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Optimization of the pretreatment step conditions for biodiesel production from waste frying oil using Box-Behnken design

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Abstract

In Tunisia, the emergence of various restaurants is generating a huge amount of waste frying oil. The valorization of this harmful waste into biodiesel production is a new trend. This valorization is necessary in order to avoid the waste's negative effects on the human health and the environment. Due to the high free fatty acid content, the pretreatment of this oil by an esterification reaction with homogenous acid in the presence of methanol was required to reduce the acid value of this oil to reach the necessary threshold in order to achieve the second step. The aim of this study was to determine the optimum conditions of the pretreatment step, using the Box-Behnken as an experimental design. The effects of the five parameters, namely, the volume of methanol, volume of sulfuric acid, stirring rate, temperature and reaction time on the acid value were studied. The optimum conditions for the pretreatment step were found as written below: reaction temperature at 60 °C, reaction time one hour, 1.02 ml of sulfuric acid, 56 ml of methanol and the stirring rate at 905 rpm. With these optimum conditions the biodiesel yield was always higher than 95%.

Keywords: Box-Behnken design, Optimization, Pretreatment, Waste frying oil, Biodiesel

1. Introduction

Human population and urbanization explosion and the new life style, are the causes responsible of the exhaustion of natural oil reserves. Thus, the issues of the awareness of environmental protection against the impact caused by emissions of greenhouse gases is a must be reviewed subject [1]. These factors have pushed the scientists to find an alternative source of energy to replace the fossil fuels (conventional sources). Biodiesel is a significant alternative to overcome the future lack of energy [1, 2]. The biodiesel production is an alternative clean fuel, biodegradable, non-toxic and renewable (environment friendly) [3]. It will reduce the CO_2 emissions by approximately 80%, carbon monoxide, and can ensure the independence towards conventional diesel [4, 5, 6]. Biodiesel can be derived from vegetable oils (refined or acid) or animal fat, combined with an alcohol (usually methanol) [3, 7]. The transformation of these products by the transesterification reaction gives reaction gives as final products biodiesel and glycerol. Biodiesel properties depend mainly of the raw material and alcohol type [3, 7].

Currently, at the industrial scale, the refined vegetable oil was used to produce biodiesel using transesterification as process in the presence of a homogeneous or heterogeneous catalysis [8, 9, 10, 11, 12]. However, this production is not economically profitable, due to the high cost of the aforementioned and the depletion of the food equilibrium [3, 7, 13]. These factors have forced the producers countries to find other sources with the lowest costs, which can minimize the global production cost [14, 15, 16, 17]. Tunisia, the valorization of the wastes (such as acidic oils) for biodiesel production constitutes a new trend for solving this problem. The waste frying oil with high level of free fatty acids was selected as a potential source for biodiesel production [14, 15, 16]. The use of this acid oil for biodiesel production has many benefits; economically (the reduction of the production's cost), environmentally (the prevention of water's pollution and the avoidance of the negative effects of the harmful waste) [18, 19]. It also allows the access to greater energy independence for low level petroleum resource countries like Tunisia. However, the high contents of free fatty acids in this oil can prevent the biodiesel manufacture. In fact, the transesterification reaction of waste frying oil in the presence of homogenous basic catalysts can cause the soap formation which blocks the installation of the production machine [20-24]. The oil's pretreatment should be relevant in order to avoid the saponification reaction and improve the production's profitability. Once the optimum conditions of the first step were determined, the second step (transesterification process) was applied in the presence of the potassium hydroxide as the basic catalyst [25, 26, 27].

In order to track the simultaneous effects of the five parameters on the acid value and the interactions between them during the pretreatment step, the response surface methodology (RSM) using Box-Behnken as experimental design and the quadratic model from the Box-Behnken design were used. In the presence of various factors (volume of methanol, volume of sulfuric acid, stirring rate, temperature and reaction time), Box-Behnken design offers some advantages compared to a factorial design or central composite design, such as a fewer number of experiments and a good optimization [28- 32].

Response surface methodology (RSM) was employed to determine the optimum conditions of these five independent variables. The relevance of these factors, their effects and interactions were tested by the analysis of variance with best confidence limits 0.05%. Thus, the values of the determination coefficient (R^2) and the adjusted coefficient (R^2 adj). The optimum conditions of the selected variables were obtained by solving the quadratic regression model, as well as by analyzing the response surface contour plots [30, 31, 32, 33].

The purpose of this work was to determine the optimum conditions which give the low acid value during the pretreatment steps of waste frying oil. The experimental data to the second-order polynomial model were fitted to the quadratic response surface models using multiple regression analysis with high coefficient of determination value (R^2) of 0.988. In the second step, the pretreated waste frying oil in the optimum conditions was directly converted into methyl ester. Finally, the physicochemical proprieties of the biodiesel obtained with the best experimental configuration were determined. Thus, the main components in the biodiesel were identified by gas chromatography-mass spectrometry (GC/MS).

2. Materials and methods

In this current study, the waste frying oil was used to produce biodiesel by a two-step process. This oil was collected from Tunisian restaurants and kept in best conditions in order to obtain the same acid value along this study (because the acid value of the waste oil always changes due to the variation in sources and preservation conditions). Methanol (98% purity) was used as alcohol for this study. Sulfuric acid (97% purity) and potassium hydroxide (98% purity) were used as acid and alkali catalysts. Around bottom flask was used as a reactor, a hot plate with a magnetic stirrer.

An experiment design (Box-Behnken design) was used in this work. The used oil had an acid value of 33 mg KOH/g-oil. In the first step, the free fatty acids were converted into fatty acids methyl ester by the pretreatment process using a sulfuric acid as catalyst in methanol excess. In the next step, the pretreated waste frying oil was converted into biodiesel by transesterification reaction, using methanol as a reagent, and potassium hydroxide as an alkaline catalyst.

2.1. *Biodiesel production from waste frying oil by two-step catalyzed process*

In the current study, different dosages of sulfuric acid (0.5-1.5 ml), different methanol-to-oil volume ratios: 40ml, 45ml, 50ml, 55ml, 60ml, 65ml and 70ml (v/v),stirring rate (300-1300 rpm), temperature (40-60 °C) and time of reaction (40-60 min) were used to track their influence on the acid value during the pretreatment step. The value ranges for these five independent variables are chosen based on the literature and the practical experience. The effects of these parameters on this oil's acid value were evaluated to determine the optimum conditions which give the lowest acid value in this step. For a five factors-three-levels, Box-Behnken design indicated that 45 experiments were required during the pretreatment step to ensure a good optimization. Before each experiment, 100 ml of waste frying oil was poured in the flask and heated up to 60 °C for 10 min. The preheated oil was mixed with different combinations of these five factors: volume of sulfuric acid, volume of methanol, reaction temperature, reaction time and stirring rate (table 3). After one hour of the occurrence of the reaction, the mixture for the first step was kept untouched for two hours in separating funnel. Afterwards, the methanol which remained at the top was removed using a separating funnel, whereas the acid value of the bottom product containing pretreated waste frying oil was further measured before carrying out the transesterification reaction. Then, the pretreated oil with optimum condition (a low acid value) was converted into biodiesel and glycerol by transesterification process (under the following optimum conditions: 20ml of methanol, 1 g of potassium hydroxide, 60 °C, 60 min and 800 rpm). After the reaction was completed, the product was allowed to settle for two hour resulting in the formation of two distinct liquid phases, the biodiesel phase at the top and the glycerol at the bottom. With this optimum combination the yield of biodiesel production was higher than 95% after purification and drying.

2.2. *Experimental design*

In the current study, the response surface methodology was used to find the optimum conditions for the pretreatment step (esterification process).The effects of five independent variables, volume of sulfuric acid, volume of methanol, stirring rate, temperature and reaction time on the acid value were investigated in the first step. The Box-Behnken design was carried in this study to minimize the number of experiments in the presence of these factors. Table 1 shows the levels of the independent variables used in this study. Such as the range of volume of methanol (X_1) volume of sulfuric acid (X_2), stirring rate (X_3), temperature (X_4) and reaction time (X_5) was fixed as: +1 and -1corresponding to the low and the high level (Table1). The dependent variable (response) was evaluated by the acid value (Y).

Table 1 Experimental range and levels of the five independent variables

Symbol code	Independent variables	Unit	Factor Levels		
			-1	0	+1
X_1	Volume of methanol	ml	40	55	70
X_2	Volume of sulfuric acid	ml	0.5	1	1.5
X_3	Stirring rate	rpm	300	800	1300
X_4	Temperature	°C	40	50	60
X_5	Time	min	40	50	60

A 5-factor, 3-level used in the design is appropriate for exploring quadratic response surfaces and constructing second order polynomial model. The second order polynomial model (polynomial equation) and response surface plots were used to relate the response (acid value) and the independent variables (five factors). The form of the quadratic model is shown in the following equation:

$$Y = b_0 + b_1 * X_1 + b_2 * X_2 + b_3 * X_3 + b_4 * X_4 + b_5 * X_5 + b_{11} * (X_1)^2 + b_{22} * (X_2)^2 + b_{33} * (X_3)^2 + b_{44} * (X_4)^2 + b_{55} * (X_5)^2 + b_{12} * (X_1 * X_2) + b_{13} * (X_1 * X_3) + b_{23} * (X_2 * X_3) + b_{14} * (X_1 * X_4) + b_{24} * (X2*X4) + b34 * (X3*X4) + b_{15} * (X_1 * X_5) + b_{25} * (X_2 * X_5) + b_{35} * (X_3 * X_5) + b_{45} * (X_4 * X_5)$$

Where: Y is the response (acid value), X_1, X_2, X_3, X_4, X_5 are the levels of the independent variables, b_0 is a constant and b_1,b_2,b_3, b_4 and b_5 are the linear coefficients. Such as, b_{11}, b_{22}, b_{33}, $b_{44}$and b_{55} are the quadratic coefficient, b_{12}, b_{13}, b_{14}, b_{15}, b_{23}, b_{24}, b_{25}, b_{34}, b_{35} and b_{45} are the interaction coefficients.

2.3. *Determination of the acid value*

The acid value of waste frying oil was analyzed using acid/base titration method. The solution was then titrated with 0.1 N of hydroxide potassium. The titration process was stopped when the solution turned into pink color. The acid value (AV) was then calculated using the following formula.

$$\text{Acid value (mg KOH/g)} = \frac{\text{Volume of KOH used} \times 56{,}1 \times 0{,}10}{\text{Weight of sample (g)}} \quad (1)$$

The conversion percentage of free fatty acids (FFA) to free fatty methyl ester (FAME) was calculated using the following formula (2).

$$\%\ \text{Conversion (conversion of FFA to FAME)} = \frac{\text{Nso} - \text{Ns}}{\text{Nso}} \qquad (2)$$

Where:
Nso: free fatty acids (FFA) content at the start of reaction (%)
Ns = free fatty acids (FFA) content at the end of pretreatment step (%)

2.4. *Determination of yield*

The yield of biodiesel produced was calculated using the equation 3. Product yield is defined as the weight percentage of the final product which is relative to the weight of the waste frying oil at the beginning of the experiment.

$$\%\text{Yield} = \frac{\text{Weight of product (g)}}{\text{Weight of raw oil (g)}} \times 100 \qquad (3)$$

2.5. *Purification*

The crude biodiesel was washed with an equal quantity of acid water with 10% and 5% phosphoric followed by a step of hot distilled water washing. The purification step aims to remove the traces of impurities and the catalyst. The moisture from the biodiesel was removed by a step of heating at 100-110°C for 20 min. After that, the physicochemical properties of the purified biodiesel were determined to evaluate its quality.

2.6. *Determination of physicochemical properties of pure biodiesel in the optimal conditions*

In this study, the most important physicochemical parameters were determined, the acid value, saponification value, density, viscosity, flash point, the cloud point, the water content, sulfur content, and potassium content. The determination of these physicochemical proprieties allowed us to evaluate the quality of biodiesel derived from the waste frying oil. The physicochemical properties of pure biodiesel are compared with those indicated in the European standard (EN14214).

2.7. *Analysis of pure biodiesel using gas chromatography-mass spectrometry (GC/MS)*

Gas chromatography coupled to mass spectrometry (GC/MS) (Trace GC Ultra-Thermo Scientific-DSQ II) was used to determine the composition of esters present in the biodiesel derived from waste frying oil. The GC was equipped with a capillary column (30 m/0.25 µm and an internal diameter of 0.25 mm). Helium was used as the carrier gas at a constant flow rate of 1.3ml/min. The analysis of biodiesel was carried out by dissolving 1 mg of biodiesel in the hexane. The mixture was stirred for 15 minutes at room temperature. Then, 1 µl diluted cold mixture was injected in the column injection. Esters content were analyzed using GC/FID; split mode of injector, and the injector temperature was kept at 250 °C.

3. Results and discussions

3.1. *Analysis of waste frying oil*

The compositions of the waste frying oil are very complex. Therefore, the essential step of this study is to find suitable methods to determine these compositions. The methylation process is one of methods can be used to analyze its content. There are a two main step used to identify the different free fatty acids in this acidic oil. The first step, the free fatty acids were converted into fatty acid methyl ester. The second step is the quantification of fatty acids methyl ester (FAME) by the gas chromatography mass spectrometry (GC/MS). After methylation, it was found that five majority compounds were detected in this oil, namely; methyl palmitate, methyl stearate, methyl oleate, methyl linoleate and methyl linolenate (figure 1). From the below chromatogram, the methyl palmitate appears at a retention time of 11.06 min followed by methyl stearate (C18:0) which appeared at a retention time of 12.80 min. The next compounds are the methyl oleate (C18:1) and methyl linoleate (C18:2) which peaks appeared at a retention time of 12.94 min and 13.32 min respectively. Finally, methyl linolenate (C18:3) appeared at a retention time of 13.85 min (figure 1).

As the methylation process does not modify the free fatty acids composition of the raw materials. In addition, this process converts the free fatty acids into fatty acids methyl ester by derivatization. Therefore, it could be concluded that there are five main components present in the waste frying oil, namely palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid. The free fatty compositions are summarized in the table 2.

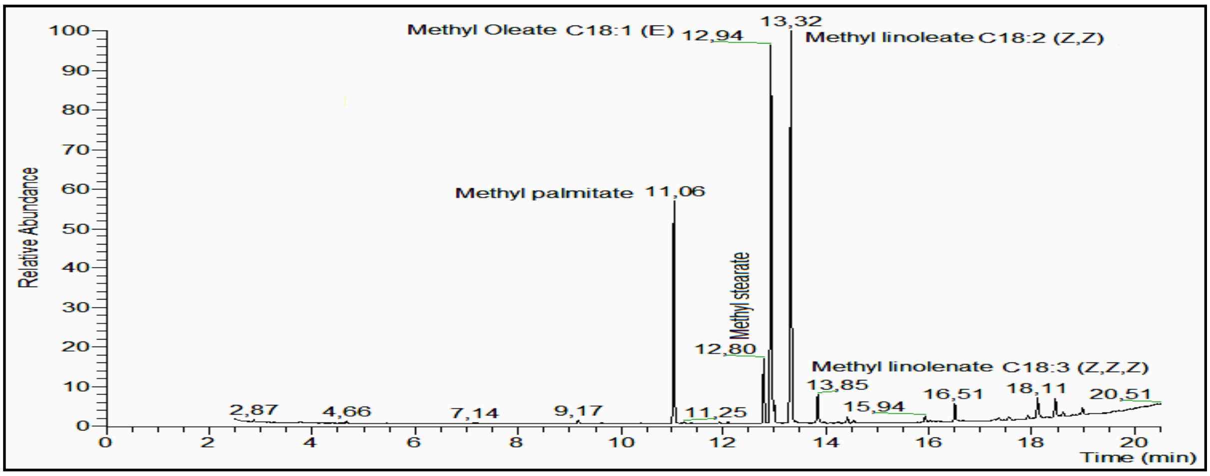

Figure1: Chromatogram showing the quantification of FAME by gas chromatography-mass spectrometry (GC/MS) after methylation process

Table 2: The main fatty acids composition in waste frying oil used in this study

Name of fatty acid	Methyl ester (trivial name / rational name)	Structure	Acid Composition (%)
Palmitic acid	Methyl palmitate /Methyl Hexadecanoate	C16:0	15.86%
Stearic acid	Methyl stearate/Methyl Octadecanoate	C18:0	4.87%
Oleic acid	Methyl oleate/ Methyl 9(E) Octadecenoate	C18:1 (E)	29.83%
Linoleic acid	Methyl linoleate /Methyl 9(Z),12(Z) octadecadienoate	C18:2 (Z,Z)	28.85%
Linolenic acid	Methyl linolenate /Methyl 9(Z),12(Z),15(Z)- octadecadienoate	C18:3 (Z,Z,Z)	2.49%

3.2. *Optimization of the pretreatment step by using an experiment design*

During the pretreatment of the waste frying oil, it is difficult to determine the interactions between five independent variables and to predict their effects on the acid value using the conventional method. For that, the statistical and mathematical tools were required to solve this problem. Therefore, Nemrod software was used in this work. In addition, the interactions between these factors and their influence on the response were studied by response surface methodology (RSM) using Box-Behnken as experimental design. Then, the quadratic model from the Box-Behnken design was used to estimate or predict the optimum conditions during the pretreatment step. The Box-Behnken design able to minimize the number of experiments performed during this step. The effect of these independent variables on the acid value (response) during the esterification reaction was investigated by the analysis of the response surface contours plots. This was achieved by a mathematical model that allowed us to trace the response surface contour plots. Consequently, several form of these contours plots are shown in two-dimensional contour response surface plots representing the variation of the acid value during this step (figure 2a-2j). After the analysis of these two-dimensional contour responses surface plots the optimal conditions during the pretreatment step can be determined. The results indicated that the five factors have significant effects on the reduction of the response, but for a specific combination. Table 3 shows that the values of the response predicted by the model are very close to the values of the experimental response; this proves that both values were accurate and reliable. In order to estimate the 21 unknown parameters of this model (the general average effect, five linear effects, five quadratic effects and 10 effects resulting of the interactions), the Box-Behnken design shows that 45 experiments should be conducted during the pretreatment step (Table 3).

From the analysis of the response of the surface contour plots, it has been found that the predicted lowest value of the acid value was 3.62 mg KOH/g-oil, however the actual experimental value was 2.24 mg KOH/g-oil.

Table 3: Observed response (Y) in Box–Behnken design

Run	Volume of methanol mL	Volume of sulfuric acid mL	Stirring rate rpm	Temperature °C	Time min	Acid value mg KOH/g-oil	
						Y experimental	Y predicted
1	40	0.50	800	50	50	13.83	13.554
2	70	0.50	800	50	50	11.85	11.663
3	40	1.50	800	50	50	10.87	11.559
4	70	1.50	800	50	50	8.24	9.018
5	40	1.00	300	50	50	22.43	22.026
6	70	1.00	300	50	50	20.74	20.635
7	40	1.00	1300	50	50	12.65	13.130
8	70	1.00	1300	50	50	9.31	10.088
9	40	1.00	800	40	50	14.73	14.164
10	70	1.00	800	40	50	11.86	11.257
11	40	1.00	800	60	50	8.91	8.264
12	70	1.00	800	60	50	7.42	6.737
13	40	1.00	800	50	40	10.89	11.436
14	70	1.00	800	50	40	7.71	7.904
15	40	1.00	800	50	60	8.29	8.468
16	70	1.00	800	50	60	7.74	7.567
17	55	0.50	300	50	50	23.49	23.494
18	55	1.50	300	50	50	21.25	21.749
19	55	0.50	1300	50	50	14.35	14.347
20	55	1.50	1300	50	50	10.96	11.452
21	55	0.50	800	40	50	14.87	15.354
22	55	1.50	800	40	50	13.43	12.649
23	55	0.50	800	60	50	9.21	9.759
24	55	1.50	800	60	50	8.54	7.824
25	55	0.50	800	50	40	12.84	12.898
26	55	1.50	800	50	40	9.16	9.023
27	55	0.50	800	50	60	10.32	9.691
28	55	1.50	800	50	60	9.75	8.926
29	55	1.00	300	40	50	23.93	24.410
30	55	1.00	1300	40	50	13.43	13.634
31	55	1.00	300	60	50	18.24	18.145
32	55	1.00	1300	60	50	9.85	9.479
33	55	1.00	300	50	40	21.64	21.692
34	55	1.00	1300	50	40	10.82	10.271
35	55	1.00	300	50	60	18.77	18.340
36	55	1.00	1300	50	60	11.35	10.319
37	55	1.00	800	40	40	11.23	10.852
38	55	1.00	800	60	40	8.17	8.382
39	55	1.00	800	40	60	10.78	11.940
40	55	1.00	800	60	60	2.24	3.990
41	55	1.00	800	50	50	6.34	6.268
42	55	1.00	800	50	50	6.23	6.268
43	55	1.00	800	50	50	6.16	6.268
44	55	1.00	800	50	50	6.32	6.268
45	55	1.00	800	50	50	6.29	6.268

3.3. *Fitting of the model*

The polynomial equation with the statistical analysis (surface plots) allows us to establish the proportionality between the response and the independent variables, in order to evaluate the effect of these independent factors on the acid value. It is required to analyze the significance and relevance of the model through the analysis of variance. Once the modeling and the optimization step have been determined, it is possible to judge the quality of the fitted model. For this, the statistical analysis of this model lead to analysis of variance (ANOVA) (Table 4). The model confirmed that the adjustment was significant and clear. Therefore the sum of squares due to residues is less than the sum of squares due to regression. Moreover, the sum of squares due to error is very small compared with the total sum. From these results it can be confirmed that the model seems to be well adjusted.

Table 4: Analysis of variance (ANOVA)

Source of variation	Sum of squares	Degrees of freedom	mean square	Ratio	Significance
Regression	1.16271E+0003	20	5.81354E+0001	10825.9605	***
Residual	1.40029E+0001	24	5.83457E-0001		
Validity	1.39814E+0001	20	6.99075E-0001	130.1815	***
Error	2.14800E-0002	4	5.37000E-0003		
Total	1.17671E+0003	44			

(***) p-value significant at 0.1% level

Another thorough study can be performed to confirm the adjustment of this model; it is done to determine the values of the determination coefficient (R^2) and the adjusted coefficient (R^2 adj.). The results indicated that these coefficients are very high and close to one previously found (R^2= 0.988) and (R^2 adj = 0.978) (Table 5). In this study, the high value of the coefficient determined ($R^2 > 98\%$) indicates the reliability of the design. In addition, the smaller p-values (lower than 0.05), which further strengthened the reliability of the models. Finally, the results validated the model. The significance of the coefficients (linear, quadratic and interaction) of the model was determined by applying the t-test and p-values of each of them as summarized in table 5. Since, the p-values of the coefficients were higher than 5% ($p > 0.05$), it implies that they are not significant. The significant of the level was estimated by three p-values: *** $P < 0.001$ (0.1%); ** $P < 0.01$ (1%) and * $P < 0.05$ (5%). The table 5 shows that the p-values of the model were always lower than 0.05, which indicated that the model was suitable and relevant to be developed in this study. From these results, the linear and quadratic effect between the different factors was considered significant, due to their low p-values which did not exceed 5% (Table 5). A positive sign of coefficient indicates a synergistic effect while a negative term indicates an antagonistic effect upon the acid value [33]. Therefore, the linear effect coefficient can be deduced and the five factors have an antagonistic effect for the reduction of the acid value during the pretreatment step. Table 5 depicted that the stirring rate had a significant linear effect, followed by temperature, sulfuric acid concentration, methanol concentration and finally the reaction time. In terms of quadratic effect, all factors have significant influences on the response due to $p < 0.001$. However, the interaction effect between the reaction time and temperature was more significant which was confirmed by the negative interaction coefficient and the low p-value level at 0.1%.

Table 5: Regression coefficients of predicted full quadratic model for the first step variables

Standard deviation of the response	0.073
R^2	0.988
R^2adj	0.978

Name	Coefficient	F.Inflation	Standard Deviation	T.exp.	Signif. %
b_0	6.268		0.033	191.26	***
b_1	-1.108	1.00	0.018	-60.49	***
b_2	-1.160	1.00	0.018	-63.32	***
b_3	-4.861	1.00	0.018	-265.32	***
b_4	-2.605	1.00	0.018	-142.19	***
b_5	-0.826	1.00	0.018	-45.10	***
b_{11}	1.945	1.27	0.026	75.70	***
b_{22}	3.236	1.27	0.026	125.95	***
b_{33}	8.257	1.27	0.026	321.37	***
b_{44}	1.892	1.27	0.026	73.66	***
b_{55}	0.631	1.27	0.026	24.55	***
b_{12}	-0.162	1.00	0.037	-4.44	*
b_{13}	-0.412	1.00	0.037	-11.26	***
b_{23}	-0.288	1.00	0.037	-7.85	**
b_{14}	0.345	1.00	0.037	9.42	**
b_{24}	0.192	1.00	0.037	5.25	**
b_{34}	0.528	1.00	0.037	14.40	***
b_{15}	0.658	1.00	0.037	17.94	***
b_{25}	0.777	1.00	0.037	21.22	***
b_{35}	0.850	1.00	0.037	23.20	***
b_{45}	-1.370	1.00	0.037	-37.39	***

(*) p-value significant at 5% level; (**) p-value significant at 1% level; (***) p-value significant at 0.1% level.

3.4. *The contour plots and response surface analysis*

The effects of the interaction between these factors on the acid value are presented in two-dimensional contour response surface plots (figure 2a-2j). It is useful to determine these effect types after analysis of the contour plots. In all figures, the five factors have a significant effect on the reduction of the acid value, provided that they are combined in a specific manner. In each two-dimensional contour response surface plots, the response (acid value) formed an optimum contour plot. In these optimal contour plots, the pretreated waste frying oil had an optimal acid value which is always lower than 4 mg KOH/g-oil. Consequently, in these conditions, the probability of soap formation in the presence of a basic catalyst in the second step is negligible. It was observed that the low volume of methanol, volume of sulfuric acid, stirring rate, reaction time and reaction temperature had no significant effect of decreasing the acid value (figure 2a-2j). In these conditions the acid values of the pretreated oil were always higher than the necessary limit to realize the second step. However, the response has decreased significantly to an optimum value while increasing the value of these parameters. According to the figures (Figure 2a-2j), the optimum response surface plots is reached when the temperature varied from 57 °C to 60 °C, the volume of methanol from 50 to 61 ml, the volume of sulfuric acid from 0.88 ml to 1.14 ml, the stirring rate from 771 to 1035 rpm and the reaction time from 58 to 60 min. It was also observed that the experimental optimum conditions were always located in the optimum contour plots which can be pointed out from the model. Figure 2c shows that the amount of catalyst and solvent are important parameters as they significantly influence the acid value, but only for a specific range. In this case, the acid value was high (8.16 mg KOH/g) at a low volume of sulfuric acid and methanol due to the poor solubility of reaction mixture (poor interaction between catalyst and free fatty acids). Although, an enhancement solubility of mixture was observed when the catalyst and solvent concentration increased. It is depicted that the acid value decreased steadily when the volume of catalyst is closer to 1ml; thereafter it was practically constant. Hence the lowest acid value was obtained at 1.02 ml of sulfuric acid and 56 ml of methanol. The results depicted that the increase of the volume of methanol, volume of sulfuric acid, the stirring rate, temperature and reaction time contributed significantly to the decrease of the acid value to an optimum value (3.62 mg KOH/oil). However, beyond this optimum value, the increase of the volume of methanol, the volume of sulfuric acid and stirring rate had no significant effect on the reduction of the acid value, which lead to the obtention of low conversion percentages of FFA to FAME) (figure 2a, 2b, 2c). This might be due to the antagonistic linear effect resulting in the negative coefficients, which was very significant at 0.1% level, as well as the quadratic effect that was significant and did not exceed 5% level (table 5). The stirring rate had the most important quadratic effect followed by the sulfuric catalyst and the volume of methanol with low p-values (P<0.001

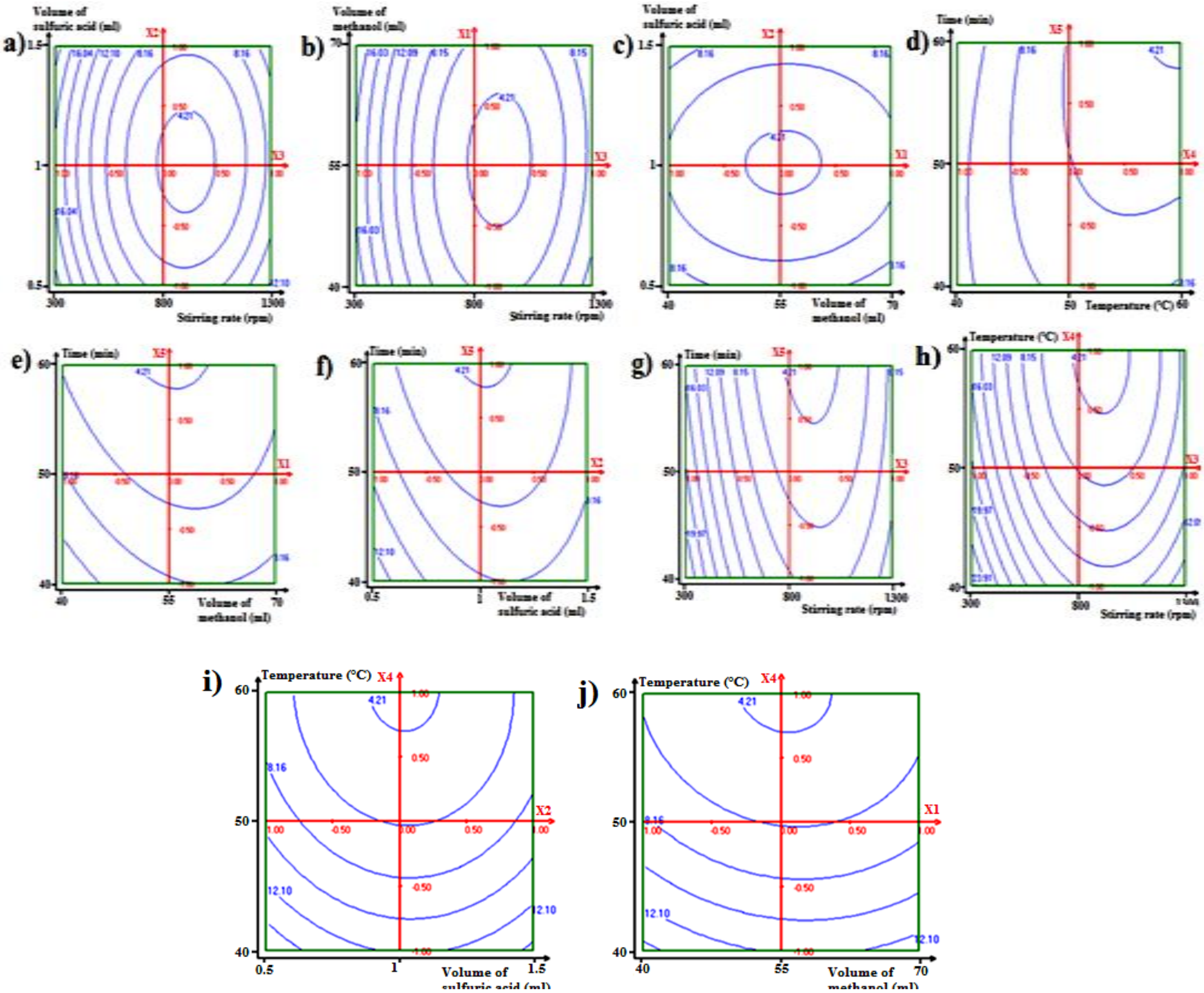

Figure.2a-2j: Response surface contour plots of the acid value, showing the effect operating parameters during the pretreatment step

). In addition, it was found that the interaction effect was significant and did not exceed 1% level (table 5). One of the parameters affecting the acid value is the reaction temperature, resulting by the negative linear coefficient and the significant p-value (P< 0.001) (table 5). The temperature should be optimized to avoid the evaporation of the methanol during the pretreatment step, for this, the reaction temperatures varied as follow (40 °C, 50 °C and 60 °C). Figure 2d shows that the increasing of reaction time and temperature contributed significantly to decrease the acid value; this is maybe due to the negative interaction coefficient between these two factors at 0.1% level (table 5). Furthermore, it was observed that at low reaction temperature and reaction time the response was higher than the optimum predicted by the experimental design. In addition, in this case, these two factors have no interaction effect, which was confirmed by the contour shapes which were always parallel to the x-axis (X_5). However, the interaction effect increased by increasing the temperature and the reaction time. Under these conditions, the interaction term can be considered significant on the reduction of the acid value due to the low p-value which is almost less than 0.1%. The contour response surface plots indicate that 60 °C and 60 min were required to realize the pretreatment step. This affirmation was confirmed by the optimum contour plots positions which are always taken in the vicinity of the optimal parameter values (60 °C and 60 min) (figure 2d-2j). Finally, all effects (linear, quadratic and interaction) of these five factors were significant for the response. The stirring rate factor has the greatest quadratic effect at 0.1%. The optimum acid value may be reached when the temperature is closer to the boiling point of methanol. The contour response surface plots are shown in the following figure (2a-2j):

3.5. *Research of the optimum conditions*

After replacement of the linear, quadratic and interaction coefficient with their values (table 5), the second order polynomial model can be changed in the following equation:

$$Y (X_1,X_2,X_3,X_4, X_5) = 6.268 - 1.108\ X_1 - 1.160\ X_2 - 4.861\ X_3 - 2.605\ X_4 - 0.826\ X_5 + 1.945\ (X_1)^2 + 3.236\ (X_2)^2 + 8.257\ (X_3)^2 + 1.892\ (X_4)^2 + 0.631\ (X_5)^2 - 0.162\ (X_1{*}X_2) - 0.412\ (X_1{*}X_3) - 0.288\ (X_2{*}X_3) + 0.345\ (X_1{*}X_4) + 0.192\ (X_2{*}X_4) + 0.528\ (X_3{*}X_4) + 0.658\ (X_1{*}X_5) + 0.777\ (X_2{*}X_5) + 0.850\ (X_3{*}X_5) - 1.370\ (X_4{*}X_5)$$

From mathematical tools, any critical point of function (Y) (each point that nullifies the partial derivatives) has the coordinates X_1, X_2 and X_3, X_4 and X_5 were solutions of the following equations system:

$$\frac{dy}{dX_1}(X_1,X_2,X_3,X_4,X_5) = -1.108 + 3.89\ X_1 - 0.162X_2 - 0.412\ X_3 + 0.345\ X_4 + 0.658\ X_5 = 0$$
$$\frac{dy}{dX_2}(X_1,X_2,X_3,X_4,X_5) = -1.160 - 0.162\ X_1 + 6.472\ X_2 - 0.288\ X_3 + 0.192\ X_4 + 0.777\ X_5 = 0$$
$$\frac{dy}{dX_3}(X_1,X_2,X_3,X_4,X_5) = -4.861 + 16.514\ X_3 - 0.412\ X_1 - 0.288\ X_2 + 0.528\ X_4 + 0.850\ X_5 = 0$$
$$\frac{dy}{dX_4}(X_1,X_2,X_3,X_4,X_5) = -2.605 + 3.784\ X_4 + 0.345\ X_1 + 0.192\ X_2 + 0.528\ X_3 - 1.370\ X_5 = 0$$
$$\frac{dy}{dX_5}(X_1,X_2,X_3,X_4,X_5) = -0.826 + 1.262\ X_5 + 0.658\ X_1 + 0.777\ X_2 + 0.850\ X_3 - 1.370\ X_4 = 0$$

The problem is to find the minimum of the multivariate function (Y) coerced to remain still in the experimental field. The optimum response was established after the determination of the optimums encoded values X_1, X_2, X_3, X_4 and X_5, which were determined by solving the system equations by using the program Matlab. The system equations could be writing under the following matrix form:

$$A{*}X = \text{Constant vector} \qquad (4)$$

Where:
A: matrix corresponding to a system
X: variable vector (X_1, X_2, X_3, X_4, X_5)
Constant vector (-1.108; -1.160; - 4.861; - 2.605; - 0.826)
To solve this system, it is necessary to determine the inverse matrix (A^{-1}) by using the Matlab program. Finally, the equation number (4) can be written as follows: inverse matrix determination

$$A^{-1}{*}A{*}\ X = A^{-1}{*}\text{constant vector} \qquad (5)$$

The results show that this equations system has a single solution, given as follow:

$$X_1 = 0.0873;\ X_2 = 0.0806;\ X_3 = 0.210;\ X_4 = 0.988;\ X_5 = 0.988$$

The results noticed that these encoded values were located in the optimum contour surface plots of the acid value. The replacement of these encoded factors in the second order polynomial model can give the optimum response predicted by the model at this critical point.

$$Y\ (X_1 = 0.0873;\ X_2 = 0.0806;\ X_3 = 0.210;\ X_4 = 0.988;\ X_5 = 0.988) = 3.99 \text{ mg KOH/g-oil.}$$

This study showed that the second order polynomial model appears correctly the studied phenomenon. It was found that the optimal experimental conditions, which minimize the acid value of the waste frying oil acid was obtained for the following coded levels: X_1= 0.0873; X_2= 0.0806; X_3= 0.210; X_4= 0.988; X_5= 0.988, from these encoded levels, the five factors take over these values (table 6):

Table 6: The optimum condition predicted by the Box-Behnken design

Encoded levels	Factors	Values
X_1	Volume of methanol	56 mL
X_2	Volume of sulfuric acid	1.02 mL
X_3	Stirring rate	905 rpm
X_4	Temperature	60 °C
X_5	Time	60 min
$Y (X_1, X_2, X_3, X_4, X_5)$	Response	3.99 mg KOH/g-oil

4. Characteristics of produced biodiesel obtained in the optimum conditions

4.1. Physicochemical properties of purified biodiesel obtained in the optimum conditions

Table 7 shows the characteristics of biodiesel derived from waste frying oil in the experimental optimum conditions. The results indicated that the physicochemical properties of this biocarburant are compliant with the European standard (EN14214). Also, the values of the physicochemical properties show that this product has better quality. It was found that the biodiesel yield is very important which is greater than 96.5%. This provided that during the transesterification, all triglycerides molecules react with the methanolic KOH liquid solution leading to form a rich methyl ester phase. From this result, it was confirmed that the remaining content of monoglycerides, diglycerides and triglycerides may be negligible in the final product. The other parameters, namely, the content of free fatty acid and water, density, viscosity, flash point, cloud point, the potassium and the sulfur content meet strict accordance with the requirement demanded by European standard (EN14214). The low cloud point can be attributed to the low percentage of saturated fatty acids in the final product (biodiesel). The sulfur and potassium are considered low compared to the limit required by the European standard EN14214. In this condition, the biodiesel can be used directly in the engine without any risk of corrosion.

Table 7: Physicochemical proprieties of the biodiesel derived from waste frying oil in the experimental optimum conditions.

Parameter	Unit	Waste frying oil	Pure biodiesel	Biodiesel (EN14214)	
				Min	Max
Viscosity at 40°C	mm^2/s	23.12	3.8	3.5	5
Density at 15°C	g/cm^3	0.91	0.87	0.86	0.9
Sulfur content	mg/kg	-	1.23	-	10
Acid value	mg KOH/g	32.82	0.428	-	0.5
Flash point	°C	-	173	101	-
Pour point	°C	-	11	-	-
Water content	ppm	1765	345	-	500
Ester conversion	%	-	98	96.5	-
Saponification value	mg KOH/g	243	194	-	-
Potassium content	mg/kg	-	0.1	-	5

4.2. *Analysis of the produced biodiesel in the optimum conditions*

Gas chromatography-mass spectroscopy was used to determine the chemical compositions of pure biodiesel in optimum conditions. The chromatogram below indicates that five main compounds were detected in pure biodiesel, namely methyl palmitate. Table 8 shows that five main compounds have been identified, namely palmitic acid the methyl ester, stearic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester and linolenic acid methyl ester. The other compounds such as arachidic acid methyl ester (C20: 0) and behenic acid methyl ester (C22: 0) are considered as minority (traces). Table 7 shows that the unsaturated fatty acids constitute about 64% out of the total fatty acid compositions, while, saturated fatty acids constituted 22%. From this result, it can be deduced that the percentage of saturated fatty acids is one third of the percentage of unsaturated acids. Therefore, the biodiesel containing more unsaturated fatty acid is less viscous and thus can be transferred more easily from the reservoir to the engine at low temperature. This result shows that the biodiesel can be considered as a better biofuel at low temperatures. Furthermore, the difficulty during the engine starting can be overcome.

Table 8 Fatty acid compositions of the biodiesel derived from waste frying in the experimental optimum conditions

Fatty acids	Formula	Structure	Percentage
Palmitic acid methyl ester	$C_{16}H_{32}O_2$	C16:0	16.71%
Stearic acid methyl ester	$C_{18}H_{36}O_2$	C18:0	5.25%
Oleic acid methyl ester	$C_{18}H_{34}O_2$	C18:1	31.69%
Linoleic acid methyl ester	$C_{18}H_{32}O_2$	C18:2	30.28%
Linolenic acid methyl ester	$C_{18}H_{30}O_2$	C18:3	2.57%
Arachidic acid methyl ester	$C_{20}H_{40}O_2$	C20 :0	0.55%
Behenic acid methyl ester	$C_{22}H_{44}O_2$	C22 :0	0.54%

Conclusions

The acid value of waste frying oil was successfully optimized using a five-factor, three-level Box–Behnken design. The most significant effects of these factors at different levels on the response (acid value) could be predicted by using the second order polynomial equation. The quadratic response surface methodology studied helped to reach the interaction effects between the combinations of these five factors. The validation of the optimization technique demonstrated the relevance and adjustment of the model. The optimum experimental conditions obtained from the classical methods (optimized formulation) were located in the optimum contour conditions predicted by the model. This optimum conditions during the pretreatment step was reached at the following combination: methanol to oil volume ratio of 56:100 ml, 1.05 ml of sulfuric acid, at 60 °C and 60 min of reaction time. From these results, it can be concluded that the statistical tools can be used for the development of the pretreatment of the acidic oil. This statistical design allows to minimize the number of experiments and to determine the different effects (linear, quadratic and interaction) present in the system. In this study, during the pretreatment step, all effects (linear, quadratic and interaction) have significant consequences on the acid value with low p-values (that do not exceed 5%). In the presence of the five factors, the best predictable optimum response was successfully accomplished by the experimental design with the fewest number of experiments.

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