D-Optimal Design Optimization of *Jatropha curcas* L. Seed Oil Hydrolysis via Alkali-Catalyzed Reactions

(Pengoptimuman Reka Bentuk D-Optimum Hidrolisis Minyak Biji *Jatropha curcas*
L. Melalui Tindak Balas Mangkin Alkali)

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ABSTRACT

Potassium hydroxide (KOH)-catalyzed reactions were utilized to hydrolyze Jatropha curcas *seed oil. In this study, the effect of ethanolic KOH concentration, reaction temperature and reaction time to the free fatty acid (FFA) produced were investigated. D-Optimal Design was employed to study the significance of these factors and optimum conditions for the technique was predicted. The results showed that ethanolic KOH concentration was a significant variable for hydrolysis of* J. curcas *seed oil. In 18 experimental designs, FFA percentage of hydrolyzed* J. curcas *seed oil increased from 1.89% to 102.2%. The optimal conditions of response were found at 1.75M of ethanolic KOH, 65 °C and at 2 h reaction time. Fourier Transforms Infrared Spectroscopy (FTIR) spectrum analysis confirmed the concentration of ethanolic KOH i.e. at 1.00, 1.50 dan 1.75 M affect the percentage yields of* J. curcas *seed oil hydrolysis process.*

Keywords: Alkaline hydrolysis; D-optimal design; Jatropha curcas *seed oil*

ABSTRAK

Tindak balas bermangkin kalium hidroksida (KOH) telah digunakan untuk hidrolisis minyak biji Jatropha curcas*. Kesan nisbah kepekatan KOH beretanol, suhu dan masa tindak balas terhadap penghasilan asid lemak bebas (ALB) telah dikaji. Reka bentuk D-Optimum telah digunakan untuk menentukan keberkesanan parameter tindak balas dan nilai optimumnya diramalankan serta ditentukan. Keputusan kajian menunjukkan bahwa kepekatan KOH beretanol merupakan parameter yang bererti bagi hidrolisis minyak* J. curcas*. Reka bentuk 18 uji kaji menunjukkan peratus ALB hasil hidrolisis minyak* J. curcas *telah ditingkatkan daripada 1.89% kepada 102.2%. Keadaan gerak balas optimum telah dicerap pada 1.75 M KOH beretanol, suhu 65°C dan 2 jam masa tindak balas. Analisis Spektroskopi Inframerah Fourier Transform (FTIR) telah membuktikan bahwa pertambahan kepekatan KOH beretanol pada 1.00, 1.50 dan 1.75 M meningkatkan peratusan hasil proses hidrolisis minyak biji* J. curcas*.*

Kata kunci: Hidrolisis beralkali; minyak biji; reka bentuk D-optimal; Jatropha curcas

INTRODUCTION

The Malaysian *J. curcas* seed oil consists of main fatty acid such as palmitic acid (13.89%), stearic acid (7.16%), oleic acid (46.40%) and linoleic acid (31.96%) (Jumat & Rozaini 2008). *J. curcas* seed oil contains high percentage (i.e about 78%) of unsaturated fatty acid. This made the oils suitable for biodiesel production. However, the chemical compositions of the oil may vary according to the climate and locality (Jumat & Rozaini 2008).

Hydrolysis of oils is the applied term to the reaction of ethanolic KOH with oil to form glycerol and fatty acids. Production of fatty acid and glycerol from oils are important especially in oleochemical industries. Glycerol and fatty acids are widely used as raw materials in food, cosmetics, pharmaceutical industries (Hermansyah et al. 2006; Serri et al. 2008), soap production, synthetic detergents, greases, lubricant, cosmetics, and several other products (Pinto & Lancas 2006).
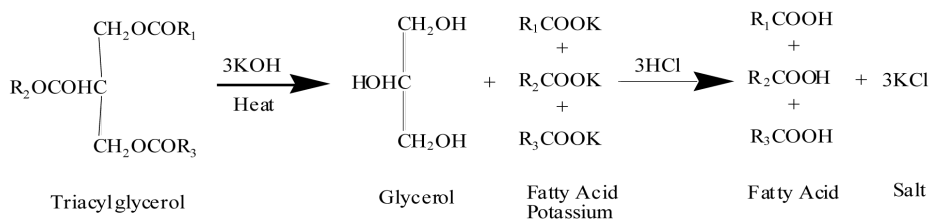

FIGURE 1. Hydrolysis of *J.curcas* seed oil using potassium hydroxide

The soap production starting from triacylglycerol and alkali was accomplished for more than 2000 years (Ackelsberg 1958). Saponification is the alkaline hydrolysis of the plant oil (triacylglycerol) as shown in Figure 1.

Researchers have used several methods to hydrolyze plant oil such as enzymatic hydrolysis using lipase from *Aspergillus niger*, *Rhizopus javanicus* and *Penicillium solitum* (Carvalho et al. 2009), *C. rugosa* (Serri et al. 2008), and subcritical water (Pinto & Lancas 2006). Nowadays, researchers have used potassium hydroxide catalyzed hydrolysis in order to reduce energy consumption (Wu et al. 2008), thermal degradation (Serri et al. 2008) and obtained highest fatty acids hydrolysis percentage (Pinto & Lancas 2006).

D-optimal designs are one form of design provided by a computer algorithm. These types of computer-aided designs are particularly useful when classical designs do not apply. D-optimal design matrices are usually not orthogonal and effect estimated are correlated. The reasons for using D-optimal designs instead of central composite and Box-Behnken designs generally due to it is much greater flexibility in selecting response surface model types. It also allows parameters to be estimated without bias and with minimum-variance (Yang & El-Haik 2009). In practical terms, D-optimal experiments can reduce the costs of experimentation.

Use of *J. curcas* seed oil, a cheap raw material for fatty acids production is available at several places in Malaysia. Hence, there is a need to search for cheaper raw materials for the production of fatty acids. The objective of this study was to determine the variables that affect the process of plant oil hydrolysis. D-optimal design was applied to relate these three important variables, i.e. ethanolic KOH concentration (X_1), reaction temperature (X_2), and reaction time (X_3) with the response (FFA%), to find the optimal combination of process variables that would maximize the FFA%.

MATERIALS AND METHODS

MATERIALS

J. curcas seeds were obtained from the Universiti Kebangsaan Malaysia Biobased Lubricant's trial plots. The ripe seeds were collected and the damaged seeds were discarded. The seeds were cleaned, de-shelled and dried in an oven at 105 °C for 30 min. The seeds were ground to powder using a grinder prior to oil extraction. All chemicals used in the study were analytical grade and used without further purification.

OIL EXTRACTION

The extraction of *J. curcas* seed oil was carried out using solvent extraction. The *J. curcas* seeds powder were extracted using hexane as a solvent for 6 h.

OIL HYDROLYSIS

Fatty acids were obtained by the hydrolysis of *J. curcas* seed oil according to methods by Syed Rahmatullah et al. (1994) and Senanayake and Shahidi (1999) with modifications. Table 1 shows different ratio of KOH, different reaction temperature and different reaction time using D-optimal design. Factors (variables) such as ethanolic KOH (M, X_1), reaction temperature (°C, X_2) and reaction time (h, X_3) were performed under the same experimental conditions in order to verify using of KOH hydrolysis of *J. curcas* seed oil. Typically, the ethanolic KOH solution was prepared at different concentrations (1.00, 1.50 and 2.00 M). A mixture containing 50 g oil and 300 mL ethanolic KOH solution was heated at different temperatures and times and the unsaponifiable matters were extracted by 100 mL of n-hexane and discarded. Distilled water was added to the mixture and then acidified with 6 N; 100 mL of hydrochloric acid until the solution reached pH 1 to release the fatty acids. These fatty acids were extracted three times, each by using 100 mL n-hexane. The combined solvent was washed with distilled water and dried over anhydrous sodium sulphate. Solvent was removed by rotary evaporator at 35°C. The fatty acids composition from hydrolysed *J. curcas* seed oil was determined according to Jumat et al. (2006). Functional groups of fatty acids were analysed using Thermo Nicolet Nexus 470 FTIR system (Madison, WI) with a Smart ARK accessory containing a 45 Ze Se trough in a scanning range of 400 – 4000 cm^{-1} for 32 scans at a spectral resolution of 4 cm^{-1} (Bashar & Jumat 2009).

EXPERIMENTAL DESIGN

A three-factor D-optimal design was employed to study the responses of the percentage of FFA [Y in %by wt, see Eq. (1)]. An initial screening step was carried out to select the major response factors and their values. The independent variables were X_1, X_2 and X_3 representing the ethanolic KOH (M), reaction temperature (°C), and reaction time (h), respectively. The settings for the independent variables were as follows (low and high values): KOH concentration of 1.0 and 2.0; reaction temperature of 50 and 70; and reaction time of 1.5 and 2.5. Each variable to be optimized was coded at three levels: -1, 0, and +1 (Table 1).

TABLE 1. Independent variables and their levels for D-optimal design

Independent variables		Variable levels		
		-1	0	+1
KOH (M)	X_1	1.00	1.50	2.00
Temperature (°C)	X_2	50	60	70
Time (h)	X_3	1.5	2.0	2.5

A quadratic polynomial regression model was assumed for predicting individual Y variables. The model proposed for each response of Y was:

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

where β_0, β_i, β_{ii} and β_{ij} are constant, linear, square and interaction regression coefficient terms, respectively, and x_i and x_j are independent variables. The Minitab software version 14 (Minitab Inc., USA) was used for multiple regression analysis, analysis of variance (ANOVA), and analysis of ridge maximum of data in the response surface regression (RSREG) procedure. The goodness fit of the model was evaluated by the coefficient of determination R^2 and the analysis of variance (ANOVA). Response surfaces and contour plots were developed using the fitted quadratic polynomial equations obtained from RSREG analysis and holding the independent variables with the least effect on the response at two constant values and changing the levels of the other two variables.

RESULTS AND DISCUSSION

EFFECT OF OPTIMIZATION VARIABLES

A three-factor response surface methodology (RSM) was employed to study the FFA% by ethanolic KOH hydrolysis of *J. curcas* seed oil. Results obtained from the experiments (observed and predicted) are summarized in Table 2 and shown in Figure 2.

The results showed the hydrolysis performances of the ethanolic KOH effects on the hydrolysis reactions when submitted to different experimental conditions. Hydrolysis reactions were carried out at various ethanolic KOH concentrations ranging from 1.00 to 2.00 M. Table 2 demonstrates the effect of ethanolic KOH concentration on the FFA%. The FFA% at 1.00 °C was low, however it increased with increasing ethanolic KOH concentration, it can be clearly seen that the maximum FFA% obtained at 1.75 M was about 102.2%.

A different observation was reported by other researchers for hydrolysis of various vegetable using *C. rugosa* lipase (Fadilloğlu & Söylemez 1998; Noor et al. 2003 and Rooney & Weatherly 2001). Increase in enzyme concentration did not give any significant changes in the reaction rate (Rooney & Weatherly 2001). Therefore, further increase in enzyme concentration did not give any improvement in the conversion.

The effect of reaction temperature on the FFA% is shown in Table 2. The FFA% increased when the temperature was increased from 50 to 70 °C. At a reaction temperature of approximately 65 °C a percentage of FFA was achieved at 102.2%. This result indicated that temperature is important in the percentage of FFA hydrolysis. This theory has been reported by Serri et al. (2008) by using enzyme *C. rugosa* lipase.

Table 2 indicates the FFA% using different reaction times (1.5, 2.0 and 2.5h) with different variables such as ethanolic KOH and reaction temperatures. The FFA% was increased with increasing reaction time as shown in Table 2. 2.0 h was chosen to obtain highest percentage of FFA (102.2%).

TABLE 2. D-optimal design optimization of *J. curcas* seed oil hydrolysis and response for FFA%

| Run no.	Coded independent variable levels			FFA, % (responses)	
	Ethanolic KOH (M, X_1)	Temperature (°C, X_2)	Time (h, X_3)	Experimental (Observed value)	Predicted Value
1	2.00	50	1.5	97.1	63.96
2	2.00	70	2.5	102.4	103.58
3	1.00	50	1.5	53.9	94.68
4	1.00	60	2.0	64.6	75.04
5	2.00	50	2.5	97.5	97.57
6	1.75	65	2.0	102.2	98.93
7	2.00	60	2.5	99.1	101.78
8	1.00	50	2.0	60.8	98.93
9	1.00	70	2.5	77.1	63.96
10	1.50	60	2.5	97.4	55.14
11	1.00	50	2.5	67.9	65.26
12	2.00	60	1.5	100.3	99.76
13	1.00	60	1.5	55.1	55.14
14	1.00	70	1.5	70.0	97.84
15	1.50	50	2.0	96.7	75.04
16	2.00	70	1.5	100.4	68.44
17	1.00	70	2.5	72.4	98.16
18	1.50	70	1.5	99.2	100.92
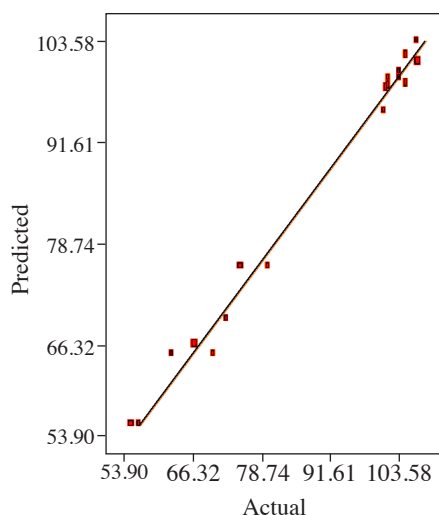
FIGURE 2. Relation between observed and predicted FFA% values

TABLE 3. Regression coefficients of the predicted quadratic polynomial model for response variables Y (FFA%)

Variables	Coefficients (β) % FFA (Y)	T	P	Notability
Intercept	96.65	144.21	0.0001	***
Linear				
X_1	17.28	889.81	0.0001	***
X_2	4.33	57.02	0.0001	***
X_3	1.91	9.52	0.0150	**
Square				
X_{11}	-15.14	130.05	0.0001	***
X_{22}	0.37	0.085	0.7777	
X_{33}	0.63	0.33	0.5838	
Interaction				
X_{12}	-3.48	30.63	0.0006	***
X_{13}	-1.40	4.02	0.0800	
X_{23}	0.11	0.023	0.8825	
R^2	0.99			

Notes: ** $P < 0.05$; *** $P < 0.01$. T: F test value
See Table 2 for a description of the abbreviations

MODEL FITTING

The quadratic regression coefficient obtained by employing a least squares method technique to predict quadratic polynomial models for the FFA% (Y) are given in Table 3. Examination of these coefficients with a T-test shows for the FFA% (Y), linear, square and interaction terms of ethanolic KOH (X_1) were highly significant (p<0.01), and linear terms of reaction temperature °C (X_2) was highly significant ($p<0.01$), while for reaction time (X_3) was significant at $p<0.05$. The coefficients of independent variables (ethanolic KOH; X_1, reaction temperature; X_2, and reaction time; X_3) determined for the quadratic polynomial models (Table 3) for the FFA% (Y) are given below:

$$Y = + 96.65 + 17.28X_1 + 4.33X_2 + 1.91X_3 - 15.14X_1^2 + 0.37X_2^2 + 0.63X_3^2 - 3.48X_1X_2 - 1.40X_1X_3 + 0.11X_2X_3$$

$$\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \text{(2)}$$

TABLE 4. Analysis of variance (ANOVA) of the response Y (FFA%) of the D-optimal design

Source	Df^a	Sum of squares	Mean square	F^b	P^c
Mean	1	1.275E+005	1.274E+005		
Linear	3	4921.05	1640.35	31.43	0.0001
Square	3	99.09	33.03	0.52	0.6756
Interaction	3	581.55	193.85	26.39	0.0002
Lack-of-fit	4	20.45	5.11	0.53	0.7205
Pure error	4	38.25	9.56		
Total	18	1.330E+005	7389.78		

Notes: (a) Df: degree of freedom; (b) F-value: distribution; (c) P-value: scale

DIAGNOSTIC CHECKING OF THE FITTED MODELS

ANOVAs for the fitted models are summarized in Tables 4. Examination of the model with an F-test and T-test indicate non-significant lack-of-fit at $p > 0.05$ relative to pure error (9.56%). The regression coefficient (R^2) for data on the FFA% was 0.99 (Table 3). These indicate that the generated models adequately explained the data variation and represented the actual relationships among the reaction parameters.

RESPONSE SURFACE PLOTTING AND OPTIMIZATION IN THE LINEAR WEIGHTING METHOD

Equation (2) shows that the FFA% have a complex relationship with independent variables that encompass both first and second-order polynomials. RSM is one of the best ways of evaluating the relationships between responses, variables and interactions that exist. Significant interaction variables in the fitted models (Table 3) were chosen as the axes (ethanolic KOH X_1, reaction temperature X_2 and reaction time X_3) for the response surface plots. The relationships between independent and dependent variables are shown in the three-dimensional representation as response surfaces. The response surfaces for the FFA% (Y) in the concentrates were given in Figures 3, 4 and 5.

In a contour plot, curves of equal response values are drawn on a plane whose coordinates represent the levels of the independent factors. Each contour represents a specific value for the height of the surface above the plane defined for combination of the levels of the factors. Therefore, different surface height values enable one to focus attention on the levels of the factors at which changes in the surface height occur. The contour plots (Figures 3(b), 4(b) and 5(b)) show the combination of levels of the ethanolic KOH and reaction temperature that can afford the same level of the FFA%. Canonical analysis was performed on the predicted quadratic polynomial models to examine the overall shape of the response surface curves and used to characterize the nature of the stationary points. Canonical analysis is a mathematical approach used to locate the stationary point of the response surface and to determine whether it represents a maximum, minimum or saddle point (Mason et al. 1989).

The model of plant oil hydrolysis was developed on the basis of the analysis of RSM. The ethanolic KOH was the most important parameter for the FFA%, which the observed value was reasonably close to the predicted value as shown in Figure 2. The conditions to get optimal response with 102.2% of FFA were found to be 1.75 M for the ethanolic KOH, 65°C for the reaction temperature and 2 h for reaction time. The process may produce highly FFA% from an economic point of view, as well as being a promising measure for further utilization of agriculture products.
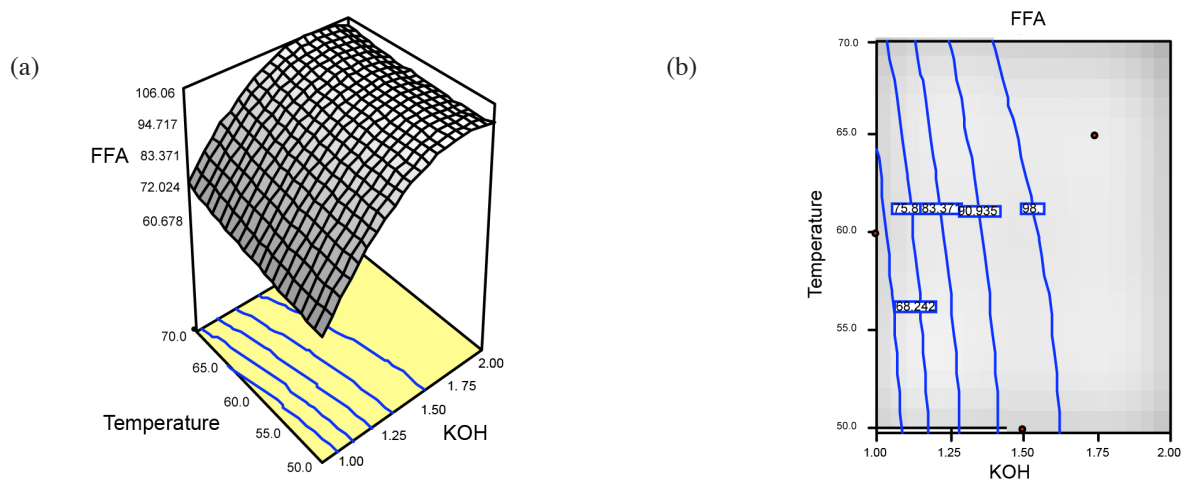

FIGURE 3. Response surface (a) and contour plots (b) for the effect of the ethanolic KOH (X_1, w) and reaction temperature (X_2, °C) on the FFA%
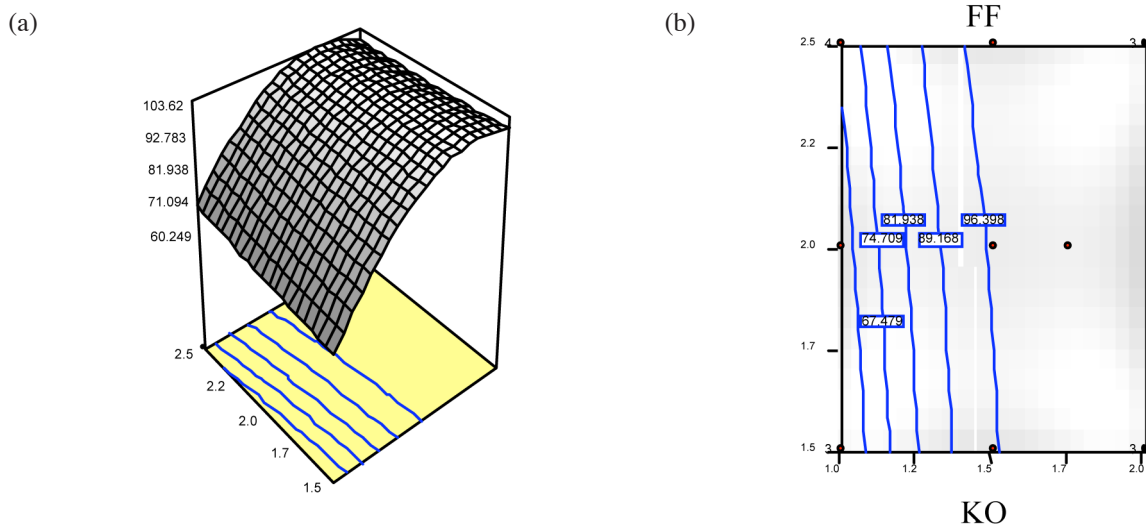
(a)

(b)

FF

KO

FIGURE 4. Response surface (a) and contour plots (b) for the effect of the ethanolic KOH (X_1, w) and reaction time $(X_3, {}^\circ C)$ on the FFA%
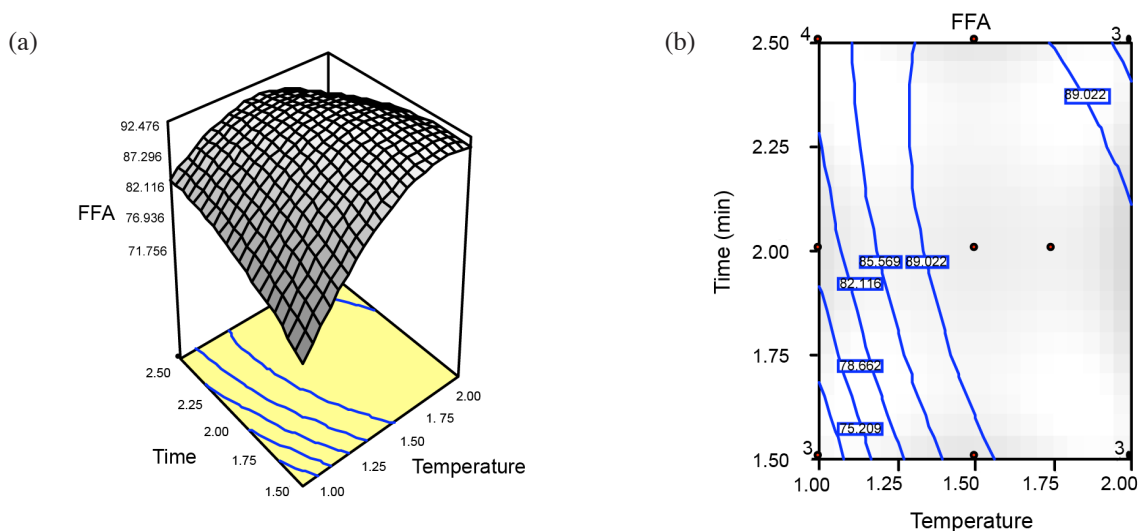
(a)

(b)

FFA

FIGURE 5. Response surface (a) and contour plots (b) for the effect of the hydrolysis reaction temperature $(X_2, {}^\circ C)$ and reaction time $(X_3, {}^\circ C)$ on the FFA%

FTIR ANALYSIS OF FATTY ACIDS

In order to prove the hydrolysis of plant oil, the spectrum from the FTIR analysis displays several absorption peaks of functional group for fatty acids as shown in Figure 6. The main peaks and their assignment to functional groups are given in Table 5.

The comparison among (a) *J. curcas* oil (b) hydrolyzed oil at 1.00 M and (c) at 1.75 M of ethanolic KOH is shown in Figure 6. For carboxylic acid carbonyl functional groups (C=O), FTIR spectrum showed absorption bands of hydrolyzed oil using 1.00 M at 1711 cm^{-1}, respectively for the stretching vibration, 1283-1285 cm^{-1} for the stretching asymmetric while at 1413 and 918-937 cm^{-1} for the bending vibration of carboxylic acid (Socrates, 2001). However

the ester carbonyl functional group still remains low. The hydrolyzed oil by using 1.75 M shows a complete disappearing of ester groups at 1746 and 1163 cm^{-1}.

Figure 6 shows the main change of functional group during the hydrolysis of oil (b and c), which (b) using 1.00 M ethanolic KOH solution. It shows low hydrolysis with low C=O (ester carbonyl) peaks at 1739 and 1180 cm^{-1} while (c) using 1.75 M ethanolic KOH which shows high hydrolysis with strong absorption. Peaks at 2925- 2854 cm^{-1} indicated the CH$_2$ and CH$_3$ scissoring while 3009 cm^{-1} for C=C bending vibration of both *J. curcas* seed oil and fatty acids which showed on Figure 6 (a), (b) and (c). FTIR spectrum also showed absorption bands at 722 cm^{-1} for C-H group vibration.
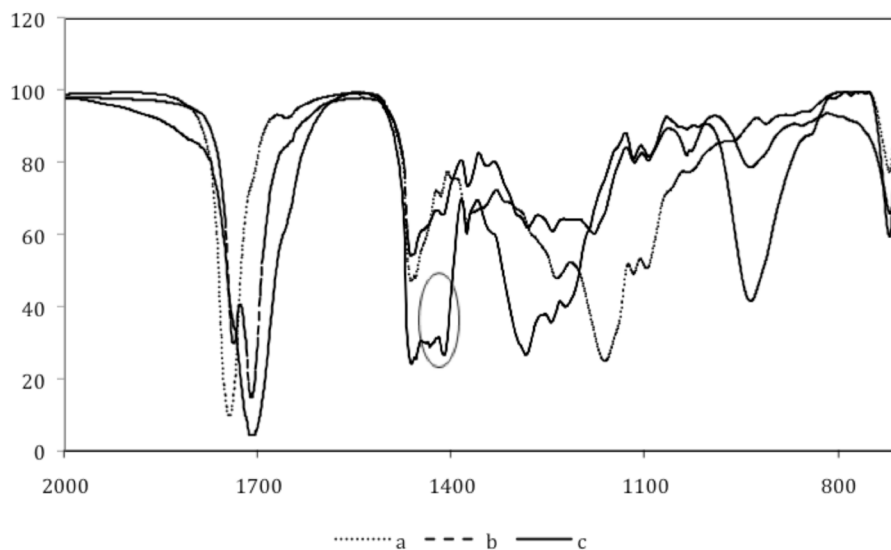
FIGURE 6. FTIR spectrum of *J. curcas* seed oil (a), fatty acid hydrolyzed at 1.00 M of ethanolic KOH (b) and hydrolyzed at 1.75 M of ethanolic KOH (c).

TABLE 5. The main wavelengths in the FTIR spectrum of hydrolyzed *J. curcas* seed oil

Wavelength of oil[a]	Wavelength of 1.00M[b]	Wavelength of 1.75M[c]	Functional groups
3009	3009	3009	C=C bending vibration (aliphatic)
2927, 2855	2925, 2854	2924, 2854	C-H stretching vibration (aliphatic)
1746	1739	-	C=O stretching vibration (ester)
-	1711	1711	C=O stretching vibration (carboxylic acid)
1463	1464	1463	C-H scissoring and bending for methylene
-	1283	1285	C-O stretching assymmetric (carboxylic acid)
1163	1180	-	C-O bending vibration (ester)
-	937	918	O-H bending vibration (carboxylic acid)
722	722	722	C-H group vibration (aliphatic)

Notes: (a) *J. curcas* seed oil; (b) hydrolysis at 1.00 M of ethanolic KOH; (c) hydrolysis at 1.75 M of ethanolic KOH

CONCLUSION

D-optimal design provided a powerful tool to optimize the hydrolysis conditions that permit an important improvement in the percentage of hydrolysis. The optimal response conditions with 102.2% of FFA% were found at 1.75 M of ethanolic KOH, 65°C and 2 h reaction time. These results implicate that the optimization using a response surface methodology based on D-optimal design was useful software in improving the optimization of FFA%.

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738

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