BABASSU OIL INTERESTERIFICATION CATALYZED BY IMMOBILIZED EVERSA® TRANSFORM 2.0

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ABSTRACT

The growing investment in renewable energy has elevated Brazil's importance in this sector, as it is one of the largest producers of biofuels globally. The most common method for producing biodiesel is through the oil vegetable transesterification with a short-chain alcohol, facilitated by a chemical catalyst. This reaction generates glycerol as a byproduct, as well as the potential for undesired reactions. In this study, fatty acid ethyl esters (FAEEs) were produced by interesterification of babassu oil with ethyl acetate. This alternative method of production aims to replace glycerol with triacetin, a product with high added value that can be used as a biodiesel additive. The catalyst used was the enzyme Eversa® Transform 2.0, which was immobilized by adsorption on Purolite® C18. After 108 hours of reaction, a maximum yield of FAEEs of approximately 53% was achieved in the 1:9 ratio via immobilized Eversa®.

Keyword 1. Biodiesel. 2. Triacetin 3. Lipase 4. Interesterification reaction 5. Babassu oil

1 INTRODUCTION

There is a clear dependence on the use of non-renewable sources in the world energy scenario, since around 80% of current demand comes from fossil fuels, accounting for three quarters of global greenhouse gas[1]. Therefore, in search of renewable and sustainable alternatives, global investment in clean energy has increased by 40% in recent years[2]. In this scenario, Brazil stands out with a production of almost 7 million m³ of biodiesel in 2023, making it one of the world's largest producers[3].

The conventional biodiesel production process involves the vegetable oil transesterification in the presence of short-chain alcohols via alkaline catalysts. However, this reaction route produces excess glycerol as a by-product, as well as soap formation when the raw material has a high content of free fatty acids [4]. Thus, it is relevant to study enzymatic interesterification reactions, since short-chain alcohols are replaced by esters and lipase enzymes are used as catalysts. As an advantage, undesired reactions are avoided [5] and triacetin is produced instead of glycerol, a high value-added co-product, because not only can it be used in various industrial segments, but it can also be an additive to the biofuel itself, improving its yield and properties.

Eversa® Transform 2.0, a lipase from *Thermomyces lanuginosus* formulated by Novozymes A/S in 2016, has been manufactured in its free form with a focus on biodiesel production. As it is a commercial enzyme and in its free form, it does not require any prior preparation. However, the use of an immobilized catalyst can be more cost-effective due to the possibility of reusing it, as well as improving the enzyme's characteristics [6].

Therefore, the aim of this study was to analyse the yield of biodiesel - fatty acid ethyl esters (FAEEs) - produced from the interesterification of babassu oil with ethyl acetate using Eversa® Transform 2.0 immobilized on Purolite® C18 support. The physical and chemical properties of babassu oil were also evaluated.

2 MATERIAL & METHODS

2.1 MATERIALS

Eversa® Transform 2.0 (*Thermomyces lanuginosus* lipase), manufactured by Novozymes A/S (Bagsvaerd, Denmark), was acquired from Sigma-Aldrich Co (St. Louis, MO, USA). Ethyl acetate was purchased from Êxodo Científica (Sumaré, SP, Brazil). Babassu oil was from COPPALJ (Lago do Junco, Maranhão, Brazil). Purolite Lifetech® ECR8806F (Purolite® C18) was kindly donated by Purolite® Lta. (Wales, UK). Other reagents were used in analytical grade.

2.2 BABASSU OIL CHARACTERIZATION

Babassu oil was characterized according to the acid value, saponification value, iodine value, humidity, density and fatty acid composition, using the Cd 3d-63, Cd 3-25, Cd 1-25, Ca 10a-25, Cc 10a-25 and Ce 1-62 methods, respectively, from the AOCS - American Oil Chemists' Society. Viscosity was measured at 35 ºC between 10 and 160 rpm by the Brookfield rheometer (DV-III ULTRA model, AMETEK Brookfield, Middleborough, MA, USA).

2.3 EVERSA® 2.0 IMMOBILIZATION

The Eversa® enzyme was immobilized by adsorption on the Purolite® C18 support in 5 mM sodium phosphate buffer solution pH 7 (1:10 support/enzyme solution ratio, g/mL) and remained under gentle stirring at room temperature for 6 hours. The enzyme load provided was 20 mg/g of support. Supernatant samples were taken every 1 hour in order to monitor enzyme activity and protein concentration. A control solution containing buffer and enzyme was kept under the same immobilization conditions. Protein concentration was measured spectrophotometrically in an Ultrospec 2000 spectrophotometer (Pharmacia Biotech, Uppsala, Sweden) using the Bradford method[7] and activity was measured by titrating the tributyrin hydrolysis with a 20mM KOH solution using an automatic titrator, according to Beisson's method[8] (Titrando 907 titrator, Metrohm (Herisau, Switzerland).

2.4. INTERESTERIFICATION REATION

The reaction was carried out in a shaker incubator (SL 222 model, Solab, Piracicaba, SP, Brazil) at 40ºC, 250 rpm, an enzyme load of 1500 TBU/g oil) and a molar ratio of 1:9 babassu oil/ethyl acetate. Samples were taken every 12 hours for a total of 120 hours. The 1:9 ratio was chosen based on the study by Muhammad et al.[9] who obtained a higher yield of fatty acids methyl esters (FAME) in a palm oil interesterification reaction with methyl acetate using the immobilized enzyme CalA. The samples were analyzed in an Agilent Technologies 7890A gas chromatograph (Santa Clara, CA, USA). Mass yield calculations of FAEEs were made in accordance with European standard EN 14103.

3 RESULTS & DISCUSSION

3.1 BABASSU OIL CHARACTERIZATION

Table 1 shows experimental values for physical and chemical properties and fatty acid composition of babassu oil. The relative density, iodine value, saponification value and viscosity values agree with those previously reported, with compliance greater than 95%[10]. Regarding the acid value, the result was around three times higher than that reported by Lima (0.500 mg KOH/g)[10], which can be explained by the different origins of the raw material. However, a high value of free fatty acids does not cause problems for the enzymatic reaction, unlike the chemical catalyst which forms unwanted reactions. The relative humidity was very low (around 1%), what is desirable for an interesterification reaction. Low humidity for babassu oil was also reported previously[11].

Babassu oil is mainly composed of lauric acid, which accounts for approximately 44% of its weight. In addition to the C12 chain, its main fatty acids are myristic acid (C14) with around 16% and oleic acid (C18:1) with 12%. The results agree very well with commercial technical data, which have reference values of 40 to 55 % for the C12 structure, 11 to 27 % for C14 and 9 to 20 % for oleic acid[12].

Table 1: Babassu Oil's Characterization

Physical-chemical Properties	Value	Fatty acid composition	% weight average	Fatty acid composition	% weight average
Density	0.9143 g/mL	C6	0.57%	C17	0.03%
Relative Humidity	1.0609%	C8	6.90%	C18	3.68%
Acid Value	1.3393 mg KOH/g	C10	5.85%	C18:1	12.22%
Saponification Value	255.531 mg KOH/g	C11	0.03%	C18:2	1.88%
Molar Mass	693.7621 g/mol	C12	43.65%	C18:2 trans	0.12%
Iodine Value	16.1614 I_2/100g	C13	0.04%	C20	0.07%
Viscosity (35°C)	31.7079 mm^2/s	C14	15.91%	C18:3	0.24%
		C16	8.73%	C:20:1	0.05%
		C16:1	0.03%		

3.1 ENZYME IMMOBILIZATION

After 4 hours of immobilizing Eversa® 2.0 lipase on Purolite (25ºC and a protein load of 20 mg/g of support), it was observed that all enzyme had been immobilized on the support, since both the protein measurement and the enzymatic activity of the supernatant were close to zero (Figure 1). The control solution of the free enzyme did not show great variations in enzyme activity or protein concentration. The immobilization yield was 97.83% (theoretically, 50000 TBU/g support).

3.3 INTERESTERIFICATION OF BABASSU OIL

In the interesterification reaction with babassu oil/ethyl acetate in a 1:9 ratio using immobilized Eversa® 2.0, with an enzyme load of 2.8 wt.% (theoretically, 1500 TBU/g oil), a maximum mass yield of 53.02 ± 3.01% was observed after 108 hours of reaction. However, taking into account the deviations, no significant increases in the conversion was observed after 96 hours of reaction (approximately 50%) (Figure 2B).

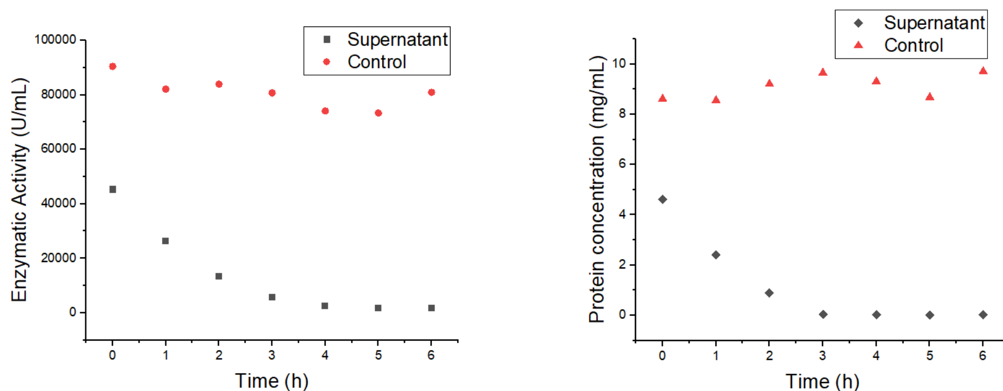

Figure 1: Immobilization profile of Eversa® Transform 2.0 on Purolite® C18. (A) (●) Enzyme activity of the control solution. (■) Enzyme activity of the supernatant. (B) (▲) Protein concentration of the control solution. (♦) Protein concentration of the supernatant.

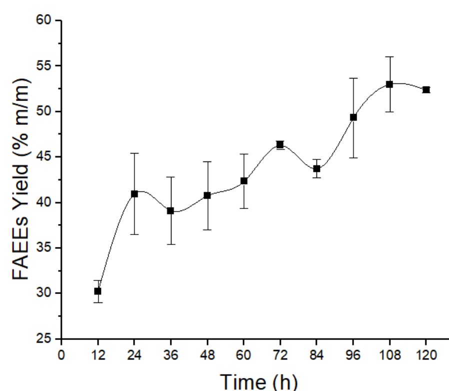

Figure 2: Yield of FAEEs (% m/m) from the interesterification of babassu oil (molar ratio 1:9 oil/ethyl acetate) during a 120-hour reaction (40ºC, 250 rpm) catalyzed by Eversa® 2.0 immobilized on Purolite® C18.

CONCLUSION

The production of biodiesel via Eversa® Transform 2.0 immobilized on Purolite® C18 support proved to be promising, since even under conditions that have not yet been optimized, conversion of around 50% was achieved. Further experiments are needed to analyze the production of triacetin, with the aim of making the reaction competitive with the conventional one. In addition, the EN14103 standard methodology is not ideal for analyzing FAEE yields, since babassu oil is mainly composed of C12 fatty acids and the European standard is effective for vegetable oils with chains between C14 and C24, requiring an adaptation or even a change in the method used.

REFERENCES

1. MASSON-DELMOTTE, V., ZHAI, P., PIRANI, A., CONNORS, S. L., PÉAN, C., BERGER, S., CAUD, N., CHEN, Y., GOLDFARB L., GOMIS, M. I., HUANG, M., LEITZELL, K., LONNOY, E., METTHEWS, J.B.R., MAYCOCK, T. K.,WATERFIELD, T., YELEKCI, O., YU, R., ZHOU, B. Cambridge Uni Press. 2021. 26.
2. International Energy Agency. World Energy Outlook, 2023.
3. ANP - Agência Nacional do Petróleo, Gás Natural e Biocombustíveis. Produção Nacional de Biodiesel Puro - B100 (m³), 2023.
4. KUSUMANINGTYAS, R. D., PURNAMASARI, I., MAHMUDATI, R., PRASETIAWAN, H.Chapter 17 : Interesterification reaction of vegetable oil and alkyl acetate as alternative route for glycerol-free biodiesel synthesis. Biofuels and Bioenergy: A Techno-Economic Approach, GURUNATHAN, B., SAHADEVAN, R. v 1.1° ed. Chennai.2022. p 435-452.
5. MASSON-DELMOTTE, V., ZHAI, P., PIRANI, A., CONNORS, S. L., PÉAN, C., BERGER, S., CAUD, N., CHEN, Y., GOLDFARB L., GOMIS, M. I., HUANG, M., LEITZELL, K., LONNOY, E., METTHEWS, J.B.R., MAYCOCK, T. K.,WATERFIELD, T., YELEKCI, O., YU, R., ZHOU, B. Cambridge Uni Press. 2021. 26.
6. KUSUMANINGTYAS, R. D., NORMALIZA, N., ANISA, E. D. N., PRASETIAWAN, H., HARTANTO, D., VENY, H., HAMZAH, F., RODHI, M. N. M. Energies. 2022. 15 (20). 7737.
7. Bradford, M. M.; Anal. Biochem. 1976, 72, 248
8. BEISSON, F. et al. Methods for lipase detection and assay: a critical review. European Journal of Lipid Science and Technology, v. 102, p. 133‑153, 2000.
9. Z. A. Muhammad, V. Harumi, H. Fazlena, N. M. R. Miradatul, C. Andri, D. Ratna, Haniif, Dhoni. Bulletin of Chemical Reaction Engineering & Catalysis, 18 (2023), 2.
10. Lima, Rafael. Ficha de informação técnica: óleo de babaçu (coco). Betim, 2018.
11. SALES, A. R. R., ALBUQUERQUE, T. N, XAVIER, L. E., SANTANA, A. G., SILVA, O. S., Costa, S. S., Fonseca, S. B., MEIREL, B. R. L. A. Brazilian Journal of Development, 2020, p. 25734–25748, 2020.
12. Campestre. Óleo de coco babaçu – ficha técnica, s.d.

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