Optimization of Response Surface Methodology by D-Optimal Design for Alkaline Hydrolysis of Crude Palm Kernel Oil

(Pengoptimuman Kaedah Gerak Balas Permukaan melalui Reka Bentuk D-Optimum untuk Hidrolisis Alkali Minyak Isirong Sawit Mentah)

MURAD BAHADI, MOHAMAD FIRDAUS YUSOFF, JUMAT SALIMON & DARFIZZI DERAWI*

ABSTRACT

Crude palm kernel oil (CPKO) was converted into its free fatty acids (FFAs) via alkaline hydrolysis. The reaction optimization was performed using Response Surface Methodology (RSM) through D-optimal design to optimize the conducted hydrolysis parameters. The optimization parameters were varied from 1.5 to 2.5 h of reaction time, 50-70°C of reaction temperature, and ethanolic KOH concentration 1-2 M. The highest percentage of free fatty acids was 98 ± 0.7 % with a percentage fatty acid yield of 84.7 ± 0.6 % under optimal conditions, which were 2.16 h of reaction time, 1.77 M ethanolic KOH concentration, and 70°C reaction temperature. High-temperature Capillary Column Gas Chromatography (GC-FID) and Nuclear Magnetic Resonance-Fourier Transform Infrared Spectroscopy (NMR-FTIR) were employed to characterize the product. The physicochemical properties of fatty acids were also tested. The acid value, iodine value, and hydroxyl value of the fatty acids obtained were 275.4 ± 0.7 mg NaOH/g, 21.8 ± 0.2 g I_2 / 100 g, and 168.2 ± 0.1 mg KOH/g, respectively.

Keywords: Crude palm kernel oil; fatty acids; hydrolysis; response surface methodology

ABSTRAK

Minyak isirong sawit mentah (CPKO) telah ditukar kepada asid lemak bebas (FFAs) melalui hidrolisis alkali. Pengoptimuman tindak balas dijalankan dengan menggunakan kaedah gerak balas permukaan (RSM) melalui reka bentuk D-optimum untuk mengoptimumkan parameter hidrolisis yang dijalankan. Parameter pengoptimuman telah berubah daripada 1.5 hingga 2.5 jam masa tindak balas, 50-70°C suhu tindak balas dan kepekatan etanolik KOH 1-2 M. Peratusan tertinggi asid lemak bebas adalah 98 ± 0.7% dengan peratus hasil asid lemak sebanyak 84.7 ± 0.6% di bawah keadaan optimum, iaitu 2.16 jam masa tindak balas, 1.77 M kepekatan etanolik KOH dan 70°C suhu tindak balas. Kromatografi Gas Kolum Kapilari Suhu tinggi (GC-FID) dan Resonans Magnet Nuklear- Spektroskopi Transformasi Fourier Inframerah (NMR-FTIR) telah digunakan untuk mencirikan produk. Sifat fizikokimia asid lemak juga telah diuji. Nilai asid, iodin dan hidroksil asid lemak yang diperoleh adalah 275.4 ± 0.7 mg NaOH/g, 21.8 ± 0.2 g I_2 / 100 g dan 168.2 ± 0.1 mg KOH/g.

Kata kunci: Asid lemak; hidrolisis; kaedah gerak balas permukaan; minyak isirong sawit mentah

INTRODUCTION

The exploration of green industrial products will protect and sustain the Earth and nature throughout its development and modernization. Global warming and fossil oil crisis have been claimed as among International issues to find out any alternative sources in replacing petrochemical products (Derawi & Salimon 2013a; Velayutham et al. 2009). Production of bio-based industrial products are getting great attention from researchers worldwide (Derawi & Salimon 2013b). Malaysia is one of the top producers of crude palm oil (CPO) and this has given the opportunity for Malaysia to develop its industrial commodities based on palm oil-based feedstocks (Derawi 2016). Mechanical screw pressing or solvent extraction is used to extract oil from the palm fruit kernel or seed. This oil is called the crude palm kernel oil (CPKO). There are many fatty acids (FAs) contained in CPKO, among which include palmitic acid, oleic acid, myristic acid, and lauric acid with percentage compositions of 7.5%, 14.8%, 15.6%, and 48.7%, respectively (Ibrahim 2013). From this composition, it can be observed that a high percentage (about 82.6%) of saturated fatty acids (SFAs) are inherent in the CPKO. These SFAs lend good properties to the CPKO, making it well suited for producing biodegradable products e.g. biodiesel, biolubricant, and bioplastics. According to Salimon and Abdullah (2008), however, the locality and climate could also affect the chemical composition of CPKO. Fatty acids and glycerol essential raw materials, particularly in oleochemical industries could be generated from hydrolyzing fat and oil (Salimon et al. 2012). Hermansyah

et al. (2006) noted that the economics of production and efficient recovery of glycerol and fatty acids could be maximized using the best reaction conditions. Besides the oleochemical industry, these materials also lend well in the cosmetics, pharmaceutical, food, and biolubricant and bioplastics industries. Catalysts such as acids, bases, or specific enzymes known as lipases could speed up the hydrolysis of oils and fats. However, the use of a catalyst is not always necessary, as long as high pressure and temperature are used during the hydrolysis reaction (Serri et al. 2008). The literature includes a wealth of studies investigating vegetable oil hydrolysis. For instance, different KOH concentrations were used to hydrolyze *Jatropha curcas* seed oil (Salimon et al. 2011). In the study, the maximum recovery (102.2%) of Free Fatty Acids (FFAs) was obtained with 1.75 M of ethanolic KOH concentration together with a 2 h reaction time at 65°C. In a similar study, Hashim and Salimon (2008) performed the hydrolysis of triacylglycerols from soybean oil using base catalysts (KOH and NaOH). The optimum condition for hydrolysis was found to occur at 60°C for 30 min of reaction time using 1 M ethanolic KOH with a 229.6 mg/g acid value. In another study, Holliday et al. (1997) hydrolyzed soybean, linseed, and coconut oils, namely the triacylglycerols in the oils, to obtain glycerol and FFAs, where the water density was 0.7 g/mL and the temperature ranged from 260 - 280°C. Hydrolysis occurred rapidly within 15 - 20 min to yield 97% recovery of the products.

Similarly, King et al. (1999) set up an experiment in a subcritical water flow reactor, where they hydrolized soybean oil to obtain FFAs. The hydrolysis achieved a yield of 97% fatty acids within a 10 to 15 min reaction time at a temperature range of 330°C to 340°C. Khor et al. (1986) used commercial lipase from *Candida rugosa* (previously identified as *C. cylindracea*) to hydrolize palm olein, stearin, and oil. The study showed that the optimal conditions for the lipase were pH7.5 and a reaction temperature of 37°C. Furthermore, Fu et al. (1995) investigated the use of lipase from *Aspergillus* sp. in the hydrolysis of lard, coconut oil, mink fat, soybean oil, palm oil, and olive oil. It was found that pH6.5 to 7.0 and a 37°C reaction temperature were optimal conditions to induce high specific activity (60,000 U/g). The data exhibited a nearly linear correlation with reaction time on the logarithmic scale within two stages. At 5 - 30 U/meq concentrations, and in 2 to 24 h, the *Aspergillus* sp. lipase effectively hydrolyzed all substrates, yielding 90% to 99% of the desired product. Serri et al. (2008) also used the *C. rugosa* lipase in the hydrolysis of triacylglycerol from palm cooking oil. The optimal conditions were 200 rpm, pH7.5, a reaction time of 90 min, a reaction temperature of 45°C, an enzyme loading of 7.46 kLU/mL, and a 01. g/mL oil concentration, all of which yielded maximum conversion.

The D-optimal design stresses an effective design solution in which very similar mean performance values and target values are emphasized. The design proves to be a useful tool, as it allows the identification of factors that are significant in the experimental condition such that the best performance is achieved. This paper details the practical use of the D-optimal method as design of an experiment to optimize the hydrolysis and characterisation of FAs from CPKO. To determine the optimum process conditions for the responses (yield and percentage of free fatty acids), the Response Surface Methodology (RSM) was used.

Malaysia consists of several locations that allow the production of cheap, raw materials to produce fatty acids (FAs) from crude palm kernel oil. Therefore, in the production of FAs in this study, only cheap raw materials were used. This study aimed to ascertain the effect of variables on the production of FAs from CPKO as shown in Figure 1. In the D-optimal design. these variables are ethanolic KOH concentration, reaction temperature, and reaction time and labeled as X_1, X_2, and X_3, respectively. Meanwhile, the percentage yield of FAs and FFA percentage composition were set as the responses. The D-optimal design ensures the maximization of the responses with the optimal combination of the process variables.
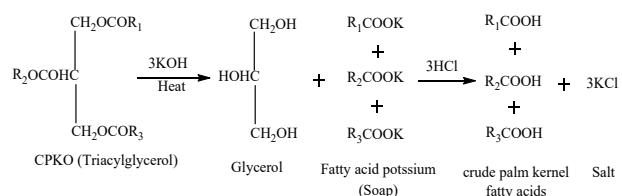
R= Mixed fatty acids (Caprylic, Capric, Lauric, Myristic, Palmitic, Stearic, Oleic and Linoleic acids)

FIGURE 1. Hydrolysis reaction of crude palm kernel oil

MATERIALS AND METHODS

MATERIALS

This study purchased potassium hydroxide (KOH) from Sigma-Aldrich. Sime Darby Sdn. Bhd. (Selangor, Malaysia) was the supplier of the crude palm kernel oil in this study. Sigma Aldrich (Steinheim, Germany) also supplied the other materials used in this study, namely hydrochloric acid 37%, n-hexane 99%, ethanol 99.5%, and anhydrous sodium sulfate.

THE CPKO HYDROLYSIS PROCEDURE

As per Japir et al. (2018), the fatty acids in this study was obtained via the hydrolysis of crude palm kernel oil (CPKO) using potassium hydroxide (KOH) as a catalyst. The reaction time and temperature and the ethanolic KOH concentration were varied using RSM, as outlined in Table 1, as part of the D-Optimal design adopted in this study. As mentioned previously, the reaction temperature (°C), reaction time (hour), and ethanolic KOH concentration (M) were denoted as X_1, X_2, and X_3, respectively. These were

varied while all other experimental conditions were kept the same. Prior to the process of hydrolysis, a mixture of crude palm kernel oil (50 g) and different concentrations of ethanolic KOH (300 mL) was placed in a flask (two-necked, round bottom), at different reaction temperatures and times. After the hydrolysis, the mixture was added with water (200 mL). Following that, 100 mL hexane was used to extract the unsaponifiable components. Approximately 60 mL of HCl 6 N was used to acidify the aqueous alcohol phase with the soap. The mixture of fatty acids (FAs) was extracted using hexane, cleansed before drying using anhydrous sodium sulphate, and then, at 45°C, a vacuum rotary evaporator was used to evaporate it. In the next stage, the % FFA is determined.

Determination of FFA Percentage

FFA percentage (as lauric acid) were determined based on the study by Japir et al. (2017, 2016). First, 5 g CPKO was prepared. Then, 5 mL of neutralized isopropanol was added to it, after which both were placed in an Erlenmeyer flask. The mixture was heated up to 40°C until it dissolved. Then 1 mL phenolphthalein was added. Next, 0.1 N NaOH was used to titrate the mixture until a light pink color was formed using 1 mL of phenolphthalein as the indicator. Equation (1) was used to calculate the % FFAs:

$$\% \text{ FFA as lauric acid} = \frac{20.0 \times N \times V}{W} \qquad (1)$$

where N is the NaOH solution normality equivalent per liter (Eq/L); V is the NaOH solution volume used (mL); and W is the sample weight (g).

Gas Chromatography Analysis of Composition of FAs

GC-FID (Gas Chromatography-Flame Ionization Detector) was used in this study to determine the fatty acids composition of CPKO and CPKFA. The model of the GC-FID was Shimadzu GC-17A. It had a BPX 70 30 m × 0.25 mm × 0.25 μm capillary column, and as per its name, also had an FID detector. The program temperature for the column was set to 3°C per minute from 120°C to 245°C, while 280°C and 260°C was set as the temperature of the detector and injector, respectively. A flow rate of 0.3 mL/min was set for the carrier gas made up of 99.99% high purity helium gas. The methods of Bahadi et al. (2016a) were referred to in the preparation of the CPKO transesterification to obtain fatty acid methyl ester (FAME) with a base catalyst. For CPKFA esterification using an acid catalyst, the methods of Ichihara and Fukubayashi (2010) were referred to. Authentic standard retention times were used to identify the peaks generated by the GC-FID.

Fatty Acid Analysis Using HPLC (High-Performance Liquid Chromatography)

The HPLC water model 1525 with an ELS (evaporative light scattering) detector was used in this study. It came equipped with a 250 mm × 4.6 mm × 5 um Spherisorb C18 column and was used to analyze the FFA, MAG, DAG, and TAG. The mobile phase comprises a mixture of acetone: acetonitrile (63.5%:36.5%), fixed at a flow rate of 1 mL/min. Bahadi et al. (2016b) were referred to for the determination of the HPLC parameters in this study. First, the sample preparation entailed the dilution of 0.1 mL sample with 1.5 mL acetone to form acetonitrile (63.5:36.5) mixture. The HPLC was immersed in the mixture and auto-injected with an overall operation time of 35 min.

Structural analysis of fatty acids

A Bruker AV400111 (USA) 400 MHz NMR spectrometer was used to record the ^{1}H and ^{13}C NMR in this study. The temperature was set to 30°C. The internal chemical shift was referred using Tetramethylsilane (Me4Si). First, the sample (about 25 mg) was dissolved in $CDCl_3$ (deuterated chloroform) in the amount of 1 mL. Shirazi et al. (2009) was referred to in carrying out the FTIR (Fourier Transform Infrared Spectroscopy) in this study. The Perkin Elmer Spectrum GX spectrophotometer was used to record the FTIR spectra of the products. The results showed a 400-4000 cm^{-1} range of the product. The functional groups can be determined from the spectra in FTIR. For the analysis, NaCl cells with an internal diameter of 25 mm and a thickness of 4 mm were covered in a very thin film of the sample.

Statistical Analysis and Design of Experiment

The two responses i.e. the percentage yield of fatty acids, Y_1 (%), and the percentage FFA, Y_2 (%) was determined via a three-factor D-optimal design, as a result of changing the independent variables, i.e. the reaction time, reaction temperature, and the ethanolic KOH concentration, denoted as X_3, X_2, and X_1, respectively. Table 1 shows the low value (−1) and high value (+1) of X_1, X_2 and X_3, equivalent to the range setting of each parameter i.e. 1-2 M for X_1, 50-70°C for X_2, and 1.5-2.5 h for X_3. The D-optimal experimental design was executed for a total of 18 runs as highlighted in Table 2. Equation (2) shows the optimization process used to predict the responses in this study, specifically based on the assumption of a linear or quadratic model:

$$Y = \beta_0 + \Sigma \beta_i x_i + \Sigma \beta_{ii} x_i^2 + \Sigma \Sigma \beta_{ij} x_i x_j \qquad (2)$$

where the constant is denoted by β0; the linear coefficient is βi; the square regression coefficient is βii; and the interaction regression coefficient is βij. The

independent variables are assigned as xi and xj. Analysis of Variance (ANOVA) and R-squared value (coefficient of determination) was used to measure the model's goodness of fit.

TABLE 1. Parameters and levels for D-optimal design of the hydrolysis reaction

Independent variables	Factor Xi	Variable levels		
			0	+1
Concentration of ethanolic KOH (M)	X_1	1	1.5	2
Reaction temperature (°C)	X_2	50	60.8	70
Reaction time (h)	X_3	1.5	2.03	2.5

TABLE 2. Experimental runs from D-optimal design and the respective responses

Run	X_1: KOH (M)	X_2: Reaction temp (oC)	X_3: Reaction time (h)	Y_1: %	Y_2: %
1	1.50	50.00	1.50	75.3	86.5
2	2.00	50.00	2.50	78.2	93.3
3	1.50	60.00	2.50	81.4	95.6
4	2.00	70.00	1.50	83.1	96.2
5	2.00	70.00	2.50	85.2	97.5
6	2.00	50.00	1.50	73.5	96.3
7	1.00	70.00	1.50	78.7	68.7
8	2.00	70.00	2.50	83.2	97.8
9	2.00	70.00	1.50	83.7	96.4
10	1.00	70.00	2.50	78.6	78.4

PHYSICOCHEMICAL ANALYSIS

The methods outlined in Salimon et al. (2006) were adopted to determine the unsaponifiable matter, saponification value, hydroxyl value, and an iodine value of CPKO and CPKFA. Meanwhile, a Moisture Analyzer model AND MX-50 was used to determine the moisture content of CPKO and CPKFA. A Lovibond Tintometer Model F/10508 was used to identify the color of the product. Viscosity analysis was conducted with the help of a Brookfield model RV DV-I+ (U.S.A) Spindle SO5. A refractometer was employed at 26.5°C to measure the refractive index.

RESULTS AND DISCUSSION

PREPARATION OF FATTY ACIDS

The fatty acids were produced via the ethanolic KOH hydrolysis of crude palm kernel oil. This procedure aims to obtain fatty acids enriched with the highest yield % and the highest percentage of free fatty acids. In this study, the variables were varied. These variables were reaction time (X_3, h.), reaction temperature (X_2, °C), and ethanolic KOH concentration (X_1, M). Its effects on hydrolysis were then investigated. The D-optimal design and RSM were used to achieve optimal parameters for the preparation of fatty acids. The % FFAs and FA yield (84.7 ±0.6 and 98 ±0.7, respectively) are presented in Table 2 for all 18 experiments. These results corresponded to the optimal conditions.

RESPONSE SURFACE METHODOLOGY (RSM)

The results show a change in the variables X_1 ethanolic KOH concentration (M), X_2 reaction temperature (°C), and X_3 reaction time (h.) significantly affected the yield of fatty acids (Y_1) and percentage of free fatty acids (Y_2). The interaction among these three factors was examined using the RSM with the D-optimal design to optimize the fatty acid preparation. The ranges of each independent factor are presented in Table 1.

D-OPTIMAL DESIGN MODEL FITTING

Tables 3 and 4 show the quadratic polynomial models of (Y_1) and (Y_2) denoting FA yield, and % FFAs, respectively. These were obtained by employing the least squares method. The t-test was conducted to examine the coefficients obtained, and a linear relationship between X_1, X_2, and X_3 was shown with high significance. The reaction temperature (X_2) had a highly significant ($P < 0.05$) quadratic relationship. A highly significant ($p < 0.01$) and quadratic relationship with Y_1 was observed for ethanolic KOH concentration (X_1) and the reaction temperature (X_2). However, reaction time (X_3) was also quadratic with Y_1 but not significant ($P > 0.05$). Among the three interactions, there was a significant interaction effect ($P < 0.05$) between X_2 and X_3 (reaction temperature and reaction time, respectively) with the yield of fatty acid (Y_1). Meanwhile, the % FFAs (Y_2) showed a significant interaction effect ($P < 0.01$) between X_2 and X_1 (ethanolic KOH concentration and reaction temperature, respectively). Reaction time (X_3) and ethanolic KOH concentration (X_1) also significantly interacted. Equations (3) and (4) were used to obtain the coefficients of the independent variables (X_1, X_2, and X_3) outlined in Tables 3 and 4, respectively, for FA yield (Y_1) and % FFAs (Y_2):

$$Y_1 = +10.00 + 2.043\,X_1 - 0.132\,X_2 + 0.450\,X_3 + 0.006\,X_{12} + 0.122\,X_{13} - 0.0113\,X_{23} - 0.797X_1^2 + 0.0014\,X_2^2 + 0.0463\,X_3^2 \tag{3}$$

$$Y_2 = -3.329 + 8.593\,X_1 + 0.141\,X_2 + 0.641\,X_3 - 0.014\,X_{12} - 0.441\,X_{13} + 0.007\,X_{23} - 1.822\,X_1^2 - 0.001\,X_2^2 - 0.0497\,X_3^2 \tag{4}$$

FITTED MODEL DIAGNOSTIC CHECKING

Table 5 shows the ANOVA for the fitted models. The two models were examined using an F-test and a P-test. Examinations of the two models (Y_1 and Y_2) resulted in a very small pure error rate of 0.0039 and 4175 %, respectively, as well as a lack of fit that was not significant ($P > 0.05$). Meanwhile, Tables 3 and 4 show that the highly significant ($P < 0.01$) regression coefficients (R^2) were satisfactory at 0.9595 and 0.9956, respectively, and the regression models. These results confirm that the data variation can sufficiently be explained using these models, and that the actual relationships between the reaction parameters are well represented.

ANALYSIS OF RESPONSE SURFACE AND OPTIMIZATION CONDITIONS

The interaction effect between the variables was illustrated using 3-D response surfaces and contour graphs. These are ethanolic KOH concentration and reaction temperature (X_1X_2), ethanolic KOH concentration and reaction time, (X_1X_3), and reaction temperature and reaction time (X_2X_3). Figures 2, 3, and 4 show the response surfaces for FA yield and %FFAs, respectively. Figure 2(A) and 2(B) shows the interaction effect between the ethanolic KOH concentration and reaction temperature (X_1X_2) on FA yield and %FFAs. The 3-D plot shows that increasing the concentration of ethanolic KOH from 1 M to 2 M continuously improved the percentage yield of fatty acids and percentage of free fatty acids, while the optimal concentration of ethanolic KOH for the maximum yield of fatty acids was 1.7 M. However, the yield of fatty acid and percentage of free fatty acid were increased when the reaction temperature increased from 50°C to 70°C.

Figure 3(A) and 3(B) shows the effect of the variable interactions on the yield of fatty acids and percentage of free fatty acids, respectively, also using the 3-D response surfaces. Figure 3(A) shows the effect of the ethanolic KOH concentration and the reaction time (X_1X_3) on the yield of fatty acids. The plot shows that the yield of fatty acids was increased with increased concentration of ethanolic KOH of up to 1.7 M, while the reaction time only slightly changed. Based on the % FFA given in Figure 3(B), the optimal conditions to obtain increased % FFA was 1 - 2 M ethanolic KOH concentration and 1.5 to 2.5 h of reaction time.

Figure 4(A) and 4(B) shows the effect of the interaction of X_2X_3 (reaction temperature and reaction time) on the yield of fatty acid and the percentage of free fatty acids, respectively. The 3-D plot shows that the increase from 50°C to 70°C in reaction temperature and the increase from 1.5 to 2.5 h in reaction time continuously improved the yield of fatty acids and percentage of free fatty acids.

Figure 5(A) and 5(B) shows the plot distribution of the actual data against the predicted FA yield and % FFA values. In this case, the predicted values were obtained from the models, while the actual data came from the initial

TABLE 3 Regression coefficients of the predicted quadratic polynomial model for response variables (yield of fatty acid) (Y_1)

Source	Coefficients (ß) (Y1)	F-value	p-value	Notability
Intercept	10.00	21.06	0.0001	***
Linear				
X_1	2.043	36.90	0.0003	***
X_2	-0.132	87.63	<0.0001	***
X_3	0.450	14.30	0.0058	***
Interaction				
X_1X_2	0.006	1.85	0.2113	
X_1X_3	0.122	2. 36	0.1633	
X_2X_3	-0.0113	8.10	0.0216	**
Quadratic				
X_1^2	-0.797	17.36	0.0031	***
X_2^2	0.0014	8.21	0.0210	**
X_3^2	0.0463	0.0475	0.8329	
R^2	0.9595			

Notes: X_1: concentration of ethanolic KOH (M); X_2: reaction temperature (ºC); X_3: reaction time (hr) ,** $P < 0.05$; *** $P < 0.01$

TABLE 4. Regression coefficients of the predicted quadratic polynomial model for response variables (percentage of free fatty acid) (Y_2)

Source	Coefficients (ß) (Y1)	F-value	p-value	Notability
Intercept	-3.329	202.37	<0.0001	***
Linear				
X_1	8.593	1548.22	< 0.0001	***
X_2	0.141	49.88	0.0001	***
X_3	0.641	38.62	0.0003	***
Interaction				
X_1X_2	-0.014	12.28	0.0080	***
X_1X_3	-0.441	35.04	0.0004	***
X_2X_3	0.007	3.77	0.0883	
Quadratic				
X_1^2	-1.822	103.44	< 0.0001	***
X_2^2	-0.001	5.17	0.0526	***
X_3^2	-0.0497	0.0623	0.8092	
R^2	0.9956			

Notes: X_1: concentration of ethanolic KOH (M); X_2: reaction temperature (ºC); X_3: reaction time (hr) ,** $P < 0.05$; *** $P < 0.01$

results obtained from the experiments conducted (Table 2). The clustering of data points around the line as a whole show that the model results and empirical data are in very good agreement. Hence, the models should forecast the values of the yield of fatty acids and the percentage of free fatty acids that remarkably coincide with the real-time experimental values. This was proven statistically by the

TABLE 5 Analysis of variance (ANOVA) for the responses (yield of fatty acid and percentage of free fatty acid)

	Source	Df	Sum of squares	Mean square	F value	P-value	Notability
	Model	9	0.9114	0.1013	21.06	0.0001	Significant
	Residual	8	0.0385	0.0048			
Y1	lack-of-fit	4	0.0229	0.0057	1.47	0.3585	Not significant
	Pure error	4	0.0156	0.0039			
	Cor Total	17	0.9499	0.0559			
	Model	9	7.69	0.8542	202.37	0.0001	Significant
	Residual	8	0.0338	0.0042			
Y2	lack-of-fit	4	0.0265	0.0066	3.64	0.1194	Not significant
	Pure error	4	1.67	0.4175			
	Cor Total	17	7.72	0.4541			

values of R^2 and adjusted R^2, implying that the prediction of the results is reliable. Therefore, at this point, the optimization tool, as part of the next level of analysis, can now be explored.

Validation of Model and Confirmation of Experiment

In preparing the fatty acids and after performing ANOVA, optimum conditions had to be first determined, and this was done by employing numerical optimization. To maximize the FA yield and % FFA, the controlling parameters were set in selected ranges, where the higher the desirability function, the more accurate the model. Ethanolic KOH concentration, reaction temperature, and reaction time were the controlling parameters. Following this, the desirability function of the predicted models was found to equal 1.00 based on the criteria selected. The estimated parameters from numerical optimization are shown in Figure 6. Using the D-optimal design, the optimum conditions predicted were an ethanolic KOH concentration of 1.77 M, a reaction temperature of 70°C, and a reaction time of 2.16 h. Under these conditions, 85.2001% FA yield, and 98.4998% of FFA percentage were obtained. As shown in Table 6, to validate the predicted model, the experiments were carried out in triplicate. This produced an average 84.7 ± 0.6 % yield of fatty acids and

98 ± 0.7 % free fatty acids. The data obtained from the model are in good agreement with these results. In summary, the D-optimal designed is confirmed as a reliable, simple, and useful approach to evaluate the optimal conditions for preparing FAs from CPKO based on ethanolic KOH hydrolysis.

FTIR Analysis of CPKO and CPKFA After Hydrolysis

FTIR spectroscopy was used to show the main peaks and corresponding functional groups that denoted the % FFA for the CPKO after hydrolysis. Figure 7 shows the FTIR spectra obtained in this study, while Table 7 shows the main peaks and corresponding functional groups. Before hydrolysis, a peak at 1739 cm^{-1} corresponding to C=O (ester carbonyl) was clearly seen. After hydrolysis, the peak of C=O of ester carbonyl disappeared and a peak at 1707 cm^{-1} appeared corresponding to the C=O of carboxylic acid. Therefore, the hydrolysis process was proven effective from the OH-stretching appearing, attributed to the fatty acids present after hydrolysis (Figure 7).

NMR analysis of Fatty Acids

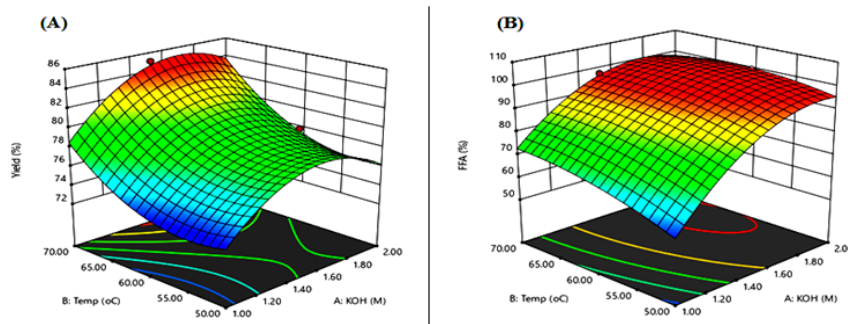The structure of fatty acids was analyzed by ^{1}H nuclear magnetic resonance (NMR) spectroscopy. Figure 8 and

FIGURE 2 Response surfaces for the concentration of ethanolic KOH (X1, M) and reaction temperature (X_2, °C) on the yield of fatty acid (Y_1) (A) and percentage of free fatty acid (Y_2) (B)
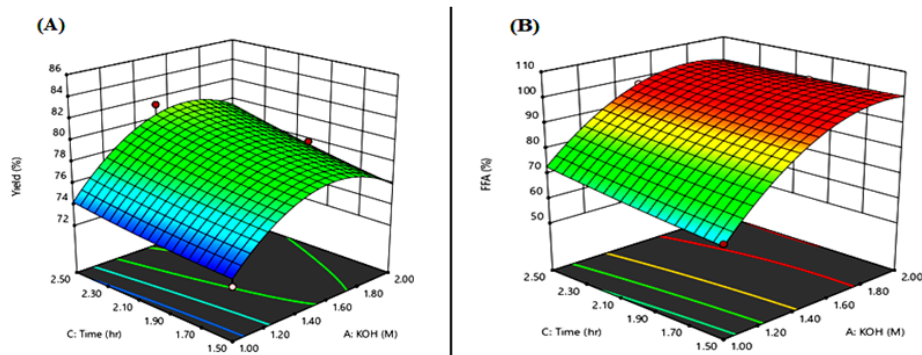
FIGURE 3. Response surfaces for the concentration of ethanolic KOH (X1, M) and reaction time (X3, h) on the yield of fatty acid (Y1) (A) and percentage of free fatty acid (Y2) (B)
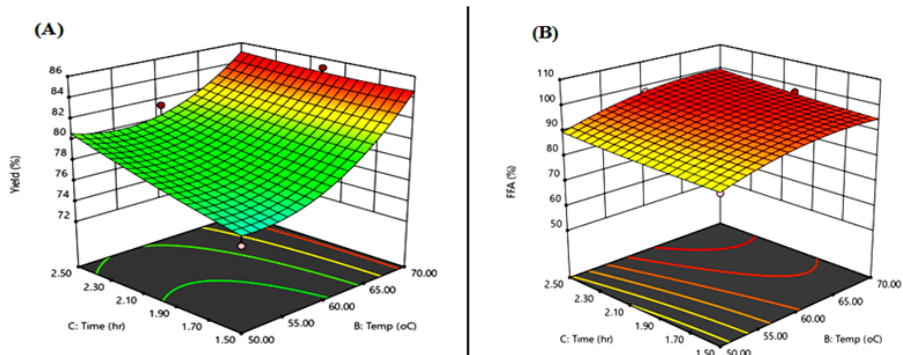

FIGURE 4. Response surfaces for the reaction temperature (X$_2$, °C) and reaction time (X$_3$, h) on the yield of fatty acid (Y$_1$) (A) and percentage of free fatty acid (Y$_2$) (B)
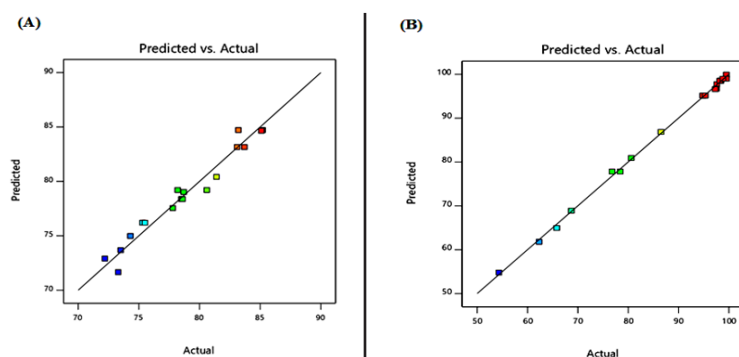

FIGURE 5. Regression plot of predicated values versus actual data of the yield of fatty acid (Y$_1$) (A) and percentage of free fatty acid (Y$_2$) (B)

TABLE 6. Validation test result of a fatty acid of optimum condition

	No	Independent variables			Responses	
		X$_1$	X$_2$	X$_3$	FAs yields (%)	FFA(%)
	1	1.77	70	2.16	84.6	97.2
Actual	2	1.77	70	2.16	85.4	98.5
	3	1.77	70	2.16	84.2	98.3
		1.77	70	2.16	84.7 ± 0.6	98 ± 0.7
Actual average Predicted		1.77	70	2.16	85.2001	98.4998

Notes: X$_1$: concentration of ethanolic KOH (M); X$_2$: reaction temperature (°C); X$_3$: reaction time (hr)
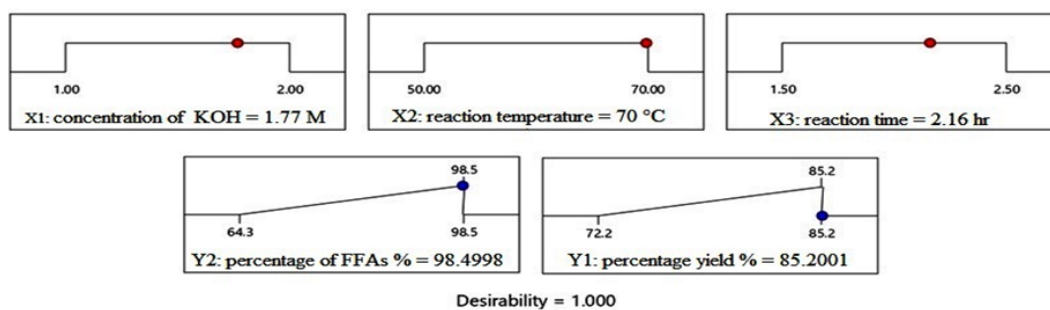
FIGURE 6. The fatty acid, as derived from the RSM predicted model using optimal conditions

TABLE 7. The functional groups of CPKO and CPKFA in terms of the main wavenumber in the FTIR

Functional group	Wavenumber (cm^{-1})	
	CPKO	CPKFA
OH stretching (FA)	-	3473
C = C bending vibration (aliphatic)	3006	3005
C-H stretching vibration (aliphatic)	2921,2853	2923, 2853
C = O stretching vibration (ester)	1739	-
C = O stretching vibration (carboxylic acid)	-	1707
C-H scissoring and bending for methylene	1465	1464
=C–H (cis) Unsaturated	1417	1417
-CH3 sym deformation	1377	1377
-C-O-Stretching vibration(ester)	1238−1165	-
C-H group vibration (aliphatic)	722	722

Notes: CPKO crude palm kernel oil, CPKFA crude palm fatty acid after hydrolysis
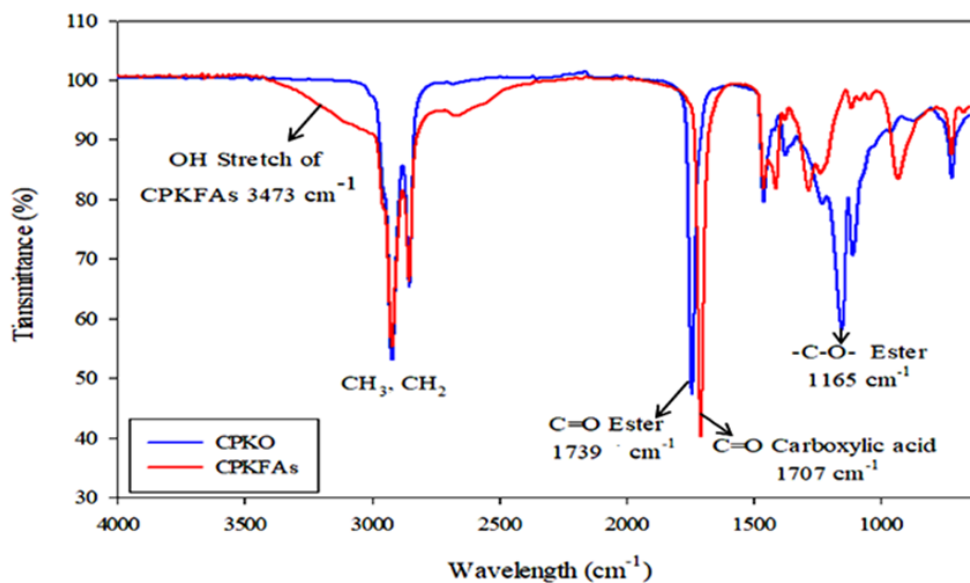
FIGURE 7. FTIR spectrum of CPKO and CPKFA

Table 8 show the ^{1}H NMR spectrum of fatty acids. In fatty acids structure, there are two types of proton which are proton of COOH and proton of -CH$_2$CO-. In this study, the NMR spectrum shows proton of COOH for fatty acids was detected at 11.39 ppm, and proton of -CH$_2$CO-.was detected at 2.33 ppm. These values were in agreement with the reference which is the chemical shift for proton COOH is at 10.5 - 12 ppm while the chemical shift for proton -CH$_2$CO- is at 2 - 2.8 ppm (Asiri & Bahajaj 2005). The fatty acids from crude palm kernel oil consist of a mixture of fatty acid groups such as caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, and linoleic acid. The presence of unsaturated fatty acids was determined by identification of the alkene group. In alkene group, there were two types of protons identifies, which are proton of the double bond (-C=C-H) at chemical shift 4.5-6 ppm and proton of methylene group which bound to double bond (-C=C-C-H) at chemical shift 1.6-2.6 ppm (Asiri & Bahajaj 2005). From Figure 8, the existence of both protons can be detected at a chemical shift 5.35 ppm (-C=C-H) and 1.99-2.06 ppm (-C=C-C-H). The presence of both peaks proved the existence of unsaturated fatty acids in fatty acids. The signal at 7.25 ppm referred to the solvent CDCl$_3$.

Spectrum ^{13}C NMR for fatty acids is shown in Figure 9 and Table 9. From Figure 9, it shows that there is the existence of the carbon atom of the carbonyl group (C=O) carbon carboxylic in fatty acid at a chemical shift of 180.68 ppm. This value was in agreement with Asiri and Bahajaj (2005) which showed that the chemical shift for carbo carbonyl (C=O) was in the range of 160-185 ppm. The presence of unsaturated fatty acids was determined by identification of the alkene group (C=C). According to Asiri and Bahajaj (2005), the chemical shift for alkene carbon (C=C) was in the range of 120 - 140 ppm. The alkene group (C=C) in unsaturated fatty acids was detected at the chemical shift of 130.14 ppm. The signal at 77.36 ppm denote the CDCl$_3$ solvent for fatty acids.

COMPOSITION OF FATTY ACIDS

Table 10 summarizes the CPKO and CPKFA FA composition after hydrolysis. The composition of CPKO prepared with base catalyst comprises lauric acid (44.5 ± 0.7 %), oleic acid (18.0 ± 0.5 %), myristic acid (16.9 ± 0.1 %), palmitic acid (9.4 ± 0.2 %), linoleic acid (2.9 ± 0.1 %), stearic acid (2.6 ± 0.1 %), caprylic acid (2.9 ± 0.1 %) and capric acid
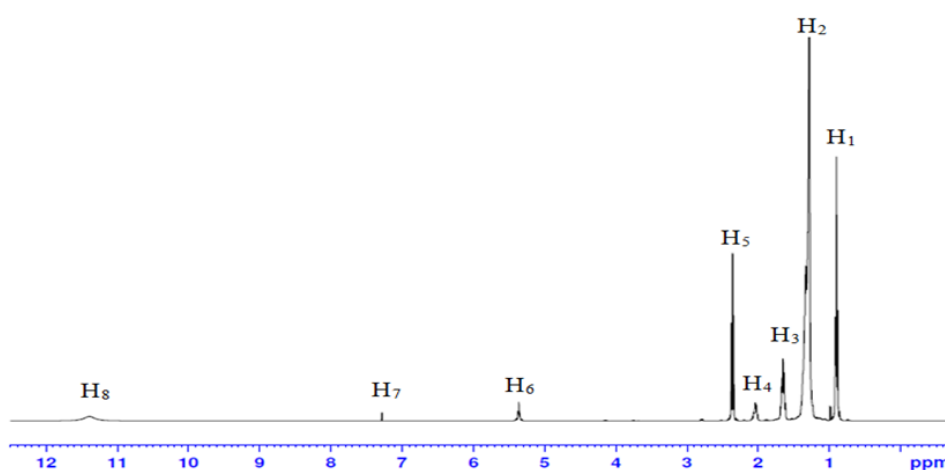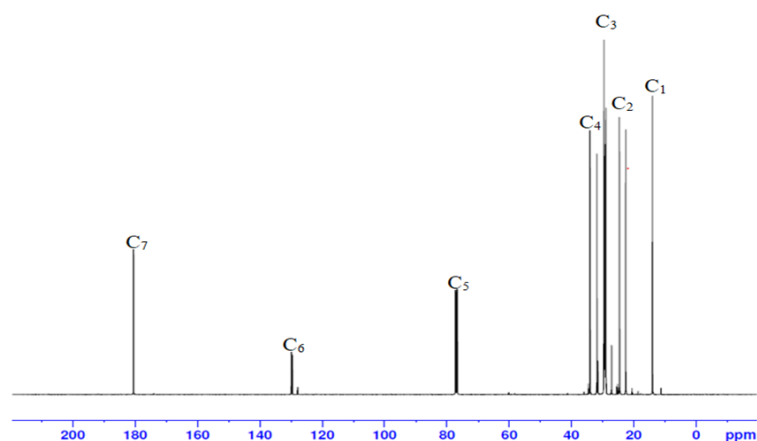

FIGURE 8. ^{1}H NMR spectra of fatty acids

TABLE 8. ^{1}H - NMR chemical shift (ppm) of fatty acids

	Compound	Chemical shift (ppm)	Theoretical chemical shift (ppm)*
H$_1$	-CH$_3$	0.89	0.7 – 1.3
H$_2$	-(CH$_2$)n-	1.23-1.32	1.2 -1.4
H$_3$	-CH$_2$-CH$_2$-C=O	1.62	1.5 - 2
H$_4$	-C=C-C-H	2.03	1.6 -2.6
H$_5$	-CH$_2$CO-	2.37	2.1 – 2.5
H$_6$	-CH=CH-	5.35	4.5 – 6.5
H$_7$	CDCl$_3$	7.25	7.25
H$_8$	-COOH	11.39	11 - 12

* Asiri and Bahajaj (2005)

FIGURE 9. ^{13}C NMR spectra of fatty acids

TABLE 9. ^{13}C - NMR chemical shift (ppm) of fatty acids

	Compound	Chemical shift (ppm)	Theoretical chemical shift (ppm)*
C_1	-CH$_3$	14.06	10 – 20
C_2	-CH$_2$-CH$_2$COOH	24.66	20 – 40
C_3	-(CH$_2$)n-	27.15-29.77	20 – 40
C_4	-CH2COOH	34.14	20.6 - 35
C_5	CDCl$_3$	77.36	77
C_6	-CH=CH-	130.14	100 - 150
C_7	-C=O	180.68	155 - 185

*Asiri and Bahajaj (2005)

(2.8 ± 0.1 %). The fatty acid composition of CPKFA after hydrolysis subjected to acid-catalyzed preparation comprised lauric acid (45.1 ± 0.4 %), oleic acid (17.9 ± 0.2 %), myristic acid (16.8 ±0.1 %), palmitic acid (9.3 ± 0.1 %), linoleic acid (2.9 ± 0.03 %), stearic acid (2.5 ± 0.03 %), caprylic acid (2.6 ± 0.1 %), and capric acid (2.9 ± 0.1 %), respectively. The GC results show that applying acid and base catalysts in the hydrolysis produced different FFA percentages and FA compositions of CPKO. That is, the FFA composition could be determined using the acid catalyst, while the base catalysts could be used to determine the oil composition.

Fatty Acid HPLC Analysis

The HPLC results show the optimum conditions of hydrolysis in this study. That is, an ethanolic KOH concentration of 1.77 M, a reaction temperature of 70°C, and 2.16 h of reaction time. The typical triacylglycerol profile for non-hydrolyzed CPKO is shown in Figure 10. In contrast, the chromatographic profile of hydrolyzed CPKO is shown in Figure 11 under optimum conditions. The HPLC chromatogram results showed that under the optimum conditions (the concentration of ethanolic KOH (M) of 1.77 M, a reaction temperature of 70°C, and 2.16 h of reaction time), the concentration of triacylglycerol

decreased while the free fatty acid composition increased. This proves that the hydrolysis process was effective.

Physicochemical characteristics

Table 11 shows the similarity and difference between the physicochemical properties of CPKO and CPKOFA. Based on the results, CPKO has a 3.7% FFA average value. After the hydrolysis of CPKO, the free fatty acid percentage of CPKFAs was increased to 98 ±0.7 %, again proving that the hydrolysis process was effective. According to Akinyeyea et al. (2011), a factor that significantly affects oil usage for human nutritional purposes or industrial applications is the acid value. In this study, CPKO and CPKFA produced acid values of 10.4 ±0.1 and 275.4 ±0.7 mg NaOH/g oil, respectively. The hydroxyl value for CPKO was 45.06 ±0.3 mg KOH/g. This value indicates the existence of free fatty acids MAG, and DAG in CPKO due to the hydrolysis of triacylglycerides resulting from humidity and temperature results. Meanwhile, the hydroxyl values for CPKFAs were increased to 168.2 ±0.1 mg KOH/g. This means that the CPKO was completely hydrolyzed into CPKFA. Table 3 shows the iodine values of the CPKO and CPKFA measured in this study, which were 20.8 ±0.6 gI2/100 g and 21.8 ±0.2 gI2/100 g, respectively. Both have different iodine values because of the USFA percentage that

TABLE 10. FA composition in CPKO with base-catalysed transesterification (%) and CPKFA after hydrolysis with acid-catalysed esterification

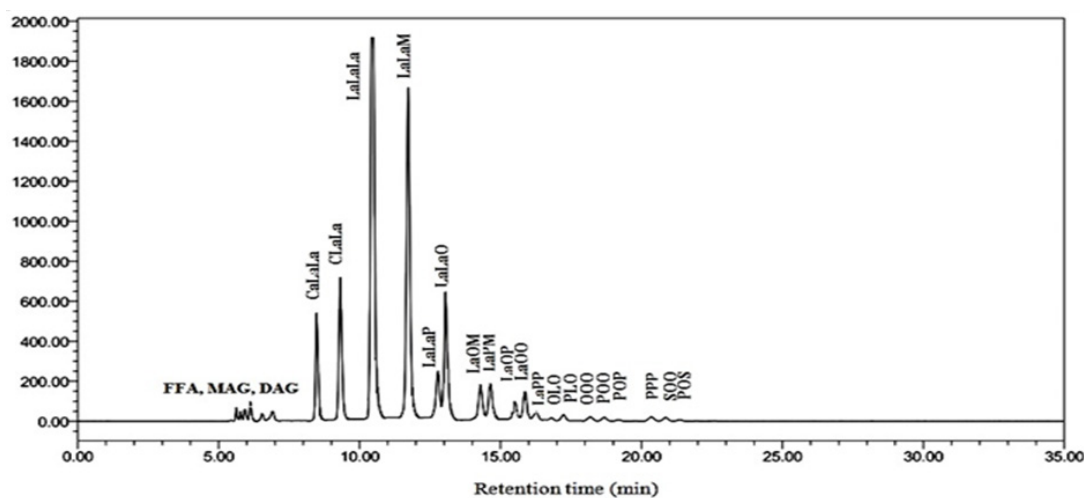| Fatty acid composition	Relative composition (%)	
	CPKO	CPKFA
Caprylic acid $C_{8:0}$	2.9±0.1	2.6±0.1
Capric acid $C_{10:0}$	2.8±0.1	2.9±0.1
Lauric acid $C_{12:0}$	44.5±0.7	45.1±0.4
Myristic acid $C_{14:0}$	16.9±0.1	16.8±0.1
Palmitic acid $C_{16:0}$	9.4±0.2	9.3±0.1
Stearic acid $C_{18:0}$	2.6±0.1	2.5±0.03
Oleic acid $C_{18:1}$	18.0±0.5	17.9±0.2
Linoleic acid $C_{18:2}$	2.9±0.1	2.9±0.03
∑ Saturated fatty acid	79.1	79.2
∑ Unsaturated fatty Acid	20.9	20.8

FIGURE 10. HPLC chromatogram of crude palm kernel oil
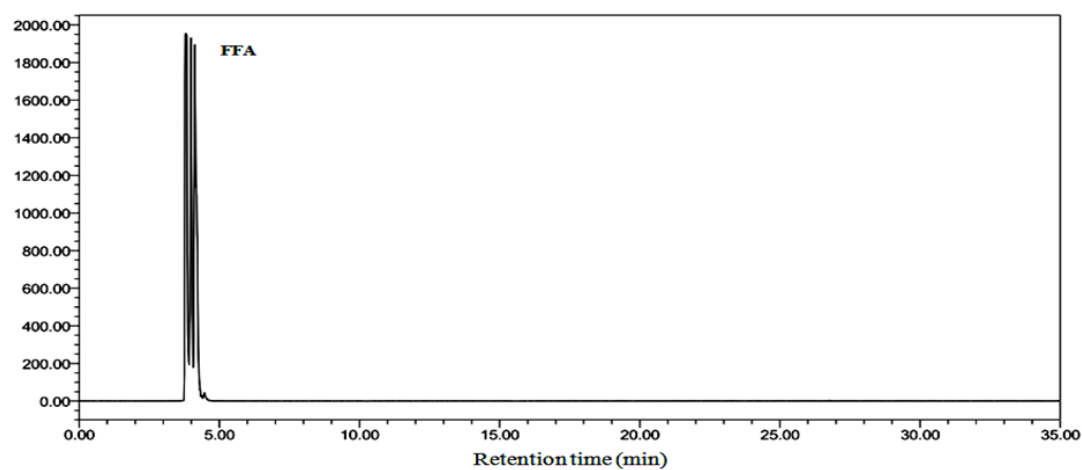

FIGURE 11. HPLC chromatogram after hydrolysis of crude palm kernel oil for producing (CPKFA)

is lower in CPKO compared to CPKFA. A low iodine value indicates low-level unsaturation in the CPKO and CPKFA (Atasie & Akinhanmi 2009). In this study, the color of CPKO and CPKFA were 0.5 red/20 yellow and 0.00 red/1.00 yellow, respectively, based on the Lovibond color. In addition to that, the viscosity at 28°C showed that CPKO exhibited the highest flow with a viscosity of 40 cP, while CPKFA showed the least resistance viscosity of 8 cP. Japir et al. (2016) explain that the high number of carbon atoms in the FA composition of CPKO and CPKFA contributes to their high refractive index. A slight difference in the density of the samples was observed i.e. from 0.9 g/mL for CPKO to 0.84 g/mL for CPKFA and the specific gravity for CPKO was 0.9 g/mL. It was found that the CPKO had similar moisture content to that of the CPKO standard. Moisture content is crucial for determining oil quality.

TABLE 11. Physicochemical properties of CPKO and CPKFAs

Parameter	CPKO	CPKFAs
FFA (as lauric acid) (%)	3.7 ±0.1	98 ± 0.7
Acid value (mg NaOH/g)	10.4 ±0.1	275.4 ± 0.7
Iodine value (Wijs) (gI2/100 g)	20.8 ±0.6	21.8 ± 0.2
Saponification value (mg KOH/g)	244.04 ±0.1	-
Unsaponifiable matter (%)	0.7 ±0.1	-
Average molecular weight (g/mol)	689.6 ±0.15	-
Hydroxyl value (mg KOH/g)	45.06 ±0.3	168.2 ± 0.1
Moisture content at 28°C (%)	0.5 ±0.1	5 ± 0.1
Refractive index at 28°C	1.455	1.44
Viscosity at 28°C (cp)	40	8
Density (g/mL)	0.9	0.84
Specific gravity (g/mL)	0.976	-
Lovibond color (R/Y) at 28°C	0.5 R –20 Y	0.00 R –1.00 Y

Conclusion

The preparation of fatty acids from crude palm kernel oil via alkaline hydrolysis was performed following the design of an experiment based on the D-optimal approach. The interaction effect between the independent variables on the fatty acid preparation was examined and the results from ANOVA showed that the experimental data and predicted data were in good agreement. Therefore, the use of D-optimal design through the Response Surface Methodology in getting the optimal condition for alkaline hydrolysis is systematically proven. The findings offer the much-needed support for validating the model in predicting the preparation of fatty acid successfully from crude palm kernel oil.

REFERENCES

Asiri, A. M. & Bahajaj, A. A. 2005. *Principles of Spectroscopic Analysis of Organic Compounds*. 1st ed. Dar Hafiz. Jeddah, KSA.

Bahadi, M. A., Japir, A. W., Salih, N. & Salimon, J. 2016a. Free fatty acids separation from Malaysian high free fatty acid crude palm oil using molecular distillation. *Malaysian Journal of Analytical Sciences* 20: 1042-1051.

Bahadi, M. A., Salimon, J. & Japir, A. W. M. 2016b. The physicochemical and thermal properties of Malaysian high free fatty acid crude palm oil. *AIP Conference Proceedings* 1784: 030002.

Derawi, D. 2016. Experimental design using response surface methods for palm olein-based hydroxy-ether systhesis. *Sains Malaysiana* 45(7): 1149-1154.

Derawi, D. & Salimon, J. 2013a. Palm olein polyols production by batch and continuous hydrolisis. *Sains Malaysiana* 42(8): 1121-1129.

Derawi, D. & Salimon, J. 2013b. Palm olein based biolubricant basestocks: Synthesis, characterisation, tribological and rheological analysis. *Malaysian Journal of Analytical Sciences* 17(1): 153-163.

Fu, X., Zhu, X., Gao, K. & Duan, J. 1995. Oil and fat hydrolysis with lipase from *Aspergillus* sp. *Journal of the American Oil Chemists' Society* 72(5): 527-531.

Hashim, H. B. & Salimon, J. 2008. Kajian pengoptimuman tindak balas hidrolisis minyak kacang soya. *The Malaysian Journal of Analytical Sciences* 12: 205-209.

Hermansyah, H., Kubo, M., Shibasaki-Kitakawa, N. & Yonemoto, T. 2006. Mathematical model for stepwise hydrolysis of triolein using *Candida rugosa* lipase in biphasic oil-water system. *Biochemical Engineering Journal* 31(2): 125-132.

Holliday, R. L., King, J. W. & List, G. R. 1997. Hydrolysis of vegetable oils in sub-and supercritical water. *Industrial & Engineering Chemistry Research* 36(3): 932-935.

Ibrahim, N. A. 2013. Characteristics of Malaysian palm kernel and its products. *Journal of Oil Palm Research* 25(2): 245-252.

Ichihara, K. I. & Fukubayashi, Y. 2010. Preparation of fatty acid methyl esters for gas-liquid chromatography. *Journal Of Lipid Research* 51(3): 635-640.

Japir, A. A. W., Salimon, J., Derawi, D., Yahaya, B. H., Bahadi, M., Al-Shuja'a, S. & Yusop, M. R. 2018. A highly efficient separation and physicochemical characteristics of saturated fatty acids from crude palm oil fatty acids mixture using methanol crystallisation method. *OCL* 25(2): A203.

Japir, A. A. W., Salimon, J., Derawi, D., Bahadi, M., Al-Shuja'a, S. & Yusop, M.R. 2017. Physicochemical characteristics of high free fatty acid crude palm oil. *OCL* 24(5): 506.

Japir, A. A. W., Salimon, J., Derawi, D., Yahaya, B. H., Bahadi. & Yusop, M. R. 2016. Purification of high free fatty acid crude palm oil using molecullar distillation. *Asian Journal of Chemistry* 28(11): 2549-2554.

Khor, H. T., Tan, N. H. & Chua, C. L. 1986. Lipase-catalyzed hydrolysis of palm oil. *Journal of the American Oil Chemists Society* 63(4): 538-540.

King, J., Holliday, R. & List, G. 1999. Hydrolysis of soybean oil in a subcritical water flow reactor. *Green Chemistry* 1(6): 261-264.

Salimon, J. & Abdullah, R. 2008. Physicochemical properties of Malaysian *Jatropha curcas* seed oil. *Sains Malaysiana* 37(4): 379-382.

Salimon, J., Abdullah, B. M. & Salih, N. 2012. D-optimal design optimization of *Jatropha curcas* L. seed oil hydrolysis via alkali-catalyzed reactions. *Sains Malaysiana* 41(6): 731-738.

Salimon, J., Abdullah, B. M. & Salih, N. 2011. Hydrolysis optimization and characterization study of preparing fatty acids from *Jatropha curcas* seed oil. *Chemistry Central Journal* 5(1): 67.

Salimon, J., Said, M., Ramli, S. & Lazim, M. 2006. *Oil and Fat Analysis.* Bangi: UKM Press.

Serri, N. A., Kamarudin, A. H. & Abdul Rahaman, S. N. 2008. Preliminary studies for production of fatty acids from hydrolysis of cooking palm oil using *C. rugosa* lipase. *Journal of Physical Science* 19(1): 79-88.

Sherazi, S. T. H., Kandhro, A., Mahesar, S. A., Bhanger, M. I., Talpur, M. Y. & Arain, S. 2009. Application of transmission FT-IR spectroscopy for the trans fat determination in the industrially processed edible oils. *Food Chemistry* 114(1): 323-327.

Velayutham, T. S., Abd Majid, W. H., Ahmad, A. B., Kang, G. Y. & Gan, S. N. 2009. Synthesis and characterization of polyurethane coatings derived from polyols synthesized with glycerol, phthalic anhydride and oleic acid. *Progress in Organic Coatings* 66: 367-371.

Murad Bahadi & Mohamad Firdaus Yusoff, Jumat Salimon & Darfizzi Derawi*
Laboratory for Biolubricant, Biofuels and Bioenergy Research
Department of Chemical Sciences
Faculty of Sciences and Technology
Universiti Kebangsaan Malaysia
43600 UKM Bangi, Selangor Darul Ehsan
Malaysia

Murad Bahadi
Faculty of Education
Hadhramout University
Hadhramout
Yemen

*Corresponding author; email: darfizzi@ukm.edu.my