



OPTIMIZATION OF SUNFLOWER OIL HYDROLYSIS USING THE D-OPTIMAL DESIGN

(Pengoptimuman Hidrolisis Minyak Bunga Matahari Menggunakan Reka Bentuk D-Optimal)

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Abstract

The hydrolysis process of sunflower oil (SFO) was carried out to produce sunflower oil fatty acids (SFOFAs). The optimization of reaction parameters was performed using response surface methodology (RSM) via D-optimal design. The optimization parameters were varied from 1.0 to 2.5 M molarity of ethanolic KOH, 50 to 70°C of reaction temperature and 0.5 to 2.5 hours of reaction time. The highest percentage of SFOFAs yield was 96.68% with a free fatty acid (FFA) of 103.23%, which was achieved at optimal conditions: 2.3 M ethanolic KOH concentration, reaction temperature of 50.14°C and 0.97 hours of reaction time. The structure of SFO and SFOFAs were confirmed using Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR). SFOFAs was monitored by the absence of C=O (ester) at 1746.33 cm⁻¹ and the existence of C=O (carboxylic acid) at 1710.23 cm⁻¹ with OH stretching at 2400-3400 cm⁻¹ in SFOFAs FTIR spectrum. Under the NMR spectrum of SFOFAs, the absence peak of ester (C=O) at 172.79 to 173.21 ppm (¹³C NMR), the emergence peak of OH at 10.61 ppm (¹H NMR) and the peak of carbon for carboxylic acid (C=O) (¹³C NMR) at 180.45 ppm showed that the fatty acid was successfully obtained.

Keywords: D-optimal design, hydrolysis, response surface methodology, saponification, sunflower oil

Abstrak

Proses hidrolisis minyak bunga matahari (SFO) telah dijalankan untuk menghasilkan asid lemak minyak bunga matahari (SFOFA). Pengoptimuman parameter tindak balas dilakukan menggunakan kaedah permukaan tindak balas (RSM) melalui reka bentuk D-optimal. Parameter pengoptimuman telah diubah daripada 1.0 hingga 2.5 M kemolaran etanol KOH, 50 hingga 70 °C suhu tindak balas dan 0.5 hingga 2.5 jam masa tindak balas. Peratusan tertinggi hasil SFOFAs ialah 96.68% dengan asid lemak bebas (FFA) sebanyak 103.23%, yang dicapai pada keadaan optimum: kepekatan KOH etanol 2.3 M, suhu tindak balas 50.14 °C dan 0.97 jam masa tindak balas. Struktur SFO dan SFOFAs telah disahkan menggunakan spektroskopi infra-merah transformasi Fourier (FTIR) dan resonans magnetik nuklear (NMR). SFOFAs dilihat dengan ketiadaan C=O (ester) pada 1746.33 cm⁻¹ dan kewujudan C=O

(asid karboksilik) pada 1710.23 cm^{-1} dengan regangan OH pada $2400\text{--}3400\text{ cm}^{-1}$ dalam spektrum FTIR SFOFAs. Di bawah spektrum NMR SFOFAs, kehilangan puncak ester (C=O) pada 172.79 hingga 173.21 ppm (^{13}C NMR), kemunculan puncak OH pada 10.61 ppm (^1H NMR) dan puncak karbon untuk asid karboksilik (C=O) (^{13}C NMR) pada 180.45 ppm menunjukkan bahawa asid lemak berjaya diperolehi.

Kata kunci: reka bentuk D-optimal, hidrolisis, kaedah permukaan tindak balas, penyabunan, minyak bunga matahari

Introduction

Nowadays, the increase in the use of petrochemical products in daily life has caused various environmental problems [1]. The petrochemicals are used in many industries like plastics, soaps and detergents, solvents, drugs, fertilizer and pesticides [2]. The use of petrochemical products is an international issue because they contribute to environmental problem, global warming and climate change due to the disadvantages of petrochemical product such as low biodegradability (20-30%), toxic to the environment, cause an increase of the greenhouse gases that affect global warming and the material come from non-renewable sources [2-6]. The use of petrochemical products needs to be changed to other alternatives that do not have a negative impact on the environment. A suitable alternative is to replace petrochemical products with biobased products such as plant oil.

Fatty acid and glycerol are essential raw materials in the oleochemical industry [4]. It is widely used as a raw material in the food, cosmetic and pharmaceutical industries. Fatty acid can be obtained from the hydrolysis of plant oil and fats via saponification as shown in Figure 1 [7, 8]. Fatty acid can also be used as a starting material in the production of biolubricant (polyol ester) [9, 10]. There are several plant oils used in the production of fatty acids, including sunflower oil. There are various type of fatty acids in sunflower oil (SFO) such as palmitic (5.2%), stearic (2.7%), oleic (37.2%), linoleic (53.8%) and linolenic (1.0%) [11], which make the ingredients suitable for hydrolysis process. The hydrolysis reaction can be catalyzed using acid, base or enzyme (lipase) [4]. Nowadays, researchers focus more on potassium hydroxide catalyzed (base catalyze) hydrolysis in order to reduce energy consumption [12].

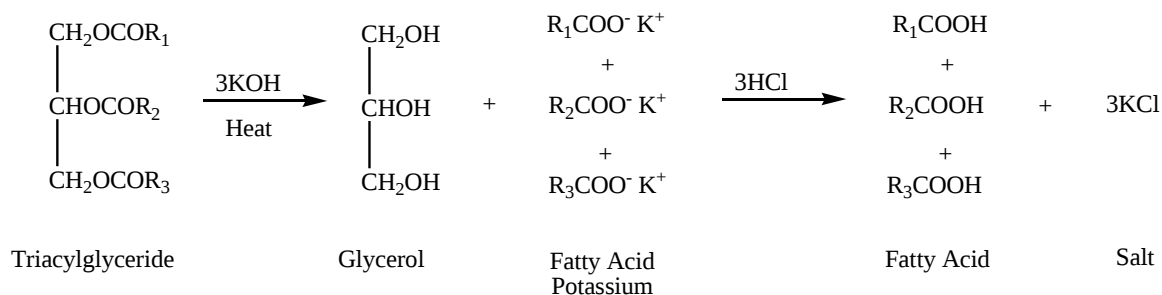


Figure 1. Hydrolysis process of triacylglycerol in plant oil

Based on the previous studies, there are several researchers who have carried out the hydrolysis process of plant oil in such as Bahadi et al. [4], who used different ethanolic KOH concentration, time and temperature for the optimization of the hydrolysis of crude palm kernel oil. The results of the study found that the free fatty acid (FFA) and yield obtained were 98% and 84.7% respectively with the optimum condition being 2.16 hours, 1.77 M ethanolic KOH at a temperature of 70 °C. Nor et al. [13] carried out

hydrolysis of RBD palm oil using base catalyst. The conditions used were 1.5 M ethanolic KOH, 2.0 hours at a temperature of 60 °C and the yield produced was 95%. Ferreira et al. [14] also conducted the hydrolysis process of cottonseed, olive and palm kernel oil by using lipase enzyme from *Geotrichum candidum* (GCL-1) as catalyst. The result showed that GCL-1 gave the highest activity of hydrolysis only for plant oil that contained high amount of unsaturated fatty acids (cottonseed and olive oil) with optimum condition at 46.8% m/m oil, 6.6

U/g of reaction mixture at 40°C. In another study, Salimon et al. [8] carried out hydrolysis for preparing fatty acids from *Jatropha curcas* seed oil. The result of the study showed that the maximum recovery of FFA was 102.2% and the optimum condition were 1.75 M ethanolic KOH, 2.0 hours at a temperature of 65°C.

This research focus on using D-optimal design from response surface methodology for optimization process for hydrolysis of sunflower oil. Previous studies on the hydrolysis of sunflower oil focus more on using enzyme as catalyst [15, 16] and the used of base catalyze (potassium hydroxide) is limited. Most of the optimization process is done using conventional methods. There is no study done using the statistical method for hydrolysis of sunflower oil via D-optimal design.

Response Surface Methodology (RSM) is a practical method that uses mathematical and statistical analysis to fit empirical models to experimentally collect data [17]. The RSM can be used to optimize the reaction process since, in comparison to conventional method, it minimizes the overall number of experimental trials, operational costs, and time [10]. Typically, the prediction of responses in optimization process are specifically based on linear and quadratic models as shown in equation (1). As a result, an optimization study can be carried out to evaluate the experimental condition.

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \sum \beta_{ij} x_i x_j \quad (1)$$

Where Y for response, β_0 for constant, β_i for linear, β_{ii} for square regression coefficient and β_{ij} for interaction regression coefficient and x_i and x_{ij} are assigned as independent variables [18, 19]. D-optimal design is a useful tool to determine the factors that are significant to the experimental condition to achieve the best performance. The concept of D-optimal design exhibits remarkable flexibility and efficiency by accommodating a wide range of factors, both in terms of quantity and characteristics, as well as diverse levels of factor configurations and model specifications. It uniquely maximizes the determinant of the information matrix associated with the model, thereby systematically

selecting the most informative experimental runs from a given set of candidates. This paper offers details on practical use for D-optimal design to optimize the hydrolysis of sunflower oil to obtain sunflower oil fatty acids (SFOFAs). D-optimal design was applied by using percentage yield and free fatty acids as responses to determine the optimal conditions for the hydrolysis.

Materials and Methods

Sunflower oil was obtained from IKO company, Negeri Sembilan, Malaysia. Potassium hydroxide (KOH), hydrochloric acid 37% (HCl), n-hexane 99%, ethanol 95% and anhydrous sodium sulphate (Na_2SO_4) were purchased from System chemical Co, Malaysia. All chemicals utilized were analytical reagent grade and used without the need for additional purification steps.

Hydrolysis of sunflower oil

The hydrolysis of sunflower oil (SFO) involves two stages which are saponification and acidification. The hydrolysis was carried out using sunflower oil (SFO) with alkaline ethanol to produce sunflower oil fatty acids (SFOFAs). At saponification stage, 12.5 grams of SFO was mixed with 75 mL of alkaline ethanol into 250 mL two neck round bottom flask equipped with a reflux condenser, thermometer and mechanical stirrer. The mixture was heated and continuously stirred at 300 rpm using magnetic stirrer. After that, the mixture undergoes acidification process where 25 mL of hydrochloric acid was added to neutralize the alkaline solution. The washing continues by adding 50 mL of distilled water and 25 mL of n-hexane. After washing, anhydrous sodium sulphate was added to the product (SFOFAs) for overnight and the product was filtered using Whatman No 1 filter paper and the solvent was removed by using rotary evaporator [20]. At the next stage, the percentage free fatty acid (FFA) of SFOFAs was determined.

Optimization of hydrolysis

Software from Design Expert version 13 was employed for this optimization. There are three parameters used in the optimization of hydrolysis, namely the molarity of catalyst used (ethanolic KOH) (A), reaction temperature (B) and reaction time (C). The range used for the molarity of ethanolic KOH was 1.0 M to 2.5 M, the range for reaction temperature was 50 to 70 °C and the

range for reaction time was 0.5 to 2.5 h as shown in Table 1. The implementation of hydrolysis reaction based on D-optimal design in response surface methodology has generated 20 runs randomly for the hydrolysis of sunflower oil (SFO). Each experiment was

carried out according to the parameter conditions set by the design model to obtain the responses, which were percentage yield and percentage FFA of SFOFAs. A regression coefficient R^2 was used to evaluate the model's adequacy and best fit [21].

Table 1. Parameter and level for D-optimal design of hydrolysis

Parameter	Unit	Symbol	Variable Level		
			-1	0	+1
Molarity ethanolic KOH	M	A	1.0	1.75	2.5
Temperature	°C	B	50	60	70
Time	h	C	0.5	1.5	2.5

Determination of percentage free fatty acids

Percentage of free fatty acids (FFA) were determined based on the study by Japir et al. [22]. 0.5 gram of oil sample was weighted, and 50 mL of neutralized isopropyl alcohol was added into the conical flask. The mixture was heated up to 40 °C until it fully dissolved. Then, 1 mL of indicator (phenolphthalein) was added to the mixture. Next, the mixture was titrated with 0.1N NaOH until light pink color appeared. The percentage FFAs as oleic acid was calculated using equation (2) below:

$$\% \text{ FFA as Oleic Acid} = \frac{28.2 \times N \times V}{W} \quad (2)$$

Where N is normality of NaOH (Eq/L); V is volume of NaOH solution used (mL); and W is weight of oil sample (g).

Verification of the model

The fitness of the constructed model was evaluated by comparing the actual values of the SFOFAs yield and SFOFAs FFA at optimum conditions with the predicted values. The model was validated using the value of Residual Standard Error (RSE), where the value cannot exceed more than 5% [17]. The percentage of RSE was calculated using equation (3).

$$\% RSE = \frac{(\text{actual value} - \text{predicted value})}{\text{predicted value}} \times 100 \quad (3)$$

Structure analysis of sunflower oil and sunflower oil fatty acids

Fourier Transform infrared spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR) were used to determine the functional groups and identify the structure of SFO and SFOFAs, respectively. Perkin Elmer Infrared spectrometer was used for FTIR in the range of 650 to 4000 cm^{-1} . NMR analysis was carried out using a JOEL-ECP 400 spectrometer at 400 MHz (^1H NMR) and 100.61 MHz (^{13}C NMR) and deuterated chloroform (CDCl_3) was used as a solvent.

Results and Discussion

Hydrolysis of sunflower oil

The hydrolysis of SFO with ethanolic KOH produced SFOFAs. The aim of this optimization is to produce a high percentage yield and high percentage FFA of SFOFAs. Table 2 shows the tabulated 20 experimental runs with results for both actual and predicted responses, namely the percentage yield of SFOFAs annotated as Y_1 and the percentage of FFA of SFOFAs annotated as Y_2 based on the conditions that have been generated from D-optimal design. The actual response values are almost equal to the predicted response values.

Table 2. Experimental runs, factors and responses of SFOFAs from D-optimal design

Run	Factors			Responses			
	Molarity Ethanol KOH (M)	Reaction Temp. (°C)	Reaction Time (h)	Yield, Y ₁ (%)		FFA, Y ₂ (%)	
				Actual	Predicted	Actual	Predicted
1	1.7	50	1.60	95.81	95.08	100.03	100.28
2	1.0	57	2.50	82.70	82.36	88.93	88.58
3	2.4	60	0.59	89.91	91.07	96.16	98.01
4	1.7	61	1.91	91.02	91.79	96.39	98.18
5	1.7	61	0.53	90.53	91.51	97.60	97.72
6	2.5	70	0.50	86.01	85.95	92.59	92.28
7	1.0	50	0.50	84.38	85.64	91.08	92.85
8	1.7	61	0.53	94.48	91.51	99.98	97.72
9	2.5	50	2.50	88.14	88.68	96.03	96.56
10	1.7	50	1.60	95.73	95.08	101.54	100.28
11	1.0	57	1.39	88.31	87.44	96.70	94.79
12	2.0	70	2.50	88.00	87.35	95.75	94.64
13	2.5	61	1.60	90.76	90.04	99.39	98.33
14	1.0	70	2.50	86.19	86.67	93.44	94.49
15	2.0	70	2.50	88.03	87.35	95.44	94.64
16	1.0	70	1.25	90.79	90.24	99.12	98.51
17	2.5	61	1.60	90.32	90.04	97.79	98.33
18	1.8	70	1.20	91.70	93.04	97.79	99.32
19	1.7	61	1.91	89.64	91.79	97.12	98.18
20	2.5	50	0.50	96.37	96.18	104.1	103.26

Model selection is made based on the highest polynomial order in which all terms are significant, and the model is not an alias. Table 3 and Table 4 show sequential model sum of squares of percentage yield and percentage FFA. By examining the sum of squares of the model for both response variables indicates that the quadratic model stands out. This is evident from the

highest F-values observed, which are 18.61 for Y₁ and 9.89 for Y₂, as well as the lowest p-values recorded, specifically 0.0002 for Y₁ and 0.0024 for Y₂. The model's F-value indicates the superior explanatory capability of the research model for both response variables.

Table 3. Sequential model sum of squares of percentage yield response

Source	Sum of Squares	dF	Mean Square	F-value	p-value	model
Mean vs Total	1.618E+05	1	1.618E+05			
Linear vs Mean	62.63	3	20.88	1.67	0.2128	
2FI vs Linear	42.48	3	14.16	1.17	0.3586	
Quadratic vs 2FI	133.33	3	44.44	18.61	0.0002	Suggested
Cubic vs Quadratic	15.03	5	3.01	1.70	0.2877	Aliased
Residual	8.85	5	1.77			
Total	1.620E+05	20	8102.50			

Table 4 . Sequential model of sum square of percentage FFA response

Source	Sum of Squares	dF	Mean Square	F-value	p-value model	
Mean vs Total	1.876E+05	1	1.876E+05			
Linear vs Mean	53.34	3	17.78	1.53	<u>0.2442</u>	
2FI vs Linear	68.04	3	22.68	2.51	0.1044	
Quadratic vs 2FI	87.86	3	29.29	9.89	0.0024	Suggested
Cubic vs Quadratic	24.04	5	4.81	4.32	0.0671	Aliased
Residual	5.57	5	1.11			
Total	1.878E+05	20	9391.57			

Model selection is also made based on the non-significant lack of fit value. Table 5 and Table 6 show lack of fit tests for percentage yield and percentage FFA. The value for lack of fit analysis is opposite to the value of the sequential analysis of the sum of squares where

the lack of fit analysis gives the smallest F-value and the largest p-value or is not significant (>0.05) Based on the analysis of lack of fit, it shows that the quadratic model is the suitable model for the both responses based on the non-significant p-values.

Table 5. Lack of Fit Tests for percentage yield

Source	Sum of Squares	dF	Mean Square	F-value	p-value model	
<u>Linear</u>	190.85	11	17.35	9.80	<u>0.0104</u>	
<u>2FI</u>	148.37	8	18.55	10.47	0.0096	
Quadratic	15.03	5	3.01	1.70	0.2877	Suggested
<u>Cubic</u>	0.0000	0				Aliased
<u>Pure error</u>	8.85	5	1.77			

Table 6. Lack of Fit Tests for percentage FFA

Source	Sum of Squares	dF	Mean Square	F-value	p-value model	
<u>Linear</u>	179.94	11	16.36	14.69	<u>0.0041</u>	
<u>2FI</u>	111.90	8	13.99	12.56	0.0064	
Quadratic	24.04	5	4.81	4.32	0.0671	Suggested
<u>Cubic</u>	0.0000	0				Aliased
<u>Pure error</u>	5.57	5	1.11			

Figure 2 shows the actual and predicted value of percentage yield (a) and percentage FFA (b) of SFOFAs. It shows that the plot is scattered along the straight

regression line. The normal probability plot proves the accuracy of the individual experimental done.

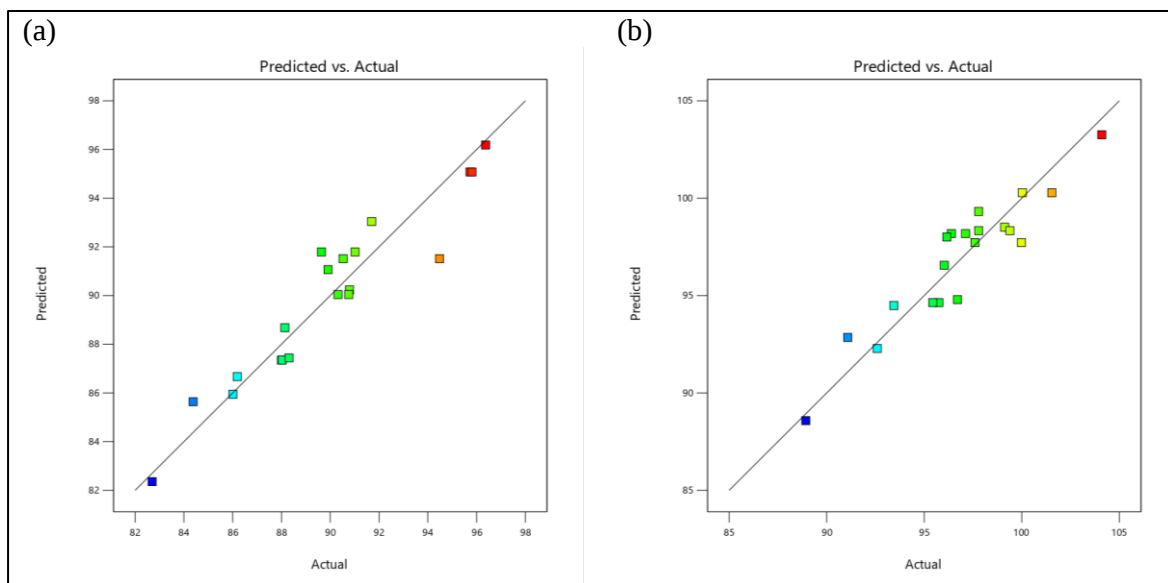


Figure 2. Actual and predicted value for a) SFOFAs yield and b) SFOFAs FFA

D-optimal design fitting

The ANOVA data analysis for percentage yield and percentage FFA of SFOFAs are shown in Table 7. The SFOFAs yield (Y_1) and SFOFAs FFA (Y_2) with p-values of 0.0004 (Y_1) and 0.0017 (Y_2), which are less than 0.05, indicate that the model for both responses are significant. The model F-values of 11.09 (Y_1) and 7.85 (Y_2) imply the model is also significant. Based on the F-values, there are only a 0.04% (Y_1) and 0.17% (Y_2) chances that an F-value this large could occur due to noise. The R^2 value for Y_1 is 0.91 and for Y_2 is 0.88. The

model shows an adequate precision ratio of 12.6511 for Y_1 and 12.0673 for Y_2 . Both adequate precision for Y_1 and Y_2 are desirable, which imply an adequate signal due to the value are greater than 4. Furthermore, the lack of fit for both Y_1 (0.2877) and Y_2 (0.0671) are not significant with p-value more than 0.05 which indicates that the model is satisfactorily fitting. The equation for regression line that are suggested by the model design for percentage yield of SFOFAs (Y_1) and percentage FFA of SFOFAs (Y_2) are shown in equation (4) and (5).

$$\text{Yield } (Y_1) = 93.35 + 1.52A - 1.28B - 1.96C - 2.90AB - 4.11A^2 - 3.58C^2 \quad (4)$$

$$\text{FFA } (Y_2) = 99.70 + 1.81A - 1.89C - 3.37AB - 2.56A^2 - 3.72C^2 \quad (5)$$

ANOVA analysis from the Table 7 shows for the Y_1 , the linear relationship between three variable parameters which are the molarity ethanolic KOH (A) and the reaction temperature (B) are significant (p-value < 0.05) and the reaction time (C) is highly significant (p-value < 0.01). The interaction between the three parameters (AB, AC, BC) has been seen to be highly significant for AB (p-value < 0.01) but the interaction between AC and BC are not significant (p-value > 0.05). For Y_2 , the linear relationship between parameters A, B and C shows that A and C are highly significant (p-value < 0.01) while B

is not significant (p-value > 0.05). The interaction between AB, AC and BC shows that only AB is highly significant while AC and BC are not significant. For both Y_1 and Y_2 , only the interaction between molarity ethanolic KOH (A) and reaction temperature (B) gives an effect in this optimization of hydrolysis.

Response surface analysis

The interaction effect between the reaction variables (A, B and C) on the percentage yield and percentage FFA of SFOFAs can be illustrated by using 3D response

surfaces and contour graphs. Figure 3 shows the interaction effect between ethanolic KOH molarity and reaction temperature (AB) on SFOFAs yield and SFOFAs FFA. Figure 3(a) shows that as the molarity of ethanolic KOH increases from 1 to 1.7 M, the percentage yield increases, but the percentage yield decreases as the molarity of ethanolic KOH increases from 1.7 to 2.5 M. As the temperature increases from 50 to 70°C, the percentage yield decreases. Increasing the molarity of ethanolic KOH and temperature cause the percentage yield to decrease. High temperatures can

cause the breaking down of molecules (thermal degradation) and using high concentration of ethanolic KOH can lead to additional unwanted reactions, like excessive saponification. Figure 3(b) shows that the higher the molarity of ethanolic KOH and the reaction temperature, the lower the percentage of FFA. The 3-D surface contour has illustrated the maximum for both SFOFAs yield (Y_1) and SFOFAs FFA (Y_2) can be reached at a lower level of 50°C reaction temperature, and at the upper level of 2.3 M for molarity of ethanolic KOH.

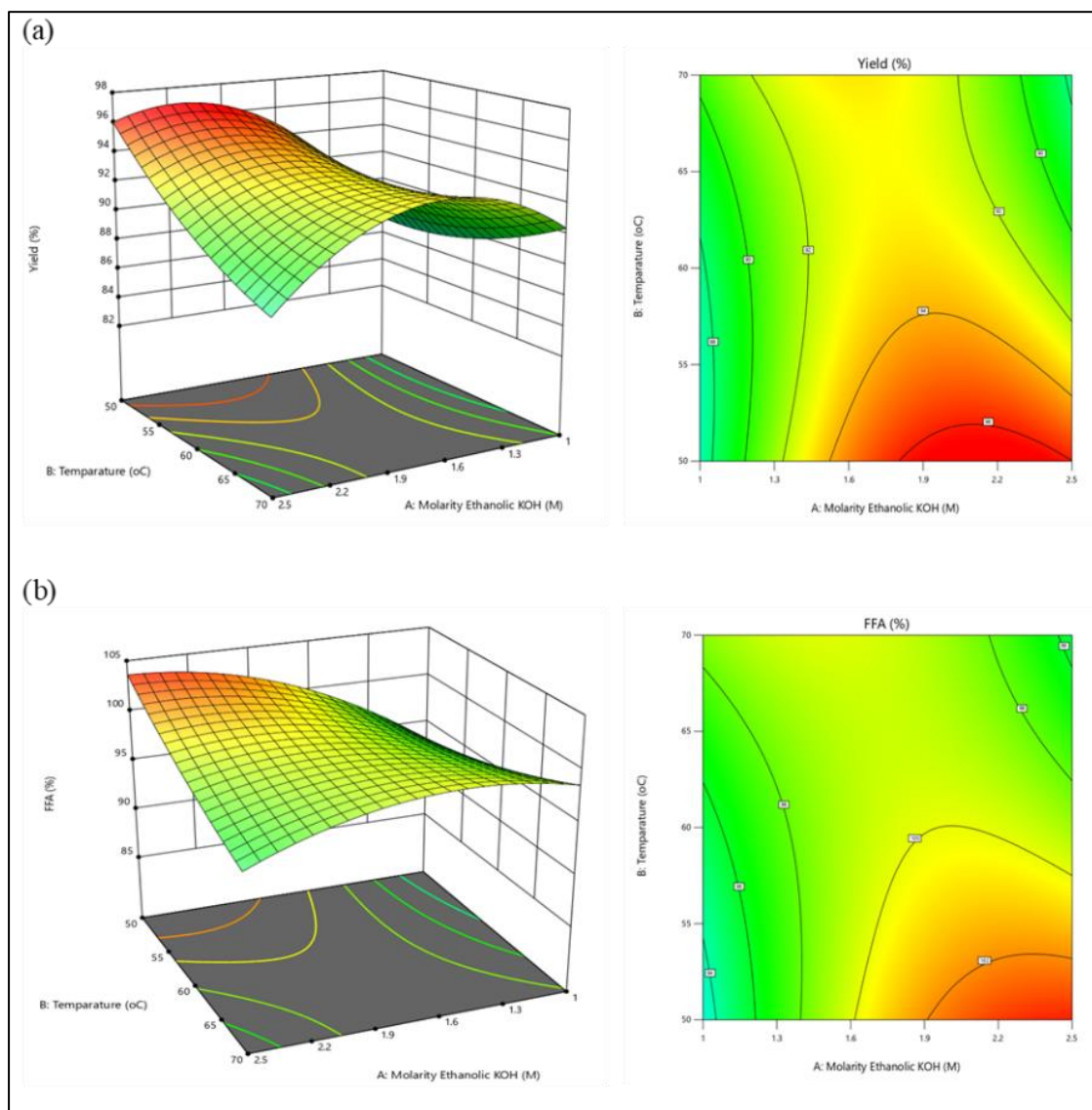


Figure 3. 3D surface and contour plot of molarity ethanolic KOH vs reaction time (AB) for a) percentage yield and b) percentage FFA

D-optimal design model verification

The numerical optimization was carried out to determine the optimum condition for hydrolysis of sunflower oil. The parameters A, B and C have been set in range to obtain the maximum percentage yield and percentage FFA of SFOFAs. The higher the desirability of the functions, the more accurate the model. Table 8 summarizes the limitation applied to obtain the optimum

condition setting for higher percentage yield and higher percentage FFA of SFOFAs. The parameters A, B and C were set in a range while the responses were set at the maximum value. The desirability of the predicted model is equal to 1.00, which indicates that the model gives the ideal value. Figure 4 shows the estimated prediction from the numerical optimization model for hydrolysis of SFO.

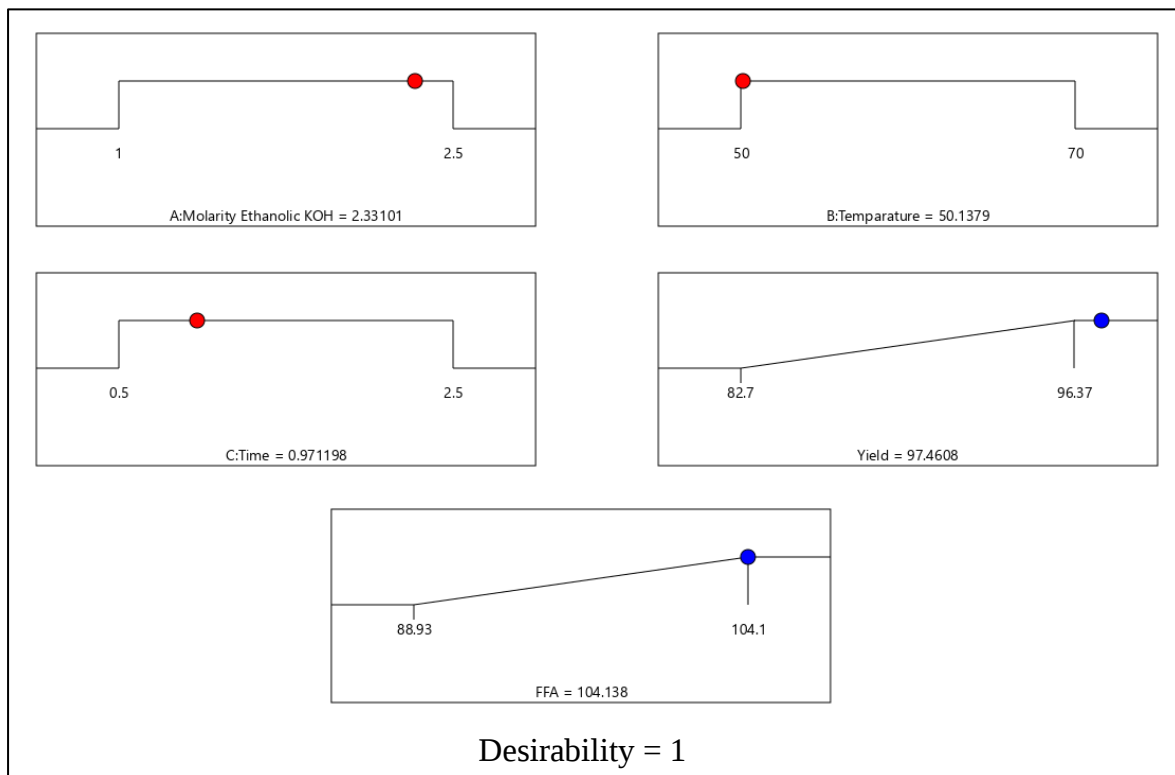


Figure 4. D-optimal predicted model at optimum condition for the hydrolysis of SFO

The suitability of the model generated from D-optimal design has been proven and verified by carrying out the hydrolysis process from three sets of reaction conditions that were chosen randomly. The actual and predicted value was compared by calculating the Residual Standard Error (RSE) value for each experiment and was only accepted if the value did not exceed 5%. The result from the Table 9 shows that all RSE values are less than 5%, indicating that this model is acceptable.

The predicted optimum condition from D-optimal design for hydrolysis of SFO was successfully acquired at 2.3 M of molarity ethanolic KOH, reaction

temperature of 50.14 °C for 0.97 hour. Under this reaction condition, 96.68% SFOFAs yield with 103.23% of SFOFAs FFA were obtained. Table 10 shows the actual and predicted data for optimum condition. The validation of the optimum condition has been done with the RSE calculated, showing that the value was less than 5% where 0.80% for SFOFAs yield and 0.87% for SFOFAs FFA.

Table 7. ANOVA analysis data and regression model for hydrolysis of SFO

Responses	ANOVA Value	Model	Factor and Their Interaction									Lack of Fit
			A	B	C	AB	AC	BC	A ²	B ²	C ²	
Yield	Sum of Squares	238.44	23.20	16.19	37.05	46.96	4.09	5.28	67.68	4.76	43.86	15.03
	df	9	1	1	1	1	1	1	1	1	1	5
	Mean Square	26.49	23.20	16.19	37.05	46.96	4.09	5.28	67.68	4.76	43.86	3.01
	F-value	11.09	9.71	6.78	15.51	19.66	1.71	2.21	28.33	1.99	18.36	1.70
	p-value	0.0004	0.0109	0.0263	0.0028	0.0013	0.2198	0.1679	0.0003	0.1885	0.0016	0.2877
	Status	***	**	**	***	***			***		***	
	R ² = 0.9089, Adequate Precision = 12.6511											
FFA	Sum of Squares	209.25	32.78	4.68	34.56	63.43	0.0047	12.21	26.15	1.57	47.16	24.04
	df	9	1	1	1	1	1	1	1	1	1	5
	Mean Square	23.25	32.78	4.68	34.56	63.43	0.0047	12.21	26.15	1.57	47.16	4.81
	F-value	7.85	11.07	1.58	11.67	21.43	0.0016	4.12	8.84	0.5311	15.93	4.32
	p-value	0.0017	0.0076	0.2370	0.0066	0.0009	0.9689	0.0697	0.0140	0.4828	0.0026	0.0671
	Status	***	***		***	***			**		***	
	R ² = 0.8761, Adequate Precision = 12.0673											

Note: A = Molarity ethanolic KOH; B = Reaction temperature; C = Reaction time; *** = Highly significant (p<0.01%); ** = Significant (p<0.05%)

Table 8. Restraints for optimization of hydrolysis of SFO parameter and responses for RSM

Parameter and Responses	Goal	Lower Limit	Upper Limit	Weight	Importance
A: Molarity ethanolic KOH	in range	1.0	2.5	1	3
B: Reaction temperature	in range	50.0	70.0	1	3
C: Reaction time	in range	0.5	2.5	1	3
Yield of SFOFAs	maximize	82.7	96.37	1	3
FFA of SFOFAs	maximize	88.93	104.1	1	3

Table 9. Actual and predicted data for verification model

Molarity Ethanollic KOH (M)	Reaction Temp. (°C)	Reaction Time (h)	Yield of SFOFAs (%)			FFA of SFOFAs (%)		
			Actual	Predicted	RSE	Actual	Predicted	RSE
2.2	55.0	1.5	92.35	94.5618	2.34	100.73	110.371	0.63
2.1	65.0	2.0	89.72	90.28	0.62	98.11	97.4848	0.64
1.9	69.0	2.1	90.67	97.63	0.04	97.27	97.5302	0.27

Table 10. Actual and predicted data for optimum condition

Molarity Ethanollic KOH (M)	Reaction Temp. (°C)	Reaction Time (h)	Yield of SFOFAs (%)			FFA of SFOFAs (%)		
			Actual	Predicted	RSE	Actual	Predicted	RSE
2.3	50.14	0.97	96.68	97.461	0.80	103.23	104.138	0.87

Comparison between current work and existing literature

Increasing temperature leads to a general acceleration of the hydrolysis rate due to heightened kinetic energy in molecules. Elevated temperatures facilitate more robust collisions between oil and hydroxide (OH) molecules, thereby promoting the cleavage of ester bonds within the oil [23]. Similarly, extending the reaction time also results in a higher yield of hydrolysis. The molarity of the catalyst plays a pivotal role in enhancing the hydrolysis yield. Elevating the catalyst's molarity increases the yield of hydrolysis by providing an alternative reaction pathway with lower activation energy. The outcomes of the experiment revealed a percentage yield of 96.68% for SFOFAs, accompanied by a 103.23% of free fatty acids (FFA) from SFOFAs, achieved under optimal conditions. These optimal conditions encompassed a molarity of 2.3 M for molarity of ethanollic KOH, a reaction temperature of 50.14°C, and a reaction duration of 0.97 hours. By comparison, the study contrasted with previous findings, by Bahadi et al. [4] wherein the yield of palm kernel oil was 84.7%, accompanied by a 98% percentage of free fatty acids, realized under optimal conditions of 2.16 hours, a temperature of 70°C, and a molarity of 1.77 M

ethanollic KOH. Notably, the temperature and reaction duration in the present experiment were observed to be lower than those of the referenced study. This discrepancy can be attributed to the utilization of a higher molarity of ethanollic KOH in the current study compared to the study conducted by [4]. This distinction underscores the influence of elevated ethanollic KOH molarity in effecting a reduction in both reaction temperature and duration for the hydrolysis process.

FTIR analysis of SFO and SFOFAs

FTIR spectroscopy was used to determine the functional groups in SFO and SFOFAs before and after hydrolysis. Figure 5 shows the FTIR spectra of SFO and SFOFAs and Table 7 shows the functional group and wavenumber of SFO and SFOFAs FTIR spectra. Before hydrolysis (SFO), a sharp peak appeared at 1746.33 cm⁻¹ indicating stretching vibration for C=O (ester carbonyl). After hydrolysis (SFOFAs), the peak for C=O (ester carbonyl) has disappeared, and a new sharp peak appeared at 1710.23 cm⁻¹ indicating stretching vibration for C=O (carboxylic acid) and broad OH peak for COOH appeared at range 2400 to 3400 cm⁻¹ which showed hydrolysis has successfully converted the ester to carboxylic acid.

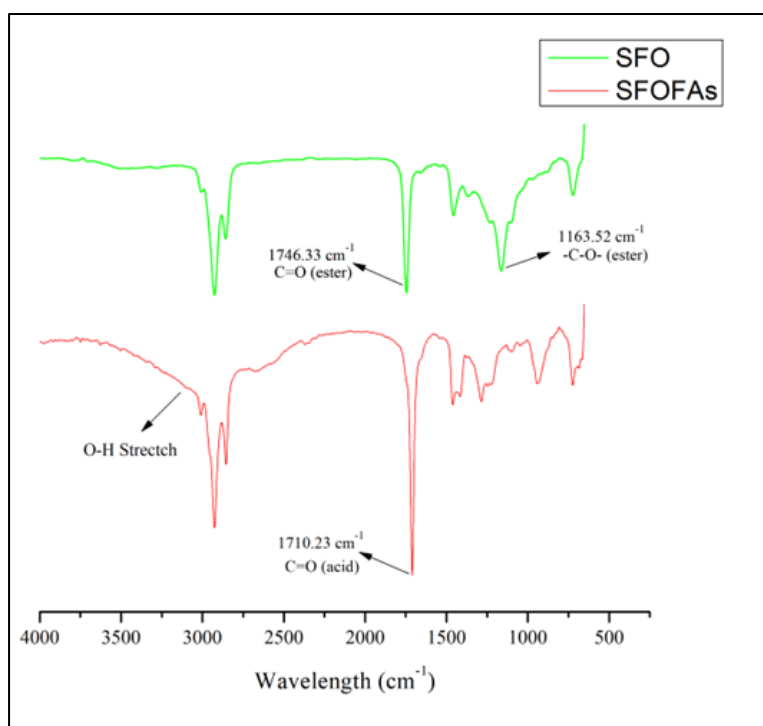


Figure 5. FTIR spectra of SFO and SFOFAs

Table 11. Functional group and wavenumber of FTIR spectra of SFO and SFOFAs

Functional Group	Wavenumber (cm ⁻¹)	
	SFO	SFOFAs
OH stretching (carboxylic acid)	-	2400-3400
C=C bending vibration (aliphatic)	3007.93	3007.93
C-H stretching vibration (aliphatic)	2926.56, 2857.78	2926.93, 2856.16
C=O stretching vibration (ester)	1746.33	-
C=O stretching vibration (carboxylic acid)	-	1710.23
-C-O stretching vibration (ester)	1232.81, 1163.52	-
-C-O stretching vibration (carboxylic acid)	-	1292.04, 1249.00

NMR analysis of SFO and SFOFAs

NMR has been used to elucidate the structure of SFO and SFOFAs. Figure 6 shows the ¹H NMR spectra of SFO and SFOFAs and ¹H NMR chemical shift of SFO and SFOFAs represented in Table 8. There are three peaks that have been focused on to determine whether the ester is successfully converted to fatty acid. From the ¹H NMR spectrum of SFO, two important peaks appeared at the range of 4.14-4.18 ppm and 4.29-4.33 ppm that signified the presence of -CH₂-COOR- and -CH-COOR- respectively. These two peaks represented

proton from glycerol backbone in SFO and these two peaks diminished in ¹H NMR spectrum of SFOFAs indicating the hydrolysis process was successfully carried out. New peak appeared at downfield region around 10.61 ppm in ¹H NMR spectrum of SFOFAs, which confirmed the formation of fatty acid (-COOH) in SFOFAs. Since both SFO and SFOFAs dissolved in deuterated chloroform (CDCl₃), the solvent peak appeared at 7.28 ppm. Another type of proton has been tabulated in Table 8.

¹³C NMR spectra and chemical shift of SFO and

SFOFAs were shown in Figure 7 and Table 9, respectively. There are several prominent peaks at 62.09 ppm ($-\text{CH}_2\text{-O}$), 68.90 ppm ($-\text{CH-O}$) and 172.79-173.21 ppm ($\text{O}=\text{C-OR}$) from SFO spectrum and peak at 180.45 ($\text{O}=\text{C-OH}$) from SFOFAs spectrum. Carbon for $-\text{CH}_2\text{-O}$ and $-\text{CH-O}$ comes from the glycerol backbone in SFO. These two peaks disappeared at SFOFAs spectrum showing that the glycerol backbone had been removed. Peak at 172.79-173.21 ppm corresponding to $\text{O}=\text{C-OR}$

ester had shifted to downfield region at peak 180.45 ppm corresponding to carboxylic acid in SFOFAs spectrum showing ester had been converted to fatty acid. Solvent peak (CDCl_3) was determined at 76.71-77.36 ppm. Table 7 shows another type of carbon present in SFO and SFOFAs. Chemical shift for peaks obtained for ^1H NMR and ^{13}C NMR are in a good agreement with the theoretical values.

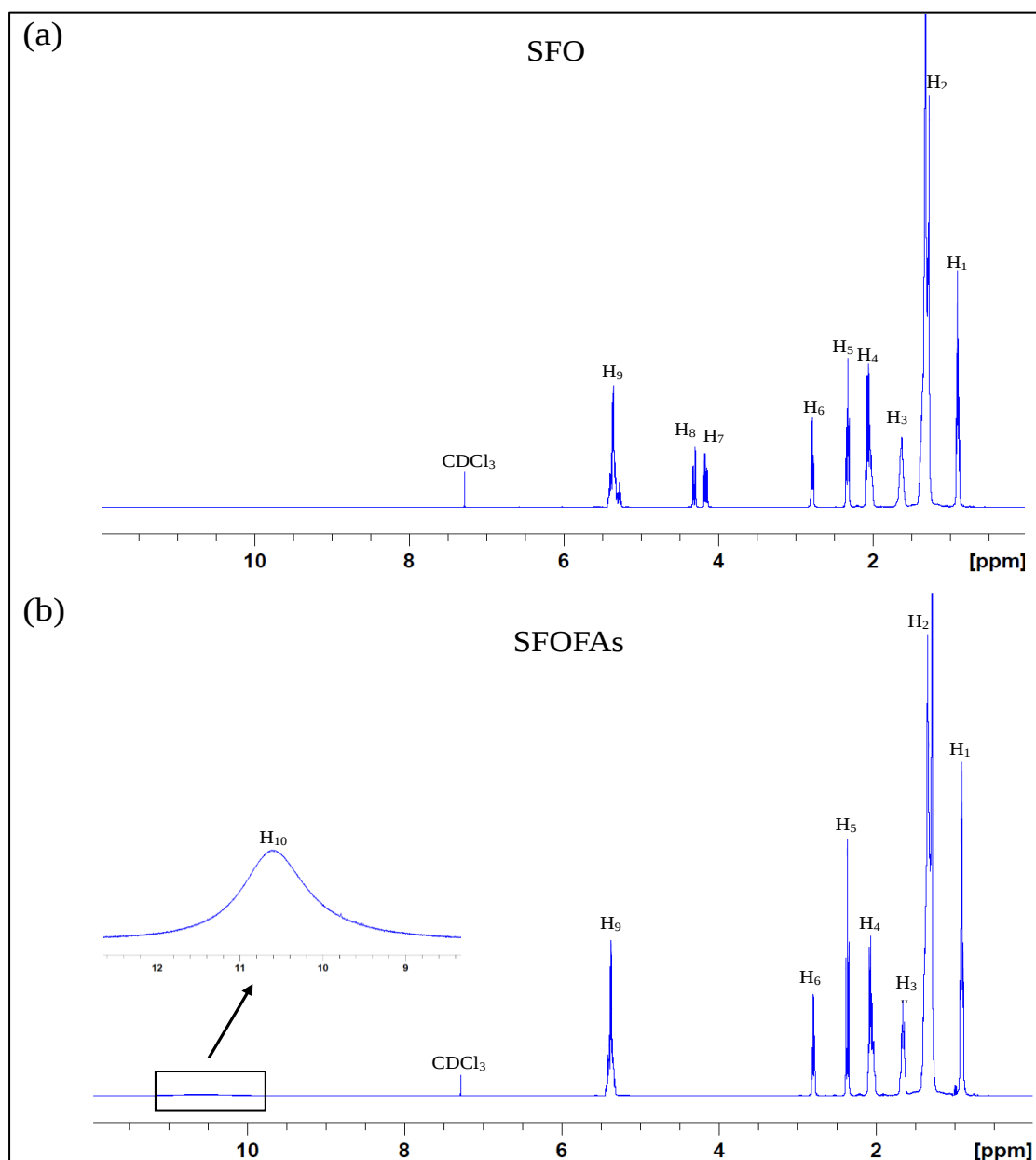


Figure 6. ^1H NMR spectra for a) SFO and b) SFOFAs

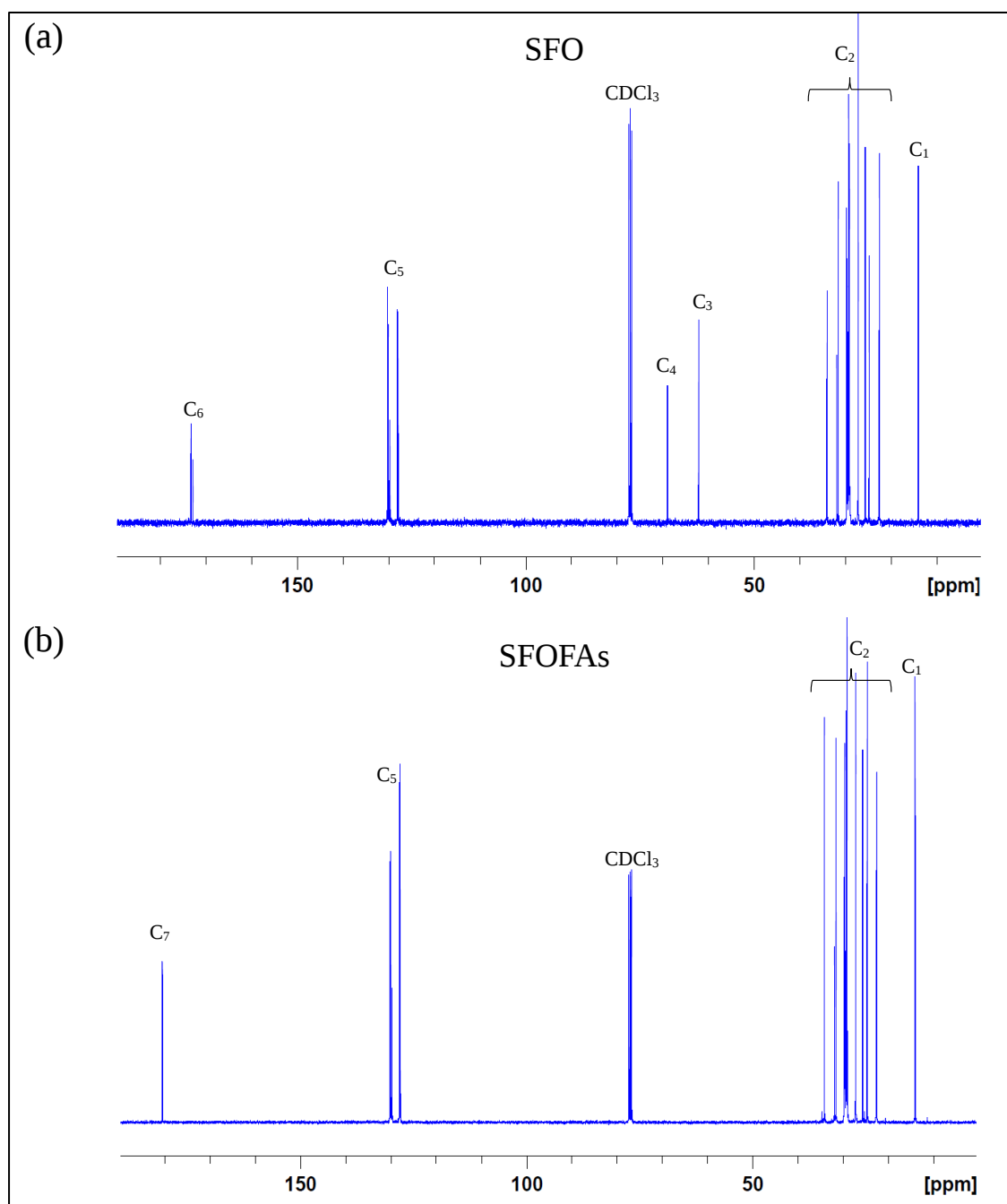


Figure 7. ^{13}C NMR spectra for a) SFO and b) SFOFAs

Table 12. Chemical shift of ^1H NMR spectra of SFO and SFOFAs

Type of Proton	Chemical Shift (ppm)		
	SFO	SFOFAs	Theoretical*
H ₁ -CH ₃	0.88-0.92	0.89-0.93	0.70-1.3
H ₂ -CH ₂ -	1.29-1.41	1.29-1.40	1.2-1.4
H ₃ O=C-CH ₂ -CH ₂ -	1.61-1.63	1.64-1.68	1.5-2.0
H ₄ -CH=CH-CH ₂ -	2.00-2.09	2.03-2.10	1.6-2.6
H ₅ O=C-CH ₂ -	2.30-2.35	2.35-2.38	2.1-2.5
H ₆ =CH-CH ₂ -CH=	2.77-2.80	2.78-2.81	2.5-3.0
H ₇ -CH ₂ -COOR- (glycerol)	4.14-4.18	-	4.0-4.5
H ₈ -CH-COOR- (glycerol)	4.29-4.33	-	4.0-4.5
H ₉ -CH=CH-	5.28-5.41	5.34	4.5-6.5
H ₁₀ COOH	-	10.61	10-12

*[24, 25]

Table 13. Chemical shift of ^{13}C NMR spectra of SFO and SFOFAs

Type of Carbon	Chemical Shift (ppm)		
	SFO	SFOFAs	Theoretical*
C ₁ -CH ₃	14.05-14.09	14.02	8-30
C ₂ -CH ₂ -	22.56-34.18	22.65-34.10	15-55
C ₃ -CH ₂ -O (glycerol)	62.09	-	40-80
C ₄ -CH-O (glycerol)	68.90	-	40-80
C ₅ -C=C-	127.90-130.20	127.90-130.17	65-90
C ₆ O=C-OR (ester)	172.79-173.21	-	155-185
C ₇ O=C-OH (carboxylic acid)	-	180.45	155-185

*[24]

Conclusion

The hydrolysis process of sunflower oil with ethanolic KOH was successfully carried out to produce the sunflower oil fatty acids. The process responses of SFOFAs yield and SFOFA FFA were improved during the optimization process using D-optimal design from response surface methodology. The optimum condition for hydrolysis of SFO was successfully acquired at 2.3 M of molarity ethanolic KOH, reaction temperature of 50.14 °C for 0.97 hour and produced 96.68% yield with 103.23% of FFA. The RSE value of less than 5% indicates that the model design produced was suitable.

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