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CIÊNCIAeNATURA

ISSN 2179-460X
OPEN ACCESS

Ci. e Nat., Santa Maria, v. 47, e88915, 2025 • https://doi.org/10.5902/2179460X88915
Submitted: 09/07/2024 • Approved: 06/24/2025 • Published: 10/24/2025

Chemistry

Innovative use of ariri coconut oil in the sustainable and rapid production of esters via enzymatic transesterification with ethanol

Uso inovador do óleo de coco ariri na produção sustentável e rápida de ésteres via transesterificação enzimática com etanol

Carlos Alberto Lira Junior [I, II], **Rafaely Nascimento Lima** [II],
Caritas de Jesus Silva Mendonça [II],
Wellington da Conceição Lobato do Nascimento [II], **Adeilton Pereira Maciel** [II]

[I] Federal Institute of Maranhão, Bacabal, MA, Brazil
[II] Federal University of Maranhão, São Luis, MA, Brazil

ABSTRACT

The present paper performed for the first time the characterisation and ethanolization of ariri coconut oil catalysed by the lipase Burkholderia cepacia. Through the Complete Factor Planning 3^2 and the Response Surface Methodology (MSR), the effects of different molar ratios of oil/ethanol (1:4, 1:6, and 1:9) and catalytic loads (2%, 5%, and 10%) were evaluated, using B. Cepacia in free and immobilised form, in a reaction time of 4 h. The chemical characterisation of the ariri coconut oil showed the predominance of saturated fatty acids (78.52%), with lauric acid (C12:0) being the majority at 31.65%. Ariri coconut oil showed typical characteristics of vegetable oils suitable for enzymatic transesterification. The experimental results showed that the best ethyl ester content (67.1%) was obtained with the lipase immobilised, with a catalytic load of 2% and in the oil/ethanol ratio of 1:4. For reactions employing the free lipase, the best results (62.8%) were obtained with a catalytic load of 10% and in the oil/ethanol ratio of 1:6. The statistical analyses indicated that the catalytic load was the most significant variable for the reactions with the free lipase. In contrast, the oil/ethanol ratio was the most significant variable for the reactions with the immobilised lipase. The results of this study establish ariri coconut oil as a innovative and promising substrate for applications in transesterification reactions.

Keywords: Biocatalysts; Burkholderia cepacia; Esters

RESUMO

O presente trabalho realizou pela primeira vez a caraterização e a etanolização do óleo de coco de ariri catalisada pela lipase Burkholderia cepacia. Através do Planejamento Fatorial Completo 3^2 e da Metodologia de Superfície de Resposta (MSR), foram avaliados os efeitos de diferentes razões molares de óleo/etanol (1:4, 1:6 e 1:9) e cargas catalíticas (2%, 5% e 10%), utilizando B. Cepacia na forma livre e imobilizada, em um tempo de reação de 4 h. A caraterização química do óleo de coco ariri mostrou a predominância de ácidos graxos saturados (78,52%), sendo o ácido láurico (C12:0) o majoritário com 31,65%. O óleo de ariri apresentou caraterísticas típicas de óleos vegetais adequados para a transesterificação enzimática. Os resultados experimentais mostraram que o melhor teor de ésteres etílicos (67,1%) foi obtido com a lipase imobilizada, com uma carga catalítica de 2% e na relação óleo/etanol de 1:4. Para as reações empregando a lipase livre, os melhores resultados (62,8%) foram obtidos com uma carga catalítica de 10% e na razão óleo/etanol de 1:6. As análises estatísticas indicaram que a carga catalítica era a variável mais significativa para as reações com a lipase livre. Em contrapartida, a relação óleo/etanol foi a variável mais significativa para as reações com a lipase imobilizada. Os resultados deste estudo estabelecem o óleo de coco ariri como um substrato inovador e promissor para aplicações em reações de transesterificação.

Palavras-chave: Biocatálise; Burkholderia cepacia; Ésteres

1 INTRODUCTION

Transesterification reactions are widely used in the formation of polymers (Leung et al., 2023), in the degradation of pollutants (Kim et al., 2021), in the formulation of pharmaceuticals (Rao et al., 2021), in the production of biolubricants, and in other bioproducts (Morales et al., 2020; Ng et al., 2020; Nie et al., 2020). However, their main application has been synthesising of esters from triglycerides (TG) from oils and fats (Luna et al., 2016; Mumtaz et al., 2017; Orege et al., 2022).

Oils and fats are transesterified by the reaction of 1 mol TG with 3 moles of alcohol produce a mixture of fatty acid esters and glycerol as a by-product. During the process, catalysts are used to make the process more efficient and economical, thus obtaining higher yields (Luna et al., 2016; Thangaraj et al., 2019).

In the transesterification of oil and/or fat, the most used catalysts are NaOH, KOH, HCl, and metal oxides; however, these catalysts present certain problems, such as difficulty of separation, contamination of the medium, sensitivity to certain TG, corrosion, leaching, soap formation, severe reaction conditions, and environmental issues, among others (Baskar et al., 2017; Rezende et al., 2021; Rizwanul Fattah et al., 2020).

In the face of problems associated with these catalysts, there has been a notable shift towards the utilisation of enzymatic catalysis in the transesterification of oils and fats.

Enzymatic catalysis has brought excellent results. Its use stands out mainly for its environmentally favourable route and the lower energy consumption caused by milder reaction conditions. Although selective, they have shown efficient catalytic activity for different types of TG since they are little influenced by the quality of the raw material. Its products have high purity, and the enzymes have greater thermal and chemical stability when immobilised (Fjerbaek et al., 2009; Guldhe et al., 2017; Santos et al., 2020; Yuzbasheva et al., 2014).

Despite their growing demand, enzymatic catalysis ou biocatalysis is still little used compared to other catalysts, mainly due to their long reaction time and the high costs to obtain them, it so that different methodological procedures have been applied to favor its use (T. A. Andrade et al., 2019; Bajaj et al., 2010; Narwal & Gupta, 2013a; Sandoval et al., 2017).

The main biocatalysts used to obtain esters are triacylglycerol lipases or simply lipases, which are enzymes belonging to the group of hydrolases, classified under E.C. 3.1.1.3, according to the International Union of Biochemistry (IUB) (Jaeger & Eggert, 2002a; McDonald & Tipton, 2023; Nelson et al., 1996; Sánchez et al., 2018).

Lipases obtained from the bacterium Burkholderia cepacia, formerly known as Pseudomonas cepacia, stand out as one of the main biocatalysts used in transesterification reactions. Their high stability and chemical specificity allows them to be applied in different reaction conditions (Bajaj et al., 2010; Jaeger & Eggert, 2002b; Porto de Souza Vandenberghe et al., 2020; Salihu et al., 2012; Thangaraj et al., 2019).

Lipases can be used in free or immobilised form to achieve different results. When immobilised, they may present greater stability, an improvement in activity and tolerance to alcohol, especially for methanol. There is a simplification in the separation of the product, and its reuse can be facilitated, thus reducing the costs linked to the process (Narwal & Gupta, 2013b; Remonatto et al., 2022; Selmi & Thomas, 1998; Zhao et al., 2015). Immobilization, although beneficial, will not always allow the best results to be obtained, so, individual assessments must be made for each set of substrates used in the reaction (Kumar et al., 2022).

In addition to the biocatalysts, the choice of alcohol used in enzymatic catalysis can have a direct impact on reaction yields. Methanol has been the most widely used alcohol in enzymatic transesterification reactions due to its high reactivity, however, its high toxicity can cause denaturation of the lipase and thus loss of catalytic activity (Lotti et al., 2015, 2018; Norjannah et al., 2016a; Rajendran et al., 2022).

Given its toxicity and the fact that it is an alcohol derived from fossil sources such as oil and natural gas, methanol has been replaced by ethanol, which is less toxic and is obtained from renewable sources (Gumbytė et al., 2018; Morin et al., 2007; Ramos et al., 2022; Véras et al., 2011).

In the context of alternative and renewable raw materials, researchers have been looking for other sources of TG that can be used in enzymatic transesterification. Ariri coconut oil, which comes from a renewable source, is a viable alternative for this application.

Belonging to the species Syagrus cocoides Martius, the ariri is an oil palm that can also be known as pati, lous, jatá, piririma, pererema, iriri, pupunha-brava, cunham-galinha, uapirima etc., depending on where it is found (Celestino, 2009; Gomes-da-Silva et al., 2022).

The ariri palm is native to the Amazon and the gallery forests of the cerrados located near the Amazon region. Its species is found in the Brazilian states of Amazonas, Pará, Tocantins, Maranhão, Piauí, Goiás and Mato Grosso. Ranging in height from 1.5 to 10 m, the ariri palm has a thin trunk and feathery leaves with well-spaced, narrow leaflets. Its ovoid or pyriform fruits measure up to 6 cm in length and have a yellowish-brown color when ripe (L. E. Andrade et al., 2018; Celestino, 2009; Gomes-da-Silva et al., 2022; Lisboa et al., 2020; Lorenzi et al., 2010; Noblick, 2017; Pinheiro et al., 1996).

To date, ariri coconut oil has not been used in transesterification reactions. Thus, this paper presents a new route for obtaining esters from the transesterification of ariri coconut oil with ethanol using biocatalysts, using a process that is environmentally friendly and has a low reaction time. The molar ratio (oil/alcohol) and the load of the biocatalyst (Burkholderia cepacia) were evaluated during the process in order to analyze their influence on the ester content obtained.

2 MATERIAL AND METHODS

2.1 Materials

Ariri oil was obtained from the coconut of the ariri palm tree by cold extraction via solvent (hexane) and used without any treatment. The coconut collection was conducted at the Dom Delgado University City, Federal University of Maranhão (UFMA), in São Luís -MA (2°33'22.6"S 44°18'29.2"W). Amano Lipase PS, from Burkholderia cepacia and Amano lipase PS-IM (immobilized on diatomite), were acquired from Merck & Sigma Brazil. The analytical-grade ethanol was purchased from Merck & Sigma Brazil.

2.2 Characterisation of ariri coconut oil

2.2.1 Physicochemical Characterization

The physicochemical properties of the oils were determined according to the Adolfo Lutz Institute, which analysed the acidity, saponification, and peroxide indexes, density, and viscosity Institute Adolfo Lutz (2008).

2.2.2 Chemical composition analysis

The chemical composition of ariri coconut oil was performed in a gas chromatograph (CG-2010) with Shimadzu Flame Ionization Detector (CG-FID), using a ZB-FFAP capillary column (30m x 0.25mm x 0.25μm). The flow of the carrier gas, helium, at a linear velocity of 47.5 cm/sec and column flow of 1.0 mL/min. The furnace programming was 120 °C for 2 min with a heating ramp from 10 °C/min to 180 °C and remained for 5 min, being heated again at a rate of 3 °C/min to 230 °C, remaining for up to 3 min. The injector and ion source temperatures were 250 °C and 200 °C, respectively. Split injection mode with 1/100 ratio. Race Time 32.67 min.

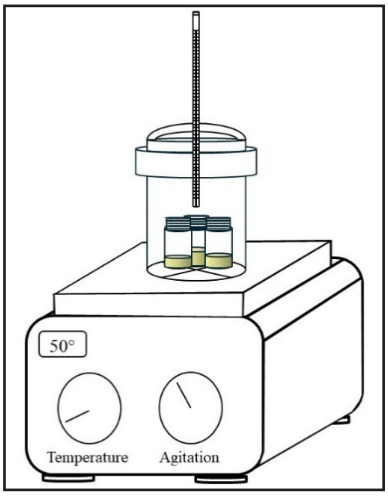
2.2.3 Infrared Spectroscopy

The samples de óleo de coco ariri were analyzed with a Fourier transform infrared spectrophotometer brand Shimadzu, model IRPrestige 21 and submitted to scanning analysis performed in the ranges of 4000-500 cm^{-1} with a resolution of 4 cm^{-1} and a number of scans of 40.

2.3 Experimental Procedure and Statistical Analysis

2.3.1 Reactions transesterification

Figure 1 – Experimental system used in transesterification reactions



Source: Authors (2025)

Enzymatic transesterification occurred under magnetic stirring at 400 rpm, 50 ºC, and 4 h duration. The mass of oil used in the reactions was 500 mg. The experimental procedure (figure 1) consisted of a 10 mL glass bottle (headspace) with a screw-on cap, a cylindrical stainless steel chamber and a magnetic stirrer with heating. At the end of the process, to separate the biocatalyst and the ethyl esters, the reaction product was transferred to a falcon tube and centrifuged at 3600 rpm for 10 min. The quantification was carried out later through Gas Chromatography (GC-FID) and the identification of the esters by Gas Chromatography with Mass Spectrometer (GC-MS).

2.3.2 Design of Experiment

Based on preliminary tests in which effective ester yields were obtained from a molar ethanol/oil ratio of 1:6 with a catalytic load of 5%, a 3^2-factorial design (three levels and two variables) was performed for each lipase, totalling 9 experiments with the central points performed in triplicate. Table 1 shows the factorial planning carried out.

Table 1 – Variables and work limits defined for the experimental routes

Variables	**Levels**		
-	-1	0	+1
Ethanol/oil molar ratio	1:4	1:6	1:9
Catalytic Load (%)	2	5	10

Source: Authors (2025)

2.3.4 Statistical Analysis

The experimental data were analysed through analysis of variance (ANOVA) with a confidence level of 95%, with significant terms $p < 0.05$ and the optimisation of the synthesis of esters performed through the response surface analysis (MSR) methodology provided by the Statistica software.

2.3.5 Chromatographic analysis of esters

The analyses of the reaction products were conducted in a Shimadzu Gas Chromatograph with Mass Spectrometer (GCMS-QP2010 SE), using an RXi1MS capillary column (30m x 0.25mm x 0.25µm). The flow of the carrier gas, helium, at a linear velocity of 37.5 cm/sec and column flow of 1.0 mL/min. The furnace programming was 120 °C for 2 min with a heating ramp from 10 °C/min to 180 °C and remained for 3 min, being heated again at a rate of 5 °C/min to 280 °C, remaining until 2 min. The injector and ion source temperatures were 250 °C and 200 °C, respectively. Split injection mode with a 1/50 ratio. Running time 33 min.

Esters were quantified according to Standard 14103-2011 (ANP), using methyl nonadecanoate (C-19 ester) and heptane as solvent as internal standard. The calculation of the percentage of esters in the samples was performed by using Equation 1:

$$\%\ \text{esters} = \left(\frac{\sum A - API}{API}\ x\ \frac{CPI\ x\ VPI}{m}\right) x\ 100 \tag{1}$$

onde:
∑A = Sum of the areas of the majority components and the internal standard;
API = Area of the internal standard (methyl nonadecanate);
CPI = Concentration in mg/mL of methyl nonadecanate;
IPV = Volume in mL of methyl nonadecanoate;
m = mass in grams of the sample.

3 RESULTS AND DISCUSSIONS

3.1 Characterisation of ariri coconut oil

3.1.1 Physicochemical Characterization

The results of the physicochemical analyses of ariri coconut oil and their comparison with other oils are illustrated in table 2.

Table 2 – Physicochemical parameters of ariri coconut oil and other oils used in enzymatic transesterification reactions

Parameters	**Ariri (a)**	**Soybean (b)**	**Palm (c)**	**Jatropha (d)**	**Babassu (e)**
Specific weight (g/cm3)	0.902	0.87 - 0.93	0.89 - 0.91	0.86 - 0.91	0.91-0.92
Acid value (%)	1.97	0.2 - 0.97	0.6 - 4	2.24 - 5.6	0.15-1.24
Saponification index (mg KOH/g)	231.4	181 - 200	190 - 208	105 - 193	245-256
Iodine content (gI2/100g)	20.4	119 - 139	47 - 56	63 - 100	10 - 18
Peroxide Index (mEg/Kg)	0.47	1.8 - 16.5	10 - 20	0.27 - 2.4	3.22 - 12.5
Viscosity (mm2/s)	30.84	36.8 - 62.2	62.75 - 65.96	32	28.79 - 38.28

Organized by the authors (2025)

Source: a - Authors (2025); b - Anwar et al. (2016); Gerde et al. (2020); Nasreen et al. (2015); Oliveira et al. (2015); Pellicano et al. (2008); Toscano & Maldini (2007); c - Koushki et al. (2015); Lin (2011); Pellicano et al. (2008); Shahedi et al. (2019); Toscano & Maldini (2007); d - Abdullah et al. (2018); Ananias Ribeiro et al. (2020); Fernandes et al. (2015); Soares et al. (2024); Yusop (2013); e - Boulifi N El et al. (2015); Kale & Ragit (2017); Sales et al. (2020); Silva Ferreira et al. (2012).

When considering using vegetable oils in transesterification reactions, we must understand that the physicochemical characteristics depend almost entirely on the existing fatty acid profiles, directly impacting the efficiency of the transesterification process and the quality of the product obtained.

As evidenced by table 3, ariri coconut oil has physicochemical characteristics within the typical ranges of vegetable oils, making it suitable for transesterification reactions.

Its acidity index of 1.97 indicates the need for a possible pre-treatment before transesterification. A high acidity index may interfere with the catalytic performance of lipases, reducing the formation of esters and interfering with the quality of the products (Jafarihaghighi et al., 2020; Neupane et al., 2021; Yeşilyurt et al., 2019).

The high saponification index implies that short-chain fatty acids are in greater quantity. Its low levels of iodine and peroxides indicate that ariri coconut oil is composed mostly of saturated fatty acids (Barbosa et al., 2009; Papin Sourou et al., 2018).

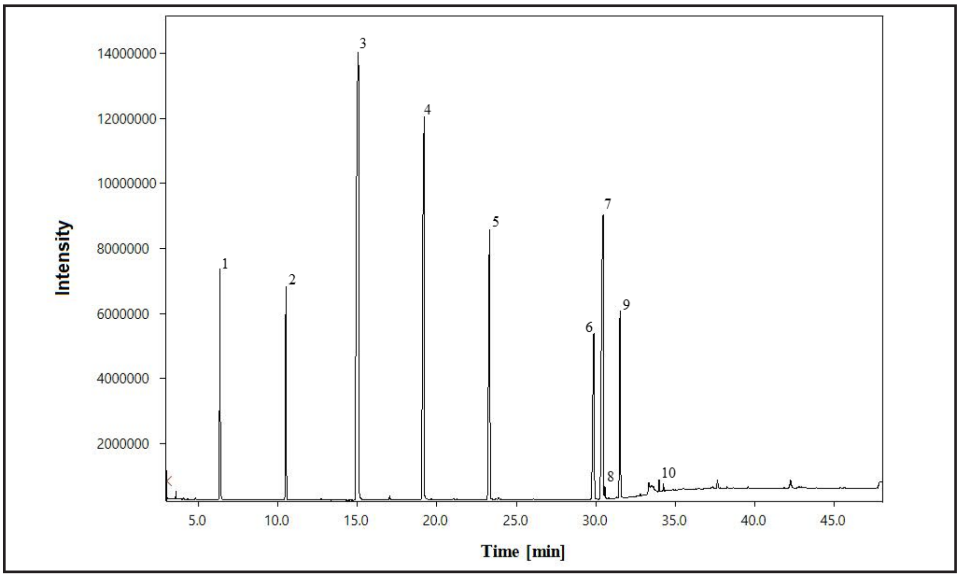
Compared to other oils such as soybean, palm, jatropha, and babassu, ariri coconut oil has competitive characteristics, especially in oxidative stability caused by saturated fatty acids. However, its high acidity index becomes a disadvantage that needs to be managed (Kwiecien et al., 2009; Moretto & Fett, 1989).

Therefore, because it has physicochemical characteristics within the typical ranges of vegetable oils, ariri coconut oil presents a viable alternative for enzymatic transesterification, especially if acidity-related issues are adequately addressed.

3.1.2 Chemical composition analysis

The free fatty acids in ariri coconut oil are shown in the total ion chromatogram, figure 3, and their indications and percentages in table 3.

Figure 2 – Spectrum in the infrared region of Ariri coconut oil



Fonte: Authors (2025)

The chromatogram of ariri coconut oil identified 10 fatty acids, most of which are chain-saturated. Lauric acid (C12:0), with a retention time of 15.02 min, was the majority, with 31.65%. In sequence, myristic acid (C14:0), oleic acid (C18:1n6c) and palmitic acid (C16:0) formed the highest fatty acid contents of ariri coconut oil with 17.96, 15.87 and 11.13%, respectively, as shown in table 3.

Table 3 – Fatty acid composition in ariri coconut oil

Peak	Acid	Formula	Name	Percentage (%)
1	C8:0	$C_8H_{16}O_2$	Octanoic acid (Caprylic acid)	4.94
2	C10:0	$C_{10}H_{20}O_2$	Decanoic Acid (Capric Acid)	5.14
3	C12:0	$C_{12}H_{24}O_2$	Dodecanoic Acid (Lauric Acid)	31.65
4	C14:0	$C_{14}H_{28}O_2$	Tetradecanoic acid (Myristic acid)	17.96
5	C16:0	$C_{16}H_{32}O_2$	Hexadecanoic acid (Palmitic acid)	11.13
6	C18:0	$C_{18}H_{36}O_2$	Octadecanoic acid (Stearic acid)	7.49
7	C18:1n9c	$C_{18}H_{34}O_2$	9-octadecenoic acid (Z) (oleic acid)	15.87
8	C18:1N9T	$C_{18}H_{34}O_2$	9-octadecenoic acid (E) (elaidic acid)	0.28
9	C18:2n6c	$C_{18}H_{32}O_2$	Linoleic acid	5.33
10	C20:0	$C_{20}H_{40}O_2$	Eicosanic acid (Arachidic acid)	0.21
			Total saturated acids	78.52
			Total unsaturated acids	21.48

Source: Authors (2025)

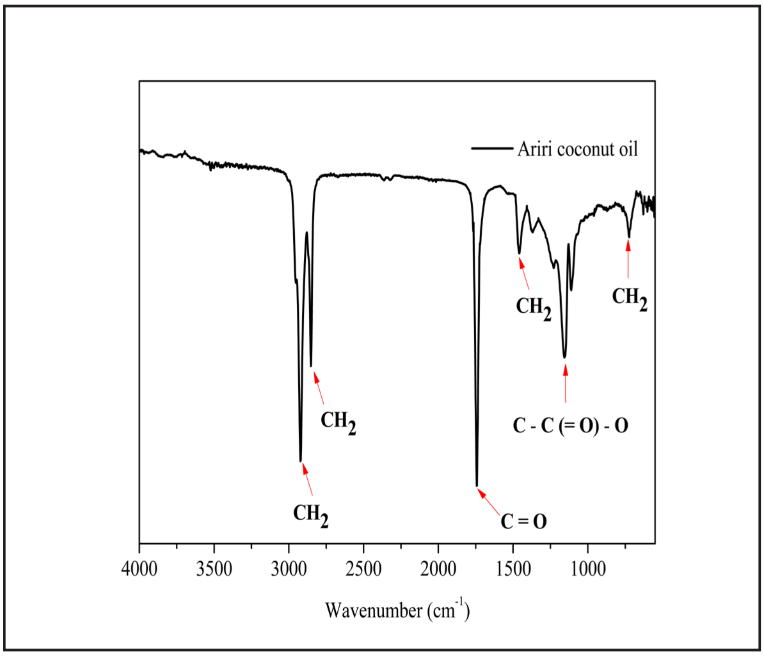
When comparing the composition of the fatty acids of ariri coconut oil with other oils used in transesterifications, such as Babassu oil, which has the greatest similarities in terms of the existing major fatty acids, mainly due to the high levels of lauric acids (Azam et al., 2010; K. S. Moreira et al., 2020a; Nascimento et al., 2009; Neto et al., 2021; Neupane et al., 2021b).

The properties of oils and their products are directly linked to the profile of existing fatty acids. Oils with high levels of saturated fatty acids tend to be more viscous, making it difficult to mix and react with the alcohol and catalyst. Still, they produce biodiesel with greater oxidative stability (Bastos, 2020; Pinto et al., 2005).

3.1.3 Infrared Spectroscopy

The spectrum obtained by infrared spectroscopy of ariri coconut oil is shown in figure 3.

Figure 3 – Spectrum in the infrared region of ariri coconut oil



Fonte: Authors (2025)

Analysing the spectrum, characteristics of the specific groups existing in ariri coconut oil can be observed. The methylene groups of the carbon chain of the esters are characterised by the absorption band related to the asymmetric stretching of CH2 in the regions of 2920 cm-1, followed by the absorption band at 2850 cm-1, referring to the symmetrical stretching of CH2, by the symmetrical strain band in the region 1462 cm-1 and angular deformations at 720 cm-1. Bands that relate to the characteristic functional groups of esters are located in the absorption band 1741 cm-1, referring to the stretching of the C=O group of the carboxyl group of esters and in 1161cm-1 referring to the deformation of the C – C(=O) – O group (Mukhametov et al., 2023; Shi et al., 2017; Silverstein et al., 2014).

3.2 Experimental procedure and statistical analysis

Transesterification reactions that use lipases as catalysts are carried out using different methodologies, so each researcher evaluates which parameters need to be verified. In this context, the complete factorial design 3^2 was used, with the following variables being the oil:ethanol ratio and the catalytic load. For each variable, three levels were considered: oil/ethanol ratios 1:4, 1:6, and 1:9, and catalytic loads of 2%, 5%, and 10%. The reaction time was kept constant at 4 h.

Table 4 presents the reaction conditions and the results obtained with their respective ester conversions.

Table 4 – Complete Experimental Design with the results for conversion into esters for enzymatic transesterification of ariri coconut oil

3*2 Planning (Three levels and two variables)						
Reaction	**Variable 1**	**Variable 2**	**Oil/ Ethanol**	**Catalytic Load**	**Ester content %)**	
					B. Cepacia free	**B. Cepacia Immobilized**
1	0	1	1:6	10%	62.8	57.0
2	1	0	1:9	5%	31.2	55.5
3	-1	-1	1:4	2%	40.3	67.1
4	-1	0	1:4	5%	47.1	66.2
5	1	1	1:9	10%	57.5	53.7
6	0	-1	1:6	2%	50.5	41.4
7	1	-1	1:9	2%	38.7	35.2
8	-1	1	1:4	10%	58.2	60.9
9	0	0	1:6	5%	21.8	39.5
9	0	0	1:6	5%	23.0	36.4
9	0	0	1:6	5%	23.3	42.3

Source: Authors (2025)

According to table 4, the results obtained from the experimental design show significant variations in the levels of esters obtained with values between 21.8 % and 67.1%. Reaction 3, in which the immobilised lipase was used, produced the largest amount of esters from the molar ratio of 1:4 (oil/ethanol) and a catalytic load of 2%.

3.2.1 Effect of oil/alcohol ratio

The oil/ethanol ratio, 1:4, presented the best results on average for both the immobilised and free lipases. The molar ratio 1:9 showed variable results, suggesting that lower catalytic loads are not ideal for forming higher ester content.

Studies show that the oil/alcohol ratio significantly affects a transesterification reaction (Norjannah et al., 2016). The alcohol content above the stoichiometric amount (1 mol of triglyceride to 3 moles of alcohol) tends to improve yield to a certain point. After that, excess alcohol can remove the water wrapped around the lipase, denaturing it and consequently reducing its catalytic efficiency (Lam et al., 2010; Ma et al., 2017; Pedro et al., 2017a; Salaheldeen et al., 2021; Tongboriboon et al., 2010).

It is worth noting that optimisations can occur in different molar ratios. Baron et al. (2014) achieved the best results with the oil/ethanol molar ratio of 1:3, while Mata et al. (2012) obtained a 1:6 ratio while Moreira et al. (2020) obtained the molar ratio of 1:18, that is, there is no oil/ethanol molar ratio that can guarantee a greater amount of esters, because this will depend on the type and form of the lipase, the catalytic content, the reaction time, among others (Baron et al., 2014a; Mata et al., 2012; K. S. Moreira et al., 2020).

3.2.2 Effect of catalytic load

The catalyst load directly influences the content of esters produced; the literature has shown that a higher catalyst load generally allows higher yields, but only up to a limit, beyond which inhibition by an excess of catalyst or particle agglomeration can occur, decreasing its efficiency (Andrade et al., 2017a, 2017b; Arana-Peña et al., 2020; Baron et al., 2014b; K. S. Moreira et al., 2020; Pedro et al., 2017a).

Data in table 4 indicate that the highest ester contents (67.1%) were achieved when using the immobilised lipase at a load of 2% and an oil/ethanol ratio of 1:4. The highest contents (62.8%) using the free lipase were obtained with a catalytic load of 10% and an oil/ethanol ratio of 1:6.

These outcomes reveal that immobilised lipase tends to be more efficient under lower catalytic loads and a low oil/alcohol ratio, while free lipase performs better with higher catalyst concentrations and a controlled oil/alcohol ratio. Such differences reflect the distinct catalytic behaviors of each lipase type in reaching optimal efficiency.

Free lipases, due to their structural flexibility, interact more freely with reactants in the reaction medium. However, this same characteristic also makes them more vulnerable to adverse effects, particularly under high ethanol concentrations. Elevated enzyme loads may result in molecular aggregation, diffusion limitations, and even substrate inhibition effects are intensified in environments rich in alcohol. Ethanol, in particular, may act as a denaturing agent at elevated levels, resulting in enzyme deactivation. Thus, while increasing the enzyme load may initially accelerate the reaction, surpassing an optimal threshold can compromise the overall efficiency of the process (Homaei et al., 2013; Maghraby et al., 2023; Martinez-Guerra & Gude, 2014).

In contrast, immobilised lipases offer greater structural stability and are more resistant to denaturation under harsh reaction conditions. However, this structural advantage comes with trade-offs: their activity is more sensitive to the oil/ethanol molar ratio due to mass transfer limitations within the immobilisation matrix. Excess ethanol may disrupt the essential water layer surrounding the enzyme, hinder substrate diffusion, or lead to enzyme deactivation, particularly at the enzyme-support interface. Therefore, fine-tuning the oil/ethanol ratio is crucial for maximising the catalytic efficiency of immobilised systems (Arana-Peña et al., 2020; Baena et al., 2022; Dutra et al., 2022; Pedro et al., 2017b; Shah & Gupta, 2007).

Overall, immobilised lipase demonstrated superior or at least comparable results to its free counterpart, appearing less sensitive to variations in the oil/ethanol ratio and catalytic load. This suggests a broader operational window and greater robustness under changing reaction conditions. On the other hand, free lipase exhibited greater variability in response to different experimental conditions, indicating lower resilience to environmental fluctuations (Homaei et al., 2013; Maghraby et al., 2023).

These findings are consistent with those of Mata et al. (2012), who reported that an excessive amount of lipase does not necessarily result in higher ester conversion, whereas an insufficient enzyme load may not be adequate to drive the reaction to completion. This reinforces the importance of identifying optimal operating conditions for each enzyme form (Mata et al., 2012).

Despite initial limitations such as diffusion restrictions and interactions with the immobiliser support, lipase immobilisation is widely recognised for enhancing enzyme stability and reusability. These improvements contribute to higher reaction yields and reinforce the practical advantages of immobilised systems in biocatalysis (Baena et al., 2022; Dutra et al., 2022; Kalita et al., 2022; Lukovic et al., 2011; Mandari & Devarai, 2022; Noureddini et al., 2005; Santos et al., 2020; Shah et al., 2004a; Thangaraj et al., 2019).

Beyond technical performance, it is also important to consider the economic and environmental aspects related to the choice of enzyme form and catalyst load. From an economic standpoint, the higher unit cost of immobilised enzymes can be offset by their longer operational lifespan and reusability, especially in continuous processes. In contrast, free enzymes, although initially cheaper, often require higher doses and cannot be reused effectively (Chapman et al., 2018; DiCosimo et al., 2013; Jegannathan et al., 2008).

3.2.3 Catalytic efficiency and optimisation of reaction time

Enzymatic transesterification reactions need an adequate time to ensure the conversion of the triglycerides present in vegetable oil into esters; in general, these reactions are usually carried out for a long time.

The search for an optimised reaction time is necessary to avoid excessive energy and resource consumption. Reactions that last longer than necessary increase operating costs and may not bring additional benefits in terms of reactions.

Table 5 presents a comparative analysis of the maximum ester contents obtained from different reaction conditions in which lipase catalysts and ethanol were used.

Table 5 – Ester content obtained in enzymatic transesterification under different experimental conditions

Time	Lipase	Oil	Catalytic Load	Oil/alcohol ratio	Ester content	Reference
1h	B. cepacia	Soy	1:15,2	4.75%	65%	(Noureddini et al., 2005)
4h	**B.cepacia**	**Ariri**	1:4	2%	**67%**	**This paper**
4h	Candida antarctica	Soy	1:3	9%	73%	(Pedro et al., 2017b)
4h	Rhizomucor miehei	Soy	1:3	9%	51.1%	(Pedro et al., 2017b)
4h	Thermomyces lanuginosus	Soy	1:3	5%	50%	(Pedro et al., 2017b)
6h	B. cepacia	Castor bean	1:3	4.5%	90%	(Baron et al., 2014a)
8h	Chromobacterium viscosum	Jatropha	1:4	10%	71%	(Shah et al., 2004)
8h	B. cepacia	Jatropha	1:4	10%	98%	(Shah & Gupta, 2007)
12h	Thermomyces lanuginosus	Corn	1:6	2.8%	98.95%	(Mata et al., 2012)
12h	Pseudomonas fluorescens	Palm	1:3	10%	>67%	(Tongboriboon et al., 2010)
24h	B. cepacia	Jatropha	1:10	52%	~70%	(Abdulla & Ravindra, 2013)
24h	Candida antarctica	Soy	~1:50	5%	89%	(Rosset et al., 2011)
24h	Thermomyces lanuginosus	Palm	1:4	20%	89.90%	(Raita et al., 2010)
24h	Pseudomonas fluorescensa	Palm	1:18	20%	~98%	(Moreira et al., 2007)
30h	B. cepacia	Soy	1:5	83%	95.42%	(Ma et al., 2017)

Organized by the authors (2025)

The results in table 5 reveal a diversity of esters produced, obtained from different combinations of oils and lipases under various experimental conditions.

Noureddini et al. (2005), using the lipase B. cepacia with soybean oil, reached a content of 65% in just 1 hour with an oil/alcohol ratio of 1:15.2 and a catalytic load of 4.75% (Noureddini et al., 2005). However, Baron et al. (2014), using the same lipase now with castor oil, obtained a 90% ester after 6 h, with a similar catalytic load, demonstrating a significantly higher efficiency obtained with increased reaction time and with substrate change, even with a lower oil/alcohol ratio (Baron et al., 2014).

Comparing these results with those obtained by the present study, it is observed that the use of the lipase B. cepacia with ariri coconut oil presented the best result (67%) in a reaction time of 4 h. Being comparable to other studies in the same reaction time, such as the one by Pedro et al. (2017), which reached 73% using Candida antarctica with soybean oil (Pedro et al., 2017). Despite a small reduction in the ester content obtained, this work used a significantly lower catalytic load (2%), indicating the high efficiency of the system used.

Other studies obtained results similar to this study. Shah et al. (2004) achieved a 71% content in the transesterification of palm oil with Chromobacterium viscosum, using an oil/alcohol ratio of 1:4 and a catalytic load of 10% in 8 h of reaction. This study employed a five-fold greater catalytic load and a two-fold longer reaction time to obtain a slightly higher result than the present work (Shah et al., 2004).

Tongboriboon et al. (2013) achieved 67% ester content by applying an oil/alcohol ratio of 1:3 and a catalytic load of 10% in the transesterification of jatropha with Pseudomonas fluorescens for 12 h. In this case, although the result is the same as in the present work, the reaction time was three times longer, and the catalytic load was five times higher (Tongboriboon et al., 2010).

Abdulla & Ravindra (2013) maximized the production of esters by approximately 70% in the transesterification of jatropha oil with B. cepacia, using an oil/alcohol ratio of 1:10 and a catalytic load of 52% in 24 h. Comparing with the results of the present work, it is observed that, although slightly higher, the ester content was achieved in a reaction time six times higher and a catalytic load twenty-six times higher (Abdulla & Ravindra, 2013).

Although the other studies presented superior results to this study, they were obtained through a high catalytic load and a long reaction time.

Shah et al. (2007) achieved an ester content of 98% with a catalytic load of 10% in a reaction time of 8 h in the transesterification of jatropha with B.

cepacia with an oil/alcohol ratio of 1:4 (Shah & Gupta, 2007). Mata et al. (2012) despite a reasonable catalytic load, needed 12 h to obtain 98.95% of esters in the transesterification of corn oil with Thermomyces lanuginosus in the oil/alcohol ratio of 1:6 (Mata et al., 2012).

For a reaction time of 24 h, Rosset et al. (2011) investigated the transesterification of soybean oil using the lipase Candida antarctica, achieving an 89% ester with an oil/alcohol ratio of approximately 1:50 and a catalytic load of 5% (Rosset et al., 2011). While Raita et al. (2010) achieved a 90% using a catalytic load of 20% and an oil/alcohol ratio of 1:4 in the transesterification of palm oil with the lipase Thermomyces lanuginosus (Raita et al., 2010). Moreira et al. (2007) used Pseudomonas fluorescens to transesterify palm oil, obtaining an approximate content of 98% esters, a 20% catalytic load, and an oil/alcohol ratio of 1:18 (Moreira et al., 2007).

Finally, Ma et al. (2017) achieved an ester content of 95%, using an oil/alcohol ratio of 1:5 and a catalytic load of 83% in 30 h of reaction. Despite the excellent result, the high catalytic load required and the long reaction time represent significant challenges in terms of cost and operational feasibility for the study mentioned above (Ma et al., 2017)

From the comparisons made in this topic, the present work stands out for the high efficiency of the catalytic system employed. This system achieves significant results in the ester content by combining a significantly lower catalytic load and a short reaction time.

Reducing the catalytic load reduces operating costs and environmental impact, making the process more sustainable and economically viable without compromising the result.

3.2.4 Statistical analysis

Tables 6 and 7 present the results of the Analysis of Variance (ANOVA) for the transesterification reactions for the free and immobilised lipases, respectively.

Table 6 – Analysis of variance (ANOVA) - B. Cepacia (Free)

Factors	Sum Quadratic	Degrees of Freedom	Average Quadratic	F	p
(1) Oil/ethanol L+Q	87.943	2	43.9714	0.569858	0,593487
(2) Catalytic load (%) L+Q	1527.469	2	763.7347	9.897810	0,012584
Error	462.972	6	77.16199		
Total	1411.642	10			
R^2	0.79672				
R^2 Adjusted	0.6612				
MS Residual	77.16199				

Source: Authors (2025)

Table 7 – Analysis of variance (ANOVA) - B. Cepacia (Immobilized)

Factors	Sum Quadratic	Degrees of Freedom	Average Quadratic	F	p
(1) Oil/ethanol L+Q	828.743	2	414.3714	6.275101	0,033838
(2) Catalytic load (%) L+Q	133.116	2	66.5580	1.007933	0,419375
Error	396.205	6	66.0342		
Total	1411.642	10			
R^2	0.71933				
R^2 Adjusted	0.53222				
MS Residual	66.03421				

Source: Authors (2025)

The data in table 6 show the p-value for the oil/ethanol ratio equal to 0.593487, which is higher than the common significance level (0.05). This indicates that the effect of the oil/ethanol ratio is not statistically significant at the level of 5% on the ester content, while the catalytic load, with a value of p = 0.01258, is statistically significant when the lipase in the free form is used.

On the other hand, when the immobilised lipase was used, the statistically significant effect at the 5% level was the oil/ethanol ratio with p = 0.033838, while the catalytic load with a value of p = 0.419375 is not statistically significant, as shown in table 7.

It is noted, therefore, that the catalytic load directly affects the obtaining of the esters produced by the reaction catalysed with free lipase; on the other hand, the oil/ethanol ratio is the variable that most affects the reaction catalysed by the immobilised lipase, that is, at the same time that the amount of catalyst and the oil/ ethanol ratio can substantially improve the content of esters produced, These same factors do not have significant effects within the levels studied.

Statistical analysis is not limited to verifying the influences caused by the variables but is complete in providing an idea of how well the model fits the data obtained.

The R^2, also known as the coefficient of determination, is extremely useful in statistical modelling and regression analysis. It assesses how independent variables explain the changes in the dependent variable (Callegari-Jacques, 2003; Seber & Lee, 2003).

Models with a higher R^2 are generally preferred, as they indicate that a large part of the variability in the dependent variable can be explained by the independent variables in the model.

R^2 is used in conjunction with other metrics, such as adjusted R^2, to ensure a complete assessment of the model's effectiveness. This provides a more accurate measure of the model's explanatory capacity, especially when comparing models with different numbers of variables (Seber & Lee, 2003).

The R^2 value for the free lipase shown in table 6 indicates that approximately 79.67% of the variability of the result can be explained by the variables studied, indicating that the model satisfactorily explains the variability of the esters obtained. The adjusted value of R^2 (0.6612) suggests that, when considering the complexity of the model, the explanation of variability is smaller but still considerable.

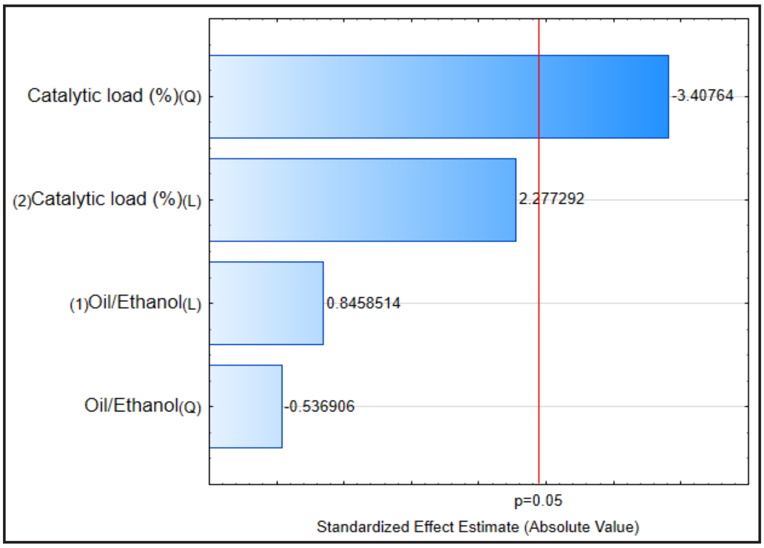
For the immobilized lipase (table 7), the R^2 value of 0.71933 indicates that approximately 71.9% of the variance in the dependent variable (esterification yield) is explained by the model, reflecting a reasonable explanatory capacity. However, the adjusted R^2 of 0.53222 suggests that, after accounting for model complexity, the proportion of explained variability is significantly reduced. This indicates that the current model, while useful for fitting the observed data, may have limited predictive power for other experimental conditions. The relatively low adjusted R^2 highlights the potential need to incorporate additional variables not considered in the current experimental design—such as water content, temperature variation, and others. Including these factors in future studies may improve the model's predictive accuracy and lead to a more comprehensive understanding of the system's behavior (Montgomery et al., 2012; Smith & Draper, 1998).

For both lipases, the adjusted R^2 is below the R^2, indicating that one of the variables may not be contributing significantly and that the model fits very well to the results found but is ineffective in predicting new experimental results, which indicates the need to include more relevant variables, improve the quality of the data, or consider other types of models or data transformations (Montgomery et al., 2012; Smith & Draper, 1998).

The effects caused by the oil/ethanol ratio and catalytic load variables are visually more observable through the Pareto graph, which provides a clearer visual representation of the data, identifying which independent variable will or will not produce an effect on the dependent variable.

On the Pareto chart, the vertical red line indicates which estimated effects are statistically significant ($p = 0.05$), and the variables that cross this line are considered statistically significant (Cavalcante et al., 2010).

Figure 4 – Pareto plot for the effect of variables on the content of esters produced - B. Cepacia (Free)



Fonte: Authors (2025)

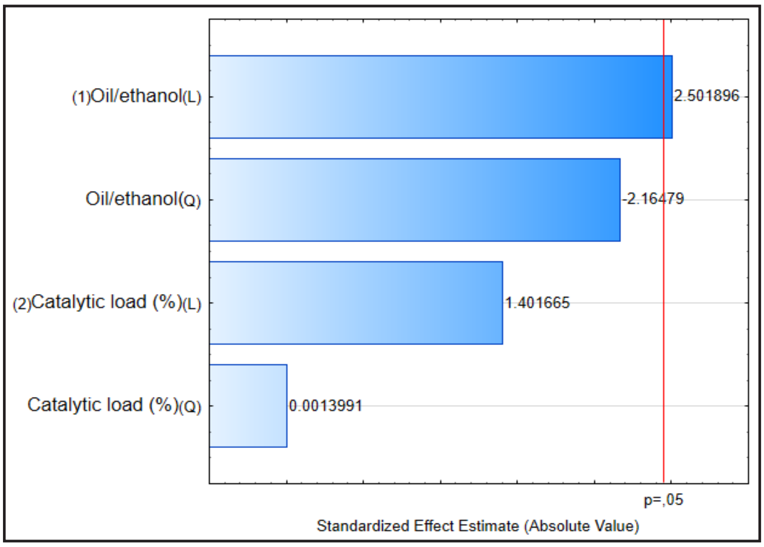
Figures 4 and 5 illustrate the Pareto diagrams for the transesterification reactions for the free and immobilised enzymes, respectively.

The graph illustrated in figure 4 shows that, it can be seen that catalytic load (Q) is the most significant variable, but with a negative effect, suggesting that increasing the charge of the free lipase will significantly reduce the ester production (Hazrat et al., 2023; Martinez-Silveira et al., 2019).

Abdulla et al. (2013), in the ethanolic transesterification of the crude oil of Jatropha curcas L. using the lipase of immobilised Burkholderia cepacia, found that a reduction in the amount of esters is related to a higher catalytic load (Abdulla & Ravindra, 2013).

The ANOVA analysis (table 6) shows that the oil/ethanol ratio does not significantly influence the ester content. Ramakrishnan et al. (2021) found that increasing the oil/ethanol molar ratio did not produce better results; in some cases, the ester content was reduced (Ramakrishnan et al., 2021). Ma et al. (2017) also noted that a further increase in the oil/ethanol molar ratio reduced the amount of esters produced (Ma et al., 2017).

Figure 5 – Pareto plot for the effect of variables on the content of esters produced - B. Cepacia (immobilised)



Fonte: Authors (2025)

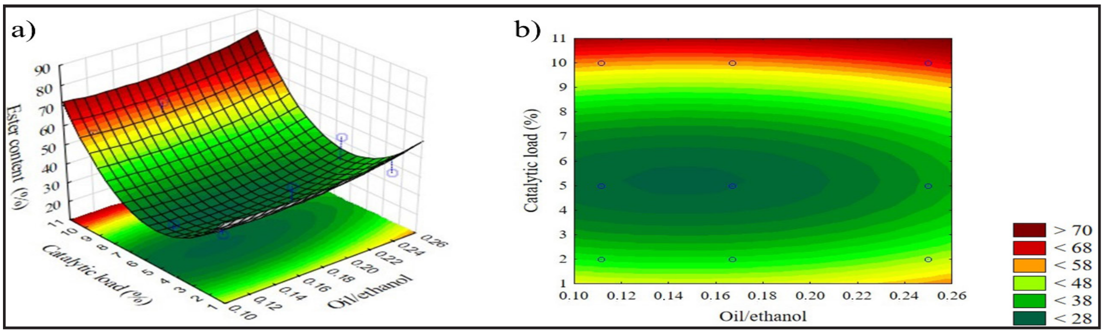
For the reactions with the immobilised enzyme, The Pareto graph, plotted in figure 5, indicates that the greatest effect is caused by the oil/ethanol (L) ratio; its positive effect indicates that, as the oil/ethanol ratio increases linearly, the content of esters produced will also increase significantly (Hazrat et al., 2023).

It is also observed that the enzyme load does not significantly influence the reaction. Some studies found that catalyst concentration did not significantly influence the reaction results compared to the other process variables and that a higher enzyme load would not cause more significant results (Baron et al., 2014; Mansourpoor, 2012; Narwal & Gupta, 2013; Santana et al., 2016).

Response surface and contour plots can be used to perform Complementary Pareto and ANOVA chart analyses. These graphs are important tools in analysing and optimising transesterification reactions, identifying the influence of each variable and the interactions between them to locate the optimal points of each reaction.

Figures 6 and 7 show the response surface and contour graphs for the transesterification reactions used.

Figure 6 – Graph of response surface (a) and contour (b) in the content of esters produced - B. Cepacia (free)

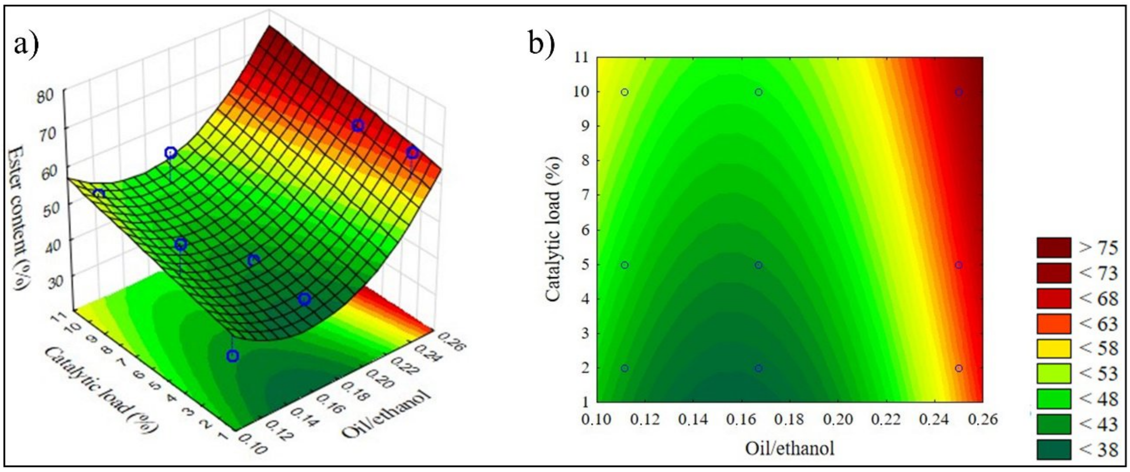


Source: Authors (2025)

Figure 6 demonstrates a clear transition region from low to high ester content, with the highest levels being achieved from a high enzyme load and for any oil/ethanol ratio used; in addition, the ester content does not vary linearly with the changes in the independent variables.

The contour curve also demonstrates a complex interaction between the independent variables, suggesting that even small changes in the variables can significantly impact the reaction medium.

Figure 7 – Graph of response surface (a) and contour (b) in the content of esters produced - B. Cepacia (free)



Source: Authors (2025)

The graphs in figure 7 indicate that the best results are initially achieved with increased catalytic load. However, this effect decreases as the catalytic load increases, so it no longer significantly influences the ester content, especially near the oil/ethanol ratio of 1:6.

Both graphs corroborate that the oil/ethanol ratio increase significantly impacts ester production, especially when combined with a high catalytic load, indicating a clear interaction between these variables.

When comparing the lipases, it is verified that free enzymes present intermediate results in most combinations of variables. At the same time, the immobilised enzyme presents a greater variation in the content of esters produced with the changes in its variables.

Thus, lipase immobilisation seems to significantly alter how enzyme load and oil/ethanol ratio influence the outcome, providing better enzyme stability or reactivity under different conditions and allowing for a greater amount of esters in a wider range of variables.

4 CONCLUSIONS

This study demonstrated the use of B. cepacia lipase in the enzymatic transesterification of ariri coconut oil with ethanol, aiming at the sustainable production of esters.

Compared to other oils such as soybean, palm, jatropha, and babassu, ariri coconut oil has competitive characteristics, especially due to its oxidative stability, which is provided by most saturated fatty acids. However, the high acidity value represents a disadvantage that must be managed properly;

The reactions indicated that lower oil/ethanol ratios and intermediate catalytic loads are more efficient, especially with the use of immobilised lipase;

The immobilised lipase proved to be more advantageous compared to the free enzyme, offering greater stability and reactivity under different conditions;

The choice of optimal conditions should consider the type of lipase used and the specific objectives of the process;

The ariri coconut oil used without treatment and solvent-free system was shown to be economically advantageous and environmentally friendly, eliminating the need for solvent recovery and recycling;

The ester content produced in a reduced reaction time and a low catalytic load underscore the potential of using enzymatic transesterification of ariri coconut oil with ethanol. This process represents an innovation since it was reported for the first time, establishing a promising basis for future studies and potential industrial applications.

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Authorship contributions

1 – Carlos Alberto Lira Junior

Industrial Chemist and PhD in Biotechnology. Professor at the Federal Institute of Maranhão, Campus Bacabal.
https://orcid.org/0000-0002-7149-9502 - carlos.lira@ifma.edu.br
Contribution: Writing - original draft, conceptualization, data acuration, formal analysis.

2 – Rafaely Nascimento Lima

Adjunct Professor, Department of Chemistry, Federal University of Maranhão, bachelor of Chemistry, PhD in Science
https://orcid.org/0000-0002-4909-6213 - rafaely.lima@ufma.br
Contribution: Writing - proofreading and editing, conceptualization, data acuration, formal analysis.

3 – Caritas de Jesus Silva Mendonça

Industrial Chemist, PhD in Food Technology, Collaborating Professor of the Professional Master's Degree in Energy and Environment at the Federal University of Maranhão
https://orcid.org/0000-0002-9479-810X - cjs.mendonca@ufma.br
Contribution: Writing - proofreading and editing, conceptualization, data acuration, formal analysis.

4 – Wellington da Conceição Lobato do Nascimento

Industrial Chemist and Chemistry PhD student
https://orcid.org/0000-0002-9979-372X - cellqmccff@gmail.com
Contribution: Writing - proofreading and editing, conceptualization, data acuration, formal analysis.

5 – Adeilton Pereira Maciel

Industrial Chemist, PhD in Chemistry
https://orcid.org/0000-0002-1787-6039 - ap.maciel@ufma.br
Contribution: Funding acquisition, project administration, conceptualization, data curation, formal analysis.

How to quote this article

Lira Junior, C. A., Lima, R. N., Mendonça, C. J. S., Nascimento, W. C. L. & Maciel, A. P. (2025). Innovative use of ariri coconut oil in the sustainable and rapid production of esters via enzymatic transesterification with ethanol. *Ciência e Natura*, Santa Maria, 47, e88915. DOI 10.5902/2179460X88915. Disponível em: https://doi.org/10.5902/2179460X88915