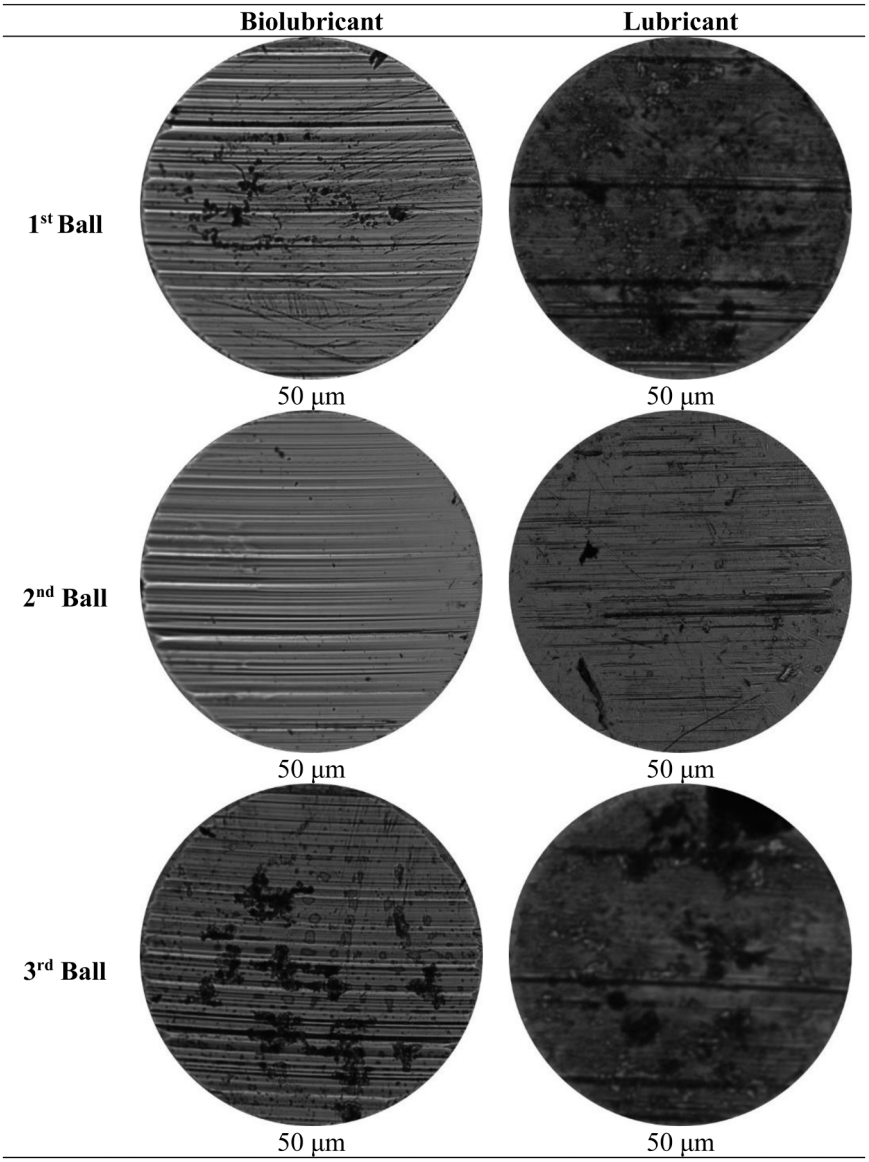

Fig. 6. Wear morphology of the surface of the balls lubricated with the biolubricant (2-ethylhexyl esters) and lubricant (20 W-50).

relatively slow. Here, further work should pursued to improve the enzyme activity, for example using immobilization strategies, which have the potential to enhance the activity, stability and even modulate the specificity of ETL, as discussed in introduction.

Beyond the production of esters from ricinoleic acid and 2-ethyl-1-hexanol, this paper shows for first time the potential of ETL to produce estolides from ricinoleic acid; however, further improvement on the biocatalysts design and process engineering must be performed to enhance its activity in this reaction to greatly improve enzyme activity (e.g., use of adequate solvents and immobilized enzymes).

CRediT authorship contribution statement

Soares Jorge B.: Writing – original draft, Investigation. **Rocha Wesley S.:** Writing – original draft, Investigation. **Fernandez-Lafuente Roberto:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Formal analysis, Data curation, Conceptualization. **de Luna F. Murilo T.:** Writing – original draft, Investigation. **de Melo Neta Maria M. F.:** Writing – original draft, Investigation. **Monteiro Rodolpho R. C.:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Vieira Rodrigo S.:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing 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 paper.

Data Availability

No data was used for the research described in the article.

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References

[1] W.G. Sawyer, Leonardo da Vinci on wear, Biotribology 26 (2021) 100160, https://doi.org/10.1016/j.biotri.2021.100160.
[2] N.A. Zainal, N.W.M. Zulkifli, M. Gulzar, H.H. Masjuki, A review on the chemistry, production, and technological potential of bio-based lubricants, Renew. Sustain. Energy Rev. 82 (2018) 80–102, https://doi.org/10.1016/j.rser.2017.09.004.
[3] Y. Meng, J. Xu, L. Ma, Z. Jin, B. Prakash, T. Ma, W. Wang, A review of advances in tribology in 2020–2021, Friction 10 (2022) 1443–1595, https://doi.org/10.1007/s40544-022-0685-7.
[4] C.T. Pinheiro, M.J. Quina, L.M. Gando-Ferreira, Management of waste lubricant oil in Europe: a circular economy approach, Crit. Rev. Environ. Sci. Technol. 51 (2021) 2015–2050, https://doi.org/10.1080/10643389.2020.1771887.
[5] X. Zhao, D. Li, H. Zhu, J. Ma, Y. An, Advanced developments in environmentally friendly lubricants for water-based drilling fluid: a review, RSC Adv. 12 (2022) 22853–22868, https://doi.org/10.1039/D2RA03888A.
[6] R.R.C. Monteiro, Á. Berenguer-Murcia, J. Rocha-Martin, R.S. Vieira, R. Fernandez-Lafuente, Biocatalytic production of biolubricants: Strategies, problems and future trends, Biotechnol. Adv. 68 (2023) 108215, https://doi.org/10.1016/j.biotechadv.2023.108215.
[7] R. Uppar, P. Dinesha, S. Kumar, A critical review on vegetable oil-based biolubricants: preparation, characterization, and challenges, Environ., Dev. Sustain. 25 (2022) 9011–9046, https://doi.org/10.1007/s10668-022-02669-w.
[8] R.V. Pawar, D.B. Hulwan, M.B. Mandale, Recent advancements in synthesis, rheological characterization, and tribological performance of vegetable oil-based lubricants enhanced with nanoparticles for sustainable lubrication, J. Clean. Prod. 378 (2022) 134454, https://doi.org/10.1016/j.jclepro.2022.134454.
[9] J.A. Cecilia, D. Ballesteros Plata, R.M. Alves Saboya, F.M. Tavares de Luna, C. L. Cavalcante, E. Rodríguez-Castellón, An overview of the biolubricant production process: challenges and future perspectives, Processes 8 (2020) 257, https://doi.org/10.3390/pr8030257.
[10] E. Barbera, K. Hirayama, R.L. Maglinao, R.W. Davis, S. Kumar, Recent developments in synthesizing biolubricants — a review, Biomass Conversion and Biorefinery, in press. https://doi.org/10.1007/s13399–022-02438–9.
[11] G. Appiah, S.K. Tulashie, E.E.A. Akpari, E.R. Rene, D. Dodoo, Biolubricant production via esterification and transesterification processes: Current updates and perspectives, Int. J. Energy Res. 46 (2022) 3860–3890, https://doi.org/10.1002/er.7453.
[12] I.C.A. Bolina, R.A.B. Gomes, A.A. Mendes, Biolubricant production from several oleaginous feedstocks using lipases as catalysts: current scenario and future perspectives, Bioenergy Res. 14 (2021) 1039–1057, https://doi.org/10.1007/s12155-020-10242-4.
[13] A.A. Mendes, C.M.F. Soares, P.W. Tardioli, Recent advances and future prospects for biolubricant base stocks production using lipases as environmentally friendly catalysts: a mini-review, World J. Microbiol. Biotechnol. 39 (2023) 25, https://doi.org/10.1007/s11274-022-03465-4.
[14] R.R.C. Monteiro, S. Arana-Peña, T.N. Da Rocha, L.P. Miranda, Á. Berenguer-Murcia, P.W. Tardioli, J.C.S. Dos Santos, R. Fernandez-Lafuente, Liquid lipase preparations designed for industrial production of biodiesel. Is it really an optimal solution? Renew. Energy 164 (2021) 1566–1587, https://doi.org/10.1016/j.renene.2020.10.071.
[15] J. Price, M. Nordblad, H.H. Martel, B. Chrabas, H. Wang, P.M. Nielsen, J. M. Woodley, Scale-up of industrial biodiesel production to 40 m^3 using a liquid lipase formulation: scale-Up of Industrial Biodiesel Production, Biotechnol. Bioeng. 113 (2016) 1719–1728, https://doi.org/10.1002/bit.25936.
[16] F.T.T. Cavalcante, A.M. Da Fonseca, J.Y.N.H. Alexandre, J.C.S. Dos Santos, A stepwise docking and molecular dynamics approach for enzymatic biolubricant production using Lipase Eversa® Transform as a biocatalyst, Ind. Crops Prod. 187 (2022) 115450–115459, https://doi.org/10.1016/j.indcrop.2022.115450.
[17] R. De Araujo-Silva, A.C. Vieira, R. De Campos Giordano, R. Fernandez-Lafuente, P. W. Tardioli, Enzymatic synthesis of fatty acid isoamyl monoesters from soybean oil deodorizer distillate: a renewable and ecofriendly base stock for lubricant industries, Molecules 27 (2022) 2692, https://doi.org/10.3390/molecules27092692.
[18] W. Xie, X. Zang, Covalent immobilization of lipase onto aminopropyl-functionalized hydroxyapatite-encapsulated-γ-Fe2O3 nanoparticles: a magnetic biocatalyst for interesterification of soybean oil, Food Chem. 227 (2017) 397–403, https://doi.org/10.1016/j.foodchem.2017.01.082.
[19] W. Xie, X. Zang, Immobilized lipase on core–shell structured Fe3O4–MCM-41 nanocomposites as a magnetically recyclable biocatalyst for interesterification of soybean oil and lard, Food Chem. 194 (2016) 1283–1292, https://doi.org/10.1016/j.foodchem.2015.09.009.
[20] W. Xie, M. Huang, Immobilization of *Candida rugosa* lipase onto graphene oxide Fe_3O_4 nanocomposite: characterization and application for biodiesel production, Energy Convers. Manag. 159 (2018) 42–53, https://doi.org/10.1016/j.enconman.2018.01.021.
[21] C. Mateo, J.M. Palomo, G. Fernandez-Lorente, J.M. Guisan, R. Fernandez-Lafuente, Improvement of enzyme activity, stability and selectivity via immobilization techniques, Enzym. Microb. Technol. 40 (2007) 1451–1463, https://doi.org/10.1016/j.enzmictec.2007.01.018.
[22] R.A. Sheldon, J.M. Woodley, Role of biocatalysis in sustainable chemistry, Chem. Rev. 118 (2018) 801–838, https://doi.org/10.1021/acs.chemrev.7b00203.
[23] R.A. Sheldon, S. Van Pelt, Enzyme immobilisation in biocatalysis: why, what and how, Chem. Soc. Rev. 42 (2013) 6223–6235, https://doi.org/10.1039/C3CS60075K.
[24] M. Bilal, M. Asgher, H. Cheng, Y. Yan, H.M.N. Iqbal, Multi-point enzyme immobilization, surface chemistry, and novel platforms: a paradigm shift in biocatalyst design, Crit. Rev. Biotechnol. 39 (2019) 202–219, https://doi.org/10.1080/07388551.2018.1531822.
[25] J. Chapman, A. Ismail, C. Dinu, Industrial applications of enzymes: recent advances, techniques, and outlooks, Catalysts 8 (2018) 238, https://doi.org/10.3390/catal8060238.
[26] C. Bernal, K. Rodríguez, R. Martínez, Integrating enzyme immobilization and protein engineering: an alternative path for the development of novel and improved industrial biocatalysts, Biotechnol. Adv. 36 (2018) 1470–1480, https://doi.org/10.1016/j.biotechadv.2018.06.002.
[27] J. Zdarta, A. Meyer, T. Jesionowski, M. Pinelo, A General overview of support materials for enzyme immobilization: Characteristics, properties, practical utility, Catalysts 8 (2018) 92, https://doi.org/10.3390/catal8020092.
[28] S. Huang, X. Kou, J. Shen, G. Chen, G. Ouyang, Armor-Plating" enzymes with metal–organic frameworks (MOFs), Angew. Chem. - Int. Ed. 59 (2020) 8786–8798, https://doi.org/10.1002/anie.201916474.
[29] C. Garcia-Galan, Á. Berenguer-Murcia, R. Fernandez-Lafuente, R.C. Rodrigues, Potential of different enzyme immobilization strategies to improve enzyme performance, Adv. Synth. Catal. 353 (2011) 2885–2904, https://doi.org/10.1002/adsc.201100534.
[30] R.C. Rodrigues, Á. Berenguer-Murcia, D. Carballares, R. Morellon-Sterling, R. Fernandez-Lafuente, Stabilization of enzymes via immobilization: multipoint covalent attachment and other stabilization strategies, Biotechnol. Adv. 52 (2021) 107821, https://doi.org/10.1016/j.biotechadv.2021.107821.

[31] J.M. Bolivar, J.M. Woodley, R. Fernandez-Lafuente, Is enzyme immobilization a mature discipline? Some critical considerations to capitalize on the benefits of immobilization, Chem. Soc. Rev. 51 (2022) 6251–6290, https://doi.org/10.1039/D2CS00083K.
[32] A.Z. Syahir, N.W.M. Zulkifli, H.H. Masjuki, M.A. Kalam, A. Alabdulkarem, M. Gulzar, L.S. Khuong, M.H. Harith, A review on bio-based lubricants and their applications, J. Clean. Prod. 168 (2017) 997–1016, https://doi.org/10.1016/j.jclepro.2017.09.106.
[33] M. Perera, J. Yan, L. Xu, M. Yang, Y. Yan, Bioprocess development for biolubricant production using non-edible oils, agro-industrial byproducts and wastes, J. Clean. Prod. 357 (2022) 131956, https://doi.org/10.1016/j.jclepro.2022.131956.
[34] M. Hajar, F. Vahabzadeh, Artificial neural network modeling of biolubricant production using Novozym 435 and castor oil substrate, Ind. Crops Prod. 52 (2014) 430–438, https://doi.org/10.1016/j.indcrop.2013.11.020.
[35] J.A.C. Da Silva, V.F. Soares, R. Fernandez- Lafuente, A.C. Habert, D.M.G. Freire, Enzymatic production and characterization of potential biolubricants from castor bean biodiesel, J. Mol. Catal. B: Enzym. 122 (2015) 323–329, https://doi.org/10.1016/j.molcatb.2015.09.017.
[36] M. Hajar, F. Vahabzadeh, Biolubricant production from castor oil in a magnetically stabilized fluidized bed reactor using lipase immobilized on Fe_3O_4 nanoparticles, Ind. Crops Prod. 94 (2016) 544–556, https://doi.org/10.1016/j.indcrop.2016.09.030.
[37] P.A.B. Diaz, F.D.A. Kronemberger, A.C. Habert, A pervaporation-assisted bioreactor to enhance efficiency in the synthesis of a novel biolubricant based on the enzymatic transesterification of a castor oil based biodiesel, Fuel 204 (2017) 98–105, https://doi.org/10.1016/j.fuel.2017.05.025.
[38] J. Greco-Duarte, E.D. Cavalcanti-Oliveira, J.A.C. Da Silva, R. Fernandez-Lafuente, D.M.G. Freire, Two-step enzymatic production of environmentally friendly biolubricants using castor oil: Enzyme selection and product characterization, Fuel 202 (2017) 196–205, https://doi.org/10.1016/j.fuel.2017.04.036.
[39] S. Sun, G. Wang, P. Wang, A cleaner approach for biodegradable lubricants production by enzymatic glycerolysis of castor oil and kinetic analysis, J. Clean. Prod. 188 (2018) 530–535, https://doi.org/10.1016/j.jclepro.2018.04.015.
[40] É.C.G. Aguieiras, E.D.C. Cavalcanti, P.R. Da Silva, V.F. Soares, R. Fernandez-Lafuente, C.L. Bessa Assunção, J.A.C. Da Silva, D.M.G. Freire, Enzymatic synthesis of neopentyl glycol-bases biolubricants using biodiesel from soybean and castor bean as raw materials, Renew. Energy 148 (2020) 689–696, https://doi.org/10.1016/j.renene.2019.10.156.
[41] R.M.A. Saboya, J.A. Cecilia, C. García-Sancho, F.M.T. Luna, E. Rodríguez-Castellón, C.L. Cavalcante, WO_3-based catalysts supported on porous clay heterostructures (PCH) with Si–Zr pillars for synthetic esters production, Appl. Clay Sci. 124–125 (2016) 69–78, https://doi.org/10.1016/j.clay.2016.02.004.
[42] R.M.A. Saboya, J.A. Cecilia, C. García-Sancho, A.V. Sales, F.M.T. De Luna, E. Rodríguez-Castellón, C.L. Cavalcante, Synthesis of biolubricants by the esterification of free fatty acids from castor oil with branched alcohols using cationic exchange resins as catalysts, Ind. Crops Prod. 104 (2017) 52–61, https://doi.org/10.1016/j.indcrop.2017.04.018.
[43] R.M.A. Saboya, J.A. Cecilia, C. García-Sancho, A.V. Sales, F.M.T. De Luna, E. Rodríguez-Castellón, C.L. Cavalcante, Assessment of commercial resins in the biolubricants production from free fatty acids of castor oil, Catal. Today 279 (2017) 274–285, https://doi.org/10.1016/j.cattod.2016.02.020.
[44] F. Luna, J. Cecilia, R. Saboya, D. Barrera, K. Sapag, E. Rodríguez-Castellón, C. Cavalcante, Natural and modified montmorillonite clays as catalysts for synthesis of biolubricants, Materials 11 (2018) 1764, https://doi.org/10.3390/ma11091764.
[45] Í.C. Rios, J.P. Cordeiro, T.B.M.G. Arruda, F.E.A. Rodrigues, A.F.J. Uchoa, F.M. T. Luna, C.L. Cavalcante, N.M.P.S. Ricardo, Chemical modification of castor oil fatty acids (*Ricinus communis*) for biolubricant applications: an alternative for Brazil's green market, Ind. Crops Prod. 145 (2020) 112000, https://doi.org/10.1016/j.indcrop.2019.112000.
[46] Í.C. Rios, J.P. Cordeiro, E.J.S. Parente, S.A. Quintella, J. Alemán, C.L. Cavalcante, F.M.T. Luna, Biodegradable base stock oils obtained from ricinoleic acid using C8 alcohols and process integration into a biodiesel industry, Biomass-.-. Convers. Biorefinery 11 (2021) 803–814, https://doi.org/10.1007/s13399-020-00942-4.
[47] P.C.F.R. Ribeiro Filho, M. Rocha Do Nascimento, S.S. Otaviano Da Silva, F. M. Tavares De Luna, E. Rodríguez-Castellón, C. Loureiro Cavalcante, Synthesis and frictional characteristics of bio-based lubricants obtained from fatty acids of castor oil, Lubricants 11 (2023) 57, https://doi.org/10.3390/lubricants11020057.
[48] A. Chowdhury, R. Chakraborty, D. Mitra, D. Biswas, Optimization of the production parameters of octyl ester biolubricant using Taguchi's design method and physico-chemical characterization of the product, Ind. Crops Prod. 52 (2014) 783–789, https://doi.org/10.1016/j.indcrop.2013.11.007.
[49] A.N. Afifah, S. Syahrullail, N.I. Wan Azlee, A.M. Rohah, Synthesis and tribological studies of epoxidized palm stearin methyl ester as a green lubricant, J. Clean. Prod. 280 (2021) 124320, https://doi.org/10.1016/j.jclepro.2020.124320.
[50] H. Islam, T. Gufrana, S. Khare, A. Pandey, P. Radha, Chicken tallow, a low-cost feedstock for the two-step lipase-catalysed synthesis of biolubricant, Biocatal. Biotransform. (2023) 1–15, https://doi.org/10.1080/10242422.2023.2176225.
[51] H. Schiefer, F. Schiefer, Statistical Design of Experiments (DoE). in: Statistics for Engineers, Springer Fachmedien Wiesbaden, Wiesbaden, 2021, pp. 1–20, https://doi.org/10.1007/978-3-658-32397-4_1.
[52] R.S. Rao, C.G. Kumar, R.S. Prakasham, P.J. Hobbs, The Taguchi methodology as a statistical tool for biotechnological applications: a critical appraisal, Biotechnol. J. 3 (2008) 510–523, https://doi.org/10.1002/biot.200700201.
[53] W.-H. Chen, M. Carrera Uribe, E.E. Kwon, K.-Y.A. Lin, Y.-K. Park, L. Ding, L.H. Saw, A comprehensive review of thermoelectric generation optimization by statistical approach: Taguchi method, analysis of variance (ANOVA), and response surface methodology (RSM), Renew. Sustain. Energy Rev. 169 (2022) 112917, https://doi.org/10.1016/j.rser.2022.112917.
[54] M. Kaieda, T. Samukawa, A. Kondo, H. Fukuda, Effect of methanol and water contents on production of biodiesel fuel from plant oil catalyzed by various lipases in a solvent-free system, J. Biosci. Bioeng. 91 (2001) 12–15, https://doi.org/10.1016/S1389-1723(01)80103-1.
[55] Ö. Köse, M. Tüter, H.A. Aksoy, Immobilized *Candida antarctica* lipase-catalyzed alcoholysis of cotton seed oil in a solvent-free medium, Bioresour. Technol. 83 (2002) 125–129, https://doi.org/10.1016/S0960-8524(01)00203-6.
[56] S. Shah, M.N. Gupta, Lipase catalyzed preparation of biodiesel from Jatropha oil in a solvent free system, Process Biochem. 42 (2007) 409–414, https://doi.org/10.1016/j.procbio.2006.09.024.
[57] C.-S. Chang, P.-L. Wu, Synthesis of triglycerides of phenylbutyric acids by lipase-catalyzed glycerolysis in a solvent-free system, J. Mol. Catal. B: Enzym. 61 (2009) 117–122, https://doi.org/10.1016/j.molcatb.2009.06.011.
[58] R.R. Sousa, A.S. Silva, R. Fernandez-Lafuente, V.S. Ferreira-Leitão, Solvent-free esterifications mediated by immobilized lipases: a review from thermodynamic and kinetic perspectives, Catal. Sci. Technol. 11 (2021) 5696–5711, https://doi.org/10.1039/D1CY00696G.
[59] E.M. Anderson, K.M. Larsson, O. Kirk, One Biocatalyst–Many Applications: The use of *Candida antarctic*a B-Lipase in organic synthesis, Biocatal. Biotransform. 16 (1998) 181–204, https://doi.org/10.3109/10242429809003198.
[60] P. Domínguez De María, C. Carboni-Oerlemans, B. Tuin, G. Bargeman, A. Van Der Meer, R. Van Gemert, Biotechnological applications of *Candida antarctica* lipase A: state-of-the-art, J. Mol. Catal. B: Enzym. 37 (2005) 36–46, https://doi.org/10.1016/j.molcatb.2005.09.001.
[61] O. Kirk, M.W. Christensen, Lipases from *Candida antarctica*: Unique biocatalysts from a unique origin, Org. Process Res. Dev. 6 (2002) 446–451, https://doi.org/10.1021/op0200165.
[62] R.R.C. Monteiro, J.J. Virgen-Ortiz, Á. Berenguer-Murcia, T.N. Da Rocha, J.C.S. Dos Santos, A.R. Alcántara, R. Fernandez-Lafuente, Biotechnological relevance of the lipase A from *Candida antarctica*, Catal. Today 362 (2021) 141–154, https://doi.org/10.1016/j.cattod.2020.03.026.
[63] R.C. Rodrigues, R. Fernandez-Lafuente, Lipase from *Rhizomucor miehei* as an industrial biocatalyst in chemical process, J. Mol. Catal. B: Enzym. 64 (2010) 1–22, https://doi.org/10.1016/j.molcatb.2010.02.003.
[64] R.C. Rodrigues, R. Fernandez-Lafuente, Lipase from *Rhizomucor miehei* as a biocatalyst in fats and oils modification, J. Mol. Catal. B: Enzym. 66 (2010) 15–32, https://doi.org/10.1016/j.molcatb.2010.03.008.
[65] R. Fernandez-Lafuente, Lipase from *Thermomyces lanuginosus*: uses and prospects as an industrial biocatalyst, J. Mol. Catal. B: Enzym. 62 (2010) 197–212, https://doi.org/10.1016/j.molcatb.2009.11.010.
[66] E.D.C. Cavalcanti, É.C.G. Aguieiras, P.R. Da Silva, J.G. Duarte, E.P. Cipolatti, R. Fernandez-Lafuente, J.A.C. Da Silva, D.M.G. Freire, Improved production of biolubricants from soybean oil and different polyols via esterification reaction catalyzed by immobilized lipase from *Candida rugosa*, Fuel 215 (2018) 705–713, https://doi.org/10.1016/j.fuel.2017.11.119.
[67] R.R.C. Monteiro, P.J.M. Lima, B.B. Pinheiro, T.M. Freire, L.M.U. Dutra, P.B. A. Fechine, L.R.B. Gonçalves, M.C.M. De Souza, J.C.S. Dos Santos, R. Fernandez-Lafuente, Immobilization of lipase A from *Candida antarctica* onto chitosan-coated magnetic nanoparticles, Int. J. Mol. Sci. 20 (2019) 4018, https://doi.org/10.3390/ijms20164018.
[68] ASTM D445, Test method for kinematic viscosity of transparent and opaque liquids (and calculation of dynamic viscosity), ASTM International, 2022. ⟨https://doi.org/10.1520/D0445–21E01⟩.
[69] ASTM D2270, Practice for calculating viscosity index from kinematic viscosity at 40 °C and 100 °C, ASTM International, 2016. ⟨https://doi.org/10.1520/D2270–10R16⟩.
[70] ASTM D5554, Test method for determination of the iodine value of fats and oils, ASTM International, 2017. ⟨https://doi.org/10.1520/D5554–95⟩.
[71] ES14112, Fat and oil derivatives. Fatty Acid Methyl Esters (FAME). Determination of oxidation stability (accelerated oxidation test), ES, 2020. ⟨https://www.en-standard.eu/bs-en-14112–2020-fat-and-oil-derivatives-fatty-acid-methyl-esters-fame-determination-of-oxidation-stability-accelerated-oxidation-test/?gclid=CjwKCAjwhJukBhBPEiwAniIcNU-LiO_4_TqKbBtEM1UD84tzCRuWdbwIAGdT9ETjnvmFmnjDcYr3ZxoC3pYQAvD_BwE⟩.
[72] J. Trivedi, M. Aila, C.D. Sharma, P. Gupta, S. Kaul, Clean synthesis of biolubricant range esters using novel liquid lipase enzyme in solvent free medium, SpringerPlus 4 (2015) 165, https://doi.org/10.1186/s40064-015-0937-3.
[73] I. Germano De Sousa, A. Valério Chaves, A.L.B. De Oliveira, K. Da Silva Moreira, P. Gonçalves De Sousa Junior, F. Simão Neto, S. Cristina Freitas De Carvalho, R. Bussons Rodrigues Valério, G. Vieira Lima, A.A. Sanders Lopes, M.C. Martins De Souza, A.M. Da Fonseca, P.B.A. Fechine, M.C. De Mattos, J.C.S. Dos Santos, A novel hybrid biocatalyst from immobilized Eversa® Transform 2.0 lipase and its application in biolubricant synthesis, Biocatalysis and Biotransformation, in press, https://doi.org/10.1080/10242422.2022.2144263.
[74] L.P. Miranda, J.R. Guimarães, R.C. Giordano, R. Fernandez-Lafuente, P. W. Tardioli, Composites of crosslinked aggregates of Eversa® Transform and magnetic nanoparticles. performance in the ethanolysis of soybean oil, Catalysts 10 (2020) 817, https://doi.org/10.3390/catal10080817.
[75] T.N. Da Rocha, D. Carballares, J.R. Guimarães, J. Rocha-Martin, P.W. Tardioli, L.R. B. Gonçalves, R. Fernandez-Lafuente, Determination of immobilized lipase stability depends on the substrate and activity determination condition: stress inactivations and optimal temperature as biocatalysts stability indicators, Sustain. Chem. Pharm. 29 (2022) 100823, https://doi.org/10.1016/j.scp.2022.100823.

[76] W.C.A. Carvalho, J.H.H. Luiz, R. Fernandez-Lafuente, D.B. Hirata, A.A. Mendes, 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 155 (2021) 106302, https://doi.org/10.1016/j.biombioe.2021.106302.

[77] J.R. Guimarães, L.P. Miranda, R. Fernandez-Lafuente, P.W. Tardioli, Immobilization of Eversa® Transform via CLEA Technology converts it in a suitable biocatalyst for biolubricant production using waste cooking oil, Molecules 26 (2021) 193, https://doi.org/10.3390/molecules26010193.

[78] Y. Chen, G. Biresaw, S.C. Cermak, T.A. Isbell, H.L. Ngo, L. Chen, A.L. Durham, Fatty acid estolides: a review, J. Am. Oil Chem. ' Soc. 97 (2020) 231–241, https://doi.org/10.1002/aocs.12323.

[79] S. Shahnazar, S. Bagheri, S.B. Abd Hamid, Enhancing lubricant properties by nanoparticle additives, Int. J. Hydrog. Energy 41 (2016) 3153–3170, https://doi.org/10.1016/j.ijhydene.2015.12.040.

[80] W. Yan, Z. Wang, C. Luo, X. Xia, Z. Liu, Y. Zhao, F. Du, X. Jin, Opportunities and emerging challenges of the heterogeneous metal-based catalysts for vegetable oil epoxidation, ACS Sustain. Chem. Eng. 10 (2022) 7426–7446, https://doi.org/10.1021/acssuschemeng.2c00617.

[81] W. Zhang, H. Ji, Y. Song, S. Ma, W. Xiong, C. Chen, B. Chen, X. Zhang, Green preparation of branched biolubricant by chemically modifying waste cooking oil with lipase and ionic liquid, J. Clean. Prod. 274 (2020) 122918, https://doi.org/10.1016/j.jclepro.2020.122918.

[82] T. Salmi, A.F. Aguilera, P. Lindroos, L. Kanerva, Mathematical modelling of oleic acid epoxidation via a chemo-enzymatic route – from reaction mechanisms to reactor model, Chem. Eng. Sci. 247 (2022) 117047, https://doi.org/10.1016/j.ces.2021.117047.

[83] R.N. Sarma, R. Vinu, Current status and future prospects of biolubricants: properties and applications, Lubricants 10 (2022) 70, https://doi.org/10.3390/lubricants10040070.

[84] API, Engine oil licensing and certification system (Annex E), American Petroleum Institute, Washington, 2022. ⟨https://www.api.org/-/media/Files/Certification/Engine-Oil-Diesel/Publications/API%201509-%2021st%20Edition.pdf⟩ (accessed November 7, 2023).

[85] D.M. Pirro, E. Daschner, Lubrication Fundamentals, 0 ed, CRC Press,, 2001, https://doi.org/10.1201/9781420029239.

[86] A.N. Afifah, S. Syahrullail, N.I. Wan Azlee, N.A. Che Sidik, W.J. Yahya, E. Abd Rahim, Biolubricant production from palm stearin through enzymatic transesterification method, Biochem. Eng. J. 148 (2019) 178–184, https://doi.org/10.1016/j.bej.2019.05.009.

[87] X. Ma, Y. Zhang, Z. Song, K. Yu, C. He, X. Zhang, Enzyme-catalyzed synthesis and properties of polyol ester biolubricant produced from *Rhodotorula glutinis* lipid, Biochem. Eng. J. 173 (2021) 108101, https://doi.org/10.1016/j.bej.2021.108101.