

STUDY OF ESTOLIDE CAPPING REACTIONS CATALYZED BY *DIUTINA RUGOSA* LIPASE

Manuella da Silva de Miranda¹, Ana Cristina de Araujo Collaço^{1*}, Elisa d'Ávila Costa Cavalcanti¹, Erika Cristina Gonçalves Aguiaras^{1,2} & Denise Maria Guimarães Freire¹

¹ Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brasil

² Campus UFRJ Duque de Caxias Professor Geraldo Cidade, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brasil

* anacriscollaco@gmail.com

ABSTRACT

Biolubricants are molecules produced from renewable sources (such as vegetable oils), and are an alternative to petroleum-based lubricants. Castor oil is composed mainly of ricinoleic acid (about 90%), which has promising characteristics for producing biolubricants (estolides). From the hydrolysis of castor oil, the ricinoleic acid of castor oil-free fatty acid (COFFA) can be esterified in the presence of biocatalysts (lipases), to obtain estolide. Thus, the aim of this work is the production of estolide using the commercial lipase of *Diutina rugosa* (Lipomod 34MDP) and the developing of capping reactions to reduce the acidity of the final product and to obtain a capped estolide. The COFFA esterification reaction generated a product with approximately 30% acidity. The capping reactions were conducted using alcohols with different chain lengths (anhydrous ethanol, isobutanol, methanol, 2-ethylhexanol, hydrous ethanol (95%), (iso)octanol, dodecanol, 1,3-propanediol, and isopropanol). After 24 h, the best conversions were obtained using anhydrous ethanol (66%), isobutanol (60.8%), and methanol (59.5%).

Keywords: 1. estolide 2. lipase 3. castor oil 4. ricinoleic acid 5. biolubricant

1 INTRODUCTION

Biolubricants derived from vegetable oils are an alternative to the use of lubricant molecules derived from petroleum, as they are biodegradable organic esters, presenting similar physicochemical properties to lubricants of mineral origin ¹. Castor oil (*Ricinus communis* L.) is a promising raw material for the synthesis of these molecules, since the fatty acid composition is 90% ricinoleic acid, giving excellent properties to the biolubricant as high molar mass, high viscosity, high miscibility in alcohol, low melting points (5°C) and very low solidification points (-12°C to -18°C), which contributes to the industrial application of its derivatives ². Esterification between the functional carbonyl, present at the carboxylic terminal of ricinoleic acid, and the hydroxyl of another ricinoleic acid is capable of forming molecules known as estolides, with excellent properties to be used as a base oil for the formulation of biolubricants ^{3,4}.

The estolide synthesis involves esterification of fatty acids and most studies use acid catalysts, such as sulfuric acid and perchloric acid, and/or high temperatures (200 °C) and pressures. However, in addition to the generation of acidic effluents, the use of strong acids can lead to the breakdown of functional groups, releasing sulfonated acid groups that decrease the pH, affecting the functioning of the fluid ⁵. Therefore, the use of biocatalysts appears to be an interesting alternative for obtaining estolides to conventional chemical methods ⁶. As enzymatic methods are very specific, almost no reaction co-products are formed, which reduces the industrial effluents and the energy consumption of the process, since enzymes act in mild reaction conditions of temperature, pH, and pressure ⁷.

However, the major challenge in the synthesis of estolides is the presence of a fatty acid at the end of the chain formed after the polymerization process, which can generate a product with a high acidity index that is not recommended for lubricating properties. To reduce the acidity index, a conversion of the free estolides into estolide esters (capping reactions) can be realized in which an esterification reaction is carried out at the free terminals of the estolide with an alcohol or fatty acid, obtaining a capped or finished estolide ⁸. Most of the works found in the literature report the use of chemical catalysts in capping reactions ^{9,10}. However, the objective of the present work is also use of the commercial *Diutina rugosa* lipase in capping reactions to obtain a capped estolide.

2 MATERIAL & METHODS

Castorbean oil hydrolysis

The castorbean oil hydrolysis was conducted in a 2 L-scale reaction using a mechanical stirred reactor. Castor oil was hydrolyzed using the ratio of castor seeds/sodium acetate buffer (100 mM; pH 4)/castor oil of 1:5:5 (w/v/v). The reaction was performed at 200 rpm and 30°C ⁶. After 24h, COFFA (castorbean oil-free fatty acid) was recovered by ethyl acetate extraction. The ethyl acetate was removed with a rotary evaporator and the COFFA content was determined by acidity.

Esterification reactions

The reactions were solvent-free and carried out in duplicate in 10-mL batch reactors with magnetic stirring and thermostated for 24 h. The estolide medium was composed of substrate COFFA (4 g), Lipomod 34 MDP (1.6 wt.%), 14% of water

content, and the reaction was carried out at 45°C¹¹. The capping reactions were conducted using alcohols with different chain lengths in magnetic stirring and thermostated reactors (40°C), in a molar ratio of 1:1 (alcohol:estolide) and 4% Lipomod 34 MDP. Different alcohols were tested such as anhydrous ethanol, isobutanol, methanol, 2-ethylhexanol, hydrous ethanol (95%), (iso)octanol, dodecanol, 1,3-propanediol, and isopropanol. The reaction progress was monitored by taking samples at fixed intervals to analyze the acidity or fatty acid ethyl ester content⁶.

3 RESULTS & DISCUSSION

The hydrolysis of COFFA generated a product with 87% acidity and only 2.5 % free ricinoleic acid. However, as the acidity attained in estolide reaction was about 30 % after 24 h, capping reactions were carried out using alcohols with different chain lengths in a molar ratio of 1:1 (alcohol:estolide) and 4 wt. % of Lipomod 34 MDP. After 24 hours, the alcohols that were able to generate the highest conversion were anhydrous ethanol (66%), isobutanol (60.8%), and methanol (59.5%) (Figure 1).

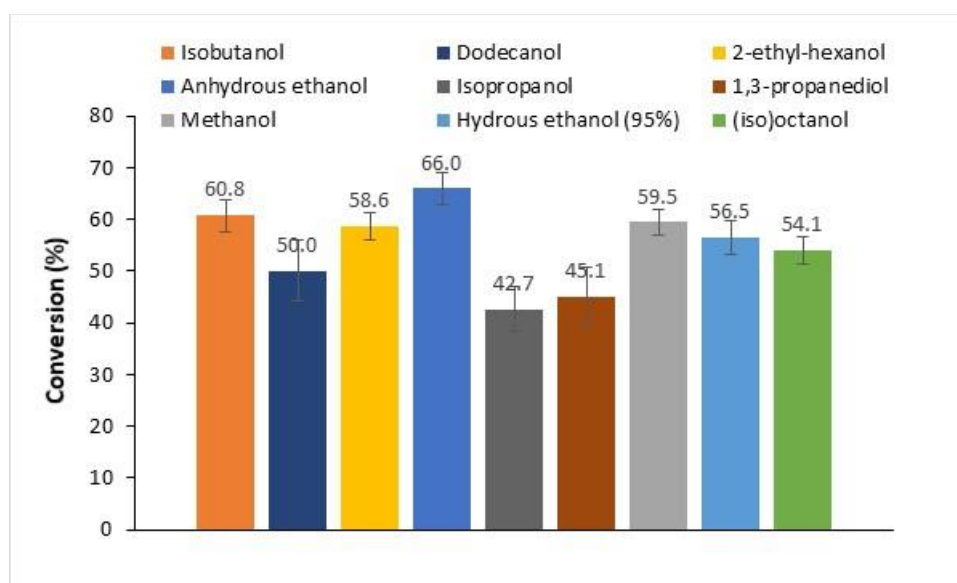


Figure 1 Conversions obtained in the capping reactions in a molar ratio 1:1 (alcohol: estolide) and 4 wt.% of Lipomod 34MDP, at 40°C.

An increase in the molar ratio was carried out in the reactions using anhydrous ethanol and hydrous ethanol. Statistical analyses were performed with a significance level of $p < 0.05\%$ following the Turkey test. For anhydrous ethanol, there was a statistical difference only in the results obtained between the molar ratios of 2:1 and 3:1. As there is no statistical difference between the molar ratio of 1:1 and 2:1, the use of a molar ratio of 1:1 is more interesting, as it uses less solvent in the reaction medium (Figure 2). On the other hand, for hydrous ethanol, a statistical difference was observed between all the molar ratios studied. Thus, a molar ratio of 2:1 seems to be the best condition using hydrous ethanol (Figure 3). The final acidity obtained in the reactions using anhydrous ethanol (molar ratio of 1:1) and hydrous ethanol (molar ratio of 2:1) was 10.3% and 13.1%, respectively.

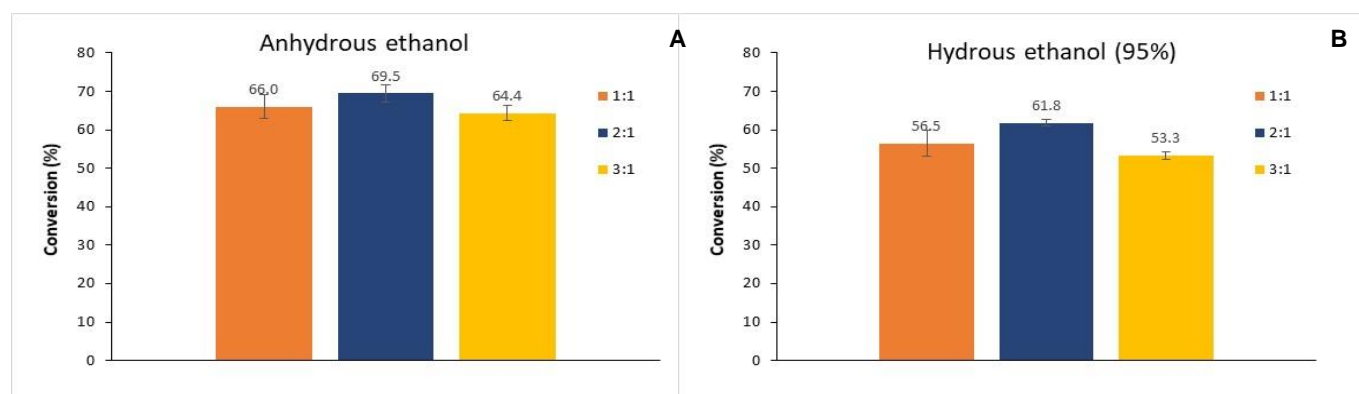


Figure 2 Conversions obtained in capping reactions in molar ratios of 1:1, 2:1, and 3:1 (ethanol: estolide) and 4 wt. % of Lipomod 34MDP, at 40°C. A) Reactions with anhydrous ethanol. B) Reactions with hydrous ethanol (95%).

4 CONCLUSION

- Results obtained show that the use of castor bean seed as a biocatalyst is promising for obtaining COFFA;
- The results showed the capacity to produce estolide using Lipomod 34 MDP as a biocatalyst;
- The best alcohols used in capping reactions were ethanol (anhydrous), isobutanol, and methanol, respectively;
- As anhydrous ethanol is the most promising alcohol in capping reactions compared with the other alcohols evaluated, subsequent reactions will be evaluated with this alcohol.

REFERENCES

- ¹ Madanhire, I., & Mbohwa, C. (2016). *Mitigating environmental impact of petroleum lubricants* (pp. 17-34). Berlin, Germany:: Springer.
- ² McKeon, T., Hayes, D. G., Hildebrand, D., & Weselake, R. (Eds.). (2016). *Industrial oil crops*. Elsevier.
- ³ Aguiéiras, E. C., Veloso, C. O., Bevilaqua, J. V., Rosas, D. O., da Silva, M. A., & Langone, M. A. (2011). Estolides synthesis catalyzed by immobilized lipases. *Enzyme research*, 2011.
- ⁴ Cermak, S. C., Isbell, T. A., Evangelista, R. L., & Johnson, B. L. (2011). Synthesis and physical properties of petroselinic based estolide esters. *Industrial Crops and Products*, 33(1), 132-139.
- ⁵ Cermak, S. C., & Isbell, T. A. (2001). Biodegradable oleic estolide ester having saturated fatty acid end group useful as lubricant base stock.
- ⁶ Greco-Duarte, J., Cavalcanti-Oliveira, E. D., Da Silva, J. A. C., Fernandez-Lafuente, R., & Freire, D. M. G. (2017). Two-step enzymatic production of environmentally friendly biolubricants using castor oil: enzyme selection and product characterization. *Fuel*, 202, 196-205.
- ⁷ Cavalcanti-Oliveira, E. D. A., Silva, P. R. D., Ramos, A. P., Aranda, D. A. G., & Freire, D. M. G. (2011). Study of soybean oil hydrolysis catalyzed by *Thermomyces lanuginosus* lipase and its application to biodiesel production via hydroesterification. *Enzyme research*, 2011.
- ⁸ Cermak, S. C., & Isbell, T. A. (2001). Biodegradable oleic estolide ester having saturated fatty acid end group useful as lubricant base stock.
- ⁹ CERMAK, Steven C. et al. Synthesis and physical properties of new coco-oleic estolide branched esters. *Industrial Crops and Products*, v. 74, p. 171-177, 2015a.
- ¹⁰ CERMAK, Steven C. et al. Synthesis and physical properties of pennycress estolides and esters. *Industrial Crops and Products*, v. 67, p. 179-184, 2015b.
- ¹¹ GRECO-DUARTE, J. Produção e caracterização de um novo biolubrificante obtido por hidroesterificação enzimática a partir do óleo de mamona. 2018. Tese (Doutorado em bioquímica) – Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro.

ACKNOWLEDGEMENTS

The authors thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAPERJ (Fundação Carlos Chagas Filho de Apoio à Pesquisa do Estado do Rio de Janeiro), PRH 20.1/ANP (Programa de Recursos Humanos – Agência Nacional do Petróleo, Gás Natural e Biocombustíveis), and PETROBRAS (Petróleo Brasileiro S/A).