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Optimization of Process Variables using D-optimal Design for Separating Linoleic acid in *Jatropha curcas* Seed Oil by Urea Complex Fractionation

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Abstract: Linoleic Acid (LA) from a mixture of free fatty acids obtained from local *Jatropha curcas* seed oil was separated using urea complex fractionation. In this study, variations of factors that affect the urea complex fractionation such as the ratio of urea to free fatty acid, crystallization temperature and crystallization time were examined to obtain optimum condition using response surface method (D-optimal design). Under the optimal conditions, the percentage of LA was 92.81 and the percentage of yield of NUCF 7.8 at urea/FFA ratio (w/w) 5, a crystallization temperature of -10°C and a crystallization time 24 h. This showed that the experimental conditions should be suitable for the preparation of high purity LA. Performing the experiment at this condition gave results that were close to the predicted values.

Key words: Fatty acid, linoleic acid, urea, D-optimal design, separation

INTRODUCTION

Jarak pagar or the scientific name is *Jatropha curcas* is live widly and belongs to Euphorbiaceae family. It is cultivated in central and south America, Southeast Asia, India and Africa (Gübitz *et al*., 1996). The *Jatropha curcas* seed oil can produce about 40-60% oil which is rich with unsaturated fatty acid. The fatty acids are palmitic (13.00%), stearic (2.53%), oleic (48.80%) and linoleic acid (34.60%). However, the chemical compositions of the oil vary according to the climate and locality (Martínez-Herrera *et al*., 2006).

Linoleic acid is an essential fatty acid; the high content of linoleic acid makes this oil very importance to the industries. The linoleic acid can be used in protective coatings, plastics, urethane derivatives, surfactant, dispersants, cosmetics, lubricants and varieties of synthetic intermediate, stabilizers in plastic formulations and in the preparations of other long chain compounds. The high content of linoleic acid in seed oil such as *Jatropha curcas* seed oil is very important to the production of oleo-chemicals (Hosamani and Katagi, 2008).

There are several method can be used to obtain unsaturated fatty acid including freezing crystallization, urea complexation, molecular distillation, supercritical fluid extraction, silver ion complexation, lipase concentration (Lui *et al*., 2006) and HPLC (high-performance liquid chromatography) (Yamamura and Shimomura, 1997). The cheapest and efficient technique to obtain linoleic acid in the form of free fatty acid is urea complex fractionation. This is well established technique to elimination of saturated and monounsaturated fatty acids (Wanasundara and Shahidi, 1999; Chen and Ju, 2001). Urea complexation has the advantage that complexes crystal is extremely stable and filtration does not necessarily have to be carried out at the very low temperatures which solvent crystallization of fatty acids would be required. This methods also favored by many researchers because complexation depends upon configuration of the fatty acid moieties due to the presence of multiple double bonds, rather than pure physical properties such as melting point or solubility (Wanasundara and Shahidi, 1999). In the urea complex fractionation, the saturated and monounsaturated fatty acids easily complex with urea and crystallize out on cooling and may subsequently be removed by filteration. The liquid and Non-Urea Complexed Fraction (NUCF) is enriched with unsaturated fatty acids and the crystals formed or Urea Complexed Fraction (UCF) is consist saturated fatty acids.

In this study, urea complex fractionation of a mixture of free fatty acids of local *Jatropha curcas* oil was carried out to obtain concentrated with high unsaturated fatty

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acids. The effects of urea/FFA ratio (w/w), crystallization temperature (°C) and crystallization time (h) on the percentage of linoleic acid and yield of linoleic acid were systematically studied.

MATERIALS AND METHODS

Materials: *Jatropha curcas* seeds were obtained from the Universiti Kebangsaan Malaysia field at 15/September/2009. 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 *Jatropha curcas* seed oil was carried out using hexane as a solvent for 6 h.

Hydrolysis: Free fatty acid (FFA) was obtained by the saponification of *Jatropha curcas* seed oil. Typically, a KOH solution was prepared by dissolving 25 g KOH in 90% aqueous ethanol. A mixture containing 50 g oil and 300 mL KOH solution was heated at different temperatures and times. One hundred milliliter of hexane and water 200 mL were then added. A 6 N hydrochloric acid was then added until the solution was at pH 1. 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.

Urea complex fractionation: The separation of unsaturated fatty acid from the hydrolyzed fatty acids of *Jatropha curcas* seed oil was carried out by Wu *et al.* (2008). FFA (10 g) were mixed with urea in 95% aqueous ethanol and heated at 60°C with stirring until the mixture was turned into a clear homogeneous solution. The ratio of urea/FFA was changed by using different amounts of urea. Initially, the urea-fatty acid adduct was allowed to crystallize at room temperature but colder temperatures were maintained later for different periods for further crystallization. The crystals formed (urea-fatty acid adducts, also referred to as the urea complexing fraction; UCF) were separated from the liquid (non-urea complexing fraction, NUCF) by fast filtration. The liquid (NUCF) was diluted with an equal volume of water and acidified to pH 2-3 with 6 N HCl; an equal volume of petroleum ether was subsequently added and the FFA were extracted. The top phase, containing liberated fatty acids, was separated from the aqueous layer containing urea. The petroleum ether layer was washed with 5% NaCl solution to remove any remaining urea and then dried over anhydrous Na_2SO_4 and the solvent was then removed at 65°C using a rotary evaporator.

Fatty acids analysis by GC-FID: The identification of the fatty acids was accomplished by gas chromatography. FFAs were converted to FAME for GC analysis according to PORIM Method. Fatty acid profile was analyzed with Shimadzu GC-17A with a BPX70 column (30 m × 0.25 mm × 0.25 μm films). Injection and detection (FID) temperatures were set at 260 and 280°C, respectively and nitrogen was used as the carrier gas with flow rate of 0.3 mL min^{-1}. split ratio was 1: 39.

RESULTS AND DISCUSSION

Experimental design and statistical analysis: A three-factor D-optimal design (Box, 1954; Cornell, 1992) was employed to study the responses, namely after urea inclusion fractionation the yield of NUCF (Y1 in % by wt., Eq. 2) and percentage of linoleic acid (Y2 in %, Eq. 3). 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 urea-to-fatty acid ratio (w/w), crystallization temperature (°C) and crystallization time (h), respectively. The settings for the independent variables were as follows (low and high values): urea-to-fatty acid ratio of 1 and 5; crystallization temperature of -10 and 10, crystallization time of 8 and 24. Each variable to be optimized was coded at four levels: -1, 0 and +1, the d-optimal design is shown in Table 1.

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 xi and xj 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 of fit of the model was evaluated by the coefficient of determination

Table 1: Independent variables and their levels for D-optimal design

	Code variable levels		
Independent variables	-1	0	+1
The urea-to-fatty acid ratio (w/w) (g/g) (X1)	1	3	5
Crystallization temperature (°C) (X2)	-10	0	10
Crystallization time (h) (X3)	8	16	24

R2 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

The initial fatty acid mixture was composed of 13.19% palmitic (16:0), 0.40% palmitoleic (16:0), 6.36% stearic (18:0), 43.32% oleic (18:1) and 36.70% linoleic (18:2). Average molecular weight of the fatty acids was 203.36 as obtained from saponification test of the original oil. The results agree with those of (Salimon and Abdullah (2008). In this chapter, the PUFA (linoleic acid) concentration has been done by using urea complex fractionation (Guil-Guerrero and Belarbi, 2001), using the FFA that was previously obtained.

The purpose of this procedure is to obtain a PUFA concentrate as enriched in linoleic acid as possible and, simultaneously, to maintain the highest yields of linoleic acid. The crystallization process with urea preferentially selects saturated and monounsaturated fatty acids and the tendency of fatty acids to combine with urea decreases with increasing chain length (Abu-Nasr *et al*., 1954).

Experimental values obtained for response, the yield percentage of NUCF and the percentage of linoleic acid for twenty five design points are given in Table 2. The results show that LA had been purified in the filtrate, while monounsaturated fatty acid (oleic acid) and saturated fatty acid (Palmitic, Palmitoleic and stearic acids) were enriched in the crystal phase. Thus these results demonstrate that oleic, palmitic, palmitoleic and stearic acids have more tendency to form urea adducts than LA.

Hayes (2006) and Hayes *et al.* (1998) have reported similar results for urea complex fractionation experiments carried out for low erucic acid rapeseed oil, canola and vegetable and fish oil. In certain experimental conditions the % of LA derived from the NUCF phase was relatively high and some even greater than 90% (Table 2). This showed that the experimental conditions should be suitable for the preparation of high purity LA. However, it is difficult to completely remove all the saturated fatty acids to obtain 100% purity of unsaturated fatty acids in the concentrate.

Ratnayake *et al.* (1988) has reported that complete removal of saturated fatty acids by urea complexation may be impossible since some of the saturated fatty acids do not bind with urea during crystallization.

Model fitting: The quadratic regression coefficient obtained by employing a least squares method technique to predict quadratic polynomial models for the yield percentage of NUCF (Y1) are given in Table 3. Examination of these coefficients with a T-test shows that for the yield percentage of NUCF (Y1)), the linear and square terms of urea-to-fatty acid ratio (X1) were highly significant ($p<0.01$) and the quadratic terms of the urea-fatty acid ratio (X1) of the yield percentage of the NUCF (Y1) was significant ($p<0.05$).

The urea-to-fatty acid ratio (X1) and the crystallization temperature (X2) for the yield percentage of linoleic acid (Y1) in the concentrate were significant at $p<0.05$ and others between any two of the four factors were not.

Table 2: D-optimal design arrangement and responses for non-urea-complexed fraction of *Jatropha curcas* seed oil

Run	Urea/FFA (X1)	Temp. (X2)	Time (X3)	Yield% (Y1)	Linoleic% (Y2)
1	1	10	8	49.0	58.23
2	3	0	24	22.2	85.16
3	2	0	16	34.9	69.41
4	3	-10	8	32.3	77.74
5	5	10	24	7.7	88.60
6	5	-10	24	7.8	92.81
7	1	10	24	50.6	54.91
8	1	-10	24	34.1	61.46
9	5	10	8	8.8	87.82
10	5	0	16	6.2	89.19
11	1	-10	16	48.1	59.85
12	1	0	8	31.3	54.63
13	5	10	24	4.1	92.14
14	3	10	16	31.6	78.42
15	1	10	8	45.6	52.53
16	5	-10	8	6.6	88.92
17	5	0	8	20.5	88.12
18	1	10	16	49.7	54.87

Table 3: Regression coefficients of the predicted quadratic polynomial model for response variables (the yield percentage of linoleic acid) in urea inclusion fractionation experiment of *Jatropha curcas* seed oil

Variables	Coefficients (β) The yield percentage of linoleic acid (Y_1)	T	p-value	Notability
Intercept	25.47	16.7	0.0003	***
Linear				
X1	-17.14	121.9	0.0001	
X2	1.97	1.61	0.2402	
X3	-1.59	0.99	0.348	
Quadratic				
X11	-3.36	0.83	0.3893	**
X22	8.71	6.25	0.0369	
X33	-3.82	1.34	0.28	
Interaction				
X12	-0.018	1.12E-04	0.9918	
X13	-0.41	0.053	0.8238	
X14	1.37	0.59	0.465	
R2	0.94			

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

Table 4: Regression coefficients of the predicted quadratic polynomial model for response variables (the percentage of linoleic acid) in urea inclusion fractionation experiment of *Jatropha curcas* seed oil

Variables	Coefficients (β) The percentage of linoleic acid (Y2)	T	p-value	Notability
Intercept	80.96	218.98	0.0001	***
Linear				
X1	16.26	1689.58	0.0001	***
X2	-1.81	20.82	0.0018	***
X3	1.83	20.29	0.002	***
Quadratic				
X11	-7.21	58.83	0.0001	***
X22	-0.76	0.73	0.4181	
X33	0.11	0.018	0.8975	
Interaction				
X12	0.91	4.53	0.0659	
X13	-0.34	0.54	0.4846	
X14	-0.73	2.58	0.1467	
R2	0.99			

**p<0.05; ***p<0.01. T: F test value. See table 2 for a description of the abbreviations

Table 4 shows the percentage of linoleic acid (Y2) for the quadratic regression coefficient obtained by employing a least squares method technique to predict quadratic polynomial models. Examination of these coefficients with a T-test shows that for the percentage of linoleic acid (Y2) the linear, square and quadratic terms of urea-to-fatty acid ratio (X1) were highly significant ($p<0.01$). Among six interactions, the urea-to-fatty acid ratio (X1) for the percentage of linoleic acid (Y2) were highly significant.

The coefficients of independent variables (urea-to-fatty acid ratio; X1, crystallization temperature; X2 and crystallization time; X3) determined for the quadratic polynomial models (Table 3) for the yield percentage of NUCF (Y1) and percentage of linoleic acid (Y2) are given below:

$$Y_1=+25.47 -17.14X_1 +1.97 X_2 -1.59X_3 -3.36 X_1^2+8.71X_2^2 -3.82X_3^2 -0.018 X_1X_2 -0.41X_1X_3+1.37 X_2X_3 \quad (2)$$

$$Y_2 = +80.96+16.26X_1-1.81X_2+1.83X_3-7.21X_1^2-0.76X_2^2 +0.11X_3^2 +0.91X_1X_2 -0.34X_1X_3 -0.73X_2X_3 \quad (3)$$

Diagnostic checking of the fitted models: ANOVAs for the fitted models are summarized in Table 5. Examinations of the two models with an F-test and P-test indicate a non-significant lack-of-fit at $p>0.05$ and pure error was 37.64%. The regression coefficient (R2) of the yield percentage of NUCF (Y1) was 0.94 (Table 3).

Table 6 shows the ANOVAs for the fitted models. Examinations of the two models with an F-test indicate a non-significant lack-of-fit at $p>0.05$ but the P-test indicate a significant lack-of-fit was 0.0486 at $p<0.05$ and pure error was 0.14%. The regression coefficients (R2) of the percentage of linoleic acid (Y2) were 0.99 (Table 4). These indicate that the generated models adequately explained the data variation and represented the actual relationships among the reaction parameters.

Table 5: Analysis of variance, showing the effect of the variables as linear, square and interactions on the response Y1 (the yield % of NUCF) of the D-optimal design

Source	df	Sum of squares	Mean square	F-value	p-value
Mean	1	13398.84	13398.84		
Linear	3	4516.14	1505.38	40.43	0.0001
2FI	3	46.62	15.54	0.36	0.783
Quadratic	3	220.08	73.36	2.31	0.1534
lack-of-fit	6	179.27	29.88	0.79	0.6507
Pure error	2	75.29	37.64		
Total	18	18436.25	1024.24		

Table 6: Analysis of variance, showing the effect of the variables as linear, square and interactions on the response Y2 (the % of linoleic acid) of the D-optimal design

Source	df	Sum of squares	Mean square	F-value	p-value
Mean	1	98998.75	98998.75		
Linear	3	3914.05	1304.68	103.44	0.0001
2FI	3	11.99	4	0.27	0.8477
Quadratic	3	148.05	49.35	23.87	0.0002
lack-of-fit	6	16.27	2.71	19.9	0.0486
Pure error	2	0.27	0.14		
Total	18	1.03E+05	5727.19		

Response surface plotting and optimization in the linear weighting method: Equation 2 and 3 showed that the yield percentage of NUCF and percentage of linoleic acid 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, 4) were chosen as the axes (urea-to-fatty acids ratio X1 and crystallization temperature X2) 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 yield percentage NUCF (Y1) were given in Fig. 1a and b.

The response surfaces of the percentage of linoleic acid (Y2) is shown in Fig. 2a and b. 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 (Wanasundara and Shahidi, 1999).

The contour plots (Fig. 1b, 2b) show the combination of levels of the urea-to-fatty ratio and crystallization temperature that can afford the same level of the yield

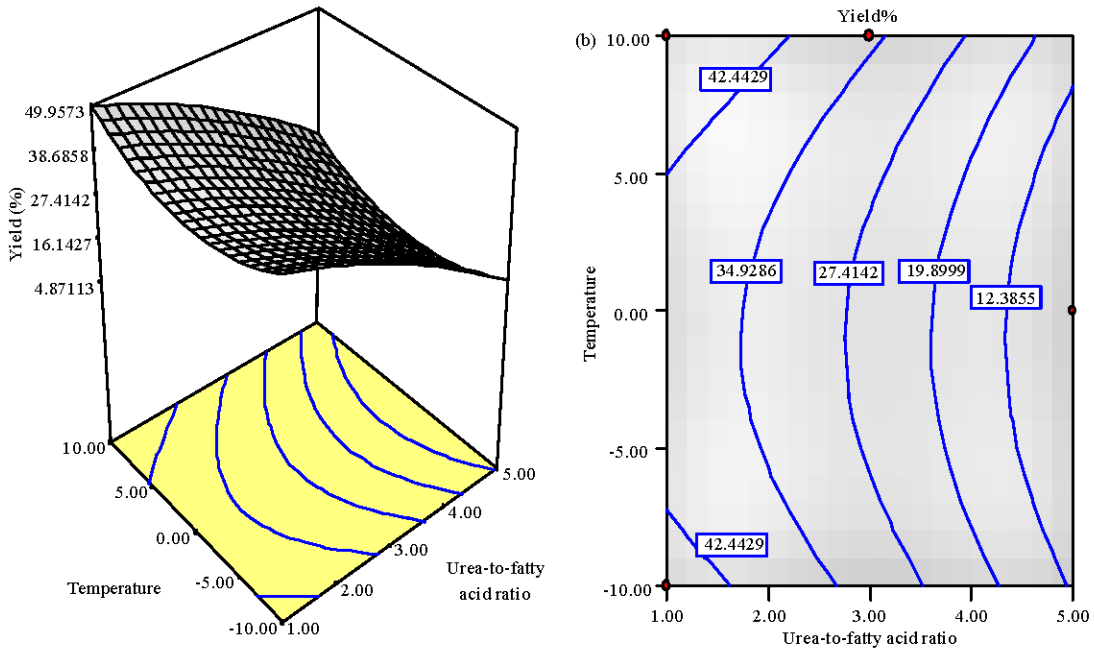

Fig. 1: (a) Response surface and (b) contour plots for the effect of the urea/FFA ratio (X1, w/w) and temperature (X2, °C) on the yield % of NUCF (Y1, %) in the NUCF

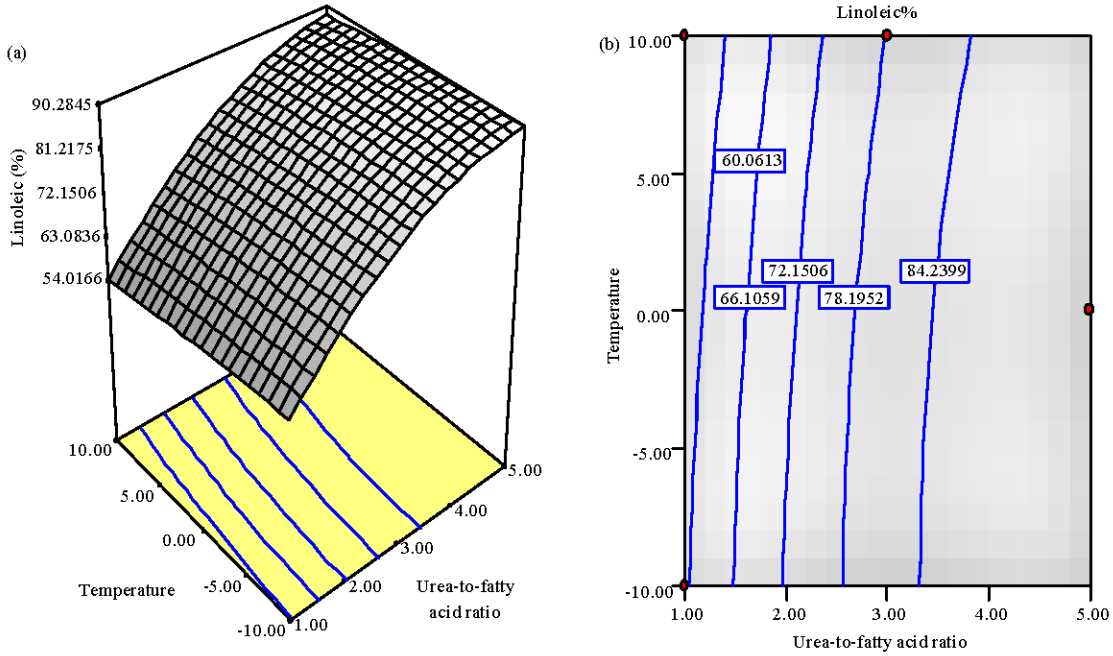

Fig. 2: (a) Response surface and (b) contour plots for the effect of the urea/FFA ratio (X1, w/w) and temperature (X2, °C) on the % of linoleic acid (Y2, %) in the NUCF

percentage of NUCF and percentage of linoleic acid. 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 (Wanasundara and Shahidi, 1999; Mason *et al*., 1989).

The model of separation of oleic acid and saturated fatty acid was developed on the basis of the analysis of RSM. The urea-to-fatty acid ratio was the most important parameter for the yield percentage of NUCF, percentage of oleic acid and percentage of saturated fatty acid. The process may be help produce highly pure of linoleic acid and yield percentage of NUCF from an economic point of view, as well as being a promising measure for further utilization of agriculture products.

CONCLUSIONS

From these results, we demonstratd that urea/FFA ratio was the most important parameter for the yield percentage of NUCF and the percentage of linoleic acid. In addition, the urea method can be used when the goal is to obtain percentage of linoleic acid. The process may be help produce highly percentage linoleic acid from an econmic point of view, as well as being a promising measure for further utilization of agriculture products.

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