

Optimization of Lipase Catalyzed Biofuel Synthesis From Waste Filter Coffee Using Response Surface Methodology

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Research Article

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Abstract

This study was executed that optimize an environmentally friendly lipase (Resinase® HT with the activity of 135,56 U/ml) catalyzed transesterification process from novel feedstock waste filter coffee oil for biofuel production. Response surface methodology (RSM) with the central composite design was performed to investigate the effect of experimental factors (enzyme content, oil/methanol molar ratio, reaction temperature) on the fatty acid methyl ester (FAME) conversion and also investigated to resolve the optimum design points. After the experimental studies the lowest FAME conversion was found as 71% when the enzyme content was 15%(%gr w/w), oil/methanol molar ratio was 0.17 and the reaction temperature value was 45 °C. According to the design response, 70.83% was observed in the same conditions. The highest FAME conversion of 97% was found when the enzyme content was 5%(%gr w/w), oil/methanol molar ratio was 0.25 and the reaction temperature value was 35 °C. The experimental run gave the FAME conversion of 96.80% at the same reaction conditions. The model fitted with the experimental values with $R^2 = 0.98$. Also, classical soxhlet extraction and Dyer method oil yields were compared. 24 % and 20 % oil removed from waste coffee grounds in traditional soxhlet and Dyer method respectively. The extraction process took 30 minutes with the soxhlet method and 45 minutes with the Dyer method. The results are promising for the application of lipase catalyst for environmentally friendly and sustainable biodiesel production from waste coffee oils all over the world.

Statement Of Novelty

Coffee grounds, which has become one of the most important biological wastes with an annual production capacity of 6 million tons worldwide; It has a very important value in terms of the economy and policies of many developing countries. Since coffee wastes are a renewable resource and the consumption of coffee worldwide increases day by day. It is claimed that it is possible to provide approximately 340 million gallons of biodiesel fuel if the coffee wastes consumed in the world are evaluated with environmentally friendly processes. According to the author's knowledge, the effects of process parameters catalyzed reaction on biodiesel conversion were optimized using the statistical test method Response Surface Methodology (RSM) for the first time in the literature.

Introduction

Coffee is one of the most popular beverages in the world and is the product with the highest trade rate after petroleum. The International Coffee Organization (ICO) has published that 8.9 billion kg of coffee was produced in 2016 and that most of this coffee was consumed in EU countries, the USA, Brazil, and Japan. Global coffee production increased by 17% from 2002 to 2014 due to increased consumption (Campos-Vega et al. 2015). This increase causes large amounts of coffee waste, which leads to waste management problems. Several wastes can be obtained throughout the coffee processing chain. These wastes are divided into two categories. First, products representing more than 50% of the coffee fruit mass-produced in the producing countries; Secondly, it is defined as "spent coffee" in the countries that consume it after beverage preparation (Cruz et al. 2012). Used coffee wastes are the result of producing

instant coffee from roasted coffee beans. In 2008–2009, the EU published the "New Waste Framework Directive" and introduced new methods for the reuse and recycling of food waste such as used coffee waste (Ravindran et al. 2017). Coffee waste occurs in two ways. The first one is the soluble coffee industry, which covers 50% of these wastes. Although the wastes are generally thrown into waste storage areas, these coffee residues should not be left directly to nature. Coffee waste occurs in two ways. The first one is the soluble coffee industry, which covers 50% of these wastes. Although the wastes are generally thrown into waste storage areas, these coffee residues should not be left directly to nature. Because these wastes; contains a high amount of eco-toxic organic compounds, such as caffeine, tannins (tannic acids), and polyolefins. Since direct disposal of used coffee wastes causes countless environmental problems in countries that have been produced for many years, many studies are being carried out for their reuse, taking into account the appropriate waste approaches and potentials. The remaining 50% of coffee consumption worldwide; is directly related to the direct preparation of beverages in cafes, restaurants, and homes. Depending on the brewing procedure applied, different brews of coffee can be obtained, and therefore coffee waste contains quantitatively different amounts of non-extracted coffee components. Coffee waste; is rich in organic compounds as an excellent raw material for the production of various added value substances such as carbohydrates, lignin, fatty acids, and antioxidant compounds and also contains different amounts of oil depending on the type of coffee (Scully et al. 2016). From dry coffee wastes, these oils can be obtained by conventional soxhlet extraction (Banerjee et al. 2013). In their study in 2013; They stated that by using n-hexane, soxhlet extraction from coffee wastes yielded 14% oil. When the literature on obtaining oil from coffee wastes is focused, it is stated that filter coffee wastes contain between 15 and 25% oil by type (Jenkins et al. 2014; Ferrario et al. 2013). Coffee grounds, which has become one of the most important biological wastes with an annual production capacity of 6 million tons worldwide; It has a very important value in terms of the economy and policies of many developing countries.

Since coffee wastes are a renewable resource and the consumption of coffee worldwide increases day by day, the conversion of oils from waste filter coffee grounds into alternative fuels with environmentally friendly processes has become the focus of attention today. It is claimed that it is possible to provide approximately 340 million gallons of biodiesel fuel if the coffee wastes consumed in the world are evaluated with environmentally friendly processes (Murthy and Naidu 2012). In another approach, it is promising to evaluate coffee wastes, which are consumed in more than 800 billion cups of coffee each year in the world (Unugul et al. 2020). As it is known, biodiesel is defined as mono-alkyl esters of long-chain fatty acids produced by the transesterification reaction from renewable oil sources (vegetable oils, waste oils, microalgae oils). In other words, it is a kind of oxygen-based fuel derived from biological sources and can be used in compression (diesel) engines. It can be mixed with petroleum diesel, in certain proportions. This rate; depends on many factors such as economy, gas emission, and combustion feature and is generally used as a mixture of 20% in European countries. Also in this study, transesterification reactions will be carried out under enzyme-catalyzed moderate reaction conditions. Enzymatic processes enable pure products to be obtained in terms of functionality, quality, and health fitness. Their high activity at room temperature and even under moderate reaction conditions allow them

to maintain their stereoselective over a wide range of substrates, resulting in enantiomerically pure products. When using enzyme catalysis, a series of separation processes brought by the chemical catalyst is not needed, since only the desired product will be synthesized. Since the immobilized lipase can be easily removed from the reaction medium, it can be easily reused in further next reactions (Yang et al. 2012).

In this study, biodiesel has been synthesized which is suitable for EN140130 standards from waste filter coffee oil by commercial lipase. According to the author's knowledge, the effects of process parameters affecting that lipase-catalyzed reaction on biodiesel conversion were optimized using the statistical test method Response Surface Methodology (RSM) for the first time in the literature. This method provides transesterification reactions that are statistically designed and experimental coefficients are represented as a mathematical model so that responses can be predicted and the accuracy of the model can be checked (Sadhukhan et al. 2016). In the present study, combined effects of enzyme content, oil/methanol molar ratio, and reaction temperature on biodiesel conversion were specified experimentally. Also, responses (biodiesel conversion) were optimized by RSM Design Expert Version 7.0.0 [Stat-Ease] Software was used to optimize responses. Besides, classical soxhlet extraction and Dyer method oil yields were compared.

Experimental

Materials

Waste filter coffee grounds were obtained from local (Kocaeli/TURKEY) Starbucks restaurants. Resinase® HT (*Aspergillus oryzae*) with the activity of 135,56 U/ml lipase biocatalyst was obtained as a gift from Novozymes (Denmark). All other chemicals (acetone, methanol, chloroform, calcium chloride, n-hexane, potassium hydroxide, ethanol, ether, methyl heptadecanoate) which were used in experiments and analysis were purchased from Merck Chemicals Turkey.

Lipase activity analysis

The reaction medium was heated to 37 ° C in an IKA brand shaking incubator with 2.5ml olive oil and 2.5ml phosphate buffer (0.1M, pH7.0) in 25ml volume flasks. 0.1 ml Resinase HT was weighed and added to the reaction medium. The hydrolysis reaction was continued for 30 min and was added to the 2.5 ml medium from the ether/ethanol mixture prepared in a 1: 1 ratio by volume to terminate. Then, 1% phenolphthalein indicator was added to the medium in 1-2 drops and titrated with 0.1M NaOH solution.

It is calculated according to Eq. 1. from the volume of spent NaOH solution. Titrations were averaged 5 times to increase reliability.

$$\frac{U}{\text{ml lipase}} = \frac{(sample, \text{ml spent} - witness, \text{ml spent})_{(\text{NaOH})} \times [\text{NaOH}] \times 1000}{(V_{lipase}, \text{ml}) \times t} \quad (1)$$

Oil extraction

Waste coffee grounds were dried in an oven at 80°C for around a day to remove moisture. Oil was obtained by using the conventional and soxhlet extraction method. n-hexane was used as the solvent to obtain oil from dried coffee wastes. 3 siphons every 10 minutes were sufficient for this process and the temperature was 70 °C. Other extraction processes were carried out according to the Bligh and Dyer method (Bligh and Dyer 1959).

Determination of fatty acid profile

The free fatty acid profile was determined using the flame ionization detector (FID) and the Agilent 7820 A model gas chromatography (GC) device with 30 m x 320 µm x 0.25 µm capillary column (CARBOWAX 20M). The detector temperature is 280°C and the split ratio is 1:50. The column temperature was adjusted to reach 230 °C with an increase of 25°C / min, 200°C to 3°C / min with an increase of 25°C / min, and to reach 280°C with an increase of 40°C / min and to stay at this temperature for 3 minutes. Fats must be converted into methyl esters for the sample to be injected into the device. For this purpose, a 0.1-gram oil sample was weighed into a 20 ml glass tube and 10 ml of n-hexane was added to it. Then, 100µl of 2N potassium hydroxide solution was added and vortexed for half a minute. The mixture is centrifuged (5000 rpm, 5 min). Then injected by taking 1µl from the upper phase to the GC (Unugul et al. 2020).

Determination of biodiesel conversion

Conversions were performed in accordance to the EU norm (EN 14103) which method is commonly used, Gas chromatography equipped with flame ionization detector (FID) HP7820A (Agilent Technologies, USA) with an autosampler (Agilent 7386B series) Separations were accomplished using a 30-m long HP-INNOWAX capillary column, (0.25 mm I.D. and 0.25 µm film thicknesses) at a constant hydrogen flow rate of 3 ml min⁻¹. Samples (1 µL) were injected in a split ratio of 1:50 with an injector temperature of 250 °C.

Transesterification reactions

The most important factors oil/methanol molar ratio, amount of lipase (%), temperature (° C) were investigated to the synthesis of fatty acid methyl esters (biodiesel) with lipase-catalyzed transesterification reaction from waste filter coffee oil. All the reactions were carried out simultaneously in 25ml closed flasks in IKA INNOVA 40 brand shaking incubator. Other reactants (methanol) and catalyst amounts were calculated based on 3g of coffee oil for each reaction. The reaction time is 24 hours, the mixing speed is 300 rpm. To eliminate the inhibition effect on the lipase, methanol was added to the reaction medium in 3 steps to 8 hours.

Biodiesel FTIR characterization

FTIR spectra were obtained at room temperature using Perkin Elmer 100 brand FTIR spectroscopy device and were obtained in the wavelength range of 650-4000 cm⁻¹. The cell was cleaned three times with

acetone before each measurement.

Optimization design by face-centered central composite

The (factors) of the present study were the enzyme content (%) (A), reaction temperature (B), and oil/methanol molar ratio (C). The limits (minimum and maximum) of the factors were specified according to the precursor experimental investigations in our biotechnology laboratory. Studies were done randomly. Both the factors and responses are given in Table 1.

Table 1 Optimization factors (most efficient variables on transesterification) and response

Run	Factor A: Enzyme content (%gr w/w)	Factor B: oil/methanol molar ratio (-)	Factor C: Temperature (°C)	Response 1 Biodiesel conversion (%)
1	15.00	0.33	45.00	72
2	10.00	0.25	45.00	70
3	10.00	0.17	35.00	75
4	10.00	0.25	35.00	78
5	15.00	0.17	25.00	75
6	10.00	0.25	35.00	80
7	5.00	0.33	25.00	92
8	10.00	0.25	35.00	78
9	10.00	0.25	35.00	77
10	15.00	0.33	25.00	78
11	5.00	0.17	25.00	88
12	5.00	0.33	45.00	90
13	15.00	0.25	55.00	83
14	10.00	0.25	35.00	78
15	5.00	0.17	35.00	89
16	10.00	0.25	45.00	78
17	10.00	0.25	25.00	74
18	5.00	0.25	35.00	97
19	10.00	0.33	35.00	82
20	15.00	0.17	45.00	71

Results And Discussion

Fatty acid profile of the waste filter coffee ground oil

As a result of extraction, the lipid content in the waste filter coffee oil was determined to be approximately 24% and the fatty acid profile was rich in margaric acid (C17:0, 35%) and linolenic acid (C18:3, 44%).

Classical soxhlet extraction versus Dyer method

Traditional Soxhlet extraction has some attractive advantages. The sample is constantly in contact with fresh solvent. Thus, fat removal from the matrix increases. As the extraction cavity is reached with the heat applied to the distillation balloon, the temperature of the system becomes higher than the room temperature. The system remains unchanged at this high temperature. Also, filtration is not required after extraction, and the amount of substance produced from the sample can be increased by performing several simultaneous extractions in parallel. Using simple low-cost equipment makes this possible. Moreover, Soxhlet extraction is a very simple methodology that requires little effort. On the other hand, In the dyer method, more various chemicals (chloroform, methanol, calcium chloride) are required for the extraction process and the extraction process requires more time. According to the experimental investigations, 24 % and 20 % of the oil were removed from waste coffee grounds in the traditional soxhlet and Dyer method respectively. Also, the extraction process took 30 minutes with the soxhlet method and 45 minutes with the Dyer method.

Determination of agitation speed for the transesterification reaction

The conversion of fatty acids methyl esters (biodiesel) from waste filter coffee oil was studied at different agitation speeds from 150 rpm to 300 rpm when the enzyme content(%) was 5 (%gr w/w), oil/methanol molar ratio was (1/4) and the reaction temperature (°C) was 35°C. Figure 1 shows the effect of agitation speed on biodiesel conversion of the transesterification reaction.

The highest biodiesel conversion (96%) was achieved at the agitation speed of 300 rpm as a result of the transesterification of the waste filter coffee oil with methanol. As the agitation speed increased, it was observed during the reactions that the enzymes in the reaction medium adhere to the walls of the reaction media (flask) and leave the medium. For this reason, the conversion at 400 rpm has decreased slightly. It was determined for this reaction that low mixing speeds were not sufficient to overcome the mass transfer restrictions. With effective mixing, mass transfer restrictions can be avoided in the reaction medium. At low mixing speeds, enzymes cannot be dispersed homogeneously in the liquid phase, or at high speeds, the adhesion sticks up from the reaction medium walls, and such mixing problems cause low yields (Xin et al. 2008).

Anova results and foresight

The experimental serial was managed, and the responses were analyzed by Design Expert 7.0.0 software. According to the designed study, the recommended model was quadratic with *an R²* of 0.98. Table 2 shows the ANOVA analysis of the transesterification reaction experimental study. The Model F value of 63.24 clarifies that the model is remarkable. Values of "Prob > F" less than 0.0500 indicate model terms are remarkable. In this case, A, B, C, AB, A², B² are remarkable model terms. Values greater than 0.1000 indicate the model terms are not remarkable. The lack-of-fit F value of 2.75 clarifies that the lack-of-fit is not significant relative to the pure error.

According to the computational experimental design, the regression equation was acquired from the Design-Expert software. Eq. 1 shows the correlation between the biodiesel (FAME) conversion and independent parameters such as enzyme content, oil/methanol molar ratio, and reaction temperature as shown below.

$$\text{Biodiesel (FAME) conversion (\%)} = + 78.50 + (-7.70)A + (-1.50)B + (+1.60)C + (-1.12)AB$$

$$+(-0.12)AC + (-0.62)BC + (+11.00)A^2 + (-7.00)B^2 + (-0.50)C^2 \quad (1)$$

Table 2
Anova analysis of transesterification reaction

Source	Sum of squares	df	Mean Square	F value	p-value	Prop > F
Model	1031.63	9	114.63	63.24	> 0.0001	significant
A-Enzyme content	592.90	1	592.20	327.12	> 0.0001	
B-temperature	22.50	1	22.50	12.41	0.0055	
C-Molar ratio	25.60	1	25.60	14.12	0.0037	
AB	10.12	1	10.12	5.59	0.0397	
AC	0.13	1	0.13	0.069	0.7982	
BC	3.13	1	3.13	1.72	0.2185	
A^2	332.75	1	332.75	183.59	< 0.0001	
B^2	134.75	1	134.75	74.34	< 0.0001	
C^2	0.69	1	0.69	0.38	0.5517	
Residual	18.13	10	1.81			
Lack of Fit	13.29	5	2.66	2.75	0.1456	Not significant
Pure Error	4.83	5	0.97			
Cor Total	1049.75	19				

According to the regression analysis, three independent variables (enzyme content, temperature, Molar ratio) were remarkable in the conversion performance. As seen from Eq. 1, the enzyme content was the most important factor affecting biodiesel conversion. The reaction temperature and molar ratio were also effective in the conversion.

In addition to this, according to Eq. 1, the increasing temperature and molar ratio could reduce the biodiesel (FAME) conversion.

Figure 2 shows the three-dimensional response of the effects of temperature and enzyme content on FAME conversion when the molar ratio was 0.17 (a), 0.25 (b), and 0.33 (c). Both figures certify that the increasing enzyme content above 5% has a significant effect on conversion at all molar ratios. The FAME conversion of 97 % was obtained when the enzyme content is 5% and the reaction temperature is 35°C. Otherwise the enzyme content, the temperature variations have a minor effect on FAME conversion. When the molar ratio increased in the reaction media, the effect of enzyme content on FAME conversion becomes significant. In Fig. 2a, the lowest and highest conversion results of 71% (temperature: 45°C, enzyme content: 15 %) and 89% (temperature: 45°C, enzyme content: 5 %) are obtained, respectively. In Fig. 2b, the lowest and highest conversion results of 70% (temperature: 45°C, enzyme content: 10 %) and 97% (temperature: 35°C, enzyme content: 5 %) are obtained, respectively. In Fig. 2c, the minimum and maximum conversion results are determined as 72% (temperature: 45°C, enzyme content: 15 %) and 92% (temperature: 25°C, enzyme content: 5 %).

Figure 3 shows the combined effects of molar ratio and enzyme content on FAME conversion when the temperature was 25°C (a), 35°C (b), and 45°C (c). The importance of the enzyme content on conversion is also validated by Fig. 3. At the enzyme content (near 5%), the effect of temperature and molar ratio on conversion is significant (Fig. 3c). As the enzyme content rises, the combined effect of temperature and molar ratio becomes insignificant. The highest FAME conversion results have been obtained for all experimental results when the temperature value is 35°C. The figure shows that when the enzyme content is high, the effect of temperature on the FAME conversion is significant. This effect decreases when a higher molar ratio is used. Indeed, it is found that the molar ratio does not affect the FAME conversion and the FAME conversion is fixed when the enzyme content is 5 %. The effect of temperature is the least effective factor for the conversion. As shown in graphs, the FAME conversion at the high enzyme content is considerably low. This result was also validated by the experimental studies. The enzyme used in large quantities adheres to the reaction medium walls and cannot adequately interact with substrates. On the other hand, they tend to punch together and collapse with each other, so they cannot show their real activity (Chowdhury et al. 2014; Syaima et al. 2015).

In Fig. 3a, the minimum and maximum FAME conversion results are obtained as 74% (enzyme content 10%, molar ratio: 0.25) and 92% (enzyme content 5%, molar ratio: 0.33) respectively. In Fig. 3b, the lowest and the highest conversions are sighted as 82% (enzyme content 10%, molar ratio: 0.33) and 97 % (enzyme content 5 %, molar ratio: 0.25) respectively. In Fig. 3c, the minimum and maximum removal results are 71 % (enzyme content 15%, molar ratio: 0.17) and 90% (enzyme content 5%, molar ratio: 0.33), respectively.

Figure 4 shows the combined effect of temperature and molar ratio on FAME conversion when enzyme content values change from 5 to 15. The results confirm the data obtained by Figs. 5 and 6. At low molar ratio values, the effect of enzyme content was more remarkable than that of temperature.

Corroboration of Regression Model

To verify the model, analogize graphs and diagnostic plots have been studied. Figure 5 shows the residual of the experiments. The residual is declared that the difference between the model data and the experimental data. The latitudes of the errors from each other can be checked from the residual plots. The location and changes of values provide information about the suitability of the model. The fitness of the model depends on the compatibility of the relationship between experimental data and actual values along a line in the plot. According to Fig. 5, The distribution of residuals along straight lines, as well as their proximity to this line, supports the compatibility and acceptability of the model.

Figure 6a represents the probability of results versus residuals. The distribution of results along a line supports the suitability and accuracy of the model. There is no noticeable deviation along the line. Figure 6b represents residues corresponding to predicted results. Although all data are within the red borders, they are far from the predicted locations. In this case, it can be assumed that the model is suitable and acceptable. Figure 6c represents actual values versus values predicted by the model. All data are clustered along the linear line.

For the validation of the designed model and the experimental results, the highest and lowest FAME conversions can be analogized. After the experimental studies, the highest FAME conversion was observed as 96.80 % when the enzyme content was 5%(%gr w/w), oil/methanol molar ratio was 0.25 and the reaction temperature value was 35°C. On the other hand, 97 % of conversion was obtained at the same conditions as the designed model. The lowest dye removal According to the designed model. The lowest FAME conversion was observed at 71 % when the enzyme content was 15%(%gr w/w), oil/methanol molar ratio was 0.17 and the reaction temperature value was 45°C. According to the design model, 70.83 % of conversion was obtained at the same experimental conditions. To check the accuracy of the proposed model, recognition was done at several points and the deviation was found approximately 1.5 %. However, Cook's distance and DFFTIS measurements of the model and both values should be less than 1 for the feasibility of the proposed model. According to these results, it could be evaluated that the FAME conversion results were controllable when the reaction conditions were implemented that the given limit values. Table 3 shows the experimental and model results similarity in comparing with residuals.

Table 3
Diagnostics Case Statistics

Standard order	Actual FAME conversion (%)	Predicted FAME conversion (%)	Residual	DFFITS	Cook's Distance
1	88.00	87.73	0.27	0.843	0.077
2	75.00	74.83	0.17	0.533	0.031
3	89.00	88.23	0.77	*2.57	0.614
4	71.00	70.83	0.17	0.533	0.031
5	92.00	92.43	-0.43	-1.322	0.185
6	78.00	79.03	-1.03	*-3.67	*1.07
7	90.00	90.43	-0.43	-1.322	0.185
8	72.00	72.53	-0.53	-1.655	0.282
9	97.00	96.80	0.20	0.194	0.004
10	83.00	81.80	1.20	1.267	0.150
11	74.00	73.00	1.00	1.027	0.105
12	70.00	70.00	0.00	0.000	0.000
13	75.00	76.40	-1.40	-1.530	0.205
14	82.00	79.60	2.40	*3.80	0.602
15	78.00	78.50	-0.50	-0.138	0.002
16	78.00	78.50	-0.50	-0.138	0.002
17	78.00	78.50	-0.50	-0.138	0.002
18	77.00	78.50	-1.50	-0.445	0.019
19	80.00	78.50	1.50	0.445	0.019
20	78.00	78.50	-0.50	-0.138	0.002

*Exceeds limit

Supporting biodiesel content with Fourier Transform Infrared Spectrophotometer (FTIR) analysis

It was also supported by FTIR analysis, where the product obtained represents biodiesel. Figure 7 shows FTIR spectra of biodiesel obtained from waste filter coffee oil. The peaks seen at 2924 cm^{-1} and 2854 cm^{-1} are the peaks formed from the vibration of the C-H bond and are the characteristic region of the methyl groups (Ghesti et al. 2006). The sharp peak seen at 1741 cm^{-1} is the characteristic ester peak arising from carbonyl groups. Peaks seen in the $1430\text{--}1460\text{ cm}^{-1}$ range are caused by the asymmetric vibration

and bending movements of the C-H bonds. Peaks seen in the range of 1000–1250 cm⁻¹ consist of asymmetric vibration of C-C (= O) -O and O-C-C bonds (Sjästam et al. 2006). The peaks seen in the range of 1100–1170 cm⁻¹ are the region of asymmetric vibrations of C-CH₂-O groups and C-O-C, C-C bonds. The sharp peaks encountered in the 700–800 cm⁻¹ range are due to the C-O groups and the deformation of the C-H groups around 1000 cm⁻¹ (Pimentel et al. 2006).

Conclusions

RSM was applied to the transesterification reaction between waste coffee grounds and methanol using an environmentally friendly lipase (Resinase® HT) catalyst. To optimize the effect on selected reaction parameters such as enzyme content, oil: methanol molar ratio, and temperature on FAME conversion. Central composite design modeling of RSM was concerted to experimental results. In the design, a quadratic model was predicted by the program with an R² of 0.98. The enzyme content was the most important factor affecting the FAME conversion. The reaction temperature and molar ratio were also effective in the conversion which was confirmed by the model equation. The increasing enzyme content above 5% has a significant effect on conversion at all molar ratios. However, as the enzyme content rises, the combined effect of temperature and molar ratio becomes insignificant. The lowest FAME conversion was found at 71% when the enzyme content was 15%(%gr w/w), oil/methanol molar ratio was 0.17 and the reaction temperature value was 45°C. The highest FAME conversion of 97% was found when the enzyme content was 5%(%gr w/w), oil/methanol molar ratio was 0.25 and the reaction temperature value was 35°C. To check the accuracy of the design model, recognition was done at several points and the deviation was found approximately 1.5 %. it is concluded that promising for the application of lipase catalyst for environmentally friendly and sustainable biodiesel production from waste coffee oils all over the world.

Declarations

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval :

Not applicable

Consent to Participate:

Not applicable

Consent to Publish :

Not applicable

Authors Contributions

Dr. Togayhan KUTLUK: Conceived and designed the analysis, Collected data, performed the analysis, and wrote the paper.

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Figures

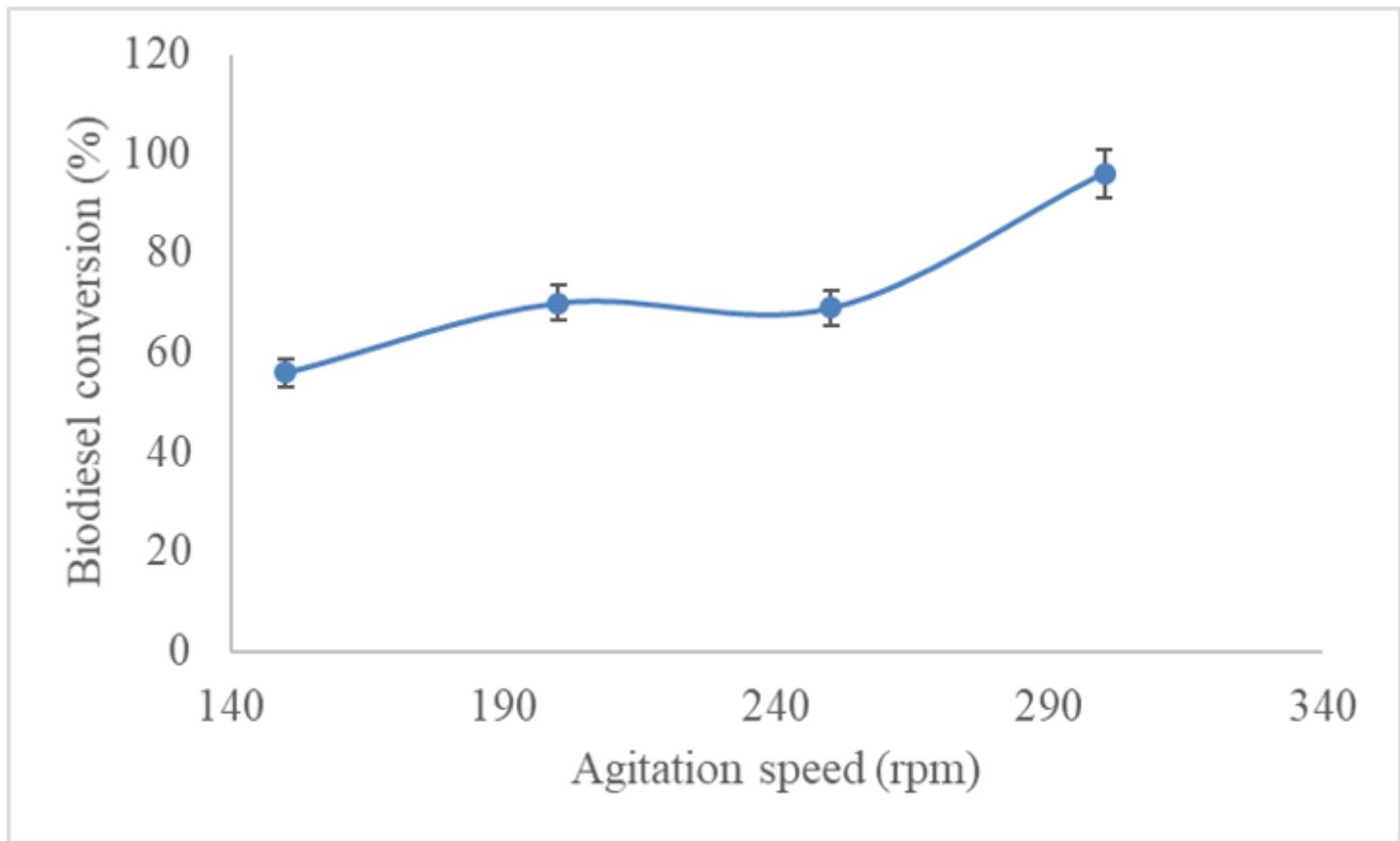


Figure 1

Effect of agitation speed on biodiesel conversion

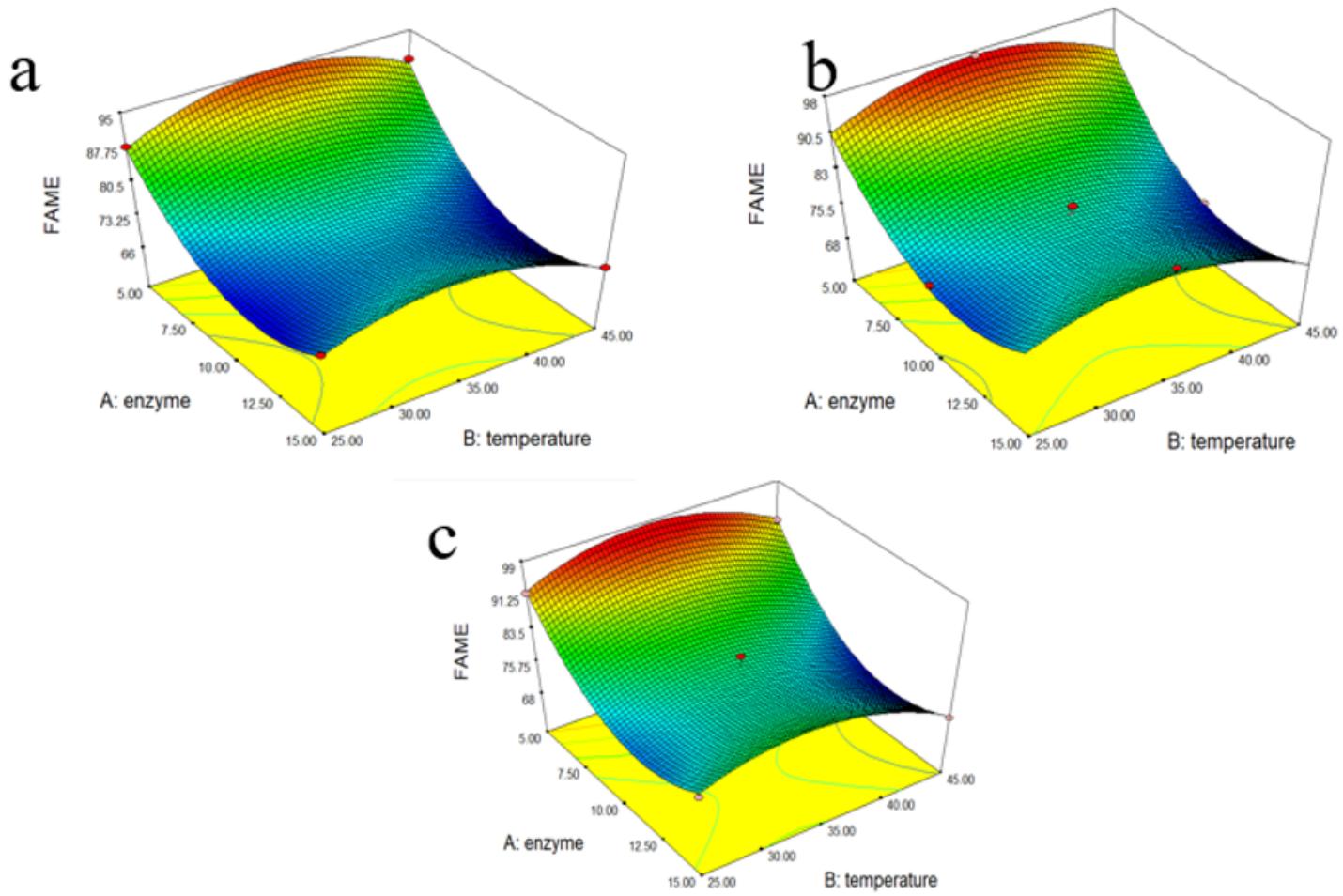


Figure 2

The effect of enzyme content and temperature on FAME conversion when molar ratio 0.17 (a), 0.25 (b), and 0.33 (c).

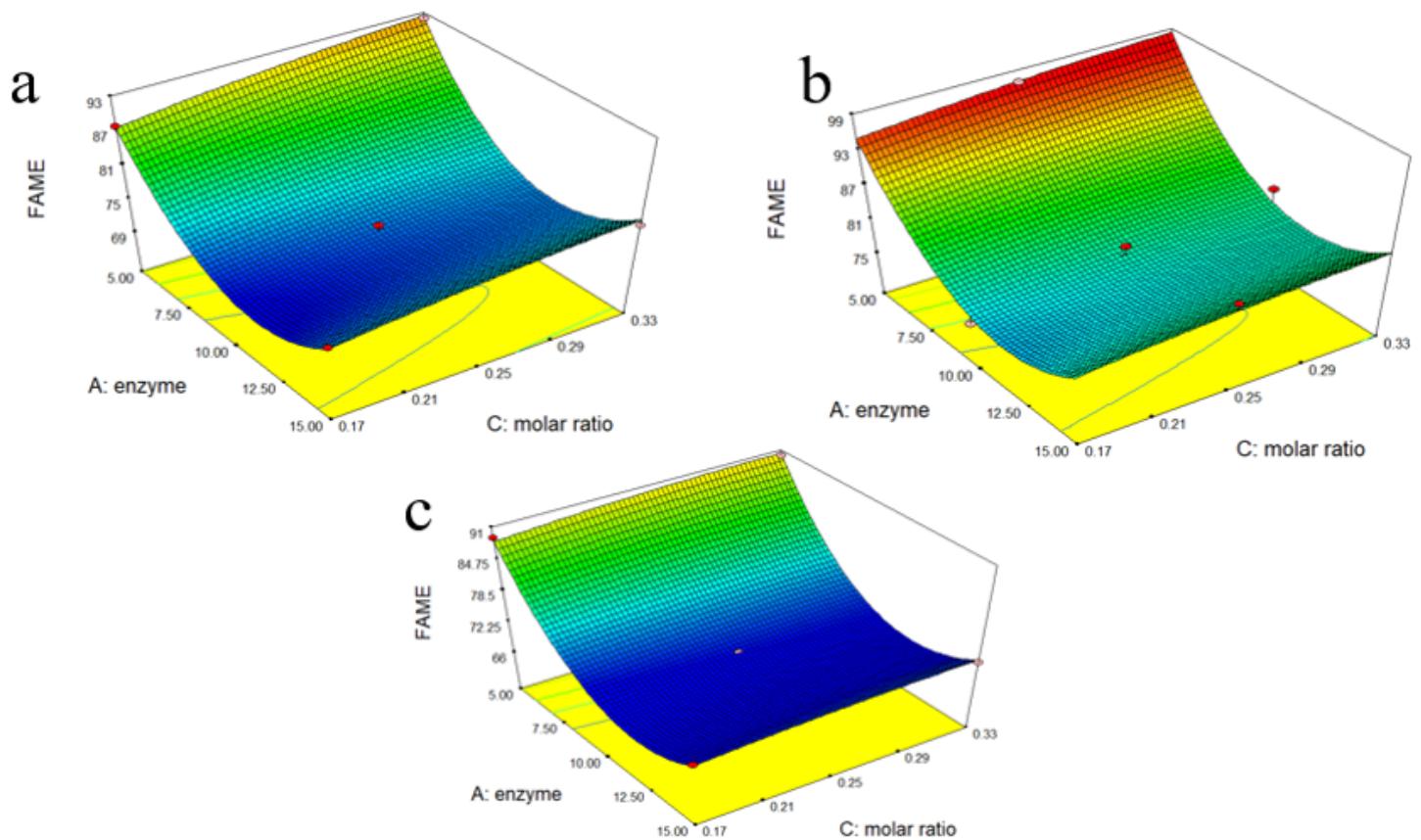


Figure 3

The effect of enzyme content and molar ratio on FAME conversion when temperature 25 °C (a), 35 °C (b), and 45 °C (c).

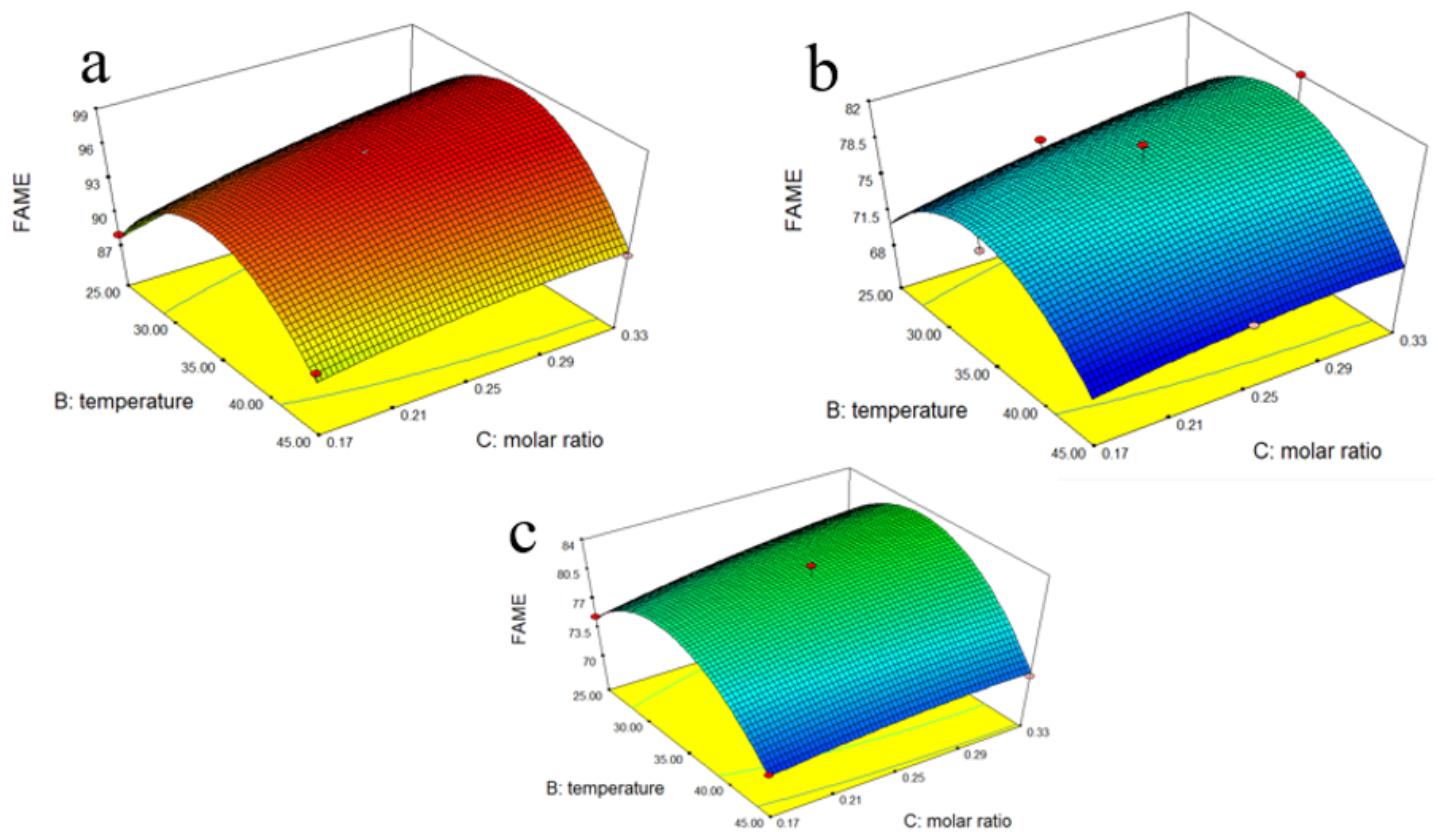


Figure 4

The effect temperature and molar ratio on FAME conversion when enzyme content 5 % (a), 10 % (b), and 15 % (c).

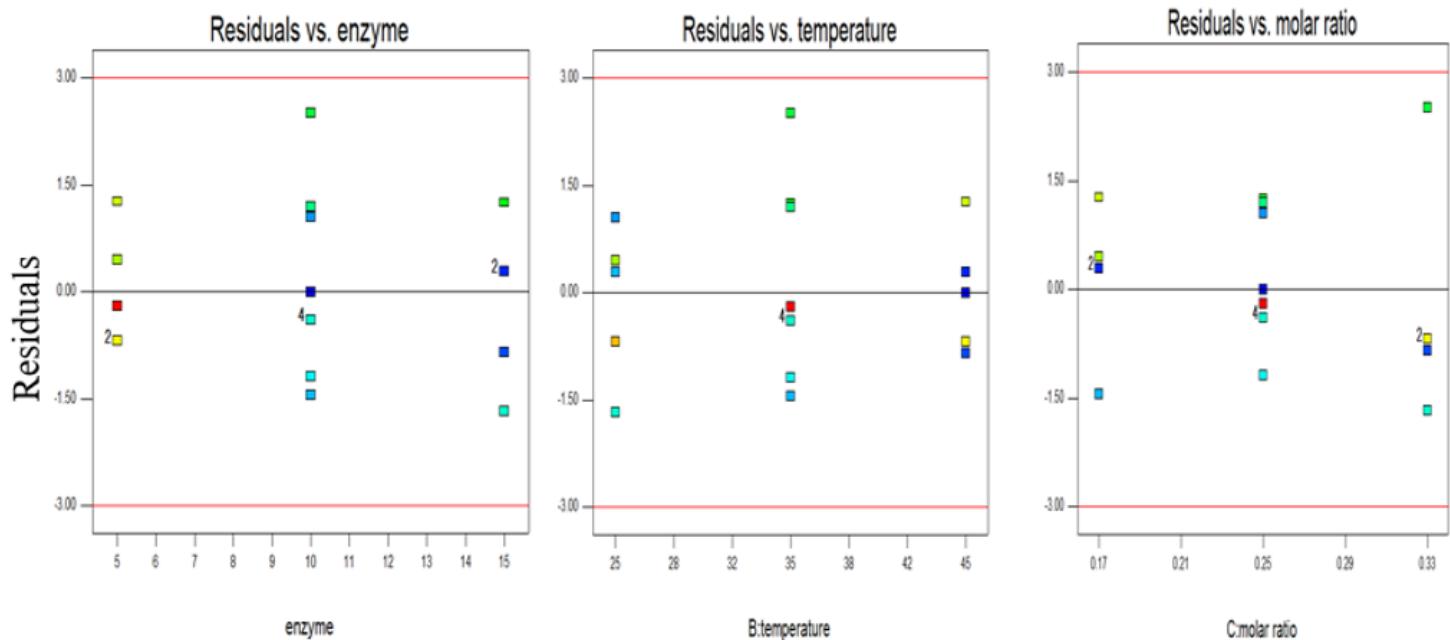


Figure 5

Diagnostic plots of residuals

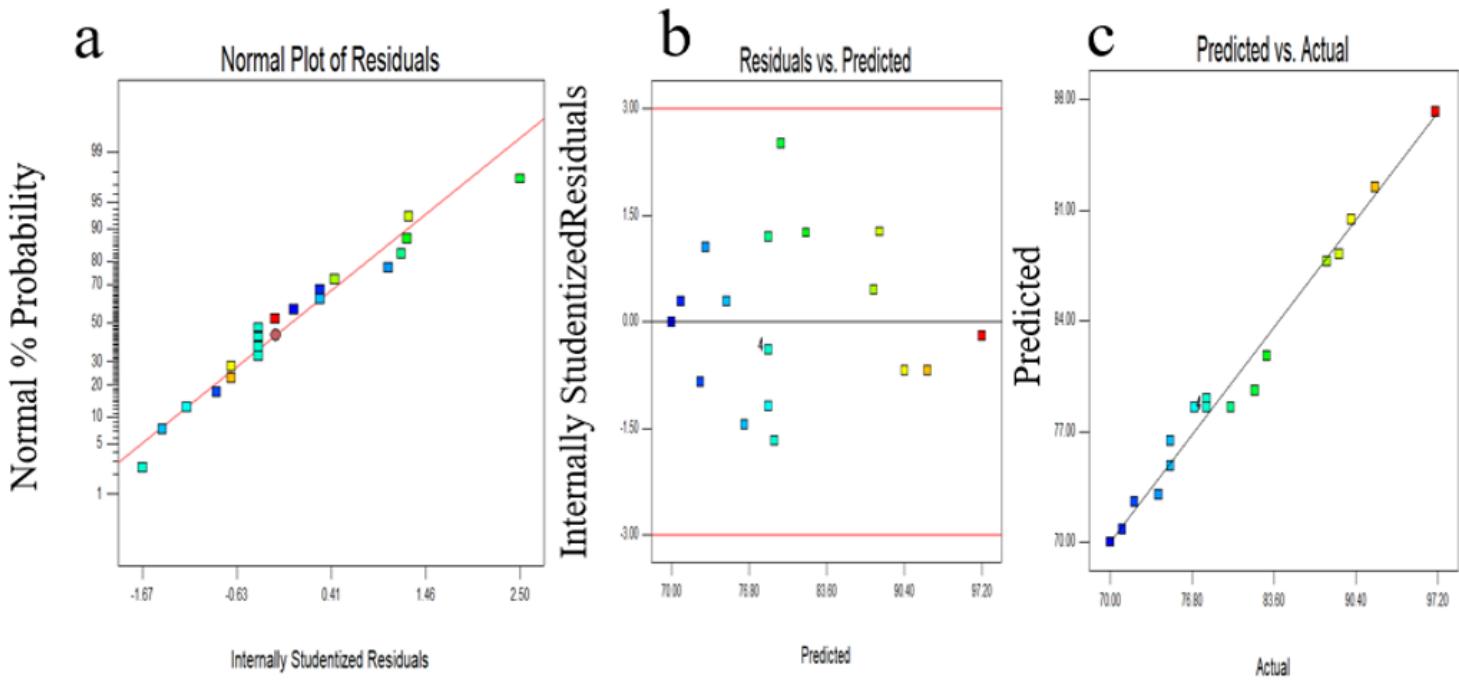


Figure 6

Plots of normal probability versus residuals (a), residuals versus predicted responses (b), predicted results versus actual results (c)

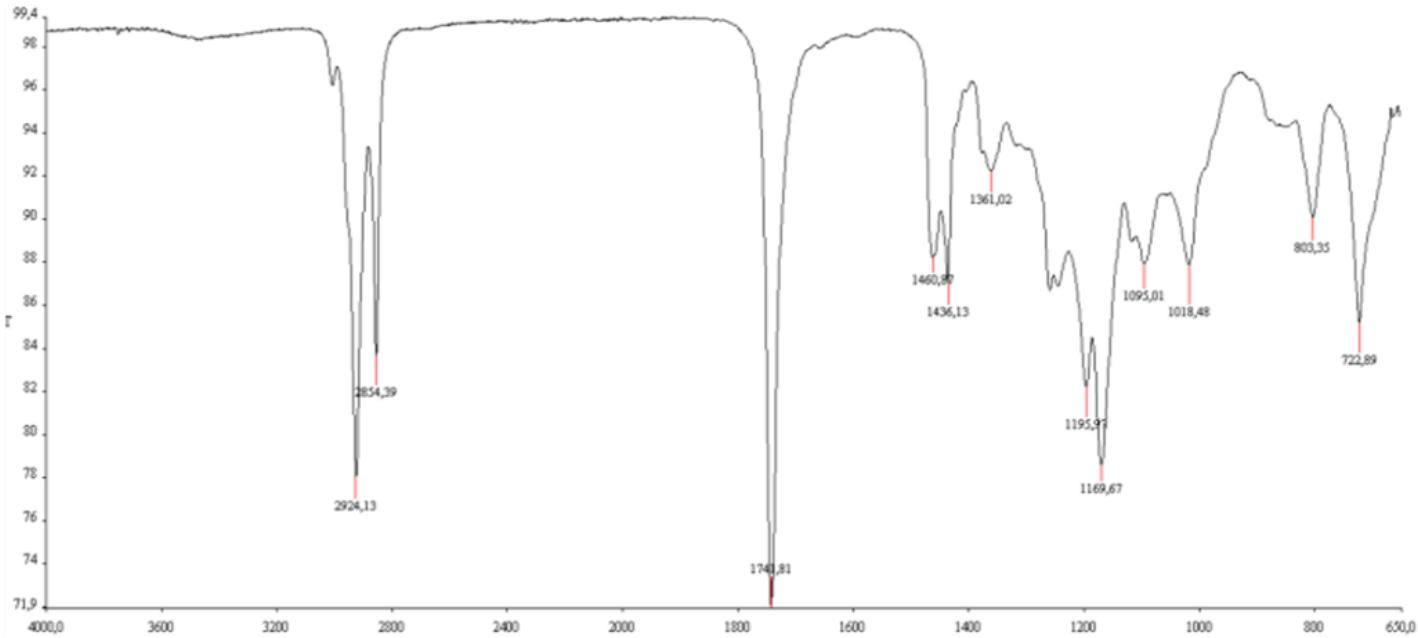


Figure 7

FTIR spectra of biodiesel obtained from waste filter coffee oil

Supplementary Files

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