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Variation in the proportion of coconut oil mixed with fish oil: an analysis of the effect on the oxidative potential and quality of the biodiesel produced

In view of the constant environmental, social and economic concerns, the biodiesel's production has proved to be a viable option as an alternative energy source substitute for fossil fuels, contributing to the reduction of greenhouse gas emissions. Whereas that the fishing industry produces a significant amount of by-products, the fish's oil use for the biodiesel production has proven to be a viable alternative for the energy matrix diversification, helping to repair the environmentally inappropriate disposal of waste generated with fish processing. The coconut oil it's an inedible oil, it has lowest cost and presents a relatively high oxidative stability, which indicates that its mixture is interesting with fish oil before the transesterification. Oxidative stability is the main impediment of using oil from fishing industry waste for biodiesel production, can be justified mainly by the presence of unsaturation in its carbon chain. Thus, this study aimed to evaluate the coconut oil effect, to fish oil from fishing activity residues of a fishmonger located in the city of Rio Grande/RS, in oxidative stability and also perform statistical tests to check the best configuration, temperature, blend, catalyst, in the biodiesel production. The results verified what addition of coconut oil, to fish oil was positive, considering it was acted as an antioxidant additive and the physical-chemical analyzes carried out showed that the biodiesel produced has good quality, since all samples analyzed fit established quality parameters, with exception of the iodine index of some samples.

Keywords: Fish oil; Coconut oil; Transesterification; Biodiesel; Physical-chemical analyzes.

Variação na proporção de óleo de coco misturado com óleo de peixe: uma análise do efeito no potencial oxidativo e na qualidade do biodiesel produzido

Diante das constantes preocupações ambientais, sociais e econômicas, a produção do biodiesel tem se mostrado uma opção viável como fonte alternativa de energia substituta aos combustíveis fósseis, contribuindo para a redução das emissões de gases de efeito estufa. Considerando que a indústria pesqueira produz uma quantidade significativa de subprodutos, a utilização do óleo de peixe para a produção de biodiesel tem se mostrado uma alternativa viável para a diversificação da matriz energética, auxiliando na reparação do descarte ambientalmente inadequado dos resíduos gerados com o processamento do pescado. O óleo de coco é um óleo não comestível, tem o menor custo e apresenta uma estabilidade oxidativa relativamente alta, o que indica que sua mistura é interessante com óleo de peixe antes da transesterificação. A estabilidade oxidativa é o principal impedimento da utilização de óleo de resíduo da indústria pesqueira para produção de biodiesel, podendo ser justificado principalmente pela presença de insaturação em sua cadeia carbônica. Assim, este trabalho teve como objetivo avaliar o efeito do óleo de coco, para óleo de peixe proveniente de resíduos da atividade pesqueira de uma peixaria localizada na cidade de Rio Grande/RS, na estabilidade oxidativa e também realizar testes estatísticos para verificar a melhor configuração, temperatura, blend, catalisador, na produção de biodiesel. Os resultados verificaram qual adição de óleo de coco, ao óleo de peixe foi positiva, visto que atuou como aditivo antioxidante e as análises físico-químicas realizadas mostraram que o biodiesel produzido possui boa qualidade, pois todas as amostras analisadas se enquadram nos parâmetros de qualidade estabelecidos, com exceção do índice de iodo de algumas amostras.

Palavras-chave: Óleo de peixe; Óleo de coco; Transesterificação; Biodiesel; Análises físico-químicas.

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INTRODUCTION

In recent years, the constant rise in the price per barrel of oil, the high demand for energy and the environmental problems related to the greenhouse gasesemission, such as global warming, boosted the search for alternative energies (CARVALHO, 2016). Biodiesel, a product from renewable and sustainable biomass, has stood out due to its high environmental, social and economic contribution (OLIVEIRA et al., 2017).

In order to incorporate biodiesel into the Brazilian energy matrix, in December 2004 the federal government launched the National Biodiesel Production and Use Program (PNPB). The legally mandatory 2% mixture (B2) came into effect in 2008 throughout the national territory. Currently, this percentage is 10.0% (B10). According to the schedule established in the resolution, the minimum biodiesel content in diesel oil will be extended 1% each year, until reach the percentage of 15% in 2023 (ANP, 2020).

Biodiesel is obtained by transesterification, a chemical reaction related to the conversion of oils and fatty acids into alkyl esters. The transesterification reaction is a chemical reaction that produces an ester from another ester (KNOTHE et al., 2017). In the transesterification reaction, a chemical reaction occurs between a triglyceride and an alcohol in the presence of a catalyst, forming ester and glycerol (by-product). Methanol is the alcohol generally used to produce biodiesel due to its lower cost and easy availability (SINGH et al., 2020). Excess alcohol is used to boost the reversible reaction in order to shift the balance to maximum biodiesel yield and assist in the separation of glycerol phases (D'AGOSTO et al., 2015).

Currently, due to inadequate disposal, the waste generated by fishing activity has been causing damage to the environment. Disposal is generally on land, ocean, rivers and lakes, and the handling of this waste is a serious problem worldwide, especially in underdeveloped countries (BALBINOT, 2015). Therefore, according to Iastiaque Martins et al. (2015a), the use of fish fat for the biodiesel production contributes to diversify the options of raw materials for the biofuel production, in addition to helping to repair the environmentally inappropriate disposal of waste generated from fish processing, bringing an environmental contribution.

In addition, according to Chiou et al. (2008), the use of fish oil as a raw material for the biodiesel production has the potential to considerably reduce production costs, however, it can be characterized by having a low oxidative stability (GARCÍA-MORENO et al., 2014). Oxidative stability is the main impediment to the use of waste oil from the fishing industry for biodiesel production, which can be justified mainly by the presence of unsaturation in its carbon chain (RODRIGUES, 2017).

Coconut oil, abundant in Asia, is a non-edible and less expensive oil. In most producing countries, Coco (Cocos nucifera L.) is used for theoil production, considering that its composition is predominantly lauric acid (ARAÚJO et al., 2009). Differently most natural oils, this acid has a short chain (C_{12} e C_{14}), which can reduce the viscosity of biodiesel (ALLEN et al., 1999). In addition, according to Silva et al. (2015), most of the fatty acids in coconut oil are saturated, giving it high resistance to oxidation. Hence, it can be a suitable oil to be mixed with fish oil before transesterification.

Therefore, this study aimed to evaluate the coconut oil effect, to fish oil from fishing activity residues of a fishmonger located in the city of Rio Grande/RS in oxidative stability and also perform statistical tests to check the best configuration, temperature, blend, catalyst, in the biodiesel production, that fits the parameters required by the established standards by National Agency of Petroleum, Natural Gas and Biofuels (ANP), European Union Standard (EU) and North-American Norm (ASTM).

METHODOLOGY

All reagents used were from the Aldrich brand and are 99% pure.

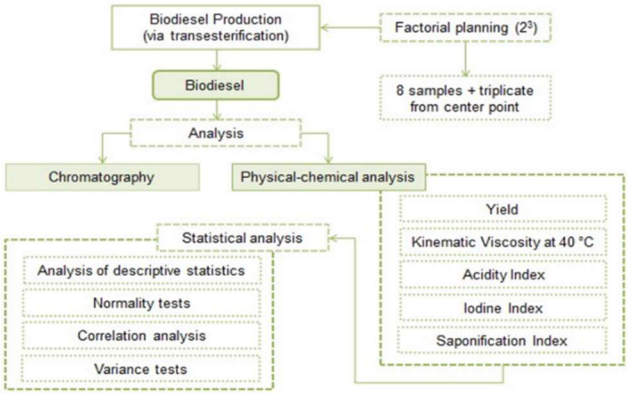

Figure 1: methodology diagram.

Factorial Design

To obtain the biodiesel samples, a 2^3 factorial design was performed (Table 1). This planning resulted in eight points with different conditions of biodiesel production varying more or less in relation to a central point (CP), from which a triplicate was performed (CUNICO et al., 2008). Variations in the conditions of the applied methodology consisted in the proportions of coconut oil in relation to fish oil, the amount of catalyst used in the reaction, as well as the temperature during the biodiesel production process (from 60 to 80ºC).

Table 1: Factorial design of the samples.

Sample	Temperature (°C)	Coconut oil (%)	Catalyst (g)
P1	60.0	1.0	1.5
P2	80.0	1.0	1.5
P3	60.0	10.0	1.5
P4	80.0	10.0	1.5
P5	60.0	1.0	2.5
P6	80.0	1.0	2.5
P7	60.0	10.0	2.5
P8	80.0	10.0	2.5
CP1	70.0	5.0	2.0
CP2	70.0	5.0	2.0
CP3	70.0	5.0	2.0

Sampling

The process of converting oils into biodiesel was carried out according to Vieira et al. (2016). The biofuel from the fish/coconut blend was obtained via methyl transesterification, using potassium hydroxide

as a catalyst. The oil conversion in to biodiesel was performed through transesterification by methanol way, due to easier fuel separation in relation to ethanol production, this process took place as follows:

Initially, in a volumetric flask (250 mL) connected to a capacitor were added 125 mL of the mixture, in proportions according to the table 1, and 30 mL of methanol, and predetermined catalyst according to the table 1, these substances are mixed in water bath with the aid of a heating plate with magnetic stirrer, for an hour at predetermined temperature (according to the table 1). In the end of this one hour, 2.25 mL diluted sulfuric acid in 15 mL of methanol are inserted, in order to neutralized the base, and then, mix another hour, obtaining a mixture like biodiesel at the end of this process.

The methanol/oil ratio is determined by the molar mass of both, the stoichiometry of the transesterification reaction requires 3 moles of alcohol per mol of triglyceride, to produce 3 moles of biodiesel and 1 mol of glycerol, therefore, this ratio must be at least 3:1 being that, upper molar ratios result in greater ester conversion in less time.

After obtaining the mixture with biodiesel, it is filtered with the aid of a funnel and filter paper, for the retention of solids, in particular the salt formed in the reaction of Sulfuric Acid with Potassium Hydroxide. The resulting liquid is the heated (around 80° C), after this, it is inserted into a ball separating funnel, where is it until the glycerin decant, and then be removed thus leaving, only biodiesel. Posteriorly, in order to remove impurities, this biodiesel is washed twice with 100 mL of distilled water, finally, it is heated again so that any water residue evaporates. This process was performed eleven times, one for each sample of the factorial design, according to the table 1. With all of them prepared, part of the physical-chemical analysis.

Yield

With the aid of a 100 mL beaker and a 10 mL beaker, the final biodiesel volume of each sample (V) was measured. The yield of conversion oil to biodiesel was found using Eq. 1.

$$Yield = \frac{V}{125} * 100 \qquad (1)$$

Physical-Chemical Analysis

Regarding the quality of biofuel, the characterization of physical-chemical analysis is of paramount importance. This step had the objective of verifying whether the kinematic viscosity, acidity index and saponification index of the biodiesel generated were within the parameters required by resolution nº 45 of August 25, 2014 of the National Agency of Petroleum, Natural Gas and Biofuels (ANP). As for the iodine index, the limitfollowed was the established by the European Union standard (EN 14.214) (EN, 2008). The determinations of the acidity index, iodine index and saponification index were performed according to the standards ASTM D664 (ASTM, 2011), ASTM (ASTM, 1997) and AOAC (AOAC, 2007), respectively. The kinematic viscosity was measured at a temperature of 40 ° C, according to the ASTM D445 (ASTM, 2006) standard, on a Saybolt Viscometer - model Q288SR.

Acidity Index

Determining the acidity index is very important, because it provides data that assesses the oil's conservation, and also about product quality, since high acidity levels can cause corrosion in the storage tank, as well as engines. This determination was made as follows:

4 grams of the sample was weighed in an Erlenmeyer, the 25 mL of a 2:1 ethyl ether-ethanol solution and two drops of phenolphthalein were added. And then this mixture was titrated with a NaOH solution (0.01 mol/L) until it turns pink color.

Subsequently, the values obtained were added in Equation 2, where: "V" is the volume of titrant used (in mL), "f" is the titrant dilution factor (in mol/L) and ''P'' is the sample weight (in grams). The value of acidity index is expressed in mg of NaOH per gram of sample, and the limit value established by ANP is 0.5.

$$Acidity\ Index = \frac{V*f*6{,}81}{P} \qquad (2)$$

Iodine Index

In an Erlenmeyer, 0.25 g of sample was added, 10 mL of cyclohexane and 25 mL of the iodine-chlorine solution (according to Wijis), and after that, the mixture was stirred, covered and kept away from light. After 30 minutes, 100 mL of distilled water were added and 10 mL of a 15% potassium iodide solution, and then with the aid of a beaker, the sample was titrated with sodium thiosulfate (0.1 mol/L), until a faint yellow color appears, at this time have been added 2 mL of a starch indicator solution 1%, and the titration was continued until the blue color disappeared.

After performing the titration, the values obtained during the analysis are inserted in Equation 3, the sample's iodine index is then obtained. In the equation we have to, "M" is molarity of the sodium thiosulfate solution, "Vb" is the volume in mL spent on white titration, "Va" is the volume in mL spent on sample titration and "P" is the weight of the sample in grams.

$$Iodine\ Index = \frac{(Vb-Va)*M*12{,}69}{P} \qquad (3)$$

Saponification Index

A 4 gram biodiesel sample mass was weighed in a flask, and 50 mL of 4% potassium hydroxide solution (KOH) in ethanol were added, after the balloon was connected to a condenser and left under reflux and agitation, per an hour, under a temperature of 90°C until complete sample saponification. Posteriorly, the sample was removed from the condenser and cooled to room temperature. Then, 2 drops of the phenolphthalein indicator were added to the flask and then the solution was titrated with hydrochloric acid 0.5 mol/L, until the pink color disappears. Lastly, a blank determination was prepared, proceeding in the same way as the sample.

After performing the above procedures, the saponification index can be obtained (SI) through the equation 4 where "A" is the volume spent on sample titration, "B" the volume spent on blank titration, "f" the factor of the HCI solution and ''P'' the sample weight used.

$$Saponification\ Index = \frac{(B-A)*f*28{,}06}{P} \qquad (4)$$

Kinematic Viscosity AT 40 °C

To determine the viscosity, the Saybolt Viscometer was used - model Q288SR. To determine the Kinematic Viscosity 40 °C one of the equipment's upper holes was closed with a stopper, which was attached by a chain. Then the Saybolt tube was filled with at least 60 mL of one the samples and was heated in a water bath until the desired temperature is reached. Thermal equilibrium reached, the stopper was removed and the flow time of 60 mL sample with the ad of a receiving flask of desired volume. The flow time (in seconds) of that sample volume, drained through the device hole, under standardized test conditions, is the Saybolt viscosity (SSU) at the temperature of the thermal equilibrium.

The procedure was repeated with all samples, and finally, from the SSU found and the Equation 5 next, kinematic viscosity is obtained at 40 °C.

$$Kinematic\ Viscosity = (0{,}224 * SSU) - \frac{185}{SSU} \qquad (5)$$

Biodiesel's oxidative stability

In a study presented by Knothe (2002), he reported that although the iodine index is part of the biodiesel specification, it is not accurate enough to assess the susceptibility of an ester to oxidation. Because of that, the author suggested an alternative method, which he named Alilic Position Equivalents (APE) abd equivalents of Bis-Alilic Position Equivalents (BAPE), for better correlation with oxidative stability. Di or tri-unsaturated fatty acids contain the most reactive sites for the inititation of auto-oxidation. The oxidation rate relates to the total number of bis-allylic sites and not with the number of double bonds. The allylic position (a methylene adjacente to a double bond) is much less reactive, which explains the much lower oxidation rate of the oleic acid ester (C18:1). Bis-allylic sites have high reactivity which leads to the formation of frees radicals. The indices will be called allylic position equivalents (APE) and equivalents of bis-allyl position (BAPE). To calculate APE and BAPE the following equations are required:

$$APE = 2 * (AC\ 18{:}1 + AC\ 18{:}2 + AC\ 18{:}3) \qquad (6)$$

$$BAPE = AC\ 18{:}2 + (2 * AC\ 18{:}3) \qquad (7)$$

The indices APE and BAPE are based on the number of reactive positions in oxidation.

Chromatography

For chromatographic evaluation, a gas chromatograph (Nitrogen) was used, equipped with a 100 m x 0.25 m x 0.25 µm column and a flame ionization detector (GC-FID) (Shimadzu QP-2010). The quantitative analysis was done by area normalization using the GC Solution software in comparison with the FAME Mix.

Statistical analysis

Statistical tests were used in order to verify the influence of variations in the factorial design (blend, temperature and catalyst) on the physical-chemical parameters obtained. The data acquired in the present study were subjected to the analysis of descriptive statistics, normality tests, correlation analysis and variance tests, using the Excel Action Stat software.

First, with the intention to suppress differences in orders of magnitude of the results of the analysis carried out, so that there is no interference with the statistical analysis, the data was standardized according to the Wilks's methodology (WILKS, 2006).

Then, to prove the normality of the data, the Kolmogorov-Smirnov (K-S) and Shapiro-Wilk test were applied with a 95% reliability interval and a significance level of 0.05. In order to ascertain the correlation of the physical-chemical parameters with each other and with the reaction parameters, the correlation matrix with the coefficient that best suited the result found in the normality test was used.

Finally, in order to verify the existence of significant variability between the results of the physical-chemical analysis applied the Kruskal-Wallis test, as well as the variation between the samples. To analyze the possible influence of the reaction parameters (blend, temperature and catalyst) on the values obtained in the analysis previously described in the methodology, used this method. The significance level used was of 0.05.

RESULTS AND DISCUSSION

Table 2 presents the results of the physical-chemical analysis obtained from the points present in the factorial design. The methodology for obtaining biodiesel demonstrated to be promising because could be observed good yield values (an average of 93.66%). The ANP does not determine a limit value for the yield, however, for this process to be viable it is expected to obtain the highest possible yield. In relation to the European standard (EN 14214), as for the yield, only points P1, P7 and P8 obtained the minimum limit established (≥ 96.50).

Table 2: Physical-chemical analysis of biodiesel.

	ν (mm^2s^{-1})	AI ($mg\ KOHg^{-1}$)	SI ($mg\ KOHg^{-1}$)	II ($g\ I_2g^{-1}$)	Yield (%)
P1	3.99	0.22	110.04	110.41	99.00
P2	4.67	0.20	111.65	123.11	86.67
P3	3.50	0.19	115.13	112.97	93.33
P4	4.34	0.22	109.98	111.84	93.33
P5	3.99	0.40	111.60	133.66	93.33
P6	3.43	0.22	110.82	106.57	86.67
P7	3.68	0.36	100.44	128.58	99.00
P8	3.32	0.23	113.49	128.91	99.00
CP1	4.64	0.20	95.77	102.05	93.33
CP2	4.40	0.24	102.54	124.58	93.33
CP3	5.49	0.20	99.93	137.52	93.33

Among the main characteristics of biodiesel, one can be highlighting is the kinematic viscosity (ν), a property that expresses the resistance offered by biofuel under the action of gravity. Furthermore, it is an

important feature to be established, as its control aims to guarantee an adequate performance of fuel injection and pump systems, in addition to preserving the lubricity characteristics of biodiesel (MARTINS et al., 2015a). The kinematic viscosity values of the 11 samples examined (Table 2) vary from 3.32 mm²/s to 5.49 mm²/s. The kinematic viscosities of points P6, P8 and PC3 marginally exceed the acceptable range specified by the European standard (EN 14214), which is 3.5 to 5 mm²/s. On the other hand, the US (ASTM D6751) and Brazil (ANP) specifications are much broader (1.9 to 6.0 mm²/s) and (3.0 to 6.0 mm²/s), respectively, making all 11 biodiesels analyzed acceptable.

The determination of the acidity index is an analysis of paramount importance for the biodiesel production, able to inform about the state of conservation and quality of the oil. High values of acidity index can cause engine corrosion and deterioration of the biofuel (MARTINS et al., 2015b). EN, ASTM and ANP establish a limit of 0.5 (KOH)/g (Biodiesel) for acidity index (IA), therefore the results of the samples obtained from the fish/coconut blends were satisfactory, presenting an average 0.24 mg (KOH)/g (Biodiesel).

Regarding the quality of biodiesel, the self-oxidation process is an issue that requires attention. In biofuel are found unsaturated compounds, which makes it susceptible to contact with air. Therefore, the iodine index was the test adopted to assess oxidation stability, since the iodine index is an indirect measure of the amount of unsaturation present in the biodiesel sample and the greater the amount of unsaturated compounds (higher iodine index), the lower is their oxidative stability (MENEGAZZA et al., 2015).

In Brazil, there is no maximum limit established for the iodine index (II), so the limit required by the European Union standard (EN 14.214), which is 120 g/100 g, was the parameter used. Thus, as observed in Table 2, the points P2, P5, P7, P8, CP2, CP3 showed values slightly above the established, however the study showed an overall average of 120.01g/100g.The results were compared with the thesis described by Vilela (2010), which produced biodiesel from oil extracted from fish residues and presented values for the iodine index between 125g/100g and 163g/100g, that is, they did not meet the limit of the established standard (iodine index <120 g/100 g sample). Vilela (2010), also suggested to test the mixture of this biodiesel with other raw materials or even with fossil diesel. In the study by Queiroz et al. (2016), biodiesel from fish also demonstrated very low oxidation stability. Therefore, Queiroz mixed fish oil with castor oil, since castor oil has a high oxidative stability, as well as the coconut oil used in the present study. The addition of castor oil biodiesel to fish biodiesel significantly improved the oxidation stability of the blend. Corroborating with Queiroz, Fadhil et al. (2017), revealed that the biodiesel production from mixture castor to fish oil (50/50) is necessary to achieve properties close to those of conventional crude oils used for the biodiesel production. According to Rodrigues (2017), oxidative stability is the main impediment to use of oil from fishing industry residues for the biodiesel production, which can be justified mainly by the presence of unsaturation in its carbon chain (high iodine index). Cunha et al. (2009), studying crude fish oil found an iodine index of 133 g I2/100g and Silva et al. (2016), found 150.55 g I2/100g.

In the present study, it can be seen that the points P1, P3, P4, P6 and PC1 presented values within the limit defined by EN 14.214. According to Mapilele (2008), the predominance of saturated fatty acids or

weak unsaturability of coconut oil indicates the low value of the iodine index and consequently a relatively high oxidative stability. However, to assess whether it was, in fact, the variation in the coconut oil percentage that caused the iodine index to fall within the EN standards, statistical analysis of correlation and significance are necessary. As can be seen in Table 2, there is no clear trend between the variation in the coconut oil percentage and the iodine index value, for the coconut oil concentrations tested in this study.

In the alternative method proposed by Knothe (2002), the use of indexes APE and BAPE instead of the iodine index implies that oxidative stability can be more strongly influenced by the presence of small amounts of more highly unsaturated fatty compounds. The allylic positions at double bonds are especially susceptible to oxidation due to the lability of hydrogens. The bis-allylic positions, common in polyunsaturated fatty acids such as linoleic acid are even more prone to oxidation, because the radical peroxides formed have stabilizing resonance structures that increase the spread of oxidation. The values found in the present study were APE 61.86; 58.26; and 59.52 for samples P1, P7 e P8, these samples were the ones that showed a better yield. The values BAPE were 4.12; 3.27 and 4.81, for the same samples. How higher the value of BAPE, greater are the unsaturations presented, in this way, the oils presented have a low reactivity and consequently a good oxidative stability.

The saponification index (SI) allows us to obtain a direct measure of the average molecular weight of biodiesel and is expressed in milligrams of KOH necessary to saponify 1g of fat. In addition, high saponification indexes mean the existence of a high percentage of fatty acids that can lead to the formation of soap during the transesterification process (SAKTHIVEL et al., 2018). Although there is no regulatory limit for it, lower rates are preferable since the SI influences the costs for the biofuel production and use (PAULA et al., 2011). The results obtained were in the range of 95.77 - 115.13 $mgKOHg^{-1}$ (Table 2), being found in the literature values that can reach 207.56 mgKOHg-1; 244.06 $mgKOHg^{-1}$ for the biodiesel of oil extracted from different fish residues (MEDEIROS, 2018) and 119.9 $mgKOHg^{-1}$ for coconut oil biodiesel (NASCIMENTO et al.; 2009), while studies found that for biodiesel from residual frying oil the saponification index was 171 $mgKOHg^{-1}$ (ROSSI et al., 2018).

Gas chromatography

The table 3 presents the fatty acid profile of 3 of 11 samples, those with the best yields. All samples analyzed showed a major composition of C16:0 and C18:1n9c.The main fatty acids present in the biodiesel produced in this study are also demonstrated in the study by Medeiros et al. (2019). The authors produced biodiesel with oil from fish processing residue and the fatty acids with the highest percentages found were palmitic (24.17%), palmitoleic (19.55%), oleic (18.40%), myristic (6.41%) and stearic (5.38%), results similar to the present study.

The presence of saturated chains presents greater oxidative stability than that of unsaturated chains (DABDOUB et al., 2009), therefore, since most of the fatty acids in the biodiesel samples obtained in this study were unsaturated, consequently oxidative stability tends to be comparatively less.

Table 3: Fatty acid profile of samples.

Fatty Acid	Carbon Chain	1	2	3
Miristic	14:0	3.63%	3.42%	3.43%
Palmitic	16:0	23.25%	22.50%	21.58%
Palmitoleico	16:1	10.51%	10.56%	10.32%
Stearic	18:0	5.61%	5.34%	5.27%
Oleic	18:1n9c	27.91%	26.81%	26.53%
Linoleic	18:2n6c	1.92%	1.37%	1.65%
Arachidic	20:0	0.36%	0.36%	0.48%
Gadolinic	20:1	1.10%	1.63%	1.83%
A-linolenic	18:3n3	1.10%	0.95%	1.58%
Arachidonic	20:4n6	2.48%	2.59%	2.89%
Eicosapentaenoic	20:5n3	8.91%	9.26%	9.26%
Docosahexaenoic	22:6n3	9.94%	10.62%	10.83%

Statistical analysis

Normality tests

First, already in possession of standardized data, in order to analyze the sample distribution of the parameters analyzed in the laboratory, two normality tests were performed as described in the methodology. The results of these tests are presented in Table 4.

Table 4: Normality tests.

Parameters	Test K-S	Test S-W
	p-values(significance)	p-value (significance)
Viscosity	0.200	0.285
Acidity Index (AI)	0.012	0.001
Saponification index (SI)	0.200	0.295
Iodine index (II)	0.200	0.587
Yield	0.001	0.000

For these tests, the null hypothesis is confirmation of normal distribution, according to the *default* of the statistical program used. Therefore, using a significance level of 0.05, the null hypothesis for parameters that obtain a p-value less than 0.05 is rejected.Therefore, with both tests in agreement, there is a non-normal distribution for the parameters AI and yieldand a normal distribution for viscosity, SI and II.

Correlation matrix

In order to analyze the correlation between the parameters analyzed in laboratory, as well as the factors that varied along the factorial design (blend, temperature and catalyst) with these parameters, proceeded with the analysis of a correlation matrix. There are several types of correlation coefficients, some used for data with normal distribution, others for data that do not follow this distribution.

Since not all parameters obtained a positive normality test for a normal distribution, the correlation coefficient chosen was Spearman's Rho coefficient, as this is a non-parametric correlation measure. The results of this correlation matrix can be seen in Table 5.

A strong correlation is considered for those parameters that obtained a Spearman coefficient equal to or greater than 0.5 in module (HELENA et al., 2000). The correlation can be strong in the same or different directions, which is why the intensity of the correlation is analyzed in module.

Table 5: Correlation Matrix.

	Temp.	Catalyst	Blend	Viscosity	AI	SI	II	Yield
Temp.	1.000							
Catalyst	0.000	1.000						
Blend	0.000	0.000	1.000					
Viscosity	0.061	-0.429	-0.252	1.000				
AI	-0.308	**0.580**	-0.045	0.216	1.000			
SI	0.141	-0.151	-0.081	0.114	0.170	1.000		
II	-0.096	0.257	0.086	0.458	**0.718**	0.426	1.000	
Yield	-0.464	0.171	0.464	-0.266	0.272	-0.177	0.151	1.000

It can be seen in Table 5 that only the catalyst-AI and AI-II correlations obtained a Spearman coefficient greater than 0.5, that is, only these are considered, according to Helena et al. (2000), as strong correlations. It is important to note that sometimes parameters and/or factors that do not have correlations considered to be strong within a pre-established limit can still have a significant influence on other parameters. The significance of the correlations shown in Table 5 can be seen in Table 6. Correlations that have a p-value of significance less than 0.05 are considered significant.

Table 6: Significance of the correlations.

	Temp.	Catalyst	Blend	Viscosity	AI	SI	II	Yield
Temp.								
Catalyst	1.000							
Blend	1.000	1.000						
Viscosity	0.764	**0.026**	0.204					
AI	0.118	**0.002**	0.822	0.280				
SI	0.482	0.451	0.689	0.573	0.396			
II	0.634	0.195	0.671	**0.016**	**0.000**	**0.027**		
Yield	**0.015**	0.394	**0.015**	0.179	0.170	0.377	0.453	

The catalyst-AI and AI-II correlations that had been considered strong are also significant. However, correlations that did not reach the limit defined by Helena et al. (2000), proved to be significant. This can be observed for the temperature-yield, catalyst-viscosity, blend-yield, viscosity-II and SI-II correlations.

These correlations, with p-value less than 0.05, but with Spearman's Rho less than 0.5, are considered as significant correlations, however of lesser intensity. This means that, observing the catalyst-viscosity and catalyst-AI correlations as an example, although the variation in the amount of catalyst causes variations in both viscosity and AI, these variations will be more pronounced in AI.

Variance tests

Another test commonly used in statistical analysis for better data interpretation is the variance tests. These tests make it possible to assess the variability significance of certain parameters within a sample space. As stated in the methodology, the Kruskal-Wallis Test was used for this study.

For this test, the factors that alternated were the same variationfactors of the factorial design, that is, these tests evaluated the significance that the variation of these factors had on the parameters analyzed in the laboratory. This test was performed with non-standard data, that is, with raw data and the results can be seen in Table 7.

Table 7: Kruskal Wallis Test.

	p-value (significance)		
	Temperature	Catalyst	Blend
Viscosity	0.170	**0.017**	0.079
AI	0.250	**0.017**	0.839
SI	**0.037**	**0.035**	**0.044**
II	0.767	0.366	0.786
Yield	0.057	0.638	0.057

The temperature variation caused significant variation only in the SI, while the variation in the amount of catalyst caused significant variation in the parameters viscosity, AI and SI. The variation in the mixture, however, incurred a significant variation, in turn, only in the SI parameter.

Influence of coconut oil percentage on oxidative potential

As previously discussed, studies show that coconut oil, due to its predominantly saturated fatty acids in its composition, can influence the oxidative potential of biodiesel produced with this oil, reducing oxidation. However, with regard to the results obtained by this study, it can be seen, through statistical methods of correlation, significance and variance, that the coconut oil percentages evaluated here (1%, 5% and 10%) had no influence significant impact on the iodine index, used as an indirect measure of oxidative potential. This could be occurred because these concentrations were too low to have any influence on the final product, or it may be due to the characteristics of the predominant raw material in this biodiesel (the residual fish oil).

CONCLUSIONS

In relation to the effect provided by adding coconut oil, the results demonstrated that this was positive, since it acted as an antioxidant additive, since the values of BAPE were 4.12; 3.27 e 4.81, for the best yielding samples (99%), values considered low, indicating little reactive activity and also the addition of coconut oil helped to reduce the iodine index and consequently in the framework European Union's standard. All samples analyzed were within the quality parameters established, with the exception of iodine index of some samples.

Regarding the results of the statistical correlation methods, significance and variance, where the percentages of coconut oil evaluated here (1%, 5% e 10%) had no significant influence on the iodine index, used as a possible indirect measure of oxidative potential. These percentages may have been very low to influence the final product or it may be due to the characteristics of the raw material prevalent in this biodiesel (residual fish oil).

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