

RESEARCH ARTICLE

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Hydrolysis optimization and characterization study of preparing fatty acids from *Jatropha curcas* seed oil

Jumat Salimon*, Bashar Mudhaffar Abdullah and Nadia Salih

Abstract

Background: Fatty acids (FAs) are important as raw materials for the biotechnology industry. Existing methods of FAs production are based on chemical methods. In this study potassium hydroxide (KOH)-catalyzed reactions were utilized to hydrolysis *Jatropha curcas* seed oil.

Results: The parameters effect of ethanolic KOH concentration, reaction temperature, and reaction time to free fatty acid (FFA%) were investigated using D-Optimal Design. Characterization of the product has been studied using Fourier transforms infrared spectroscopy (FTIR), gas chromatography (GC) and high performance liquid chromatography (HPLC). The optimum conditions for maximum FFA% were achieved at 1.75M of ethanolic KOH concentration, 65°C of reaction temperature and 2.0 h of reaction time.

Conclusions: This study showed that ethanolic KOH concentration was significant variable for *J. curcas* seed oil hydrolysis. In a 18-point experimental design, FFA% of hydrolyzed *J. curcas* seed oil can be raised from 1.89% to 102.2%, which proved by FTIR and HPLC.

Background

Hydrolysis of oils and fats is the applied term to the operation in which ethanolic KOH reacts with oil to form glycerol and fatty acids (FAs). Production of FAs and glycerol from oils are important especially in oleochemical industries. FAs and glycerol are widely used as raw materials in food, cosmetics, pharmaceutical industries [1,2], soap production, synthetic detergents, greases, cosmetics, and several other products [3].

The soap production starting from triglycerides and alkalis is accomplished for more than 2000 years by the [4]. Saponification is the alkaline hydrolysis of triacylglycerol Figure 1. These reactions produce the FAs that are the starting point for most oleochemical production. As the primary feedstocks are oils and fats, glycerol is produced as a valuable byproduct. Reaction routes and conditions with efficient glycerol recovery are required to maximize the economics of large-scale production [5].

Lipid hydrolysis is usually carried out in the laboratory by refluxing oils and fats with different catalysts [6]. The

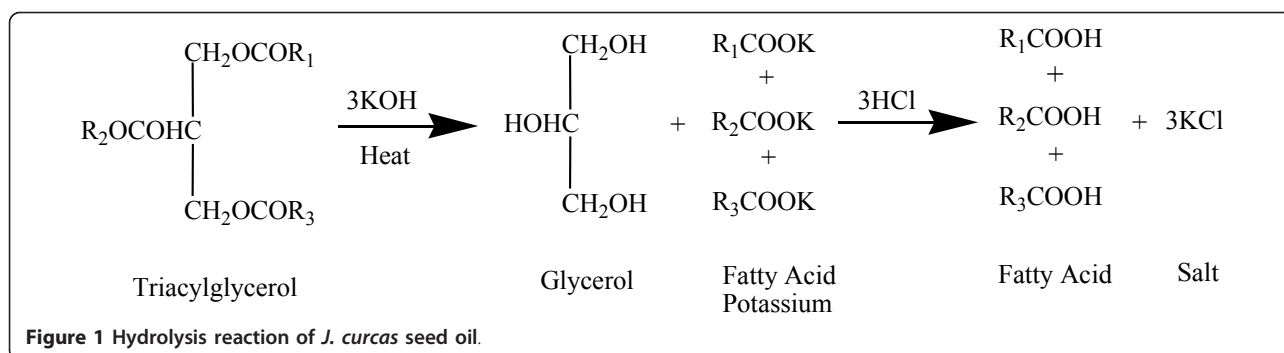
reaction can be catalyzed by acid, base, or lipase, but it also occurs as an un-catalyzed reaction between fats and water dissolved in the fat phase at suitable temperatures and pressures [7].

Researchers have been used several methods to prepare FAs and glycerol such as enzymatic hydrolysis using lipases from *Aspergillus niger*, *Rhizopus javanicus* and *Penicillium solitum* [8], *C. rugosa* [1], and subcritical water [3]. Nowadays, researchers have used potassium hydroxide catalyzed hydrolysis of esters is sometimes known as saponification because of its relationship with soap making. There are two big advantages of doing this. The reactions are one-way rather than reversible, and the products are easier to separate as shown in [3]. On a laboratory scale, alkaline hydrolysis is carried out with only a slight excess of alkali, typically potassium or sodium hydroxide in ethanol, refluxing for 1 h, and the FAs recovered after acidification of the reaction mixture. This is a sufficiently mild procedure that most FAs, including poly-unsaturates, epoxides, and cyclopropenes, are unaltered [9].

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Today ethanol is given emphasis over methanol in the world. Methanol was preferred in the seventies and eighties but the interest for methanol ended and instead ethanol programs were initialized. The grain-ethanol production, which today dominates the Europe alternative fuel market, may decide the technological path for decades to come. Today some European countries have commercial plants and pilot plant on ethanol production but no plant on methanol production [10].

Several reports have appeared on the hydrolysis of oils and fats using enzymes. Fats and oils can hydrolyze in the presence of natural enzymes. Enzyme reactions require milder conditions, less solvent, and give cleaner products attributes of green chemistry. There is increasing interest in the use of lipase enzymes for large-scale reactions. Reaction generally occurs under milder conditions of temperature and pH and there is reduced danger of undesirable side-reactions [6].

Though several studies which have appeared on the use of enzymes for hydrolysis of fats and oils. These studies have used to hydrolysis of FAs depends on the types of catalysts, types of vegetable oils and also depends on the different variables such as temperature and time to achieve 100% hydrolysis of vegetable oils. Hydrolysis of *J. curcas* seed oil by using sodium hydroxide NaOH [11] and potassium hydroxide KOH [12] have been studied. The literatures [11,12] showed that with using methanol and NaOH, the experiment was conducted with optimum molar ratio (6:1) keeping the catalyst concentration (1% NaOH), reaction temperature (65°C) and reaction time (1 h). But by using KOH, the experiment was conducted with optimum molar ratio (8:1) keeping the catalyst concentration (1% KOH), reaction temperature (70°C) and reaction time (31/2 h).

This study is executed for the factors that affect the process of hydrolysis of *J. curcas* seed oil. D-optimal design was used to evaluate the effect of three factors, such as concentrations of ethanolic KOH concentration, temperature and time reaction were studied for the optimum hydrolysis.

Results and discussion

Effect of Process Parameters and Statistical Analysis

D-optimal design optimization was employed to study the percentage of free fatty acid (FFA%) by ethanolic KOH concentration hydrolysis of *J. curcas* seed oil. Experimental results of FFA% for the ethanolic KOH concentration effects to *J. curcas* seed oil hydrolysis are given in Table 1.

The results show the hydrolysis performance 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 1 demonstrates the effect of ethanolic KOH concentration on the FFA%. The FFA% at 1.00 M 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 [13-15]. Increase in enzyme concentration did not give any significant changes in the reaction rate [15].

The effect of the reaction temperature on the FFA% is shown in Table 1. 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 indicates that the temperature is important factor in the hydrolysis of *J. curcas* seed oil. This theory was reported by [1] using enzyme *C. rugosa* lipase.

Table 1 indicates the FFA% using different times (1.5, 2.0 and 2.5h) with different variables, such as concentration of KOH and reaction temperatures. The FFA% increased with an increase in the reaction time, as shown in Table 1; 2.0 h was chosen to obtain the highest percentage of FFA (102.2%).

The quadratic regression coefficient was obtained by employing the least squares method technique to predict quadratic polynomial models for the FFA% (Y), as shown in Table 2.

Table 1 D-optimal design optimization of *J. curcas* seed oil hydrolysis and response for FFA%.

Run no.	Coded independent variable levels			FFA, % (responses)
	Ethanol KOH (M, X_1)	Temperature ($^{\circ}$ C, X_2)	Time (h, X_3)	
1	2.00	50	1.5	97.1
2	2.00	70	2.5	102.4
3	1.00	50	1.5	53.9
4	1.00	60	2.0	64.6
5	2.00	50	2.5	97.5
6	1.75	65	2.0	102.2
7	2.00	50	2.5	99.1
8	1.00	50	2.5	60.8
9	1.00	70	2.5	77.1
10	1.50	60	2.5	97.4
11	1.00	50	2.5	67.9
12	2.00	60	1.5	100.3
13	1.00	50	1.5	55.1
14	1.00	70	1.5	70.0
15	1.50	50	2.0	96.72
16	2.00	70	1.5	100.4
17	1.00	70	2.5	72.4
18	1.50	70	1.5	99.2

Examination of these coefficients with a *T*-test shows that the FFA% (*Y*), linear, square and interaction terms of ethanolic KOH (X_1) were highly significant ($p < 0.01$), and that the linear terms of reaction temperature $^{\circ}$ C (X_2) were highly significant ($p < 0.01$), while the 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 2) for the FFA% (*Y*) are given below:

Table 2 Regression coefficients of the predicted quadratic polynomial model for response variables *Y* (FFA%).

Variables	Coefficients (β) % FFA (<i>Y</i>)	<i>T</i>	<i>P</i>	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 1 for a description of the abbreviations

$$Y = +96.65 + 17.28X_1 + 4.33X_2 + 1.91X_3 - 15.14X_1^2 + 0.37X_2^2 + 0.63X_3^2 - 3.48X_1X_2 - 1.40X_1X_3 + 0.11X_2X_3 \quad (1)$$

ANOVAs for the fitted models are summarised in Table 3. The examination of the model with an *F*-test and *T*-test indicate a 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 2). This indicates that the generated models adequately explained the data variation and represented the actual relationships among the reaction parameters [16].

Equation (1) showed 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 [16]. Significant interaction variables in the fitted models (Table 2) were chosen as the axes (concentration of ethanolic KOH; X_1 , reaction temperature; X_2 and reaction time; X_3) for the response surface plots. The relationships

Table 3 Analysis of variance (ANOVA) of the response *Y* (FFA%) of the D-optimal design

Source	<i>Df</i> ^a	Sum of squares	Mean square	<i>F</i> ^b	<i>P</i> ^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: *Df*: degree freedom (a); *F*-value: distribution (b); *P*-value: scale (c).

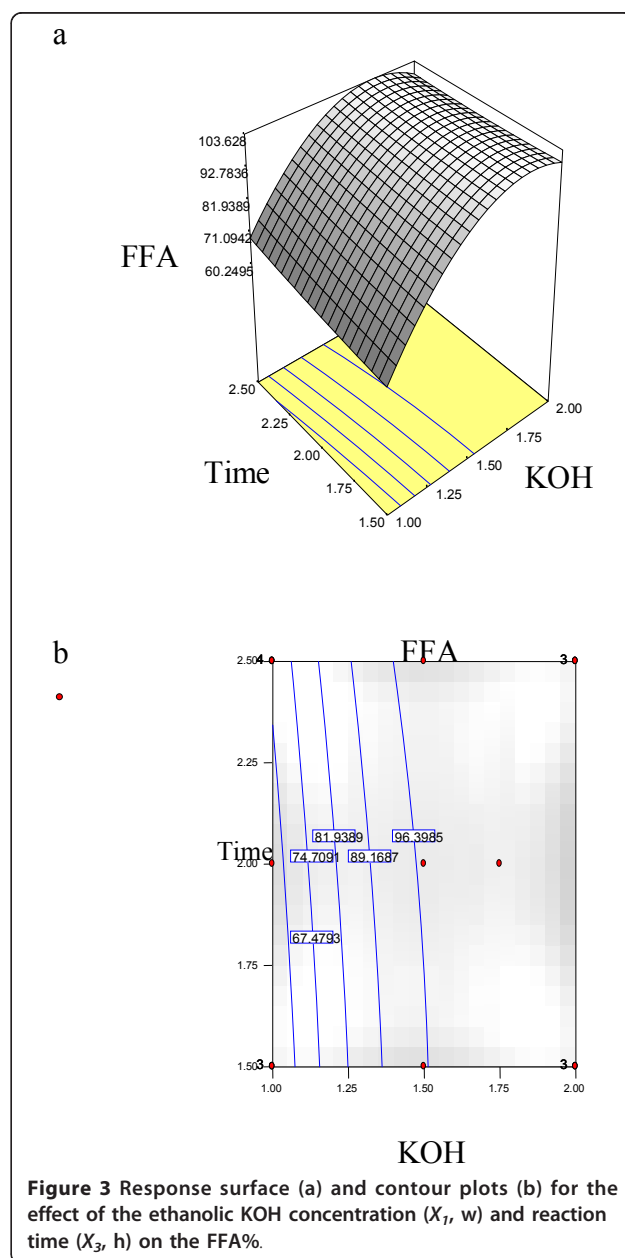
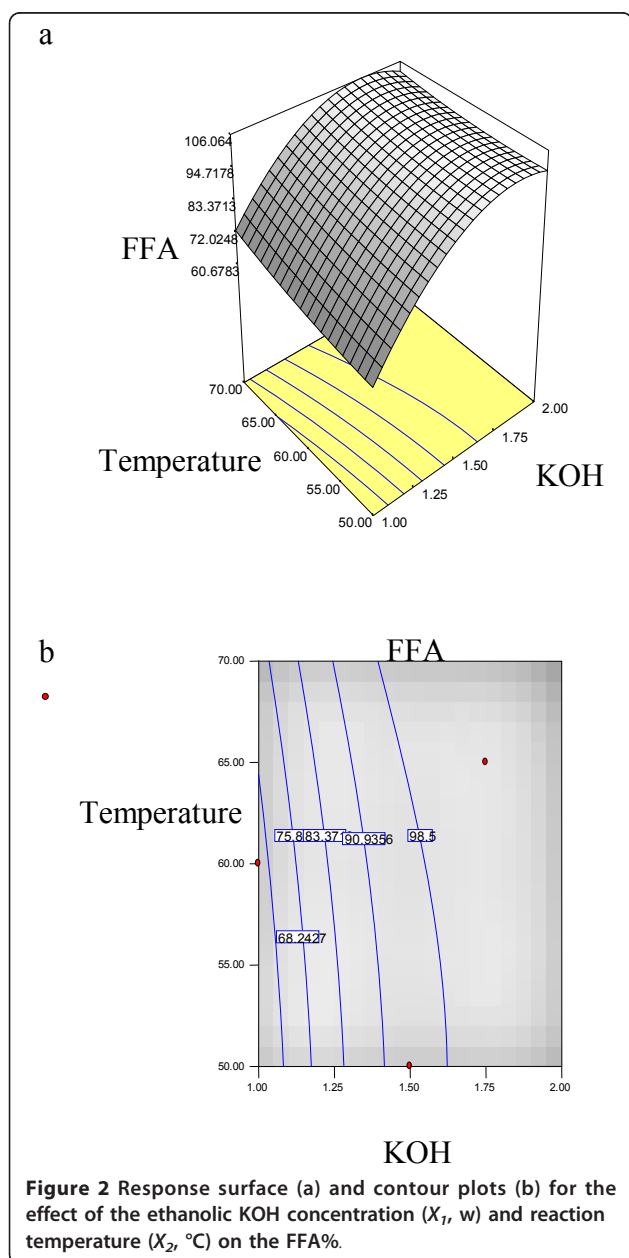
between independent and dependent variables are shown in the three-dimensional representation as response surfaces. The response surfaces for the FFA% (Y) in the concentrates were given in Figures 2, 3 and 4.

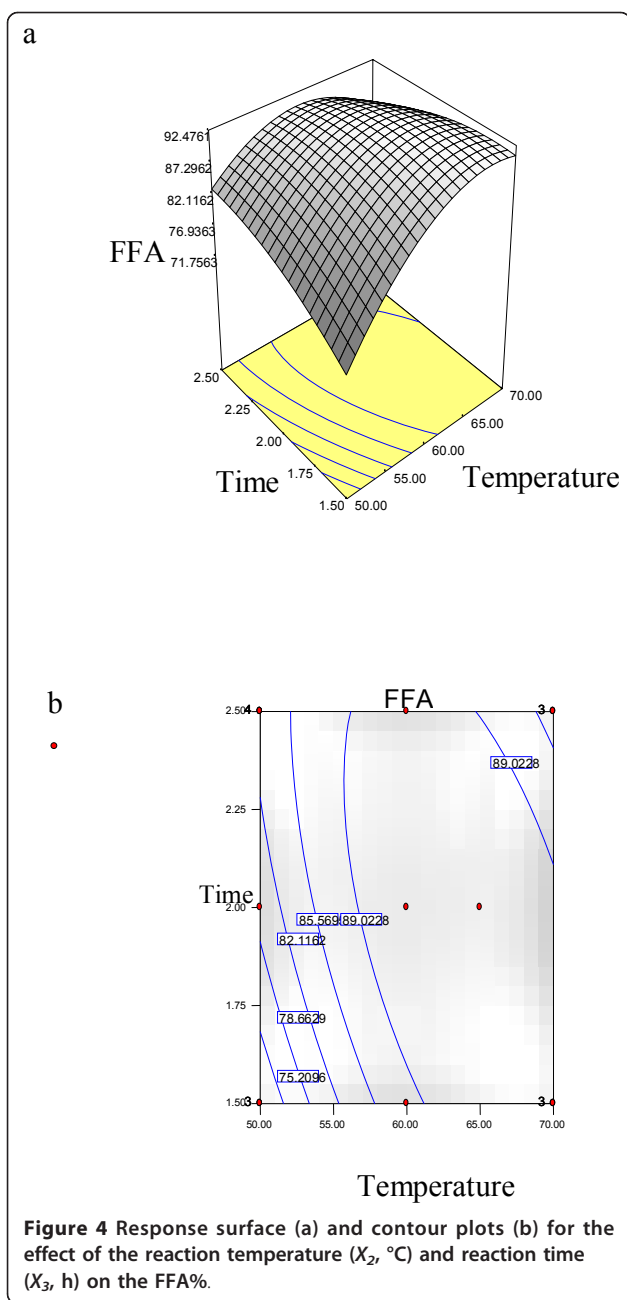
The contour plot (Figures 2b, 3b and 4b) shows the combination of levels of the concentration of KOH and reaction temperature that can provide the same FFA%. Canonical analysis was performed on the predicted quadratic polynomial models to examine the overall shape of the response surface curves and used to characterise 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 [16,17]. Ethanolic KOH concentration was the most important factor for FFA%, which the observed value was reasonably close to the predicted value as shown in Figure 5.

GC-FID Analysis of Fatty Acids Composition

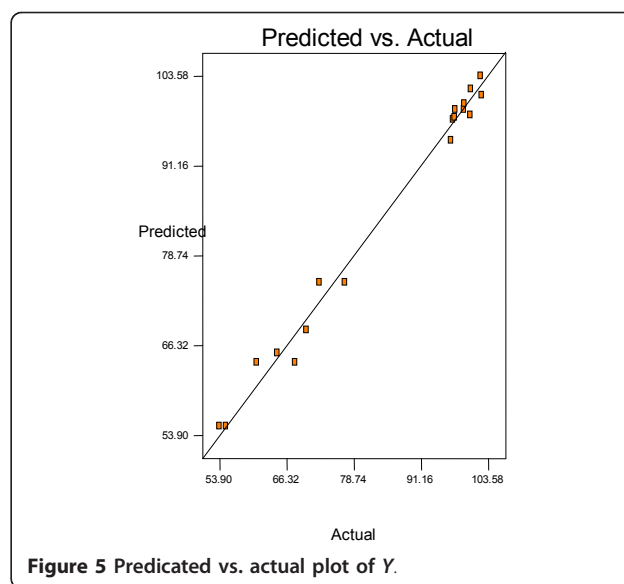
Response surface methodology (RSM) was employed to study the composition of FFA by ethanolic KOH concentration of *J. curcas* seed oil hydrolysis through FAMES analysis before and after the hydrolysis. The analyses made by GC-FID had a positive identification





of FAs. Experimental results of the percentage of the composition of FAs for ethanolic KOH reactions with *J. curcas* seed oil are given in Table 4. The comparative data indicate that no significant difference under the optimum conditions $p < 0.05$.

Table 4 shows a comparison of the FAs composition before the hydrolysis (a) and after the hydrolysis at different ethanolic KOH concentration (b and c, respectively), as determined directly by GC-FID, through FAMES analysis. Intermediate products formed in the hydrolysis, as well as the methyl esters by FAMES [3].



The comparative data indicate that the hydrolysis does not cause the decomposition of the FAs.

FTIR Analysis of Fatty Acids

In order to prove the *J. curcas* seed oil hydrolysis, FTIR spectroscopy supported the FFA% by showing the main peaks and their functional groups of the *J. curcas* seed oil. The comparison between *J. curcas* seed oil (a), hydrolysis at 1.00M (b) and at 1.75M of ethanolic KOH concentration (c), FTIR spectra is shown in Figure 6. The main peaks and their assignment to functional groups are given in Table 5.

For carboxylic acid carbonyl functional groups ($C = O$), FTIR spectrum showed absorption bands of hydrolysis oil (b and c) at 1711 cm^{-1} for stretching vibration, $1283\text{-}1285\text{ cm}^{-1}$ for stretching asymmetric while at 1413 and $918\text{-}937\text{ cm}^{-1}$ for bending vibration of carboxylic acid [18]. The hydrolysis of *J. curcas* seed oil at 1.75M show disappeared completely of ester groups at 1746 and 1163 cm^{-1} .

Figure 6 show the main change of hydrolysis *J. curcas* seed oil (b and c), which (b) 1.00M ethanolic KOH solution show low hydrolysis with $C = O$ (ester carbonyl) at 1739 , 1180 cm^{-1} while (c) at 1.75M ethanolic KOH solution shows high hydrolysis with strong absorption. Peaks at $2925\text{-}2854\text{ cm}^{-1}$ indicated the CH_2 and CH_3 scissoring of both *J. curcas* seed oil and hydrolysis oil which showed on Figure 6(a), (b) and 6(c). FTIR spectrum also showed absorption bands at 722 cm^{-1} for ($C\text{-H}$) group vibration.

HPLC Analysis of Fatty Acids

The results by using higher performance liquid chromatography (HPLC) show the hydrolysis performances of

Table 4 Fatty acid composition before and after *J. curcas* seed oil hydrolysis

Fatty acids	FA% before hydrolysis ^a	FA% after hydrolysis 1.00M ^b	FA% after hydrolysis 1.50M ^c	FA% after hydrolysis 1.75M ^d
Palmitic	13.19	13.55	13.06	13.07
Palmitoleic	0.40	0.64	0.56	0.55
Stearic	6.36	4.52	6.78	6.80
Oleic	43.32	43.94	43.97	43.03
Linoleic	36.70	37.32	36.46	36.51

Notes: *J. curcas* seed oil (a), hydrolysis at 70°C (b), (c) and (d)

the ethanolic KOH concentration effects on the hydrolysis reaction when submitted to different concentrations of the ethanolic KOH (1.0, 1.5 and 1.75M). The study of variation yield of the hydrolysis *J. curcas* seed oil has been showed in Figures 7, 8, 9 and 10.

Figure 7 illustrates a typical profile of triacylglycerol obtained of non hydrolyzed *J. curcas* seed oil. Figures 8, 9 and 10 illustrate the variation of the chromatographic profile of hydrolyzed *J. curcas* seed oil as a function of the ethanolic KOH concentration effects at 1.00, 1.50 and 1.75M, respectively.

HPLC chromatogram results showed, that with increasing ethanolic KOH concentration an increase in the FFA% and decreases of the concentration of the triacylglycerol is observed, fact that supports the hydrolysis model under investigation. A different observation was reported by other researchers for hydrolysis of various vegetable using *C. rugosa* lipase [13-15]. Increase in enzyme concentration did not give any significant changes in the reaction rate [15]. Therefore, further increase in ethanolic KOH solution concentration show improvement in the conversion.

Experimental

Procedure of *J. curcas* Seed Oil Hydrolysis

FFA was obtained by the hydrolysis of *J. curcas* seed oil, as carried out by [19]. Table 6 shows different ethanolic KOH concentration, different reaction temperature and

different reaction time using RSM (D-optimal design). Factors such as ethanolic KOH concentration (M, X_1), temperature (°C, X_2) and time (h, X_3) were performed under the same experimental conditions. In a typical experiment, *J. curcas* seed oil 50 g was mixed in the reactor with 300 mL of saponifying solution comprising of ethanolic KOH concentration (1.00-2.00 M), and ethanol (300 mL: 90% v/v). The hydrolysis was carried out in a 500 mL temperature-controlled reactor at different reaction temperature 50-70°C and different reaction time 1.5-2.5 h as shown in Table 6. After the hydrolysis, 200 mL water was added to the mixture. Unsaponifiables were separated by extraction with 100 mL of hexane. The aqueous alcohol phase, containing the soaps, was acidified to pH 1 with HCl 6N, and the FFA was recovered by extraction with hexane. The extract was washed with distilled water to neutral pH. The resulting lower layer was removed using a separating funnel and discarded. The FFA-containing upper layer was dried with anhydrous magnesium sulfate, and solvent was evaporated in a vacuum rotary evaporator at 35°C. The FFA% was measured.

Determination of the FFA%

The FFA% of the hydrolysis of *J. curcas* seed oil was determined according to [20]. Approximately 50 mL of isopropanol was placed into the flask, and about 0.5 mL phenolphthalein was added and was neutralised by addition of sodium hydroxide (NaOH, 0.02N) until a permanent pink colour was obtained. The neutralised isopropanol was added to the 5 g of FFA, which was placed into an Erlenmeyer flask, and about 0.5 mL of phenolphthalein was added. After shaking the mixture gently, the mixture was neutralised by the addition of (NaOH, 0.02N) until the first permanent pink colour was obtained. The FFA% was calculated by using equation 2.

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

Gas Chromatography Method Analysis of Fatty Acids Composition

Gas chromatography method (GC) analysis was performed on Shimadzu equipped with flame ionization

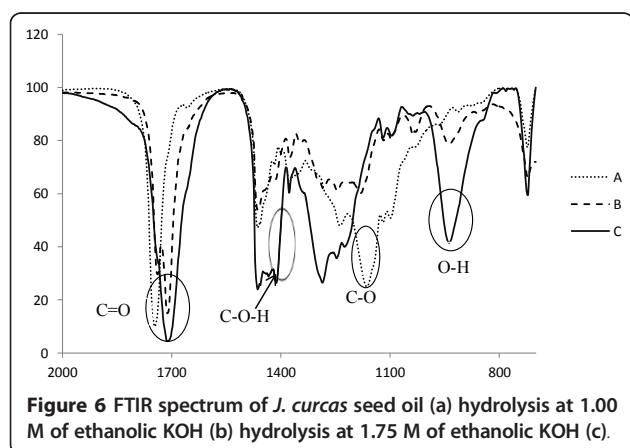


Table 5 The main wavelengths in the FTIR functional groups of *J. curcas* seed oil hydrolysis

Wavelength of oil ^a	Wavelength of 1.00M ^b	Wavelength of 1.75M ^c	Functional group
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 asymmetric (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: *J. curcas* seed oil (a), hydrolysis at 1.00 M of ethanolic KOH (b), hydrolysis at 1.75 M of ethanolic KOH (c).

detector and capillary column (30 m × 0.25 mm × 0.25 mm film). The parameters of GC have been carried out according to [21].

Fourier Transforms Infrared Spectroscopy analysis of Fatty Acids

Fourier transforms infrared spectroscopy (FTIR) has been carried out according to [21]. FTIR of the products was recorded on a Perkin Elmer Spectrum GX spectrophotometer in the range 400-4000 cm⁻¹. FTIR was used to measure functional groups of FA. A very thin film of FA was covered on NaCl cells (25 mm.i.d × 4 mm thickness) and was used for analysis.

High Performance Liquid Chromatography Method Analysis of fatty Acids

High performance liquid chromatography (HPLC) was performed on waters model 1515 equipped with refractive index detector and Spherisorb C18 column (250 mm × 4.8 mm × 3 mm) was used for analysis the TAG, DAG, MAG and FFA. The parameters of HPLC have been carried out according to [21]. The samples were

dissolved in 10 mL of the mixture acetone: acetonitrile before 20 mL of the sample inject into HPLC.

Experimental Design (D-Optimal) and Statistical Analysis

A three-factor D-optimal design was employed to study the responses of FFA% [Y in % by wt, see Eq. (2)]. An initial screening step was carried out to select the major response factors and their values. The independent variables were X₁, X₂ and X₃ representing the concentration of 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 1.00 and 2.00 M; hydrolysis temperature of 50 and 70°C; and hydrolysis time of 1.5 and 2.5 h. Each variable to be optimized was coded at three levels: -1, 0, and +1. Six replicates the D-optimal design is shown in Table 6. A quadratic polynomial regression model was assumed for predicting individual Y variables. The model proposed for each response of Y was:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \sum \beta_{ij} x_i x_j \quad (3)$$

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

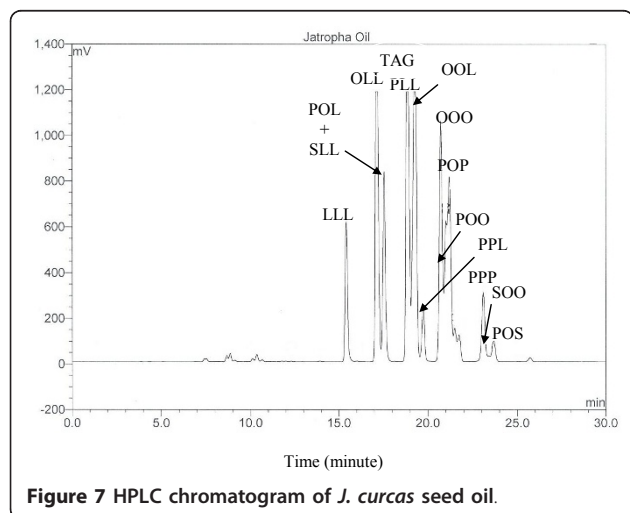


Figure 7 HPLC chromatogram of *J. curcas* seed oil.

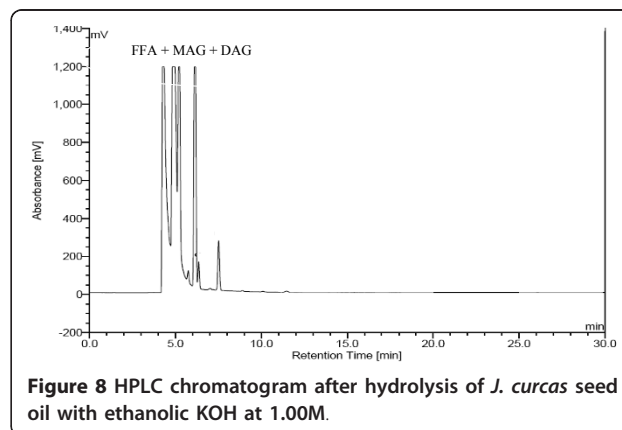


Figure 8 HPLC chromatogram after hydrolysis of *J. curcas* seed oil with ethanolic KOH at 1.00M.

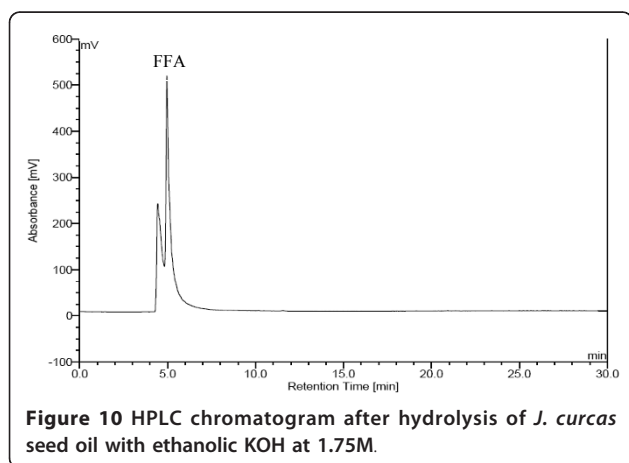
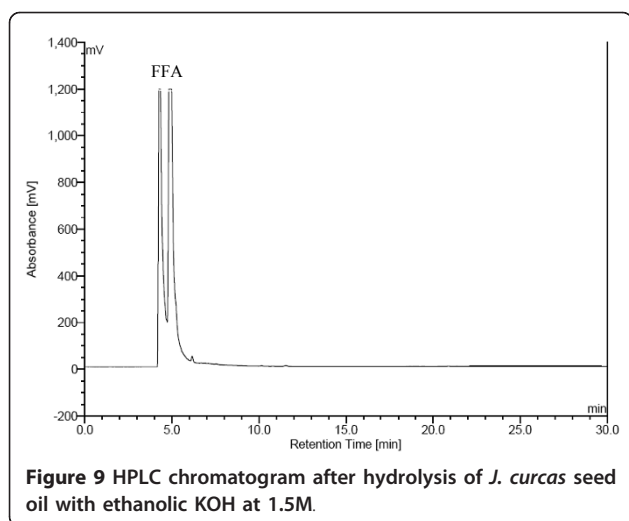


Table 6 Independent variables and their levels for D-optimal design of the hydrolysis reaction

Independent variables		Variable levels		
		-1	0	1
KOH (M)	X_1	1	1.5	2
Temperature (°C)	X_2	50	60	70
Time (h)	X_3	1.5	2	2.5

multiple regression analysis, analysis of variance (ANOVA), and analysis of ridge maximum of data in the response surface regression (RSREG) procedure. The goodness of fit of the model was evaluated by the coefficient of determination R^2 and the analysis of variance (ANOVA) [16].

Conclusion

J. curcas seed oil hydrolysis, under optimum conditions, a highest hydrolysis was achieved. Hydrolysis occurs rapidly at 1.75M of ethanolic KOH, yielding 102.2% of

the FFA. The analyses made by GC-FID had a positive identification of FAs composition and there is no significant difference under the optimum conditions $p < 0.05$. FTIR analyses showed strong absorption of carboxylic acid peaks at 1711 and 1285 cm^{-1} . The results by using HPLC showed with increasing in ethanolic KOH concentration shows improvement in the *J. curcas* seed oil hydrolysis.

Acknowledgements

We would like to thank UKM and the Ministry of Science and Technology for research grant UKM-GUP-NBT-08-27-113 and UKM-OUP-NBT-29-150/2011.

Authors' contributions

JS developed the concept, analyzed the data and drafted the manuscript. BMA performed the hydrolysis reaction optimization and its characterization study. NS advised on the methods of tests. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 1 August 2011 Accepted: 1 November 2011

Published: 1 November 2011

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doi:10.1186/1752-153X-5-67

Cite this article as: Salimon *et al.*: Hydrolysis optimization and characterization study of preparing fatty acids from *Jatropha curcas* seed oil. *Chemistry Central Journal* 2011 **5**:67.

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