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UTILIZATION OF IMMOBILIZED LIPASES AS CATALYSTS IN THE TRANSESTERIFICATION OF NON-EDIBLE VEGETABLE OILS WITH ETHANOL

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Abstract - This work reports the use of commercially available immobilized lipase preparations (Novozym® 435 and Lipozyme TL IM, both from Novozymes, and Lipase PS IM from Amano) as catalysts in the transesterification reaction of different alkyl-chain triglycerides with ethanol. The ethanolysis of native oils from Brazilian Amazon plants andiroba (*Carapa guianensis*), babassu (*Orbignya* sp.), jatropa (*Jatropha curcas*), and palm (*Elaeis* sp.) was studied in a solvent-free system. In a typical reaction, the immobilized preparations were added to the mixture of vegetable oil-to-ethanol in a molar ratio of 1:9. The reactions were performed at 50 °C for a maximum period of 48 h. Under the conditions used, all the immobilized lipase preparations were able to generate the main esters of fatty acids present in the tested feedstocks, and both the reaction rate and ester yield were dependent on the source of lipase and vegetable oil. The viscosity values for the samples obtained in each reaction displayed a consistent reduction in relation to their original feedstocks, which also confirms the high conversion of triglycerides to ethyl esters (99.8-74.0%). The best performances were obtained with Amano PS IM and Novozym® 435, with the biodiesel samples from the babassu and jatropha oils exhibiting viscosity values in accordance with those predicted by the technical standards of ASTM D6751 (1.9-6.0 mm^2/s). Lipozyme TL IM displayed an unsatisfactory performance, indicating that the conditions of the transesterification reaction should be improved. This comparative study using different catalysts and several vegetable oil sources with varying fatty acid compositions is particularly important for all tropical countries with a diversity of native vegetable oil sources.
Keywords: Lipase; Biocatalyst; Non-edible feedstock; Biodiesel; Ethanol.

INTRODUCTION

The current increased global demand for liquid fuels in addition to global warming, energy security, and political development in the fields of agriculture and energy have opened new areas of interest and opportunities for research in both academia and industry because these points of concern are responsible for renewed interest in biofuels (Abbaszaadeh *et al.*, 2012; Knothe *et al.*, 2005).

Biodiesel derived from triglycerides or free fatty acids by transesterification or esterification with short-chain alcohols has attracted considerable attention during the past decade as a renewable, biodegradable, and nontoxic fuel (Abbaszaadeh *et al.*, 2012). Although biodiesel has been successfully produced chemically, there are still several process issues that require further development, such as glycerol recovery and the removal of inorganic salts. The disadvantages caused by chemical catalysts are largely avoided by using lipases as catalysts (Zhang *et al.*, 2012; Fjerbaek *et al*., 2009).

Lipases, also known as triacylglycerol hydrolases (E.C.3.1.1.3), are enzymes that preferentially cata-

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lyze the hydrolysis of acylglycerols at the oil/water interface. The hydrolytic reactions catalyzed by lipases are generally reversible, and the potential of these enzymes in organic synthesis has been well studied and recently reviewed by Kapoor and Gupta (2012), who emphasize the versatility of these enzymes as biocatalysts. Such versatility is also evident by the number of processes implemented on a large scale, particularly in the oleochemical industry, which has a permanent interest in controlling the physical, functional, and organoleptical properties of the products. Furthermore, recent interest in the use of lipases in the form of free or immobilized enzymes is gaining increasing attention for the production of biodiesel (Ribeiro *et al.*, 2011; Narwal and Gupta, 2013).

Different lipase sources have been utilized in biodiesel syntheses, and many have been found to have limited performance because of their fatty acid chain-length specificity, substrate specificity, and regioselectivity. However, the majority of lipases are capable of converting triglycerides, diglycerides, monoglycerides, and free fatty acids into methyl or ethyl fatty esters. The most active lipases for biodiesel production include those derived from *Candida antarctica* B, *Rhizomucor miehei*, *Burkholderia* (*Pseudomonas*) *cepacia*, and *Thermomyces lanuginosus* (Ribeiro *et al,* 2011; Adlercreutz, 2013).

In addition to the lipase source, other important variables of the enzymatic process include temperature, reaction time, oil-to-alcohol molar ratio, type of acyl acceptor, and stirring intensity. Moreover, the type of vegetable oil and chain length are also important parameters that influence the overall efficiency of the process (Fjerbaek *et al*., 2009; Ribeiro *et al*, 2011).

Methanol is commonly used an acyl acceptor because of its suitable physical and chemical properties and low cost (Marjanović *et al*., 2010). However, problems such as the toxicity of methanol and the risk of explosion motivate the search for alternatives, and the use of ethanol has emerged as an interesting alternative (Stamenković *et al.*, 2012). Furthermore, ethanol is a larger and heavier alcohol than methanol, leading to a mass yield gain in the enzymatic synthesis of fatty acid ethyl esters (FAEEs) that results in a higher biodiesel mass per unit mass of oil (Vieitez *et al*., 2010). In some countries, ethanol is sold at lower prices than methanol; thus, the alcohol component is always significantly less expensive than the oil component. Accordingly, the extra mass or volume gain when using ethanol instead of methanol could become a major sales argument, particularly for the Brazilian market (Stamenkovic *et al*., 2011; Brunschwig *et al*., 2012).

Vegetable oil production in Brazil is anticipated to increase because the country has approximately 150 million hectares that could be incorporated into agricultural production, 90 million related to new borders, and 60 million for land and pastures (Takahashi and Ortega, 2010). These areas are distributed throughout the country, and oilseed production requires edaphoclimatic conditions that are very favorable in Brazil. Therefore, crops such as soybeans, corn, peanut, sunflower, babassu, and palm could be exploited for the production of biodiesel (Bergmann *et al.*, 2013). It is also clear that the search for beneficial biodiesel sources should focus on feedstocks that do not compete with food crops, do not lead to land clearing, and provide greenhouse-gas reductions. These feedstocks include such high-yielding (Table 1), non-edible tropical crops as andiroba (*Carapa guianensis*), babassu (*Orbignya* sp.), jatropha (*Jatropha curcas*), and palm trees (*Elaeis guineensis*). Large amounts of these non-edible oil plants are available in several regions of Brazil (Bergmann *et al*, 2013) at a low exploration cost.

Based on the aspects mentioned above, this study aimed to assess the most appropriate source of lipase among a selection of available enzymes to synthesize biodiesel from different non-edible vegetable oils in the presence of ethanol, considering the overall efficiency and productivity of the reaction as process evaluation parameters. The lipases chosen are commercially available in immobilized form and include non-specific lipases (derived from *Candida antarctica* B and *Burkholderia cepacia*) and 1,3 specific lipases (derived from *Thermomyces lanuginosus*), allowing comparisons with the published data.

MATERIALS AND METHODS

Materials

Andiroba oil (Formil Chemistry Florabrasil-Aracaju, SE, Brazil), babassu oil (Cognis, Jacarei, SP, Brazil), jatropha oil (IAPAR, Londrina, PR, Brazil), and palm oil (Agropalma, Belém, PA, Brazil) were used as the feedstocks. Ethanol (minimum 99%) from Cromoline (São Paulo, SP, Brazil) was used as the acyl acceptor. Several commercial immobilized lipase preparations, including Lipase PS IM purchased from Amano Pharmaceuticals (Nagoya, Japan) and Lipozyme TL IM and Novozym® 435 kindly donated by Novozymes Latin American (Araucaria, PR, Brazil), were tested as biocatalysts; additional information on these preparations is provided in Table 2 based on manufacturer-supplied information. Other reagents and solvents were of standard laboratory grade.

Table 1: Characteristics of the selected tropical crops used in this work.

Feedstock	Region	Oil content (%)	Oil yield L/ha/year	Plant cycle	Available cultivation area
Andiroba (*Carapa guianensis*)	W Antilles, Central America south of Honduras, many parts of the Amazon and tropical region Brazilian and Africa	43	1420	2–3-year cycle	Brazilian Amazon has 25.7 trees per hectare on dry land forest and 14.6 in inundated forest.
Babassu (*Orbignya* sp.)	Northeast, North and Midwest Brazilian	45-60	2689	perennial plant	In Brazil babassu production area occupies about 17 million ha. The largest concentration area of babassu is the Northeast, with the State of Maranhão being the major producer.
Jatropha *(Jatropha curcas* L.)	Mexico, Central America, Africa, India, Brazil, Argentina and Paraguay	40-50	1892	perennial plant	The total cultivation area of Jatropha is 900,000 ha globally among which 84.4% (760,000 ha) is in Asia, 13.3% (120,000 ha) in Africa and 2.23% (20,000 ha) in Latin America.
Palm (*Elaeis* sp.)	Nearly 80% is located in Malaysia and Indonesia. West Africa and is now widespread throughout the tropical areas of America and South East Asia	30-60	5950	perennial plant	Together, all palm oil producing countries account for a cultivated area of 2 million hectares. Brazil has palm oil production of 1,292,713t in 108,919 ha

Sources: Atabani *et al*., 2012; Bergman *et al*., 2013; Klimas *et al*., 2007; Lopes and Neto Steidle, 2011.

Table 2: Some characteristics of the microbial lipases used in the present work.

Trade name	Lipase PS IM	Lipozyme® TL IM	Novozym® 435
Supplier	Amano	Novozymes	Novozymes
Microorganism	*Burkholderia cepacia*	*Thermomyces lanuginosus*	*Candida antarctica*
Support	Diatomaceous earth	porous silica granules	macroporous acrylic resin bead-shaped
Substrate specificity	Nonspecific	1-3-specific	Nonspecific
Temperature optimum (°C)	25-60	40-60	40-60

Biodiesel Synthesis

The reactions were performed in a jacketed cylindrical glass reactor (60 mm high X 40 mm internal diameter, with a capacity of 100 mL) containing 20 g of substrate consisting of vegetable oil and ethanol mixtures at a molar ratio of 1:9, without the addition of solvent. Mixtures were incubated with immobilized lipase preparations at the fixed proportion of 250 units of activity per gram of vegetable oil. The reactions were performed at 50 °C for a maximum period of 48 h under constant magnetic agitation of 150 rpm. For the time-course studies, an aliquot of the reaction medium was removed at various time intervals and diluted in *n*-hexane for GC analysis.

Purification of Biodiesel

When the reaction was completed, the lipase was separated from the medium, and the organic phase was washed twice with water to remove both the remaining ethanol and the free glycerol as a by-product. Any residual water was removed by rotary evaporation to attain the final fatty acid ethyl ester product.

Vegetable Oil Characterization

The physicochemical properties (iodine value, saponification value, acid value, peroxide value, and viscosity) were determined following the methods described by the American Oil Chemists' Society (AOCS, 2004). Fatty acid composition was determined by capillary gas chromatography using a CGC Agilent 6850 Series GC System with the following capillary column: DB-23 Agilent (50% cyanopropyl) with methylpolysiloxane (size 60 m, Ø int 0.25 mm, 0.25 mM) film. Helium was used as the carrier gas at a rate of 1.00 mL/min, with a linear speed of 24 cm sec^{-1}. The temperatures of the detector and injector

were set at 280 ºC and 250 ºC, respectively. The column temperature was kept at 100 °C for 5 min, heated to 215 ºC at 5 ºC min^{-1}, and kept constant for 34 min. The volume injected was 1.0 μL.

Monitoring Ethyl Ester Formation

The formation of ethyl esters from the ethanolysis of different feedstocks was analyzed by gas chromatography using a Varian CG 3800 GC (Inc. Corporate Headquarters, Palo Alto, CA, USA) equipped with a flame-ionization detector and 5% DEGS CHR-WHP 80/100 mesh (6 ft 2.0 mm ID and 1/8'' OD; Restek, Frankel Commerce of Analytic Instruments Ltd., SP, Brazil). Nitrogen was used as the carrier gas at a flow rate of 25 mL min^{-1}. Temperature programming was performed: the column temperature was kept at 90 °C for 3 min, heated to 120 ºC at 25 ºC min^{-1}, and kept constant for 10 min; the column temperature was then programmed at 25 ºC min^{-1} to 170 ºC and kept constant for 15 min. The temperatures of the injector and detector were set at 250 ºC. The data collection and analyses were performed using the software Galaxie Chromatography Data System version 1.9. The calibration curves were generated with standard ethyl esters using hexanol as the internal standard. The reaction yield was calculated by taking into account the mass of ester content obtained by GC analysis and the total theoretical ester mass based on the initial amount of both oil and ethanol (Urioste *et al*., 2008; Silva *et al.*, 2012).

Viscosity and Density Determinations of Purified Fatty Acid Ethyl Esters (FAEEs)

The absolute viscosity of biodiesel was determined using an LVDV-II cone and a plate spindle Brookfield viscometer (Brookfield Viscometers Ltd, England) with a CP 42 cone. A circulating water bath was used to maintain the temperature for each analysis at 40 °C, with an accuracy of 0.1 °C. The shear stress measurements were taken as a function of the shear rate, and the dynamic viscosity was determined as the slope constant. Samples of 0.5 mL were used, and the measurements were replicated three times. The density of biodiesel was determined with a DMA 35N EX digital densimeter (Anton Paar). The temperature was maintained at 20 °C during the assays. Biodiesel samples of 2.0 mL were used, and the measurements replicated three times (Silva *et al.*, 2012).

RESULTS AND DISCUSSION

Physicochemical properties, including the fatty acid profile, are important for determining the suitability of a feedstock for the production of biodiesel. Indeed, the feedstock quality influences both the transesterification reaction and the quality of the biodiesel generated. The feedstocks used in this study were characterized according to the methodology recommended by official methods, and the most important properties are shown in Table 3.

Table 3: Physicochemical properties and fatty acids composition of non-edible vegetal oils for biodiesel production.

	Andiroba	Babassu	Jatropha	Palm
Kinematic viscosity at 40 °C (mm^2/s)	40.6	29.5	34.5	36.8
Acid value (mg KOH/g of oil)	0.8	0.65	0.30	0.33
Saponification value (mg KOH/ g of oil)	194	238	141	198
Iodine value (g I_2/ 100 g of oil)	72	25	101	98
Peroxide value (meq/ kg of oil)	30.0	1.82	4.2	2.1
Fatty acid composition (% wt)				
Caprylic acid (C8)		3.5		
Capric acid (C10)		4.5		
Lauric acid (C12)	0.1	44.7	0.1	0.10
Myristic acid (C14)	0.1	17.5	0.1	1.20
Palmitic acid (C16)	29.0	9.7	12.9	46.8
Stearic acid (C18)	10.0	3.1	5.6	3.8
Oleic acid (C18:1)	47.0	15.2	39.8	37.6
Linoleic acid (C18:2)	10.7	1.8	40.0	10.5
Linolenic (C18:3)	0.3		0.2	
Arachidic (C20:0)	1.3		0.2	
Gadoleic (C20:1)	0.1		0.1	
Behenic (C22:0)	0.3		0.1	
Lignoceric (C24:0)	0.2		0.1	
Saturated (%wt)	41.1	83.0	19.0	51.9
Unsaturated (%wt)	58.9	17.0	80.9	48.1

Acidity and peroxide values reveal the deterioration of the feedstock in terms of hydrolytic rancidity according to the acidity (concentration of free fatty acids) and oxidative rancidity according to the peroxide index. The saponification index is useful for determining the average molecular mass and the expected deterioration rate, and the iodine index reveals the extent of feedstock unsaturation.

The estimated acidity index ranged from 0.3 to 0.8 mg KOH/g, displaying no traces of hydrolytic rancidity, thereby allowing a high yield and preventing corrosion problems in diesel engines (Bancovik-llic *et al.*, 2012).

The peroxide contents of all the feedstocks were adequate, except for the andiroba oil, which displayed a high peroxide content (30 meq/ kg) that can be associated with the long storage period in the laboratory. This value may have a negative influence on biochemical catalysis, as reported in the literature, which also recommends values lower than 5 meq/ kg to avoid enzymatic inhibition (Ibrahim *et al.*, 2007).

The iodine index indicates the unsaturation level of the feedstock, and this value varied between 25 and 101 gI_2 100 g^{-1}, satisfying an important characteristic of a material to be used as a feedstock in biofuel production. The highest content of unsaturated fatty acids was found for jatropha oil, with a value of 101 g I_2 100 g^{-1}. Studies conducted by Knothe (2002) revealed that a value higher than 115 g I_2 100 g^{-1} indicates a low oxidative stability, which may limit industrial application.

The saponification index of babassu oil was 238 mg KOH g^{-1}, and jatropha oil had the lowest value (141 mg KOH g^{-1}). This difference may be explained by the lower molecular weight fatty acid composition of babassu oil. The saponification value found for the babassu oil is compatible with the literature (Lima *et al*., 2007).

Fatty acids may differ according to their carbon chain length and number of double bonds (unsaturation level), and the physical and chemical properties of biodiesel depend on the type of feedstock and the fatty acid composition.

The fatty acid profiles of the selected feedstocks (Table 3) indicate a well-diversified composition, allowing verification of the biocatalyst performance in terms of the reaction selectivity. The size and number of carbon chain unsaturations are also a decisive factor when determining some biodiesel properties (Knothe *et al*., 2005).

The fatty acid (FA) profile of the vegetable oils characterized by gas chromatography yielded 13 FAs with carbon chains ranging from C8 to C24 and different degrees of unsaturation (17-81%). The majority of FAs were palmitic acid (C16:0), ranging from 9.7% to 46.8%, and oleic (C18:1) and linoleic (C18:2) acids, ranging from 15.2 to 47.0 % and from 10.5 to 40.0%, respectively.

Andiroba oil displayed 41.2% saturated FAs and 58.8% unsaturated FAs, with a greater proportion of oleic (46.9%), linoleic (30.9%), and palmitic (23.5%) acids. Babassu oil had approximately 83% saturated FAs, with a higher proportion of lauric (44.7%), myristic (17.5%), and palmitic (9.7%) acids; the unsaturated FAs obtained were 15.2% oleic (C18:1) and 1.8% linoleic (C18:2) acids. An antagonistic profile was found for jatropha oil, which contained 81% unsaturated and 19% saturated FAs, with linoleic acid being present at a higher proportion (40%), followed by oleic (39.7%) and palmitic (12.9%) acids. It is important to note that babassu oil has the highest concentration of short-chain fatty acids (lower than C12), which are reported to interact more effectively with the acyl agent and catalyst (Lima *et al.*, 2007). Palm oil had 51.9% saturated FAs (46.8% palmitic and 3.8% stearic) and 48.1% unsaturated FAs, with oleic (37.8%) and linoleic (10.5%) acids in the higher proportions.

Biodiesel Enzymatic Synthesis

The feasibility of using vegetable oils to yield biodiesel using ethanol as an acyl acceptor was assessed using three commercially immobilized lipase preparations. Experiments were conducted under a preliminary set of reaction conditions (oil-to-ethanol proportion of 1:9; 50 °C, and 250 units of immobilized lipase per gram of vegetable oil) that may not have been the optimum set for all the feedstocks and lipases tested. The results regarding the transesterification yield as a function of time are shown in Figure 1(a-d) for each of the tested vegetable oils, and the main results are displayed in Table 4. Because Table 4 presents the reaction yield attained by all the biocatalysts for the same period of time under the same conditions, it is possible to compare their activities directly using this parameter.

As observed in Figure 1(a-d), both the reaction rate and ester yield were dependent on the source of lipase and the vegetable oil. It was verified that Amano PS IM and Novozym® 435 had similar initial rates for babassu and palm oils and a slightly slower initial rate for jatropha oil. The lowest performance was attained with Lipozyme TL IM, except in the case of jatropha oil.

The reactions with andiroba oil were found to produce the lowest final yield (Figure 1a and Table 4), which can be attributed to certain features of this

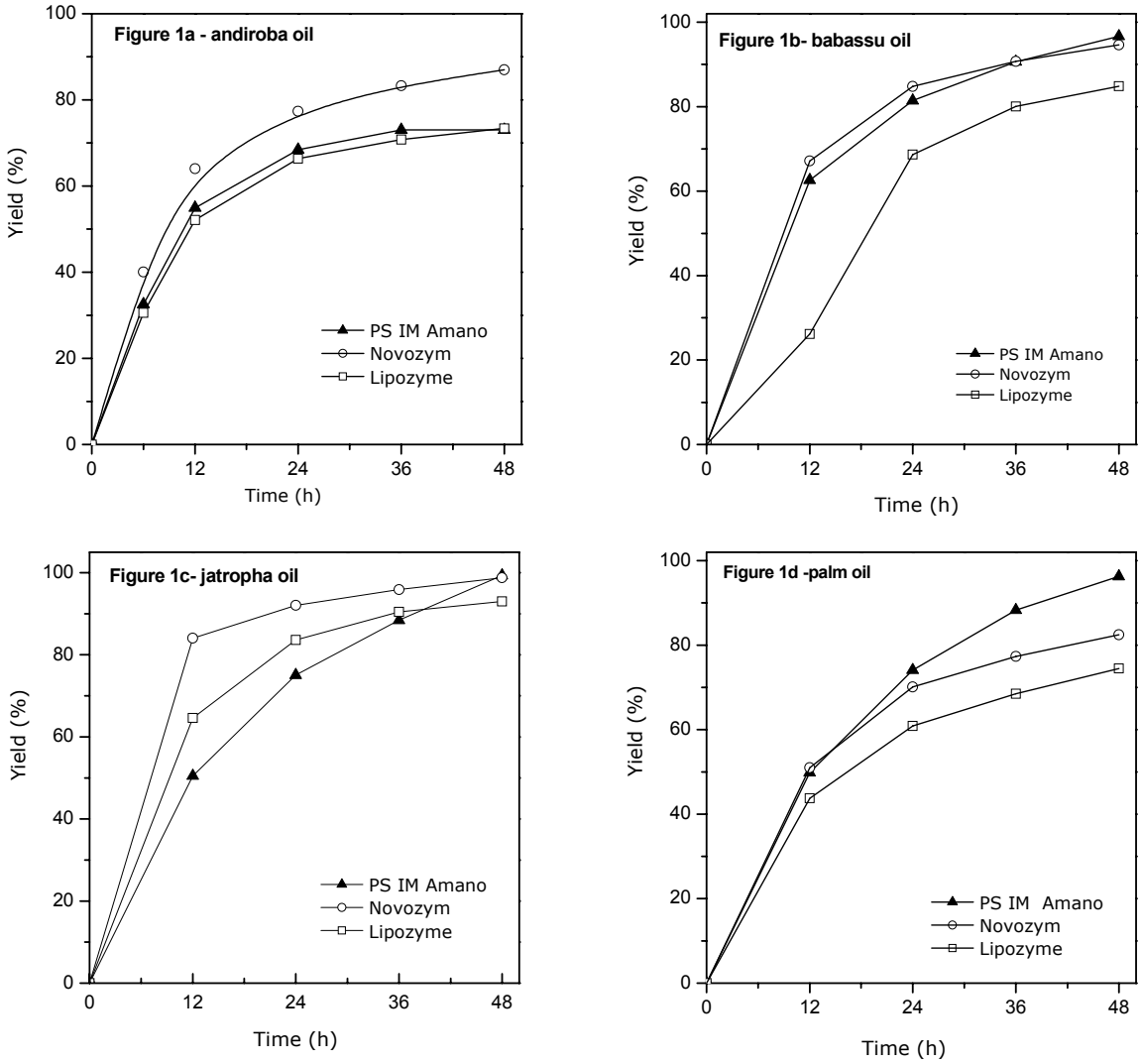

Figure 1: Transesterification yield from non-edible vegetal oils using different immobilized lipases as a function of reaction time: (a) andiroba oil, (b) babassu oil, (c) jatropha oil and (d) palm oil.

Table 4: Transesterification yield, productivity and kinetic viscosity attained in the enzymatic ethanolysis of different feedstock sources.

Vegetal oil	Enzyme	Ethyl ester concentration in the reaction medium (%wt)								Yield (%)	Kinematic viscosity (mm^2/s)
		C8	C10	C12	C14	C16	C18	C18:1	C18:2		
Andiroba	Novozym 435	-	-	-	-	23.4	6.3	28.0	6.8	87.0	8.5
	Lipozyme TL IM	-	-	-	-	21.8	5.8	22.1	5.0	73.8	11.6
	PS Amano IM	-	-	-	-	22.8	6.3	20.2	4.7	72.8	14.8
Babassu	Novozym 435	2.9	3.2	37.2	10.4	5.2	0.1	7.3	1.3	94.9	4.8
	Lipozyme TL IM	3.0	2.9	31.0	8.9	5.1	0.1	7.8	1.4	84.6	6.2
	PS Amano IM	3.6	3.6	38.7	11.7	6.6	0.5	8.4	1.9	96.9	4.9
Jatropha	Novozym 435	-	-	-	-	11.5	9.4	24.6	26.6	98.8	6.3
	Lipozyme TL IM	-	-	-	-	10.8	8.1	24.0	20.0	94.0	7.4
	PS Amano IM	-	-	-	-	12.1	3.0	25.2	39.1	99.8	6.9
Palm	Novozym 435	-	-	-	0.1	28.8	3.1	24.0	5.2	82.7	7.3
	Lipozyme TL IM	-	-	-	-	26.6	2.9	21.0	4.2	74.0	8.2
	PS Amano IM	-	-	-	0.2	37.1	4.4	24.7	5.2	96.7	6.2

feedstock, such as a long storage time during which the oil had oxidized, thereby decreasing the enzyme activity in the transesterification reaction. When using this oil, the highest yield (87.0%) was attained with Novozym® 435, and the performances of Amano PS IM and Lipozyme® TL IM were lower, at 72.8% and 73.8%, respectively. These results are in agreement with the data reported by Wang and Gordon (1991), Posorske *et al*. (1988), and Ohta *et al*. (1989), who indicated that lipases are inactivated by lipid oxidation products in nonaqueous media. In addition, the activity of Lipozyme was found to decrease more rapidly in soybean oil than in olive oil, and this was ascribed to the effect of the lipid oxidation products in the more unsaturated soybean oil (Posorske *et al*., 1988).

The highest yields were achieved with babassu (Figure 1b) and jatropha oils (Figure 1c) for all the tested lipases. For babassu oil, both Amano PS IM and Novozym® 435 displayed similar rates for ethyl ester production, with yields of 98.8% and 94.9%, respectively, whereas Lipozyme® TL IM was much less efficient (84.6%). The jatropha oil yields were 98.9% with Amano PS IM; 98.9% with Novozym® 435, and 94.0% with Lipozyme® TL IM. For jatropha oil (Figure 1c), at the beginning of the reaction, Amano PS IM was the slowest ethyl ester producer but attained the level of Novozym® 435 at 48 h; these two enzymes provided the highest yields, which can be explained by the fact that both lipase preparations are not specific enzymes. In contrast, Lipozyme® TL IM exhibited different behavior with these two oils because it is a 1,3-specific lipase; nonetheless, the results were satisfactory. The lower yield can also be attributed to the role of water in the reaction, which, despite being essential for maintaining the conformational flexibility of the enzyme, may have contributed to reducing the activity of this biocatalyst.

For palm oil, the efficiency of biodiesel production was in the following order: Amano PS IM > Novozym® 435 > Lipozyme® TL IM, with yields of 96.7, 82.7, and 74.0%, respectively (Figure 1d). As opposed to the other transesterification reactions in this study, the synthesis using Amano PS IM had the highest initial rate, and the activity of Novozym® 435 in biodiesel production was again higher than that of Lipozyme® TL IM.

Similar results were reported by Rodrigues *et al.* (2008) in assessing the activity of Lipozyme® TL IM and Novozym® 435 in biodiesel production by ethanolysis of different raw materials, achieving the best results for reactions using Novozym® 435. The lower performance obtained by the Lipozyme® TL IM lipase can be attributed either to its specificity or the role of water in the reaction. The sn-1,3 specificity of lipases can lead to reduced conversion, creating 2-monoacylglycerols as byproducts, and this was proposed as the reason for the lower conversion obtained with the sn-1,3-specific enzyme Lipozyme® TL IM compared to the less regioselective Novozym® 435. The high yields obtained for the conversion of fatty acids in Novozym® 435-catalyzed reactions in the present study can be explained in terms of thermodynamic considerations and the greater resistance of this particular lipase to deactivation in comparison to Lipozyme® TL IM. Novozym® 435 is more robust in the presence of low molecular weight alcohols, which thereby facilitates its ability to catalyze the ethanolysis reaction for longer times. Moreover, the hydrophilic character of the Lipozyme immobilizing support also played a role in the reaction by modifying the partitioning of water between the liquid and solid phases, which also results in drastic changes in the enzyme activity. As Lipozyme consists of a lipase preparation immobilized on an ion exchange resin, it is expected that its use will be accompanied by a high amount of water absorption on the solid enzyme phase, mainly for high-polarity substrates, such as the oil/ethanol system (Silva *et al.*, 2009). This helps to explain the consistently higher yields achieved in the reaction systems catalyzed with both nonspecific lipases compared to the reactions catalyzed with Lipozyme® TL IM.

As different lipases convert different substrate molecules present in the reaction mixture at different rates, it can be advantageous to use a combination of lipases to catalyze the process, for example, a combination of Lipozyme® TL IM and Novozym® 435.

Regarding the acyl acceptor to oil ratio employed, high ratios usually imply greater oil transformation to biodiesel and higher reaction rates when the acyl acceptor does not deactivate the enzyme. By using ethanol as an acyl acceptor, ratios as high as 18 can be utilized to obtain yields higher than 95%, without any inhibitory effect detected (Moreira *et al.*, 2007); however, the feasibility of this depends on the lipase source. Hernandez-Martín and Otero (2007) studied the influence of the molar ratio of ethanol to fatty acid in the deactivation of lipase due to the contact of the enzyme with the immiscible polar organic phase formed because of a lack of complete solubility of the alcohol, in addition to the glycerol byproduct, in the oil phase, with maximum conversion in the trials involving Lipozyme® TL IM. Nonetheless, for the trials involving ethanolysis catalyzed by Novozym® 435, the reaction proceeded to a greater extent in the presence of a large excess of ethanol (Hernandez-

Martín and Otero, 2007).

As observed in Table 4, all the lipases were able to form the main esters of fatty acids present in the tested vegetable oil sources under the conditions used. Additionally, all the lipases displayed similar behavior in terms of selectivity for the fatty acids present in higher concentrations in each material. Although the conditions used in this work were not optimized for the highest reaction yield, they still provide a means to compare the catalytic activities of the immobilized lipases.

The viscosity values for the purified samples obtained in each reaction indicated a consistent reduction in relation to their original feedstock, which also confirms the conversion of triglycerides into ethyl esters (Table 4). The viscosity values for the feedstocks were between 29.5 and 40.6 mm^2/s and sharply decreased upon the transesterification reaction. The transesterification yields ranged from 98.5 to 72.8%, corresponding to viscosity values between 4.8 to 14.8 mm^2/s. Under the conditions tested, the best performance was obtained using Amano PS IM and Novozym® 435 with biodiesel samples from babassu and jatropha, exhibiting values of viscosity that meet the standards established by ASTM D6751 (Knothe *et al.*, 2005).

CONCLUSION

Lipase PS IM displayed very similar behavior to Novozym® 435, the lipase most used for mediating typical lipase reactions for biodiesel production. Therefore, our results suggest the potential application of Lipase PS IM in those types of reactions. Under the conditions tested, the best performance was obtained for the reaction catalyzed by Lipase PS IM and Novozym® 435 using babassu oil, which may have resulted from the shorter chains of this feedstock interacting more effectively with the enzyme and alcohol. The samples obtained using these enzymes exhibited viscosity values within the standards required by ASTM D6751 (range of 1.9-6.0 mm^2/s). The enzyme Lipozyme® TL IM presented unsatisfactory performance, indicating that the transesterification reaction conditions should be optimized when using this enzyme. The high viscosity of the product obtained using andiroba oil can be explained by the low conversion reaction due to a possible inhibition of the lipase by the high level of peroxide in this raw material, a situation that arose from the inadequate and extended storage conditions.

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