USE OF THE ELETRONIC NOSE ASSOCITED WITH CLASSICAL TECHNIQUES FOR ETHYL ESTERS ANALYSIS

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ABSTRACT

This paper aims to synthesize biodiesel via the ethanolysis process applied to babassu oil, employing *Candida antarctica* Lipase B (Novozym® 435) as the catalyst. This entailed the production of ethyl esters through a batch reaction conducted at 45°C, within a solvent-free environment. The resultant product underwent comprehensive analysis, encompassing viscosity and density assessments, culminating in a remarkable 98% yield in ethyl esters. Additionally, this research introduced the utilization of an electronic nose as a novel analytical approach for biodiesel evaluation, juxtaposed against conventional methodologies. To validate its efficacy, the olfactory profiles of Babassu Biodiesel were compared with those of Babassu oil, with 10 and 20 readings respectively. Leveraging decision trees trained with a dedicated validation set, it was discerned that a remarkable 100% distinction could be achieved between Babassu Biodiesel and Babassu oil.

Keywords: Biodiesel. Biocatalysis. Energy. Eletronic Nose. Lipase.

1 INTRODUCTION

Over the past decades, the increasing societal concern for environmental sustainability has driven the exploration of energy sources with reduced environmental impacts. Consequently, renewable energy technologies have garnered significant attention due to their capacity for continuous renewal and minimal ecological footprint. Within this domain, biomass has emerged as a prominent energy matrix[1]. Specifically, the substitution of conventional diesel with biodiesel—a fuel derived from the transesterification of short-chain alcohols and vegetable oils—presents a promising alternative. Nonetheless, for biodiesel production to be economically viable and socially acceptable, it is imperative that the feedstock oils do not compete with food resources to prevent escalation in food prices[2]. In the Brazilian context, babassu oil, which is extensively cultivated in the northeastern region of the country, represents a promising lipid source. Concurrently, ethanol, abundantly produced in Brazil, serves as an optimal short-chain alcohol for this process[3].

For efficient biodiesel production, the implementation of heterogeneous catalysts is essential, as they facilitate easier purification and recovery of the catalyst while maintaining high performance. In this context, the use of lipases presents a viable alternative, given their ability to catalyze both esterification and transesterification reactions. Specifically, *Candida antarctica* Lipase B (Novozym® 435) has demonstrated efficacy in biodiesel production, achieving satisfactory conversion levels[4].

This study aimed to produce biodiesel through the enzymatic ethanolysis of babassu oil. The resulting biodiesel was analyzed using classical characterization techniques and compared with the analysis of the ester profile obtained via an electronic nose.

2 MATERIAL & METHODS

Feedstock and catalyst

Babassu oil was utilized as the source of lipid material, while anhydrous ethanol served as the acyl group acceptor. The enzymatic syntheses were conducted employing *Candida antarctica* Lipase B (Novozym® 435) as the catalyst.

Synthesis

The reaction was conducted in a jacketed cylindrical glass reactor (6 mm height × 4 mm internal diameter, 50 mL capacity) equipped with a reflux condenser. The reactor contained 30 g of a substrate composed of feedstock and ethanol at a molar ratio of 1:15, without the addition of solvents. The mixtures were incubated with Candida antarctica Lipase B (Novozym® 435) at a specified enzyme-to-reaction medium ratio. The reaction proceeded for 48 hours under constant agitation (150 rpm) at a temperature of 45°C.

Purification

Upon completion of the reaction, the reactor contents were removed and fractionated. A 1.5 mL aliquot of the raw biodiesel was isolated for analysis using an electronic nose. The remaining sample was transferred to a Falcon tube and centrifuged for 7 minutes at 2600 rpm. The supernatant, containing ethyl esters, was separated from the enzyme, which settled at the bottom of the tube. Glycerol, formed as a by-product, was removed by dry washing with chamotte clay. Excess ethanol was subsequently evaporated using a rotary evaporator at 80°C for 30 minutes[5].

Analysis

Density values were measured using a digital densimeter (Model DMA 35n EX, Anton Paar) at a temperature of 20°C, with a sample volume of 2 mL. Viscosity measurements were performed with a Brookfield Viscometer (Model LVDVII, Brookfield Viscometers Ltd, England) at 40°C. The determination of ethyl fatty acid esters (FAEE) followed the methodology previously established by Urioste et al. (2008)[6].

The olfactory profile analysis was performed using an electronic nose (Model Cyranose 320, Smith Detection). For this analysis, 1 mL aliquots of babassu oil and purified biodiesel were prepared in five series. In total, 10 olfactory profile readings were conducted for babassu oil and 20 for the biodiesel compound. Utilizing the model developed by Siqueira et al. (2018)[7], seven parameters (a, b, c, k, p, m, and 1/k) were obtained for each olfactory profile from the 32 sensors available in the Cyranose. To classify the olfactory profiles, the statistical technique of decision trees was employed using Minitab 22 software. The decision tree model was trained with a training set comprising 5 biodiesel olfactory profiles and 9 babassu oil profiles, and tested with a test set consisting of 5 biodiesel olfactory profiles and 11 babassu oil profiles.

3 RESULTS & DISCUSSION

The properties measured for both the oil and the biodiesel are detailed in Table 1.

Table 1 Proprieties of babassu oil and biodiesel

Properties	Babassu oil	Biodiesel
Viscosity (mm^2/s)	29,59$\pm$ 0,01	3,5$\pm$ 0,02
Density (g/cm^3)	0,8709 $\pm$ 0,03	0,8698$\pm$ 0,03
Yeld(%)	-	98 $\pm$ 0,04

The data obtained reflect the quality of the analyzed compounds. According to the National Petroleum Agency (ANP), biodiesel should exhibit a kinematic viscosity between 3.0 and 6.0 mm^2/s. The produced biodiesel conforms to this standard, with a viscosity of 3.5 mm^2/s. In contrast, babassu oil exhibited a kinematic viscosity of 29.59 mm^2/s. The effectiveness of the transesterification reaction is demonstrated by the substantial reduction in the viscosity of babassu oil, thereby meeting the ANP's specified parameters.

Regarding the olfactory profile, Figure 1 illustrates the characteristic behavior of the olfactory profile obtained during the analysis.

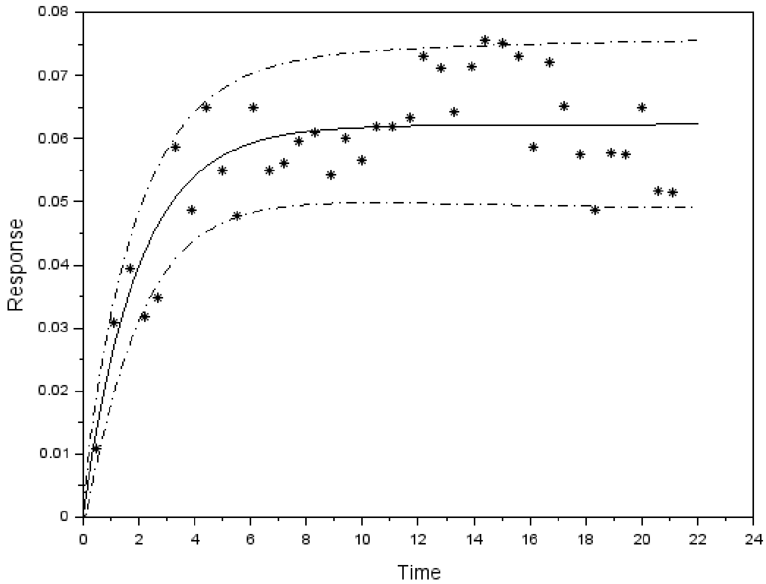

Figure 1 Olfactory profile generated by Crynose for the Biodiesel sample.

The response is measured as the variation in electrical resistance when volatile material adheres to the sensor surface. The solid line represents the average behavior as predicted by the model developed by Siqueira et al. (2018), while the dashed lines denote the 95% confidence interval.

Following the training and testing of the decision trees, the selected model was developed exclusively based on the value of parameter m from sensor 6. The corresponding confusion matrix is presented in Table 2.

Table 2 Confusion matrix

	Predicted class (Training)				Predicted class (Test)			
Real Class	Count	1	0	Correct (%)	Count	1	0	Correct (%)
1 (Event)	5	5	0	100.0	5	5	0	100.0
0	9	0	9	100.0	11	0	11	100.0
All	14	5	9	100.0	16	5	11	100.0

Statistics	Training (%)	Test (%)
True positive rate (sensibility or recall)	100.0	100.0
False positive rate (type I error rate)	0.0	0.0
False negative rate (type II error rate)	0.0	0.0
True negative rate (specificity)	100.0	100.0

The analysis reveals that the chosen decision tree demonstrated exceptional efficacy in identifying the origin of the odor profile, achieving a perfect classification accuracy of 100%, with no occurrences of false positives or false negatives.

4 CONCLUSION

The alignment of the produced biodiesel with the standards stipulated by the National Petroleum Agency (ANP) substantiates the efficacy of the transesterification process applied to babassu oil. This notable decrease in viscosity underscores the viability of babassu oil as a promising feedstock for biodiesel production and underscores the efficiency of the employed biocatalyst. Despite the modest sample size, the olfactory profile recognition technique demonstrates considerable potential, particularly when considering that the classification method relied solely on data from a single parameter of sensor 6 of the device.

REFERENCES

1 DA SILVA, Simão Pereira et al. A importância da biomassa na matriz energética brasileira. Pensar Acadêmico, v. 19, n. 2, p. 557-583, 2021.

2 ZAMBARE, Vasudeo et al. Recent advances in feedstock and lipase research and development towards commercialization of enzymatic biodiesel. Processes, v. 9, n. 10, p. 1743, 2021.

3 CRUZ, Glauber et al. Biofuels from oilseed fruits using different thermochemicalprocesses: opportunities and challenges. Biofuels, Bioproducts and Biorefining, v. 14, n. 3, p. 696-719, 2020.

4 TONGBORIBOON, Ketsara et al. Mixed lipases for efficient enzymatic synthesis of biodiesel from used palm oil and ethanol in a solvent-free system. Journal of Molecular Catalysis B: Enzymatic, v. 67, n. 1-2, p. 52-59, 2010.

5 CARVALHO, Ana Karine F. et al. Sustainable enzymatic approaches in a fungal lipid biorefinery based in sugarcane bagasse hydrolysate as carbon source. Bioresource technology, v. 276, p. 269-275, 2019.

6 URIOSTE, Daniele et al. Synthesis of chromatographic standards and establishment of a method for the quantification of the fatty ester composition of biodiesel from babassu oil. 2008.

7 SIQUEIRA, A. F.; MELO, M.P.; GIORDANI, D.S.; GALHARDI, D.R.V.; SANTOS, B.B.; BATISTA, P.S.; FERREIRA, A.L.G. Stochastic modeling of the transient regime of an electronic nose for waste cooking oil classification. Journal of Food Engineering, v. 221, p. 114-123, 2018.

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