

Response surface for biodiesel production from soybean oil by ethylic route

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Abstract. Petroleum has been the most consumed energy source in the world, but it tends to run out due its non-renewable character. Among biofuels, biodiesel has emerged as the main candidate to substitute petroleum diesel. The present study aimed to identify the maximum yield point of biodiesel production by generating a response surface using molar ratio, temperature and agitation time as independent variables, and yield as a dependent variable. From the response surface, it is observed that the increase in temperature and reaction time leads to reduced yield. The configuration that resulted in maximum yield of 93.30% was 12:1 molar ratio, 30 °C temperature and 30-minute reaction time. From the chromatographic analysis it was possible to identify five different fatty acids in the composition of the biodiesels. Total saturated fatty acids (palmitic and stearic acids) ranged from 41.53% to 42.09% and total unsaturated fatty acids including monounsaturated and polyunsaturated fatty acids (oleic, linoleic and linolenic acids) ranged from 57.92% to 58.48%. According to the results of the physicochemical analyses, the specific mass at 68°F is in agreement with Brazilian, American and European specifications, ranging from 877.46 kg m⁻³ to 879.64 kg m⁻³. The kinematic viscosity at 104 °F ranged from 4.49 mm² s⁻¹ to 4.82 mm² s⁻¹. The acid value obtained did not vary within the limits established by the norms, and values between 0.54 and 2.74 mg KOH g⁻¹ were observed.

Key words: biofuels, ethanol, optimization, transesterification.

INTRODUCTION

The most consumed energy source in the world is fossil fuel, derived from petroleum, but this raw material tends to run out due to its non-renewable nature (AMBAT et al., 2018). In addition, its use has been causing severe damage to the environment, from the extraction process to the final consumption. Thus, the study of alternative, clean and renewable energy sources is extremely important.

Dantas et al. (2016) state that the use of biomass has been gaining significant relevance due to the possibility of being used in the production of heat, either for industrial thermal use, or for electricity generation and/or for the possibility of being transformed into other forms of solid energies (charcoal), liquid energies (bioethanol, biodiesel, bio-oil) and gaseous energies (biogas).

For Razack & Duraiarasan (2016), among biofuels, biodiesel has become increasingly important as a substitute for petroleum diesel. This is because, according to Ambat et al. (2018), as it is derived from renewable raw materials, such as vegetable oils and animal fats, biodiesel promotes sustainable development through energy savings, reduces the need to import diesel oil, in addition to presenting characteristics such as low toxicity and low emission of polluting gases.

For Knothe et al. (2006), biodiesel can be obtained through several technologies, namely: cracking, esterification and transesterification, which involve the management of variables such as the alcohol:oil molar ratio, temperature, reaction time and catalyst quantity, which are decisive for the efficiency of the production process of this fuel. According to Bet-Moushoul et al. (2016) the transesterification process has been the most used industrially. Transesterification consists of the reaction of triglycerides in vegetable oil and an alcohol, in the presence of a catalyst, which can be acidic or basic, and generates glycerol as a by-product (MUSA, 2016).

Biodiesel production can be carried out from a wide variety of raw materials, which include most vegetable oils and animal fats, as well as residual oils and fats (Sirviö et al. (2018). Among these raw materials, soy stands out, which in January 2017 was responsible for approximately 65% of the biodiesel production in Brazil (ANP, 2017).

According to Musa (2016) and Jokiniemi & Ahokas (2013), the most used alcohols for the transesterification of biodiesel are methanol and ethanol. Other types of alcohol can also be used, as, for example, propanol, butanol, isopropanol, tert-butanol, octanol and branched alcohols, but the cost is much higher. Still according to the author, despite the several advantages of using methanol over ethanol (shorter reaction time, greater reactivity, lower reaction consumption, etc.), its use demands greater care in handling due to its greater volatility and to its highly toxic character. The use of ethanol, on the other hand, has the advantages of producing a 100% renewable fuel, a greater number of ketanes in ethyl esters and greater oxidative stability.

According to Meneghetti et al. (2013), the basic homogeneous catalysts NaOH and KOH have been the most used for the industrial biodiesel production. In homogeneous catalysis, both the catalyst and the reagents are in the same phase, forming a uniform mixture and, thus, the catalyst effectively participates in the reaction, but at the end of the process it is not consumed.

According to Victorino et al. (2016), the composition of the glycerin generated as a by-product may vary according to the raw material chosen for transesterification. Containing from 50% to 60% of glycerol and other substances inherent to the process, glycerin has several uses, from its utilization in the food industry to its use as a substrate in fermentation processes.

The molar ratio directly influences the yield of the transesterification reaction, and although the stoichiometric ratio is 3:1 (three moles of alcohol to one mole of triglyceride), it is necessary to use excess alcohol (6:1, 9:1, etc.) so that the balance of the reaction is disturbed and favors the formation of esters, as observed in the works of Borges et al. (2014), Peiter et al. (2018) and Giuffrè et al. (2017), the reaction temperature also has great influence on transesterification, since, in addition to promoting high percentages of biodiesel conversions, it also reduces the time in which these reactions are carried out. Temperatures of alcohols below the boiling point are the most used, as observed in the work of Joshi et al. (2017), who worked with temperatures in the range of 40 °C, 50 °C and 60 °C.

Knothe et al. (2006) explain that the biodiesel quality determines the good functioning and useful life of diesel engines. The fuel must present appropriate physicochemical properties to ensure complete combustion and adequate engine performance. At the laboratory level, the most observed features for the characterization of produced biodiesel are specific mass at 20 °C, kinematic viscosity at 40 °C, acid value, saponification, ester content, cold filter plugging point and flash point.

Aiming to optimize biodiesel production by ethylic route from soybean oil, the present work aims to determine the biodiesel production process with the best yield using the response surface methodology (RSM) by varying the production factors (molar ratio, reaction time and temperature).

MATERIAL AND METHODS

Experiments realization site

Biodiesel production and analysis were performed at the Agricultural Machinery Laboratory (LABMAQ) of the Agricultural and Environmental Engineering Department and at the Teaching Laboratory of the Chemical and Petroleum Engineering Department (LENTEQ), both located at the School of Engineering at the Federal Fluminense University (UFF).

Raw materials used

The oil used in the present work was industrialized soybean oil. The ethanol and the catalyst used were Ethanol P.A. with 99.5% purity and sodium hydroxide (NaOH) in micropearls, respectively.

Experiment planning

The following factors were evaluated: molar ratio alcohol:oil, temperature and reaction time for three levels, according to Table 1. The values of the production factors were obtained through a bibliographic survey of previous studies on biodiesel production by ethylic route. The experiments were carried out in triplicate.

The amount of oil used in the procedures was fixed at 100 g in order to be able to determine the amount of alcohol used for each molar ratio. The amount of catalyst used was 1% in relation to the oil mass. The average molar mass of soybean oil was presented by Almeida (2016) with a value of 874.9 g mol⁻¹. The chemical composition in fatty acids of soybean oil is shown in Table 2. The molar mass of ethanol is informed by the manufacturer with a value of 46.06 g mol⁻¹.

Table 1. Variation sources used in the work

Oil	Soybean		
Catalyst	Sodium Hydroxide (NaOH)		
Molar ratio (Ethanol:Oil)	6:1	9:1	12:1
Reaction Temperature (°C)	30	40	50
Reaction Time (min)	30	60	120
[mbo] Oil Mass (g)	100	100	100
Catalyst Mass (g)	1	1	1
[mba] Ethanol Mass (g)	31.588	47.381	63.175

Transesterification

Biodiesel was produced through the transesterification process in the presence of a basic catalyst. Initially, the necessary quantities of raw materials (oil, ethanol and sodium hydroxide) were weighed according to the treatment. The oil was then placed in the oven to be preheated to the treatment temperature. Meanwhile, NaOH was diluted in ethanol using a magnetic stirrer at a temperature of 45 °C, producing sodium ethoxide. As soon as the mixture of ethanol with NaOH became homogeneous and the oil reached the preheating temperature, the latter was slowly placed in sodium ethoxide and the treatment temperature was controlled with a thermometer inserted in the Erlenmeyer.

After the transesterification process, the mixture was placed in a separating hopper, remaining for 24 hours for the separation of glycerol from biodiesel. At the end of the separation phase, the glycerin was packed in safe containers for proper disposal and the biodiesel needed to go through purification steps as it presented impurities such as unreacted glycerol, ethanol and catalyst residues.

The first stage of purification consists of washing the 'dirty biodiesel' with distilled water at a temperature of 50 °C and hydrochloric acid, allowing it to rest for 20 minutes before removing the washing liquid. This procedure was performed three times, following the proportions of 30 mL of distilled water and 3 drops of hydrochloric acid (HCL) for each 100 mL of biodiesel.

According to Mendow & Querini (2013) using different acids in the biodiesel washing process, concluded that the biodiesel samples obtained by any of treatments have similar acid values. A similar phenomenon took place during the washing with hydrochloric, carbonic, citric or phosphoric acid solution. For this reason, the biodiesel samples obtained by any of treatments have similar acid values and are considerably higher than the maximum permitted by the international standard (EN 14214 - acid value = 0.5 mg KOH g⁻¹). higher than the maximum permitted by the international standard.

In the second stage, the biodiesel went through a drying process in which it was placed in an oven at a temperature of 105 °C ± 3 °C for 2 hours, so that the rest of the distilled water could evaporate. In the last stage, the biodiesel was slowly inserted into a funnel with filter paper, in order to remove the last residues present in the fluid, thus obtaining clean biodiesel.

Table 2. Chemical composition in fatty acids of soybean oil used in the work

Fatty Acid	M/M (%)	
C14:0	Miristic	0.08
C15:0	Pentadecanoic	0.05
C16:0	Palmitic	10.82
C16:1	Palmitoleic	0.09
C17:0	Margaric	0.09
C17:1	Cis-10-heptadecenoic	0.06
C18:0	Stearic	3.69
C18:1	Oleic	24.18
C18:2 trans	t-linoleic	0.10
C18:2	Linoleic	52.72
C18:3 trans	t-linolenic	0.30
C18:3	Linolenic	6.56
C20:0	Arachidic	0.37
C20:1	Eicosenoic	0.26
C22:0	Behenic	0.46
C24:0	Lignoceric	0.17

SOURCE: Adapted from Scamilhe; Pimenta; Pereira (2016).

Production yield

The yield of each treatment was obtained through Equation 1, used by Ambat et al. (2018), just evaluating the values of the oil mass and the clean biodiesel mass produced in each batch.

$$\text{Yield} = \frac{\text{biodiesel mass}}{\text{soybean mass}} \quad (1)$$

Physicochemical characterization

The physicochemical characterization of the produced biodiesel was conducted in accordance with Brazilian, American and European regulations, as indicated by Resolution No. 45 of August 25, 2014 of the Brazilian National Agency of Petroleum, Natural Gas and Biofuels (Agência Nacional do Petróleo, Gás Natural e Biocombustíveis - ANP, 2014). For the characterization of the biodiesel at a laboratory level, the specific mass at 20 °C, the kinematic viscosity at 40 °C and the acid value were used. The reference values adopted, according to the aforementioned Resolution, were: specific mass at 20 °C with a limit between 805 to 900 kg m⁻³; kinematic viscosity at 40 °C with a limit between 3.0 to 6.0 mm² s⁻¹ and acid value (max.) with a limit of 0.50 mg KOH.g⁻¹, as shown in Table 3 (ANP, 2014).

Table 3. Biodiesel specifications established by Brazilian, American and European regulations

Characteristic	Unit	Limit	Method		
			ABNT NBR	ASTM D	EN/ISO
Aspect	-	LII	-	-	-
Specific Mass at 20 °C	Kg m ⁻³	850 to 900	7,148	1,298	EN ISO 3675
Kinematic viscosity at 40 °C	mm ² s ⁻¹	3.0 to 6.0	14,065	4,052	EN ISO 12185
Acid value, máx.	mg KOH.g ⁻¹	< 0.50	10,441	445	EN ISO 3104
			14,448	664	EN 14104

SOURCE: Adapted from ANP, 2014.

The specific mass was determined in LENTEQ, using the Anton Paar digital densimeter, model DMA 500.

The procedure for obtaining the kinematic viscosity was also carried out in LENTEQ, using the digital controller Schott Gerate, model ASV350, the thermostatic bath Schott Gerate, model CT52 at 40 °C ± 0.05 °C and a CANNON-Fenske viscometer capillary tube n°100 and constant C equal to 0.01481 mm² s⁻². The kinematic viscosity, ν , was calculated from the measured flow time, t , and the instrument constant, C , using Eq. 2:

$$\nu = C \cdot t \quad (2)$$

where ν – kinematic viscosity, in mm² s⁻¹; C – viscosimetric tube calibration constant, in mm² s⁻²; t – flow time, in seconds.

The procedure to determine the acid value consists of using 0.1M solution of potassium hydroxide (KOH) to titrate the free fatty acid in the sample. In each sample, 2 g of oil were weighed in 10 mL of ether and ethanol solution (2:1) and, after homogenization of the solution, it is titrated with a phenolphthalein indicator. The acidity calculation is determined by Eq. 3:

$$I = \frac{V \cdot 0.1 \cdot f \cdot 56.1}{P} \quad (3)$$

where I – acid value; f – correction factor for the KOH solution; V – volume of KOH to titrate the sample; 56.1 – gram equivalent of KOH; P – number of grams of the sample.

Analysis by Gas Chromatography coupled to Mass Spectrometry

The samples were qualitatively analyzed in the Gas Chromatograph coupled to Mass Spectrometry (CG-EM) GCMS-QP2010 (Shimadzu, Tokyo, JP) using the following conditions: injection with flow division in a 1:20 ratio; DB5-MS column (30 m × 0.25 mm D.I. and 1 μm of 5% phenyl-polydimethylsiloxane); the carrier gas used was He (99.999% pure) under a constant flow of 3.0 mL min⁻¹; oven temperature setting was 50 °C – 180 °C with heating rate of 8 °C min⁻¹, 180 °C – 230 °C with heating rate of 5 °C min⁻¹, 230 °C – 310 °C with heating rate of 20 °C min⁻¹ followed by isotherm for 15 minutes. The chromatographic profiles were made by comparison with the Nist 147 library (US National Institute of Standards and Technology 147), indicating the presence of some methyl esters in the samples. The distribution of the observed substances was determined by normalizing the area of each peak, that is, in percentage of relative chromatographic area.

Statistical analysis

For the statistical analysis, the program SISVAR version 5.3 (FERREIRA, 2014) was used, applying the Tukey test at the significance level of 5%. The statistical analysis was performed using the experimental data obtained in the present study, with a total of 81 observations, estimating the effects on the biodiesel production yield of the studied variables and their interactions.

Making the response surface

To make the response surface, the Matlab R2015b software (academic version) was used. Initially, all data was tabulated using electronic spreadsheets, in which all input and output variables were inserted. After data tabulation, the file was exported to Matlab through a script to generate the response surface based on interpolation.

The response surface was obtained based on the three input variables ((molar ratio (x), stirring time (y) and temperature (z)) and as the output variable the yield. Therefore, the yield was a function of the three input variables, according to Eq. 4:

$$\text{Yield} = f(xq, yq, zq) \quad (4)$$

After obtaining the response surface, the maximum yield point was determined based on the inputs.

RESULTS AND DISCUSSION

Physicochemical characterization of Biodiesel

Specific mass at 20 °C [kg m⁻³]

Table 4 presents the variance analysis of the effect of the studied variables and their interactions in the specific mass determined with digital densimeter. The obtained results show that the molar ratio and reaction time, as well as the molar ratio*time and time*temperature interactions cause significant changes in specific mass at 20 °C. The

significance of each effect is considered by the probability value (*p-value*). The *p-value* was set at 0.05 or 5% for a 95% confidence level in this study. In this analysis, the effects with a *p-value* of less than 0.05 are considered significant effects.

Table 4. Variance analysis of the effect of the studied variables and their interactions on the specific mass determined with digital densimeter

<i>F.V.</i>	<i>G.L.</i>	<i>SQ</i>	<i>QM</i>	<i>FC</i>	<i>P-value</i>
Molar ratio	2	5.542141	2.77107	62.768	0.0000*
Time	2	0.854719	0.42736	9.680	0.0073*
Temperature	2	0.178319	0.08916	2.020	0.1950 ^{ns}
Molar ratio * Time	4	0.949570	0.23739	5.377	0.0212*
Molar ratio * Temperature	4	0.659437	0.16486	3.734	0.0533 ^{ns}
Time * Temperature	4	0.882659	0.22067	4.998	0.0257*
Error	8	0.353185	0.044148		
Total Corrected	26	9.420030			
<i>CV</i>	0.02				
<i>Overall Average</i>	878.4437				

F.V. = variation source; *G.L.* = degree of freedom; *SQ* = sum of squares; *QM* = mean square; *FC* = calculated *F*; *Pr > Fc* = hypothesis test; * Significant; *ns* = not significant.

From the statistical analysis of the molar ratio*reaction time interaction (Table 5), it is possible to observe that the highest specific mass values 879.14, 878.95 and 879.09 kg m⁻³ occurred for a 12:1 molar ratio, with no significant differences for reaction times. The lowest values of specific mass 877.63 and 877.72 kg m⁻³ were observed for a 6:1 molar ratio and the times of 60 and 120 minutes, respectively, with no significant differences between them.

In the analysis of biodiesel by Peiter et al. (2018), the authors determined the specific mass at 20 °C of biodiesel produced from soybean oil by ethylic route using the molar ratio parameters of 1:10 oil:alcohol and reaction time of 30 minutes, and it was observed that the value of 873.4 kg m⁻³ is in accordance with ANP Resolution No. 45/2014. In the work of Borges et al. (2014), the authors determined the specific mass of soybean biodiesel produced by ethylic route using molar ratios of 9:1 and 12:1 and reaction times of 30 and 60 minutes, making it possible to observe that the specific mass values 870.0 kg m⁻³ and 891.8 kg m⁻³ are also in accordance with ANP Resolution No. 45/2014. Thus, the results obtained in the present study do not differ from those found by Peiter et al. (2018) and Borges et al. (2014) and are in accordance with the parameters of ANP Resolution No. 45/2014.

Kinematic Viscosity at 40 °C [mm² s⁻¹]

Table 6 presents the variance analysis of the effect of the studied variables and their interactions on kinematic viscosity. The results obtained show that the molar ratio, as

Table 5. Statistical analysis for specific mass determined with densimeter considering molar ratio and reaction time

Time (min)	Molar Ratio		
	6:1	9:1	12:1
30	878.60 Ab	878.32 Aa	879.14 Ba
60	877.63 Aa	878.21 Ba	878.95 Ca
120	877.72 Aa	878.32 Ba	879.09 Ca

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

well as the molar ratio*time interaction cause significant changes in the kinematic viscosity at 40 °C. The significance of each effect is considered by the probability value (*p-value*). The *p-value* was set at 0.05 or 5% for a 95% confidence level in this study. In this analysis, effects with a *p-value* of less than 0.05 are considered significant effects.

Table 6. Variance analysis of the effect of the studied variables and their interactions on kinematic viscosity

<i>F.V.</i>	<i>G.L.</i>	<i>SQ</i>	<i>QM</i>	<i>FC</i>	<i>P-value</i>
Molar ratio	2	0.055563	0.027782	9.147	0.0086*
Time	2	0.009289	0.004644	1.529	0.2739 ^{ns}
Temperature	2	0.009641	0.004821	1.587	0.2627 ^{ns}
Molar ratio * Time	4	0.048839	0.012210	4.020	0.0447*
Molar ratio * Temperature	4	0.024089	0.006022	1.983	0.1902 ^{ns}
Time * Temperature	4	0.015276	0.003819	1.257	0.3615 ^{ns}
Error	8	0.024297	0.003037		
Total Corrected	26	0.186994			
<i>CV</i>	1.18				
<i>Overall Average</i>	4.659339				

F.V. = variation source; *G.L.* = degree of freedom; *SQ* = sum of squares; *QM* = mean square; *FC* = calculated *F*; *Pr > Fc* = hypothesis test; * Significant; *ns* = not significant.

The interaction molar ratio*reaction time (Table 7) makes it possible to observe that the kinematic viscosity was higher for the molar ratio of 6:1 and time of 30 minutes, in which the value of 4.80 mm² s⁻¹ was found, differing significantly from other times and molar ratios.

The analysis of the molar ratio*temperature interaction (Table 8) highlights that the molar ratio of 9:1 and the temperature of 30 °C present the lowest value of kinematic viscosity 4.52 mm² s⁻¹, with significant differences for the other molar ratios.

Table 7. Statistical analysis for kinematic viscosity considering molar ratio and reaction time

Time (min)	Molar Ratio		
	6:1	9:1	12:1
30	4.80 Bb	4.59 Aa	4.66 Aa
60	4.64 Aa	4.60 Aa	4.69 Aa
120	4.64 Aa	4.59 Aa	4.72 Aa

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

Table 8. Statistical analysis for kinematic viscosity considering molar ratio and temperature

Temperature (°C)	Molar Ratio		
	6:1	9:1	12:1
30	4.71 Ba	4.52 Aa	4.69 Ba
40	4.71 Aa	4.65 Ab	4.69 Aa
50	4.65 Aa	4.61 Aab	4.68 Aa

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

In the work of Borges et al. (2014), the authors produced soybean biodiesel by ethylic route and obtained kinematic viscosity values of 4.3 and 4.4 mm² s⁻¹. The kinematic viscosity value of 5.0625 mm² s⁻¹ obtained by Peiter et al. (2018) also complies with Brazilian and European standards, which establish values from 3.0 to 6.0 mm² s⁻¹. Thus, the results obtained in the present study do not differ from those found by Peiter et al. (2018) and Borges et al. (2014), and are in accordance with the standards.

Acid value [mg KOH g⁻¹]

Table 9 presents the variance analysis of the effect of the studied variables and their interactions on the acid value. The results obtained for the acid value show that only the molar ratio causes significant changes. The significance of each effect is considered by the probability value (*p-value*). The *p-value* was set at 0.05 or 5% for a 95% confidence level in this study. In this analysis, effects with a *p-value* of less than 0.05 are considered significant effects.

Table 9. Variance analysis of the effect of the studied variables and their interactions on acid value

<i>F.V.</i>	<i>G.L.</i>	<i>SQ</i>	<i>QM</i>	<i>FC</i>	<i>P-value</i>
Molar ratio	2	7.915563	3.957781	52.733	0.0000*
Time	2	0.158141	0.079070	1.054	0.3925 ^{ns}
Temperature	2	0.145341	0.072670	0.968	0.4202 ^{ns}
Molar ratio * Time	4	0.456770	0.114193	1.521	0.2839 ^{ns}
Molar ratio * Temperature	4	0.253704	0.063426	0.845	0.5343 ^{ns}
Time * Temperature	4	0.166393	0.041598	0.554	0.7022 ^{ns}
Error	8	0.600430	0.075054		
Total Corrected	26	9.696341			
<i>CV</i>		24.87			
<i>Overall Average</i>		1.101482			

F.V. = variation source; *G.L.* = degree of freedom; *SQ* = sum of squares; *QM* = mean square; *FC* = calculated *F*; *Pr > Fc* = hypothesis test; * Significant; *ns* = not significant.

As shown in Tables 10 and 11, for the 6:1 and 9:1 molar ratios, the values do not present significant differences among them. The highest values of acid value were found for the 12:1 molar ratio, differing significantly from the values of the 6:1 and 9:1 molar ratios. The reaction time and temperature did not cause significant changes in the acid value.

Table 10. Statistical analysis for acid value considering time and molar ratio

Time (min)	Molar Ratio		
	6:1	9:1	12:1
30	0.70 Aa	0.70 Aa	1.84 Ba
60	0.59 Aa	0.85 Aa	2.18 Ba
120	0.65 Aa	0.84 Aa	1.57 Ba

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

Table 11. Statistical analysis for acid value considering temperature and molar ratio

Temperature (°C)	Molar Ratio		
	6:1	9:1	12:1
30	0.59 Aa	0.83 Aa	1.59 Ba
40	0.65 Aa	0.80 Aa	2.07 Ba
50	0.70 Aa	0.76 Aa	1.93 Ba

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

During the analysis of the biodiesel acid value, values were found well above the limit established by Table 3. This fact probably results from the procedure used to wash the biodiesel using three drops of hydrochloric acid and 30 mL of distilled water for each 100 mL of dirty biodiesel.

This procedure can work satisfactorily for washing biodiesel produced with methanol, because, according to Gomes (2011), it has smaller molecules (compared to ethanol), so the bonds between alcohol and glycerin are broken more easily and quickly, while ethanol has more stable bonds with glycerin, and therefore requires a more

appropriate washing procedure. It is also worth noticing that the acid value of the soybean oil used with raw material was checked and that it had an acid value of $1.08 \text{ mg KOH g}^{-1}$, which, possibly, may also have contributed to the high acid value of biodiesels. According to Santos et al. (2017), the acid value is directly related to the oil's conservation status and the latter is influenced by the nature and quality of the raw material, the purity of the oil, the processing and, mainly, by the storage conditions, since the decomposition of glycerides is accelerated by heating and light.

Thus, the washing procedure adapted from Abbaszadeh et al. (2014) was tested, which consists of standardizing a 1 molar hydrochloric acid solution and using a 1:1 water: biodiesel ratio to wash. The molar ratio used in these tests was 6:1, with a temperature of $40 \text{ }^{\circ}\text{C}$ and 30 minutes of reaction.

In the first test, only two drops of the solution were added to the washing water in the first wash. In the other washes, distilled water was used without adding the hydrochloric acid solution. Biodiesel was washed until the waste water from the wash had a clear appearance. In the second test, the first two washes were made only with water and in the third wash two drops of the solution were added to the water and the biodiesel continued to be washed until the waste water from the wash had a clear appearance. In the third test, in every wash, two drops of the solution were added to the water and the biodiesel continued to be washed until the waste water from the wash was clear. Table 12 shows the number of times that each test took until the waste water had a clear appearance.

Table 12. Result of biodiesels washing tests

Variable	Test 1	Test 2	Test 3
Addition of two drops of the HCl solution	In the 1st wash	In the 3rd wash	In all washes
Number of washes	9	14	19
Yield (%)	88.37	87.48	87.91
Specific Mass (Kg m^{-3})	878.68	879.16	879.74
Kinematic viscosity ($\text{mm}^2 \text{ s}^{-1}$)	5.05	5.10	5.11
Acid value (mg of KOH g^{-1})	0.54	0.54	0.60

The tests showed positive results in relation to the acid value, as it was possible to observe that washing the biodiesel with distilled water and two drops of the HCl solution in the first wash resulted in a biodiesel with a lower acid value than the one previously produced, with a lower number of washes.

Observing the physicochemical characteristics of biodiesel, it is noticed that the values are close to those previously obtained. It is believed that this procedure is also capable of producing biodiesel that complies with the limits established in ANP Resolution No. 45/2014 for the other molar ratios of 9:1 and 12:1.

Analysis by Gas Chromatography coupled to Mass Spectrometry

For the Chromatography analysis, only the molar ratio was taken into account, therefore, the samples (of each molar ratio) were prepared by mixing all treatments of each molar ratio.

In the chromatographic profile of biodiesel produced from soybean oil by ethylic route with a 6:1 molar ratio, the signs of interest related to fatty esters are included in the interval from 16 to 21 minutes of elution (or retention), as shown in Table 13 and Fig. 1. The fatty acids that present the largest areas are Oleic and Stearic acids, with areas of 52.26% and 30.69%, respectively.

Table 13. Fatty acids table for a 6:1 molar ratio

Fatty acid	Nomenclature	Retention time (min)	Area (%)
C16:0	Palmitic	16.962	11.40
C18:0	Stearic	19.433	30.69
C18:1	Oleic	20.040	52.26
C18:2	Linoleic	20.645	5.30
C18:3	Linolenic	20.755	0.36

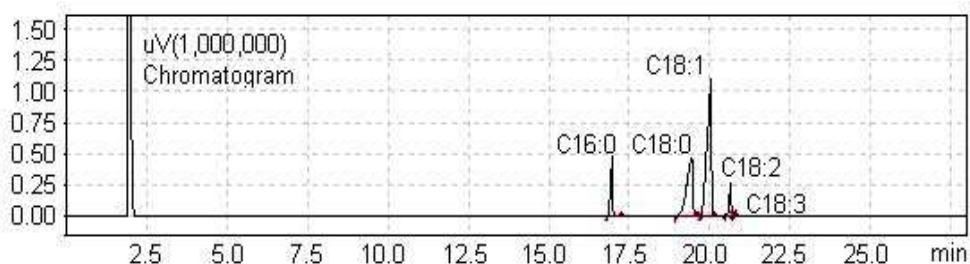


Figure 1. Chromatogram for a 6:1 molar ratio.

The Chromatogram of the biodiesel sample produced from soybean oil by ethylic route with a 9:1 molar ratio shows, in the interval between 16 and 21 minutes, the retention times of the fatty esters that most contributed to the biodiesel composition, as shown in Table 14 and Fig. 2. It can be seen that biodiesel with a 9:1 molar ratio has Oleic and Stearic acids as those with the largest areas, with 52.41% and 30.34%, respectively.

Table 14. Fatty acids table for a 9:1 molar ratio

Fatty acid	Nomenclature	Retention time (min)	Area (%)
C16:0	Palmitic	16.966	11.19
C18:0	Stearic	19.488	30.34
C18:1	Oleic	20.052	52.41
C18:2	Linoleic	20.651	5.59
C18:3	Linolenic	20.757	0.48

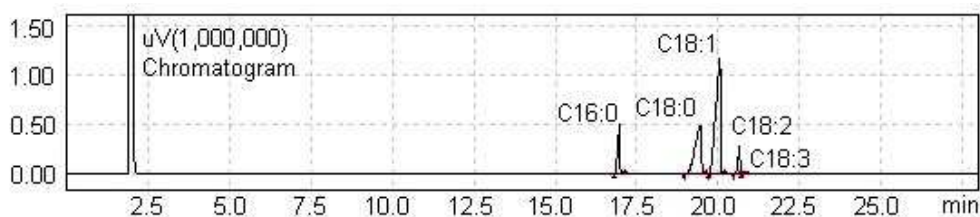


Figure 2. Chromatogram for a 9:1 molar ratio.

In the chromatographic profile of biodiesel produced from soybean oil by ethylic route with a 12:1 molar ratio, the signs of interest related to fatty esters are included in the interval from 16 to 21 minutes of retention, as shown in Table 15 and Fig. 3. It can

be seen that biodiesel with a 12:1 molar ratio presents Oleic and Stearic acids as those with the largest areas, with 52.22% and 30.61%, respectively.

With the chromatographic analysis, from the determination of the elution times, it was possible to identify five different fatty acids in the composition of the biodiesels. Total saturated fatty acids (palmitic and stearic acids) ranged from 41.53% to 42.09% and total unsaturated, including monounsaturated and polyunsaturated (oleic, linoleic and linolenic acids) ranged from 57.92% to 58.48%.

Table 15. Fatty acids table for a 12:1 molar ratio

Fatty acid	Nomenclature	Retention time (min)	Area (%)
C16:0	Palmitic	16.972	11.25
C18:0	Stearic	19.468	30.61
C18:1	Oleic	20.069	52.22
C18:2	Linoleic	20.659	5.45
C18:3	Linolenic	20.761	0.47

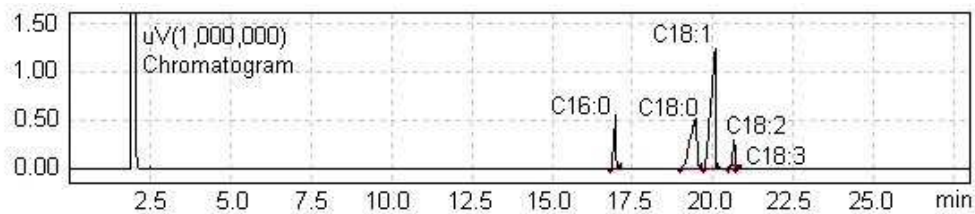


Figure 3. Chromatogram for a 12:1 molar ratio.

From the analysis of Table 16, it can be seen that biodiesel with a 6:1 molar ratio has, on average, a higher percentage of saturated fatty acids 42.09%, while the 9:1 molar ratio has the lowest percentage of saturated fatty acids 41.53%, and all molar ratios differ significantly from each other. On the other hand, the 9:1 molar ratio has the highest percentage of unsaturated fatty acids 58.48%, differing significantly from the other molar ratios.

Yield

Table 17 presents the variance analysis of the effect of the studied variables and their interactions on the production yield. The results obtained show that the molar ratio, reaction time and temperature, as well as the molar ratio*temperature interaction, cause significant changes in yield. The significance of each effect is considered by the probability value (*p-value*). The *p-value* was set at 0.05 or 5% for a 95% confidence level in this study. In this analysis, effects with a *p-value* of less than 0.05 are considered significant effects.

Table 16. Statistical analysis for fatty acids areas

Fatty acid	Molar Ratio		
	6:1	9:1	12:1
Stearic	30.69 C	30.34 A	30.61 B
Linoleic	5.30 A	5.59 C	5.45 B
Linolenic	0.36 A	0.48 C	0.47 B
Oleic	52.26 B	52.41 C	52.22 A
Palmitic	11.40 C	11.19 A	11.25 B

Averages followed by the same letter on the line do not differ from each other at the level of 5% probability according to the Tukey test.

Table 17. Variance analysis of the effect of the studied variables and their interactions on the production yield

<i>F.V.</i>	<i>G.L.</i>	<i>SQ</i>	<i>QM</i>	<i>FC</i>	<i>P-value</i>
Molar ratio	2	39.54295	19.771474	8.855	0.0004*
Time	2	62.07995	31.039974	13.902	0.0000*
Temperature	2	359.37242	179.686211	80.475	0.0000*
Molar ratio * Time	4	19.23380	4.808450	2.154	0.0847 ^{ns}
Molar ratio * Temperature	4	28.77455	7.193638	3.222	0.0182*
Time * Temperature	4	17.55756	4.389390	1.966	0.1108 ^{ns}
Error	62	138.43403	2.232807		
Total Corrected	80	664.99526			
<i>CV</i>	1.71				
<i>Overall Average</i>	87.28216				

F.V. = variation source; *G.L.* = degree of freedom; *SQ* = sum of squares; *QM* = mean square; *FC* = calculated *F*; *Pr > Fc* = hypothesis test; * Significant; *ns* = not significant.

From the analysis of the molar ratio * reaction time interaction (Table 18) it is possible to observe that the highest yields 89.72% and 88.37% were obtained for the time of 30 minutes and for the molar ratios of 9:1 and 12:1, not differing significantly from each other. It is also observed that the increase in time leads to a lower yield in production, except for the 6:1 molar ratio, whose yield does not differ significantly among times.

The molar ratio*temperature interaction (Table 19) highlights that the highest yields 91.16% and 89.51% were obtained for the temperature of 30 °C and for the molar ratios of 9:1 and 12:1, not significantly differing between them. It can be seen that the increase in temperature leads to a lower yield in production.

Table 18. Statistical analysis for yield considering time and molar ratio

Time (min)	Molar Ratio		
	6:1	9:1	12:1
30	87.12 Aa	89.72 Bb	88.37 ABb
60	86.55 Aa	87.71 Aa	87.29 Ab
120	86.31 ABa	87.35 Ba	85.13 Aa

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

Table 19. Statistical analysis for yield considering temperature and molar ratio

Temperature (°C)	Molar Ratio		
	6:1	9:1	12:1
30	88.61 Ab	91.16 Bc	89.51 ABb
40	87.58 ABb	88.70 Bb	86.16 Aa
50	83.80 Aa	84.92 Aa	85.12 Aa

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

In the interaction temperature*reaction time (Table 20), the maximum yields 90.87% and 90.09% are observed for the temperature of 30 °C and times of 30 and 60 minutes, and do not differ significantly from each other. It is also observed that the increase in temperature and reaction time leads to a decrease in yield, except for the temperature of 50 °C, in which the yield does not differ significantly between times.

Moradi et al. (2013) obtained the maximum yield of 93.2% for the 9:1 molar ratio and reaction temperature of 60 °C. In the work of Rahimi et al. (2014), the authors obtained the maximum yield of 94.78% using the 9:1 molar ratio and it was observed

that the increase in the molar ratio from 6:1 to 9:1 causes an increase in yield; on the other hand, the increase in the molar ratio from 9:1 to 12:1 results in reduced yield, the same behavior observed in the present study.

Joshi et al. (2017) investigated the effects of the molar ratio on the biodiesel yield, keeping the catalyst quantity, temperature and reaction time constant. The authors observed that there is a constant increase in the biodiesel yield up to a 10:1 molar ratio; however, after 10:1, the yield remains practically constant. They attribute this to the fact that the glycerol produced during the reaction is dissolved in the excess alcohol, affecting the reaction balance.

Morais et al. (2013) also observed that the increase in the molar ratio from 1:8 to 1:12 caused a reduction in yield, and associated this problem with excess alcohol and its boiling point, as it is likely that in larger molar ratios the evaporation of alcohol is more pronounced, causing the loss of part of the reagent and consequent reduction in conversion to esters.

Response Surface

The Response Surfaces present the behavior of the variables manipulated in this work (molar ratio, temperature and reaction time), as well as their interactions with the biodiesel production yield from soybean oil.

As shown in Fig. 4, the increase in temperature results in a decrease in yield. As represented by the dark blue color, the yields of biodiesels produced at a temperature of 50 °C were the lowest for all molar ratios and all stirring times, with values varying between 83% and 85%. Temperatures between 30 °C and 40 °C showed higher yields compared to the temperature of 50 °C. This fact can be explained, according to Morais et al. (2013), by the loss of alcohol during the reaction, as the increase in this variable favors the alcohol volatilization. On the other hand, Joshi et al. (2017), using a reflux condenser coupled to the system, observed an increase in the reaction yield when the temperature goes from 40 °C to 50 °C, because with the use of the reflux condenser the evaporated alcohol returns to the reaction.

The Fig. 5 shows another perspective of the same response surface. It is possible to notice that the molar ratios of 9:1 and 12:1 were the ones that presented the highest yields. The 9:1 molar ratio showed yields above 90% (90.85%, 91.19% and 91.44%) for 30, 60 and 120 minutes, respectively, with the temperature at 30 °C. With the temperature at 40 °C, the 9:1 molar ratio also showed yields above 90% (91.78%) for the time of 30 minutes. The 12:1 molar ratio showed the maximum yield of 93.30%, for the temperature of 30 °C and time of 30 minutes, represented by the dark red color.

Table 20. Statistical analysis for yield considering temperature and reaction time

Temperature (°C)	Time (min)		
	30	60	120
30	90.87 Bc	90.09 Bc	88.32 Ab
40	89.09 Bb	87.34 Ab	86.01 Aa
50	85.24 Aa	84.13 Aa	84.46 Aa

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

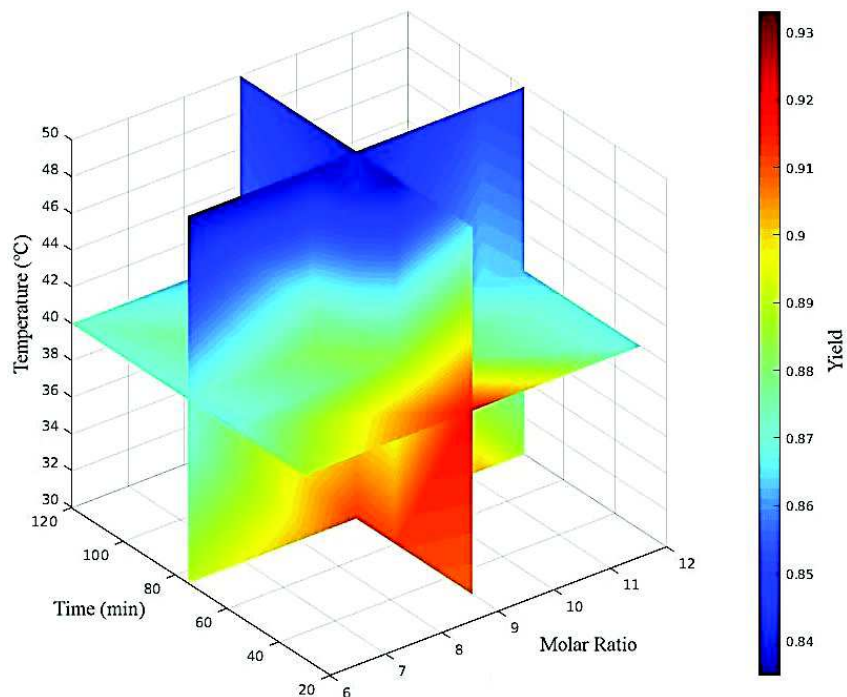


Figure 4. Response Surface of behavior of the variables manipulated (molar ratio, temperature and reaction time) at production yields effects.

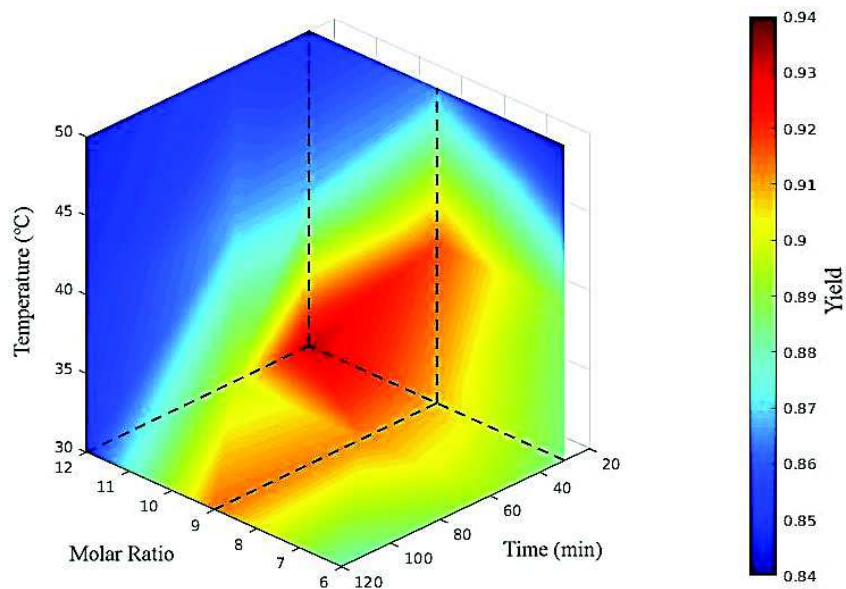


Figure 5. Response Surface - another perspective.

The increase in the molar ratio shows an increase in yield due to the greater amount of alcohol present in the reaction and consequently to the greater conversion into esters.

However, the increase in the molar ratio from 9:1 to 12:1 for higher temperatures showed a reduction in yield, a fact that according to Morais et al. (2013) is related to alcohol volatilization. Joshi et al. (2017) also explain that the transesterification reaction using high molar ratios favors the solubilization of glycerol in alcohol, which generates an imbalance in the reaction and consequently a reduction in yield. Although the 12:1 molar ratio was the one with the highest yield, this was only observed for the temperature of 30 °C and time of 30 minutes, and in the other times and temperatures this molar ratio showed unsatisfactory yields.

The Fig. 6 shows the normalized distribution of production yields, and it is possible to notice that for the 12:1 molar ratio, few values reached yields of 92% to 95%, and these refer to the region of maximum yield observed in Fig. 5. On the other hand, the 9:1 molar ratio showed a significantly greater amount of values between 90% and 93%. These values are justified by the better performance of the 9:1 molar ratio (in relation to the 12:1 ratio) at the different reaction times studied, as shown in Fig. 5.

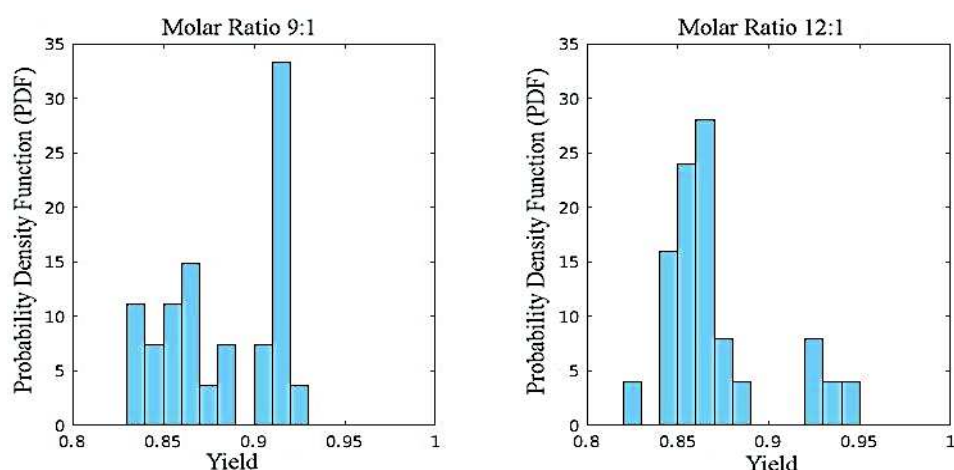


Figure 6. Normalized Probability Distribution of production yields for 9:1 and 12:1 molar ratio.

This study clearly shows that the response surface methodology was an adequate method to determine the best operating conditions, in order to maximize the production of ethyl esters. Higher yield results in less glycerin produced, therefore, in lower environmental impact. By determining the best yield point, the amount of alcohol used is optimized, which results in efficiency in both production cost and yield. Regarding the reaction time and temperature, it is possible to state the condition for obtaining greater yield in the biodiesel production and with low energy consumption.

CONCLUSIONS

In this work, the yield of biodiesel production from soybean oil was analyzed during the variation of the parameters molar ratio alcohol: oil, temperature and reaction time in laboratory conditions, making it possible to conclude that the configuration that results in maximum yield of 93.30% is a 12:1 molar ratio, temperature of 30 °C and reaction time of 30 minutes. The 6:1 molar ratio showed a higher yield of 89.30% at a

temperature of 30 °C and a reaction time of 60 minutes, while the 9:1 molar ratio showed a maximum yield of 91.78% at a temperature of 40 °C and reaction time of 30 minutes. From the analysis of the response surface, it is observed that the increase in temperature and reaction time leads to a reduction in yield, a fact explained by the favor of alcohol volatilization, which impacts on the formation of ethyl esters.

According to the results of the physicochemical analyses, the specific mass at 20 °C is in accordance with Brazilian, American and European specifications, varying between 877.46 kg m⁻³ and 879.64 kg m⁻³. The kinematic viscosity at 40 °C varied between 4.49 mm² s⁻¹ and 4.82 mm² s⁻¹, and is also in accordance with the limits established by the standards. The acid value obtained did not vary within the limits established by the standards. Values between 0.54 and 2.74 mg of KOH g⁻¹ were observed, but according to the standards presented, this value could not exceed the maximum limit of 0.5 mg of KOH g⁻¹.

Based on the results of the study, it can be said that the use of ethanol (as a substitute for methanol) combined with soybean oil, results in the production of a fuel from 100% renewable sources (ethanol derived from sugarcane and soybean oil), in addition to using a low toxicity reagent when compared to methanol.

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