Research Article

Estolides Synthesis Catalyzed by Immobilized Lipases

Erika C. G. Aguieiras, Cláudia O. Veloso, Juliana V. Bevilaqua, Danielle O. Rosas, Mônica A. P. da Silva, and Marta A. P. Langone

1 Escola de Química, Universidade Federal do Rio de Janeiro, Avenida Athos da Silveira Ramos, 149, 21949-909 Rio de Janeiro, RJ, Brazil
2 PPGEQ, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 524, PHLC, Iq, sl. 427, 20559-900 Rio de Janeiro, RJ, Brazil
3 Petróleo Brasileiro, and Petrobras Biocombustível S. A., Avenida Presidente Vargas, 3131, Cidade Nova, 20210-030 Rio de Janeiro, RJ, Brazil
4 PDAB/HPE, CENPES/PETROBRAS, Avenida Horácio Macedo, 950, 21941-915 Rio de Janeiro, RJ, Brazil

Correspondence should be addressed to Erika C. G. Aguieiras, erikaaguieiras@gmail.com and Marta A. P. Langone, langone@uerj.br

Received 13 January 2011; Revised 6 March 2011; Accepted 7 April 2011

Academic Editor: D. M. G. Freire

Copyright © 2011 Erika C. G. Aguieiras et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Estolides are vegetable-oil-based lubricants obtained from oleic acid or any source of hydroxy fatty acids. In this work, the estolides synthesis from oleic acid and methyl ricinoleate (biodiesel from castor oil), using immobilized commercial lipases (Novozym 435, Lipozyme RM-IM, and Lipozyme TL-IM) in a solvent-free medium was investigated. Acid value was used to monitor the reaction progress by determining the consumption of acid present in the medium. Novozym 435 showed the best performance. Water removal improved the conversion. Novozym 435 was more active at atmospheric pressure. Novozym 435 was reused four times with conversion reaching 15% after the fourth reaction at 80°C. Estolides produced under the reaction conditions used in this work presented good properties, such as, low temperature properties as pour point (−24°C), viscosity (23.9 cSt at 40°C and 5.2 cSt at 100°C), and viscosity index (153).

1. Introduction

Estolides are a class of polyesters based on vegetable oils that are formed when the carboxylic acid functionality of one fatty acid reacts at the site of unsaturation of another fatty acid [1] or by covalent ester bonds between hydroxyl moiety of one hydroxyl acid and the carboxyl moiety of another hydroxyl acid molecule [2]. These compounds have a variety of potential applications as greases, plastics, inks, cosmetics, viscosity controller for chocolate, emulsifier in margarine, and lubricants [3–5]. As lubricants, estolides have been developed in order to overcome deficiencies associated with some characteristics of vegetable oils, which are known to have poor thermal oxidative stability, low hydrolytic stability, and poor low temperature properties [6]. These vegetable-oil-based lubricants and derivatives have excellent lubricity and biodegradability properties and currently out-perform the commercially available industrial products such as petroleum-based hydraulic fluids, soy-based fluids, and petroleum oils [6, 7].

The conventional chemical route of estolides synthesis using high temperatures (205–210°C) or strong acids as catalysts leads to a lower selectivity, undesired byproducts, colouring, and malodorous products and cause corrosion of equipments and produce acid effluent [8].

The enzymatic synthesis of ricinoleic acid estolides using lipases (triacylglycerol ester hydrolases E.C.3.1.1.3) have been investigated as an alternative to overcome the common problems that occur in a conventional route. Most of lipases act under mild reaction conditions (low temperature and pressures, and neutral pH), which prevent degradation of
2. Experimental

2.1. Materials. The commercial immobilized lipases used were Novozym 435, Lipozyme TL-IM, and Lipozyme RM-IM, all kindly supplied by Novozymes Latin America (Paraná, Brazil). Oleic acid (extra pure) and ethanol (95 wt.%) were purchased from Merck (Darmstadt, Germany). Biodiesel from castor oil was provided by Cenpes/Petrobras (Rio de Janeiro, Brazil). Acetone P.A., butanol (99 wt.%), and sodium hydroxide were supplied by Vetec Química Fina Ltda (Rio de Janeiro, Brazil). Molecular sieves (3 Å) were purchased from SIGMA/Aldrich (St. Louis, USA).

2.2. Reaction System. The enzymatic reaction was carried out in a 15 mL closed batch reactor magnetically stirred and coupled to a condenser. Temperature of the medium was kept constant by circulating hot ethylene glycol through the reactor jacket. A thermostatic bath allowed a close control over the process temperature. The reaction medium contained oleic acid, biodiesel from castor oil, and immobilized lipase.

2.3. Measurement of Lipase Activity. Esterification activities of Lipozyme RM-IM, Lipozyme TL-IM, and Novozym 435 were measured by the consumption of oleic acid at 45°C during the esterification reaction with butanol (0.030 mol oleic acid and 0.030 mol butanol) using an enzyme concentration of 3 wt.%. The reactions were carried out in a 20 mL open batch reactor magnetically stirred. The reactor was kept at the desired temperature by a thermostatic bath. Samples of 100 μL were withdrawn at 0, 5, 10, 15, 20, 30, 40, 50, and 60 min, diluted in 30 mL of acetone/ethanol (1:2) and titrated with NaOH 0.02 mol L⁻¹, using an automatic titrator Mettler model DL25. One esterification unit was defined as the amount of lipase that consumed 1 μmol of oleic acid per minute per gram of enzymatic preparation under the experimental conditions described herein. Esterification enzymatic activity was calculated using the formula below:

\[
A(\mu\text{mols} \cdot \text{min}^{-1} \cdot \text{g}^{-1}) = \frac{(V_1 - V_2) \times C}{t \times m} \times 1000 \times \frac{V_m}{V_a}.
\]

A is esterification activity (μmols·min⁻¹·g⁻¹); \(V_1\) is volume of NaOH consumed in titration of the sample taken at time zero reaction (mL); \(V_2\) is volume of NaOH consumed in titration of the sample taken at time \(t\) minutes of reaction (mL); \(C\) is NaOH concentration (mol·L⁻¹); \(t\) is reaction time (min); \(m\) is enzyme mass used in the reaction (g); \(V_a\) is volume of sample (mL); \(V_m\) is volume of medium (mL).

The enzymatic activities values were maintained during the experimental work.

2.4. Use of Molecular Sieves. Amounts of adsorbent (3 Å molecular sieves) were added into the reaction system to adsorb the byproduct water. Before each reaction, molecular sieves were dried at 300°C during 3 h, and then they were added to the reaction medium at the beginning (0 h), after 6 h or 24 h of reaction. The effect of adsorbent amount was studied using 100, 250, 500, and 650 mg of molecular sieves. Reactions were carried out at 80°C, using molar ratio of reagents equal to 1 (0.015 mols of each reagent) and 6 wt.% of Novozym 435 during 48 h.

2.5. Reactions Under Vacuum. The system consisted of a closed batch reactor, equipped with magnetic stirring and coupled to a condenser. A vacuum pump (Edwards Model RV3) was connected to the condenser giving a constant pressure of 0.6 mbar (0.45 mmHg). To evaluate the effect of water removal two strategies were used: the pump was switched on either at the beginning (\(t = 0\) h) or after 6 h of reaction. Reactions were carried out at 80°C, using molar ratio of reagents equal to 1 (0.015 mols of each reagent) and 6 wt.% of Novozym 435 during 48 h.

2.6. Effect of Pressure. The effect of pressure was tested in three levels: atmospheric pressure, 100 and 250 psi using a 50 mL Parr Reactor Model 4843. Nitrogen was used for the system pressurization. Stirring speed was kept constant at 550 rpm. Reactions were carried out at 80°C, using molar...
ratio of reagents equal to 1 and 6 wt.% of Novozym 435. The reaction medium was approximately 38 mL (75% of reactor volume).

2.7. Reuse of Novozym 435. The reusability of Novozym 435 was carried out using reagents in a molar ratio of 1 : 1 (0.015 mols of each reagent), 6 wt.% of immobilized lipase and 80°C. After 48 h of reaction, the lipase was separated from the reaction medium by decantation and washed with n-hexane. After that, it was vacuum-filtered. Enzyme was then reused in a new batch.

2.8. Measurement of Reaction Extension. Acid value (AV) was used as an index to show the degree of reaction [10]. The acid value is the number of milligrams of potassium hydroxide necessary to neutralize the free acids in 1 g of sample. AV corresponds to the carboxyl group concentration in the reaction mixture, which decreases due to the condensation of fatty acid. This analysis was chosen to measure the reaction conversion due to the difficulty to identify the reaction products and to quantify methyl ricinoleate consumption. Reaction progress was monitored by taking duplicate samples (100 µL) of reaction medium that was dissolved in 30 mL of acetone/ethanol (1 : 1). Free fatty acids present in reaction medium samples were analyzed by titration with NaOH 0.02 mol·L⁻¹ using a Mettler DL 25 autotitrator.

The conversion was calculated using the formula below

\[
\text{Conversion} \% = \left( \frac{\text{AV}_{\text{initial}} - \text{AV}_{\text{final}}}{\text{AV}_{\text{initial}}} \right) \times 100. \quad (2)
\]

Reproducibility of conversion data was found to be within 2–5%. A blank test was carried out at 100°C in the absence of biocatalyst and no reduction was observed in AV after 24 h of reaction.

2.9. Physical Properties of the Estolides. Physical properties such as viscosity at 40 and 100°C [11], viscosity index [12], pour point [13], acid value [14], and copper strip corrosion [15] were determined for the lubricant obtained in this work.

3. Results and Discussion

3.1. Lipase Activity. The activities obtained for Lipozyme RM-IM, Lipozyme TL-IM and Novozym 435 were 1909, 699, and 3824 µmols of acid·min⁻¹·g⁻¹ of lipase, respectively.

3.2. Lipase Type Effect. In this work, the synthesis of estolides from oleic acid and methyl ricinoleate was studied using three commercial immobilized lipases: Lipozyme RM-IM, Lipozyme TL-IM, and Novozym 435.

Under the experimental conditions used in this work, the main reaction is the esterification between oleic acid and methyl ricinoleate producing estolide and water. However other side reactions could occur such as the self-condensation of methyl ricinoleate, the transesterification between oleic acid and methyl ricinoleate forming methyl oleate and ricinoleic acid, and the self-condensation of ricinoleic acid.

The reactions were carried out using oleic acid/methyl ricinoleate molar ratio equal to 1, at 80°C and enzyme content of 6 wt.%.. Results are shown in Figure 2. After 48 h, the highest conversion was attained with Novozym 435 (33%).

Conversions obtained with the 1,3-positionaly specific enzymes (Lipozyme RM-IM and Lipozyme TL-IM) were below 15%, which may be related to the specificity of the lipases studied. It has been reported that reactions involving estolide formation from ricinoleic acid or hydrolysis of estolides have been successfully catalyzed by “random lipases”, which present no 1,3-positional selectivity (Candida rugosa, Chromobacterium viscosum, Pseudomonas sp., and Geotrichum candidum), but are not well catalyzed by 1,3-specific lipases (Rhizopus, Rhizomucor miehei, pancreatic lipase, etc.) [4, 5]. According to Pélaez et al. [16] 1,3-selective lipases from Rhizopus delamari and R. miehei did not catalyze formation of estolide. Similar results were obtained by Hayes and Kleiman [9] in reactions with lesquerolic acid (14-hydroxy-11-eicosanoic) and octadecenoic acid.

3.3. Effect of Substrate Molar Ratio. The effect of oleic acid/methyl ricinoleate molar ratio on the conversion was investigated. Reactions were carried out for 24 h, at 80°C and using 6 wt.% of Novozym 435. Figure 3 shows that higher conversion (22%) was attained with stoichiometric molar ratio of reagents. However, molar ratio of reagents did not have significant effect on conversion.

![Figure 2: Effect of lipase type on conversion after 48 h at 80°C, using 6 wt.% of immobilized lipase and substrate molar ratio of 1.](image)

![Figure 3: Effect of substrate molar ratio (oleic acid/methyl ricinoleate) on conversion after 24 h at 80°C, using 6 wt.% of immobilized lipase.](image)
The conditions of 6 wt.% of lipase, stoichiometric molar ratio of reagents, and 80°C were used to study the influence of reaction time during 24, 48, 72, and 100 h of reaction. Higher conversions were obtained after 72 and 100 h, as expected (Figure 4). The conversion obtained after 72 and 100 h of reaction (46%) indicates that equilibrium was reached. One of the most common reactions to produce estolides is the self-condensation of ricinoleic acid. Bódalo et al. [8] investigated this reaction using immobilized lipase from Candida rugosa and obtained an acid value of 50 after 150 h (72% of conversion), using 16.6 wt.% of lipase at 40°C. These results indicate the applicability of lipases for estolides synthesis through different pathways.

3.4. Molecular Sieves Effect. In esterification reactions catalyzed by lipases water play multiple roles. It is necessary for the catalytic function of lipases because it participates, directly or indirectly, in all noncovalent interactions that maintain the conformation of the catalytic site. On the other hand, the reaction reaches equilibrium and stops when the water content in reaction mixture increases as result of the water formed during the dehydration-condensation reaction [4]. Adsorbents such as alumina, silica gel, and zeolites can be adopted to control water concentration during the reaction process. Molecular sieves present superior drying ability because they cannot coadsorb large hydrocarbon molecules [20].

In order to verify the effect of water removal, reactions were carried out with 500 mg of 3 Å molecular sieves added to the system at the beginning of the reaction, or after 6 or 24 h. The reaction conditions were stoichiometric ratio of reagents and 6 wt.% of Novozym 435 at 80°C. Results are shown in Figure 5. The addition of molecular sieves at the beginning of the reaction (t = 0 h) did not improve the final conversion. It was observed that higher conversion values were obtained when molecular sieves were added after 6 or 24 h of reaction. This result can be explained due to excessive stripping of the essential water needed for enzyme activity by the desiccant agent [21].

The effect of adsorbent amount was also studied. The reactions were carried out with 100, 250, 500, and 650 mg of adsorbent added after 6 h of reaction. Results are shown in Figure 6 and it can be seen that conversion increased with increasing molecular sieve amount up to 500 mg.

3.5. Reactions under Vacuum. The use of vacuum as an alternative to removing water from the reaction medium was investigated in reactions using stoichiometric molar ratio of reagents (0.015 mols of each reagent), 6 wt.% of Novozym 435 at 80°C during 48 h. In order to evaluate the effect of water removal two strategies were used: the pump was switched on either at the beginning (t = 0 h) or after 6 h of reaction. Results are shown in Figure 7. The use of vacuum (0.45 mmHg) increased the conversion after 48 h in more than 10%. No difference was observed when vacuum was applied in the beginning or after 6 h of reaction. Bódalo et al. [2] have also observed that the use of a vacuum system (160 mmHg) was efficient to remove water produced on the estolides synthesis from ricinoleic acid catalyzed by Candida rugosa lipase. The use of vacuum on estolides synthesis from ricinoleic acid using immobilized lipase from Candida rugosa was also investigated by Yoshida et al. [3]. The condition of 110 mmHg was the most advantageous.

The conversion attained after 48 h using 500 mg of molecular sieves added after 6 h of reaction (44%) was similar to that obtained in reactions carried out with vacuum
3.6. Effect of Pressure. The hydrostatic pressure has been used to increase the activity and/or stability of several enzymes in different solvents [22]. Lipases stability and activity under high pressures have been investigated in compressed or supercritical fluids and gases including carbon dioxide, propane, butane, and mixtures of butane and propane [22].

The effect of high pressure on estolides synthesis from oleic acid and methyl ricinoleate was studied in a Parr reactor at $80^\circ$C operating during 48 h, using reagents in a molar ratio of 1:6, 6 wt.% of Novozym 435 and stirring speed of 550 rpm. The pressures investigated were 100 and 250 psi (6.8 and 17 atm, resp.). Nitrogen was used to pressurize the system. A pressure increase led to a small reduction in the conversion (less than 10%). These results indicate that the catalytic activity of lipase was not favored by pressure increase in the reaction system.

3.7. Effect of Temperature. Temperature influences the enzymatic reaction rate, enzyme stability, and the velocity of water evaporation from the reaction medium and its viscosity [2]. An increase in temperature can reduce the mixture viscosity, enhance mutual solubility, and improve substrates diffusion process, thus reducing mass transfer limitations and favoring interactions between enzyme particles and substrates. However, high temperatures can lead to higher lipase deactivation [23].

The effect of temperature was studied in a range of 50–100°C. The reactions were carried out using 0.015 mols of each reagent and 6 wt.% of Novozym 435, during 24 h. Results are shown in Figure 8. An increase in temperature until 90°C improved the conversion. Even at 100°C, 30% of conversion was obtained.

3.8. Effect of Catalyst Amount. The effect of enzyme concentration on the fatty acid conversion was studied in a range of 4 and 16 wt.% of Novozym 435 at 90°C using stoichiometric reagents. Results are shown in Figure 9. A linear correlation between catalyst amount and conversion was found until 14 wt.% of lipase (conversion of 53%). Loading 16 wt.% of lipase caused significant conversion decrease (43%) that can be explained taking into account the phenomenon of enzyme agglomeration.

Similar results were obtained by Bódalo et al. [2] that studied the influence of the enzyme concentration on estolides synthesis from ricinoleic acid catalyzed by immobilized Candida rugosa lipase (concentrations of 8.3; 16.6 and 33 wt.%). The authors observed that when the concentration of immobilized enzyme was increased, the reaction progressed faster and a lower acid value was reached. However, changes in reaction rate and final acid value were more noticeable when the enzyme concentration varied from 8.3 to 16.6 wt.% than when the enzyme concentration was increased to 33 wt.%.

The behavior of leveling-off of esterification at higher enzyme concentration has also been reported in other enzymatic reactions [23]. Clumping leads to aggregate formation and inhomogeneous enzyme distribution. Then, just the fraction of the catalyst that remains on the outer surface of the agglomerates is truly available for catalysis. Substrates have reduced access to catalytic molecules inside agglomerates, being the efficiency of the milligram of catalyst added to reaction mixture seriously reduced [24].
Table 1: Comparison between the properties of the product obtained after reaction with oleic acid and methyl ricinoleate using Novozym 435 (6 wt.%) at 80°C and those of estolides reported in the literature.

| Lubricant | Pour point (°C) | Viscosity at 40°C (cSt) | Viscosity at 100°C (cSt) | Viscosity index |
|-----------|----------------|-------------------------|--------------------------|----------------|
| Product of this study (oleic acid + methyl ricinoleate) | −24 | 23.9 | 5.2 | 153 |
| Cermak and Isbell [17]a | −27 | 123.4 | 16.7 | 146 |
| Cermak et al. [18]b | −54 | 34.5 | 7.6 | 196 |
| Hayes and Kleiman [9]c | | 126.3 | 23.11 | 191 |
| Cermak and Isbell [19]d | −24 | 389.1 | 37.7 | 143 |

aCuphea-oleic estolides.  
bCastor oil estolides.  
cOleic acid estolides.  
dOleic acid estolides + octanoic acid.

Table 2: Comparison between the properties of the product obtained after reaction with oleic acid and methyl ricinoleate using Novozym 435 (6 wt.%) at 80°C and those of commercial lubricants.

| Lubricant | Viscosity at 40°C (cSt) | Viscosity at 100°C (cSt) | Viscosity index |
|-----------|-------------------------|--------------------------|----------------|
| Product of this study (oleic acid + methyl ricinoleate) | 23.9 | 5.2 | 153 |
| Light neutral solvent | 30–40 | 5 | 125 |
| Medium neutral solvent | 40–50 | 7 | 95 |
| Heavy neutral solvent | 70–300 | 10–20 | 95 |
| Mineral oil Spindle 09 | 10.7 | 2.7 | 95 |
| Lubrax Unitractora | 54 | 9.3 | 156 |

Biodegradable synthetic ester marketed by BR for tractors.

Figure 10: Effect of repeated batch reactions on conversion after 48 h for 6 wt.% of Novozym 435 at 80°C.

3.9. Reuse of Novozym 435. Immobilization provides an attractive possibility for consecutive use of the same biocatalyst [9]. Catalyst reusability was studied with reactions at 80°C, using 6 wt.% of Novozym 435 and reagents in a molar ratio of 1:1 during 48 h (Figure 10). After each batch reaction, the enzyme was recovered, washed with n-hexane, and dried under vacuum before reuse.

As shown in Figure 10, in the first batch experiment, the conversion attained was 33%. The conversion has decreased after successive use of the catalyst. After the fourth batch reuse, the conversion was half that obtained in the first experiment. The decrease on lipase activity when it was reused was also observed by other authors [3, 9].

3.10. Physical Properties of the Product. The physical properties of some estolides reported in the literature [9, 17–19] and of the product obtained after reaction between oleic acid and methyl ricinoleate using Novozym 435 (6 wt.%) at 80°C are presented in Table 1. The kinematic viscosities obtained at 40 and 100°C (23.9 and 5.2 cSt, resp.) were lower than the values reported in the literature [9, 17–19]. The viscosity index was higher than the desirable value for lubricants (150). Pour point (−24°C) showed a value similar to that obtained by Cermak and Isbell (−27°C) [17] indicating the potential application of the biolubricant at lower temperatures. According to the corrosion test on copper strip, the product presents no corrosiveness. Table 2 shows a comparison between the properties of some commercial lubricants and the product obtained in this study. According to the viscosity data, the estolide from oleic acid and biodiesel from castor oil is similar to light neutral solvent. However, the product obtained in this study has a high acid value (56.3 mg KOH·g⁻¹) that was due to the presence of oleic acid.

4. Conclusions

Estolides synthesis from oleic acid and biodiesel from castor oil catalyzed by three immobilized lipases was studied and can be performed in a solvent-free system. Novozym 435 was the most efficiently biocatalyst tested. Novozym 435 was reused four times, however with partial loss of activity. Despite the high acid value of the product, the reaction route investigated to obtain a lubricant using a clean technology (enzymatic catalysis) allowed us to obtain a noncorrosive product with good low temperature properties, which can be proved by comparing its properties to data reported in the literature regarding the same subject. Therefore, it was possible to establish some conditions for the biolubricant synthesis from reagents present in abundance in Brazil.
Acknowledgments

The authors would like to acknowledge Cenpes/Petrobras, FAPERJ, and CNPq for financial support and Novozymes Latin America Ltda for kindly providing the enzyme for this research. E. C. G. Agueiras thanks Cenpes/Petrobras and FAPERJ for the scholarship.

References

[1] S. C. Cermak and T. A. Isbell, “Synthesis of estolides from oleic and saturated fatty acids,” Journal of the American Oil Chemists’ Society, vol. 78, no. 6, pp. 557–565, 2001.

[2] A. Bódalo, J. Bastida, M. F. Máximo, M. C. Montiel, M. D. Murcia, and S. Ortega, “Influence of the operating conditions on lipase-catalysed synthesis of ricinoleic acid estolides in solvent-free systems,” Biochemical Engineering Journal, vol. 44, no. 2-3, pp. 214–219, 2009.

[3] Y. Yoshida, M. Kawase, C. Yagamuchi, and T. Yamane, “Enzymatic synthesis of estolides by a bioreactor,” Journal of the American Oil Chemists’ Society, vol. 74, no. 3, pp. 261–267, 1997.

[4] A. Bódalo, J. Bastida, M. F. Máximo, M. C. Montiel, and M. D. Murcia, “Enzymatic biosynthesis of ricinoleic acid estolides,” Biochemical Engineering Journal, vol. 26, no. 2-3, pp. 155–158, 2005.

[5] D. G. Hayes, “The catalytic activity of lipases toward hydroxy fatty acids—a review,” Journal of the American Oil Chemists’ Society, vol. 73, no. 5, pp. 543–549, 1996.

[6] S. C. Cermak, G. Biresaw, and T. A. Isbell, “Comparison of a new Estolide oxidative stability package,” Journal of the American Oil Chemists’ Society, vol. 85, no. 9, pp. 879–885, 2008.

[7] S. C. Cermak and T. A. Isbell, “Synthesis and physical properties of mono-estolides with varying chain lengths,” Industrial Crops and Products, vol. 29, no. 1, pp. 205–213, 2009.

[8] A. Bódalo, J. Bastida, M. F. Máximo, M. C. Montiel, M. Gómez, and M. D. Murcia, “Production of ricinoleic acid estolide with free and immobilized lipase from Candida rugosa,” Biochemical Engineering Journal, vol. 39, no. 3, pp. 450–456, 2008.

[9] D. G. Hayes and R. Kleiman, “Lipase-catalyzed synthesis and properties of estolides and their esters,” Journal of the American Oil Chemists’ Society, vol. 72, no. 11, pp. 1309–1316, 1995.

[10] AOCS Te 1a-64, “Official methods and recommended practices of the American oil chemists’ society,” AOCS Industrial Oils and Derivatives, 1997.

[11] ASTM, “Standard test method for kinematic viscosity of transparent and opaque liquids (the calculation of dynamic viscosity),” Designated D445-09, 2000.

[12] ASTM, “Standard practice for calculating viscosity index from kinematic viscosity at 40 and 100 degrees C,” Designated D2270-04, 2004.

[13] ASTM, “Standard test method for pour point of petroleum products,” Designated D97-07, 2007.

[14] ASTM, “Standard test method for acid and base number by color-indicator titration,” Designated D974-08, 2008.

[15] ASTM, “Standard test method for corrosiveness to Copper from petroleum products by Copper strip test,” Designated D130-04, 2004.

[16] M. Peláez, C. Orellana, A. Marqués, M. Busquets, A. Guerrero, and A. Manresa, “Natural estolides produced by Pseudomonas sp. 42A2 grown on oleic acid: production and characterization,” Journal of the American Oil Chemists’ Society, vol. 80, no. 9, pp. 859–866, 2003.

[17] S. C. Cermak and T. A. Isbell, “Synthesis and physical properties of cuphea-oleic estolides and esters,” Journal of the American Oil Chemists’ Society, vol. 81, no. 3, pp. 297–303, 2004.

[18] S. C. Cermak, K. B. Brandon, and T. A. Isbell, “Synthesis and physical properties of estolides from lesquerella and castor fatty acid esters,” Industrial Crops and Products, vol. 23, no. 1, pp. 54–64, 2006.

[19] S. C. Cermak and T. A. Isbell, “Physical properties of saturated estolides and their 2-ethylhexyl esters,” Industrial Crops and Products, vol. 16, no. 2, pp. 119–127, 2002.

[20] J. Giacometti, F. Giacometti, C. Milin, and D. Vasic-Racki, “Kinetic characterization of enzymatic esterification in a solvent system: adsorptive control of water with molecular sieves,” Journal of Molecular Catalysis B, vol. 11, pp. 921–928, 2001.

[21] S. Tarahomjoo and I. Alemzadeh, “Surfactant production by an enzymatic method,” Enzyme and Microbial Technology, vol. 33, no. 1, pp. 33–37, 2003.

[22] M. J. Eisenmenger and J. I. Reyes-De-Corcuera, “High pressure enhancement of enzymes: a review,” Enzyme and Microbial Technology, vol. 45, no. 5, pp. 331–347, 2009.

[23] Z. Q. Duan, W. Du, and D. H. Liu, “Novozym 435-catalyzed 1,3-diacylglycerol preparation via esterification in t-butanol system,” Process Biochemistry, vol. 12, pp. 1923–1927, 2010.

[24] M. L. Foresti and M. L. Ferreira, “Solvent-free ethyl oleate synthesis mediated by lipase from Candida antarctica B adsorbed on polypropylene powder,” Catalysis Today, vol. 107-108, pp. 23–30, 2005.