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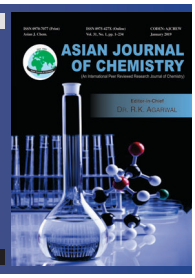

Optimization of Low-Temperature Methanol Crystallization for Unsaturated Fatty Acids Separation from Crude Palm Fatty Acids Mixture Using Response Surface Methodology

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This current work studies the separation of unsaturated fatty acids from crude palm fatty acids mixture by methanol crystallization. Separation conditions of unsaturated fatty acid were optimized through the adoption of the response surface method and the optimal model was developed. The percentages of unsaturated fatty acids recovered under the optimal condition were higher than 95 ± 0.3 % with the yield of product percentage 55.2 ± 0.8 %. The optimized condition comprised of three essential aspects methanol-to-fatty acids ratio of 15:1 (mL:g), crystallization temperature of –15 ºC and crystallization time of 20.8 h, respectively. The unsaturated fatty acids composition isolated from crude palm fatty acids mixture consisted of 76.3 % oleic acid (C18:1), 18.3 % linoleic acid (18:2) and 0.2 % linolenic acid (C18:3). Only 4.9 ± 0.3 % of saturated fatty acids was discovered in the product. The iodine value of separated unsaturated fatty acids had increased from 54.0 ± 0.1 mg/g to 94.4 ± 0.2 mg/g. The results have demonstrated that low-temperature methanol crystallization is very active, low cost, stable and obtainable, and comparatively ease to recover for the separation of unsaturated fatty acids from an oil mixture of fatty acids.

Keywords: Unsaturated fatty acids, Crude palm oil, Methanol crystallization, D-optimal Design.

INTRODUCTION

Crude palm oil (CPO) has almost equal quantities of saturated fatty acids (palmitic acid 44 % and stearic acid 4 %) and unsaturated fatty acids (oleic acid 41 % and linoleic acid 9.6 %) [1]. Unsaturated fatty acids (USFAs) accounts for one or more double bonds in the fatty acid chain [2]. A monounsaturated fatty acid molecule has one double bond in its fatty acid chain and molecules with more than one double bond are characteristically polyunsaturated [3]. The unsaturated fatty acids content of crude palm oil is significant for diverse scientific and industrial uses. Industrial applications have been dependent upon the use of USFA [4,5]. Likewise, unsaturated fatty acids is also integral to the preparation of other long chain ester compounds [6]. There is a scarcity of works that have sought to emphasize USFAs systematically. Each method came up with varied percentages yields such as adsorption chromatography, fractional or molecular distillation and urea inclusion techniques [7]. In present study, the simplest and most efficient technique to obtain USFAs concentrates in the form of fatty acids is low temperature solvent crystallization, a prevalent technique has been discussed that can be used to remove saturated fatty acids [8].

The low-temperature solvent crystallization stands prominently as a perfect method of separating the saturated from the unsaturated fatty acids [9]. This comes from the fact that the long carbon chain saturated fatty acids are much less soluble in solvent compared to their corresponding unsaturated fatty acids. The low temperature crystallization works well to partially or fully separate the unsaturated fatty acids from the saturated fatty acids. Notwithstanding this fact, the solubility of any given acid is very much linked with its melting point and it is dependent on the nature of the solvent up to a certain extent [10]. Fractionation by crystallization through the differences of melting point of fatty acids comes in two fractions. They are the non-crystallized fraction (liquid) of concentrated unsaturated

fatty acids and the crystallized fraction containing the saturated fatty acids [11].

The low-temperature solvent crystallization of mixed fatty acids was later studied using several solvents *viz*. methanol, acetone, methyl formate and propane at different temperatures (–60 ºC to 10 ºC). The results have singled out methanol (due to its polarity) as comparatively effective and much better than both acetone and ethanol in terms of the separation of saturated and unsaturated components of the fatty acid mixture. As far as all cases with solvent crystallization are concerned, the non-crystallized fraction consists of the concentrated unsaturated fatty acids, whereas the crystallized fraction comprises saturated fatty acids and is removed by filtration later [12].

Optimization through experimental design proves to be a successful way to reduce the number of experimental runs not to mention the production cost. To date, several experimental designs (DOE) methods are applied to make the synthesis procedures better or more efficient [13]. Several research studies reported that D-optimal is a perfect method to separate and purify the experimental designs [6,14]. The D-optimal is a very convenient tool, which offers some practical solutions on a design by stressing on a mean performance value that is very close to the target value. This way, significant factors that leave some significant impacts on the experimental condition could be acknowledged and the optimal performance ascertained. This paper details the practical use of D-optimal method for the design of experiments in looking into the optimization of low-temperature methanol crystallization to isolate the unsaturated fatty acids from crude palm fatty acids mixture. The effects of different process conditions with regard to the responses (yield and purity of unsaturated fatty acids) were optimized and determined facilitated by the response surface methodology (RSM).

EXPERIMENTAL

Sime Darby Sdn. Bhd. (Selangor, Malaysia) was the supplier of crude palm oil in this study. The chemicals such as sodium hydroxide, *n*-hexane, Wijs solution, sodium thiosulphate, potassium iodide, hydrochloric acid, potassium hydroxide and methanol (95 % v/v) were purchased from Sigma-Aldrich. Fatty acid methyl esters (standard) were bought from Sigma-Aldrich Chemical Co. Inc. (St. Louis, MO, USA).

Preparation of crude palm oil fatty acids mixture: Crude palm fatty acids mixtures from crude palm oil were obtained through hydrolysis in batches manually prior to the separation of USFAs as cited by Salimon *et al*. [15]. A two-necked round bottom flask was filled with 50 g crude palm oil (CPO) and 300 mL of ethanolic KOH solution (2 M). The solution was mixed together prior to the process of hydrolysis at the temperature of 65 ºC that lasted for 2 h. Post-hydrolysis, 200 mL water was added into the mixture. Unsaponifiable components were extracted using 100 mL of hexane. The aqueous alcohol phase with the soap was acidified to pH 1 with HCl 6N (~ 60 mL). Hexane was used to extract the fatty acids mixture and it was cleanzed before drying using anhydrous sodium sulphate and the hexane was evaporated in a vacuum rotary evaporator at 45 ºC. As the next process, free fatty acids percentage (% FFAs) was determined.

Determination of free fatty acids percentage: The estimation of acidity of crude palm fatty acids mixture (CPFAMs) was performed by AOCS method Ca 5a-40 [16] and Bahadi *et al*. [17]. Isopropanol (50 mL) and phenolphthalein (0.5 mL) were poured into a flask, and later on neutralized by the addition of sodium hydroxide (0.1N) until a permanent pink colour was produced. The neutralized isopropanol was added to 5 g CPFAMs and afterwards placed in an Erlenmeyer flask. The mixture was heated up to 40 ºC to the point of dissolution. The mixture was then titrated with 0.1N NaOH until the light pink color was formed using 1 mL of phenolphthalein which plays its part as the indicator. The percentage of free fatty acid was calculated using the following eqn. 1:

$$\text{FFA as palmitic acid (\%)} = \frac{25.6 \times N \times V}{W} \quad (1)$$

where, V is the volume of NaOH solution used (mL); N is normality of NaOH solution (eq/L); W is the weight of the sample (g).

Separation of unsaturated fatty acids: There has been an attempt to separate USFAs from the CPFAMs obtained in the preceding process using low-temperature methanol crystallization with a super cooling facility in a refrigerator and this allows for the temperature control. A homogenous solution was formed by having 10 g of CPFAMs mixed in 95 % (v/v) methanol and heated at 60 ºC and it is stirred continuously. To grasp the key factors determining the yield and purity of USFAs, three parameters were chosen: methanol-to-CPFAMs ratio, crystallization temperature and crystallization time. The assessment of each variable was done by varying their values within a minimum (-1) and maximum (+1) value as provided in Table-1. Once the reaction is complete, two fractions were observed. The crystallized fraction containing the saturated fatty acids was removed through the process of filtration using vacuum filtration. The residue resulting from this was a non-crystallized fraction (liquid) of concentrated USFAs. Methanol was removed from the non-crystallized fraction under reduced pressure using a vacuum rotary evaporator at 55 ºC. This procedure has been done in 18 series given different conditions based on the experimental design.

TABLE-1
PARAMETERS AND LEVELS FOR D-OPTIMAL DESIGN OF THE SEPARATION USFAs

Independent variables	Factor X_i	Variable levels		
		-1	0	+1
Methanol-to-CPFAMs ratio (mL/g)	X_1	5	10	15
Crystallization temperature (°C)	X_2	-15	0	5
Crystallization time (h)	X_3	8	16	24

Iodine value: The iodine value of crude palm fatty acids (CPFAs) before and after separation was calculated consistently with the AOCS method Cd 1-25 [18] and the method proposed by Salimon *et al*. [19]. Approximately, 0.4 g of sample was poured in a 500 mL flask, added by 15 mL of cyclohexane (oil solvent). 25 mL of Wijs solution was added as well, and then the flask was corked with a stopper. The flask containing mixture was shaken in a gentle manner and placed without exposure to light for 1 h. After 1 h of incubation, 20 mL of

10 % potassium iodide solution and 150 mL of distilled water were added to the mixture. The mixture was then titrated with sodium thiosulphate (0.1N) until a yellow colour was observed and this means that the iodine is almost disappearing completely. Next, 1 mL of starch solution (1 %) was added, then the continuous titration took place until the blue colour vanished after the flask was shaken hard. The blank was treated with the same condition. Iodine value was determined based on the eqn. 2 that follows:

$$\text{Iodine value} = \frac{12.69 \times N(V_b - V_s)}{W} \quad (2)$$

where, N is the exact normality of $Na_2S_2O_3$ solution used (eq/L); V_b is the volume of $Na_2S_2O_3$ solution used for blank test (mL); V_s is the volume of $Na_2S_2O_3$ solution (mL) and 12.69 serves to transfer an equivalent thiosulphate to g (iodine). The molecular mass unit or relative molecular unit of iodine is found to be 126.9.

Preparation of fatty acid methyl ester (FAME): Fatty acid methyl ester (FAME) was prepared with base-catalyzed transesterification for crude palm oil using the procedural method of Salimon *et al*. [19]. Hexane (1 mL) was added to 0.1 mL of HFFA-CPO. 1 mL of sodium methoxide (1.55 g of NaOH in 50 mL methanol) solution was then added to the oil mixture. The solution was stirred vigorously for 10 s using a vortex stirrer and then kept for 10 min for phase separation; the clear FAME solution and the cloudy aqueous layer. The upper FAME layer was carefully decanted. While, fatty acid methyl ester (FAME) with acid-catalyzed esterification for CPFAMs was prepared [20]. The CPFAMs (2 g) was dissolved in 1 mL of toluene followed by the addition of 7.5 mL methanol and 1.5 ml of the reagent solution (2.5 mL HCl, 37 % diluted with 10 mL methanol). The tube was agitated and afterwards heated at 65 ºC for 1.5 h. After cooling, the mixture was transferred to a separatory funnel. As the next step, 10 mL of hexane and 10 mL of water were added to the mixture to extract methyl esters present in the hexane phase which was then dried with anhydrous sodium sulphate. Finally, in a manual way, 1 µL was injected into a GC-FID.

GC-FID analyses: Gas chromatography (Shimadzu GC-17A) equipped with a capillary column BPX 70 (30 m × 0.25 mm × 0.25 m) and the FID detector functioned to identify the fatty acid composition of CPFAMs. The column temperature was adjusted at 120 ºC with a regular increase of about 3 ºC/min for 57 min. Nevertheless, the detector and the injector temperature were programmed at 280 and 260 ºC, respectively. Helium gas was utilized as the gas carrier with a flow rate of 0.3 mL/min. The parameters of GC were carried out according to Bahadi *et al*. [21]. The peaks were identified by drawing a comparison with the retention times of the authentic standards.

Experimental design and statistical analysis: A three-factor D-optimal design was employed to delve into the effect of methanol-to-CPFAMs ratio, crystallization temperature and crystallization time on the responses: (a) yield of USFAs, Y_1 (%); (b) percentage of USFAs, Y_2 (%); and (c) percentage of saturated fatty acids (SFAs), Y_3 (%). These responses were represented using equations 1, 2 and 3, respectively. The independent variables were labeled as X1 for methanol-to-CPFAMs ratio (mL/g), X_2 for crystallization temperature (ºC) and X_3 for crystallization time (h). The low value (-1) and high value (+1) of X_1, X_2 and X_3 as can be seen from Table-1, which were equivalent with the range setting of each parameter: 5-15 mL/g for X_1, −15-5 ºC for X_2 and 8-24 h for X_3. The execution of the experimental design with D-optimal generated 18 runs as highlighted in Table-2. For the prediction of the responses, a quadratic or linear model was assumed for the optimization process as expressed in eqn 3:

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

Given that β_0 is a constant, β_i a linear coefficient, β_{ii} a square regression coefficient, β_{ij} is the interaction regression coefficient, x_i and x_j are independent variables. The coefficient of determination (R-squared) and ANOVA test served to assess the goodness of fit of the model.

TABLE-2
EXPERIMENTAL RUNS FROM D-OPTIMAL DESIGN AND THE RESPECTIVE RESPONSES

Run No.	Variables levels X			Responses Y		
	MeOH to CPFAMs[a] (X_1)	Temp[b] (X_2)	Time[c] (X_3)	Y_1 Yield of USFAs	Y_2, USFA (%)	Y_3, SFA (%)
1	15	5	24	57.5	80.63	19.37
2	15	-15	24	42.1	94.88	5.12
3	12.50	0	16	52.15	83.11	16.89
4	5	5	24	43	87.26	12.74
5	5	-15	24	26.5	95.52	4.48
6	5	5	24	42.75	89.45	10.55
7	5	5	8	59.65	83.46	16.54
8	10	-5	24	26.7	91.92	8.08
9	5	-15	8	34.08	86.89	13.11
10	15	-5	8	43.87	88.79	11.21
11	15	5	8	53.65	81.62	18.38
12	5	-5	16	50.15	85.86	14.14
13	5	-15	8	44.08	92.83	7.17
14	15	-15	8	48.75	91.49	8.51
15	5	-15	24	25.95	94.88	5.12
16	15	-15	24	51.1	95.62	4.38
17	10	5	8	45.65	79.35	20.65
18	10	-15	16	43.33	93.29	6.71

Notes: USFA: unsaturated fatty acid (C16:1, C18:1, C18:2, C18:3) SFA: saturated fatty acid (C12, C14, C16, C18) [a]Methanol-to-CPFAMs ratio (mL/w) [b] crystallization temperature (°C), [c]Crystallization time (h)

RESULTS AND DISCUSSION

CPFAMs recovery following hydrolysis and determination of FFAs: CPFAMs was produced *via* the hydrolysis of crude palm oil (CPO), with glycerol as a side product. Table-3 shows the free fatty acid (FFA) percentage of CPFAMs and percentage yield of CPFAM after 3 runs. The data from the 3 runs were averaged to give a CPFAMs average percentage yield of 88.1 ± 1% and a FFA average percentage yield of 99.3 ± 1 %. The hydrolysis of CPO into CPFAMs will be more complete with a higher percentage of FFA in CPFAMs. From the hydrolysis, the main product is CPFAMs while the side product is glycerol.

TABLE-3
YIELD (%) OF CPFAMS AND FFAs (%) DETERMINATION AFTER HYDROLYSIS OF CPO

Run	CPO weight (g)	CPFAM weight (g)	Yield (%)	FFA (%)
1	50	44.5	89.0	100.2
2	50	43.7	87.4	99.5
3	50	44.0	88.0	98.3

Fatty acid composition of CPO and CPFAM: The fatty acid composition of CPO and CPFAMs subjected to base and acid catalyzed preparation comprise palmitic acid (45.7 %, 42.3 %), oleic acid (39.5 %, 42.2 %), and linoleic acid (9.4 %, 9.9 %), stearic acid (4.3 %, 4.3 %), myristic acid (0.9 %, 0.9 %), lauric acid (0.2 %, 0.2 %), linolenic acid (0.0 %, 0.2 %), respectively (Table-4). The fatty acid compositions of CPO and CPFAMs are relatively different due to the fatty acid in CPO, which only refers to oil composition. Nonetheless, fatty acids composition in CPFAM represents fatty acids and FFAs composition because of the hydrolysis process.

Response surface methodology: The response surface methodology approaches supplements and provides a means of a simple and systematic way to optimize low-temperature crystallization to obtain USFAs. In general, RSM based on the D-optimal design for optimization processes involves mainly four major steps: (a) statistically design experiments following the experimental plan; (b) suggest the mathematical model in reference to the experimental results and elaborate upon the analysis of variance (ANOVA); (c) check the model adequacy through diagnostic plots and (d) foresee the model's response and validity.

TABLE-4
FATTY ACID COMPOSITION (%) CPO AND CPFAMs AFTER HYDROLYSIS

Fatty acid composition	CPO (%)	CPFAMs (%)
Lauric acid ($C_{12:0}$)	0.2	0.2
Myristic acid ($C_{14:0}$)	0.9	0.9
Palmitic acid ($C_{16:0}$)	45.7	42.3
Stearic acid ($C_{18:0}$)	4.3	4.3
Oleic acid ($C_{18:1}$)	39.5	42.2
Linoleic acid ($C_{18:2}$)	9.4	9.9
Linolenic acid ($_{C18:3}$)	0	0.2
Σ Saturated fatty acid	51.1	47.7
Σ Unsaturated fatty acid	48.9	52.3

Model fitting of D-optimal design: The effects of independent variables on yield and percentage of unsaturated fatty acids and the percentage of saturated fatty acids were represented using a quadratic polynomial model, estimated based on the experimental results with the respective coefficients as given in eqns. 4-6. Herein, Y_1, Y_2 and Y_3 are the yield, percentage of USFAs and percentage of SFAs, respectively. The variables X_1, X_2 and X_3 represent methanol volume (mL), crystallization temperature (ºC) and crystallization time (h), respectively. The analysis of variance (ANOVA) for this regression model is provided in Tables 5-7. All the models were well described within the range of the independent variables. The F-value of 13.27, 9.83 and 9.80 indicated that the models were significant in order to elaborate on the separation of USFAs with adequate precision of 11.813, 9.075 and 9.064.The adequacy of the signal to noise ratio was considered desirable with a value greater than 4 [22]. Model Y_1 (yield of USFA) was reasonably significant with all variables X_1, X_2 and X_3 showing $p < 0.05$ (Table-3). The term X_1 for model Y_2 (percentage of USFA) and Y_3 (SFA) has highlighted insignificance with $p > 0.1$, despite the fact that X_2 and X_3 were relatively significant (Tables 4 and 5). All models 'lack of fit showed F-values of 0.20, 1.03 and 1.03 that respectively appears to be insignificant relative to the pure error [23]. From Tables 3-5, we can explicitly state that the correlation coefficients of R^2 and adjusted R-squared

TABLE-5
ANALYSIS OF VARIANCE (ANOVA) OF YIELD PERCENTAGES USFAs (Y_1)

Source	Sum of square	Degree of freedom	Mean square	F-value	P-value
Model	1627.58	9	180.84	13.27	0.0007[a]
X_1	280.35	1	280.35	20.58	0.0019[a]
X_2	555.77	1	555.77	40.80	0.0002[a]
X_3	161.14	1	161.14	11.83	0.0088[a]
X_1^2	239.80	1	239.80	17.60	0.0030[a]
X_2^2	83.13	1	83.13	6.10	0.0387[a]
X_3^2	334.58	1	334.58	24.56	0.0011[a]
X_{12}	69.65	1	69.65	5.11	0.0536[a]
X_{13}	175.52	1	175.52	12.88	0.0071[a]
X_{23}	0.046	1	0.046	3.410×10^{-3}	0.9549
Residual	108.99	8	13.62		
Lack of Fit	18.30	4	4.58	0.20	0.9249
Pure Error	90.68	4	22.67		
Cor Total	1736.57	17			

$R^2 = 0.9372$, R^2adj = 0.8666, adequate precision = 11.813. [a]Significant at < 0.05 % level.

TABLE-6
ANALYSIS OF VARIANCE (ANOVA) OF USFA PERCENTAGES (Y_2)

Source	Sum of square	Degree of freedom	Mean square	F-value	P-value
Model	460.74	9	51.19	9.83	0.0019[a]
X_1	6.22	1	6.22	1.19	0.3063
X_2	346.22	1	346.22	66.45	< 0.0001[a]
X_3	47.34	1	47.34	9.09	0.0167[a]
X_1^2	0.20	1	0.20	0.038	0.8498
X_2^2	1.69	1	1.69	0.32	0.5846
X_3^2	9.46	1	9.46	1.82	0.2148
X_{12}	25.16	1	25.16	4.83	0.0592
X_{13}	13.04	1	13.04	2.50	0.1524
X_{23}	0.50	1	0.50	0.095	0.7654
Residual	41.68	8	5.21		
Lack of Fit	21.16	4	5.29	1.03	0.4884
Pure Error	20.52	4	5.13		
Cor Total	502.42	17			

R^2 = 0.9170, R^2adj = 0.8237, adequate precision = 9.075. [a]Significant at < 0.05 % level

TABLE-7
ANALYSIS OF VARIANCE (ANOVA) OF SFA PERCENTAGES (Y_3)

Source	Sum of square	Degree of freedom	Mean square	F-value	P-value
Model	461.01	9	51.22	9.80	0.0019[a]
X_1	6.23	1	6.23	1.19	0.3067
X_2	346.38	1	346.38	66.30	< 0.0001[a]
X_3	47.36	1	47.36	9.06	0.0168a
X_1^2	0.20	1	0.20	0.038	0.8513
X_2^2	1.70	1	1.70	0.32	0.5843
X_3^2	9.44	1	9.44	1.81	0.2158
X_{12}	25.22	1	25.22	4.83	0.0592
X_{13}	13.08	1	13.08	2.50	0.1523
X_{23}	0.50	1	0.50	0.096	0.7645
Residual	41.80	8	5.22		
Lack of Fit	21.22	4	5.30	1.03	0.4885
Pure Error	20.58	4	5.14		
Cor Total	502.81	17			

R^2 = 0.9169, R^2adj = 0.8234, adequate precision = 9.064. [a]Significant at < 0.05 % level.

(R^2 adj) for each three responses were considerably high, suggesting that there is a good fit between the regression model and the experimental values.

$$Y_1 = 43.56 + 4.68X_1 + 6.59X_2 - 3.37X_3 + 10.67X_1^2 + 6.28X_2^2 - 14X_3^2 - 2.50X_1X_2 + 3.80X_1X_3 + 0.062X_2X_3 \quad (4)$$

$$Y_2 = 87.06 - 0.70X_1 - 5.20X_2 + 1.83X_3 - 0.31X_1^2 - 0.90X_2^2 + 2.35X_3^2 - 1.50X_1X_2 - 1.04X1X3 - 0.20X_2X_3 \quad (5)$$

$$Y_3 = 12.94 + 0.70X_1 + 5.20X_2 - 1.83X_3 + 0.31X_1^2 + 0.90X_2^2 - 2.35X_3^2 + 1.50X_1X_2 + 1.04X_1X_3 + 0.20X_2X_3 \quad (6)$$

Adequacy check of the model: The model validation must be performed *via* adequacy check to confirm the accuracy of the model. A valid mathematical model that is highly accurate would help improve the real process or otherwise the there will be poor or misleading results given by the analysis [24]. The studentized residuals were plotted against the predicted yield, percentage of USFAs and the percentage of SFAs as highlighted in Fig. 1. The studentized plots of residual *versus* the value of a fitted response show off the distribution of points that is scattered about the boundary of 0 to ± 4.506 at random, suggesting that the variance is constant for all response values. Thus, it is confirmed that the models were appropriate for application without any transformations for the purpose of reducing the scatter [25]. Conversely, Fig. 2 showed the plot distribution for the actual data against the predicted values of yield, purity of USFAs and percentages of SFAs. The actual data came in the form of the initial results obtained from the experiments (Table-2) and the predicted values were obtained from the models. Through observation, all data points cluster around the line indicating that there is a perfect agreement between the models and the empirical data. As it is, the models should forecast the values of USFA yields, its percentages and the percentages of SFA that remarkably coincide with the real-time experimental values. This was proven statistically by the values of R^2 and R^2adj as given in Tables 3-5 implied that the prediction of results is reliable. Hence, the idea of proceeding with the next stage of analysis *via* the optimization tool is seen as desirable.

Response surface analysis and optimization conditions: The 3-D response surfaces and contour graphs make the illustration of the effect of interaction between variables on the low-temperature crystallization for separation of USFAs possible. The response surfaces in Fig. 3 highlight the effect of methanol-to-CPFAMs ratio and crystallization temperature on the USFAs separation. There is a need to supervise the proliferation of yield and percentages of USFA (Fig. 3a-b) and the reduction

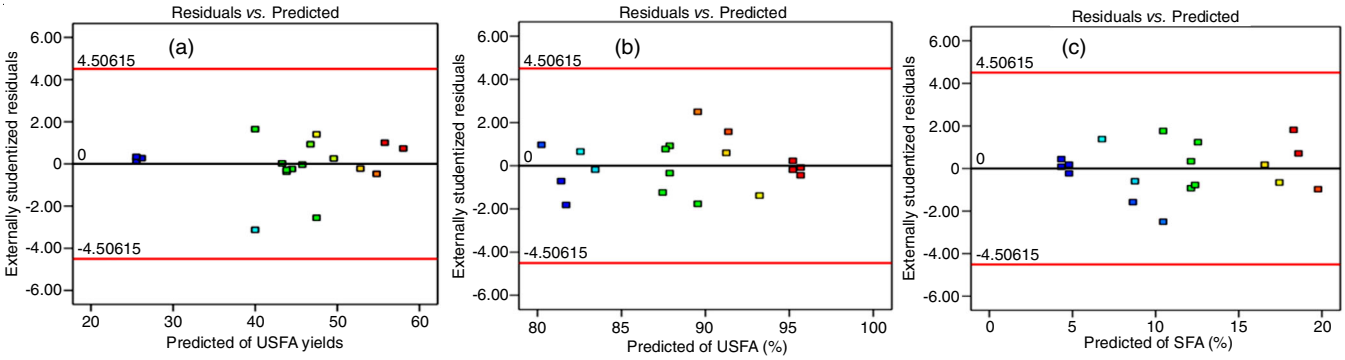

Fig. 1. Studentized residuals *versus* the predicted USFA yields (a), predicted USFA percentages (b) and predicted SFAs percentages (c)

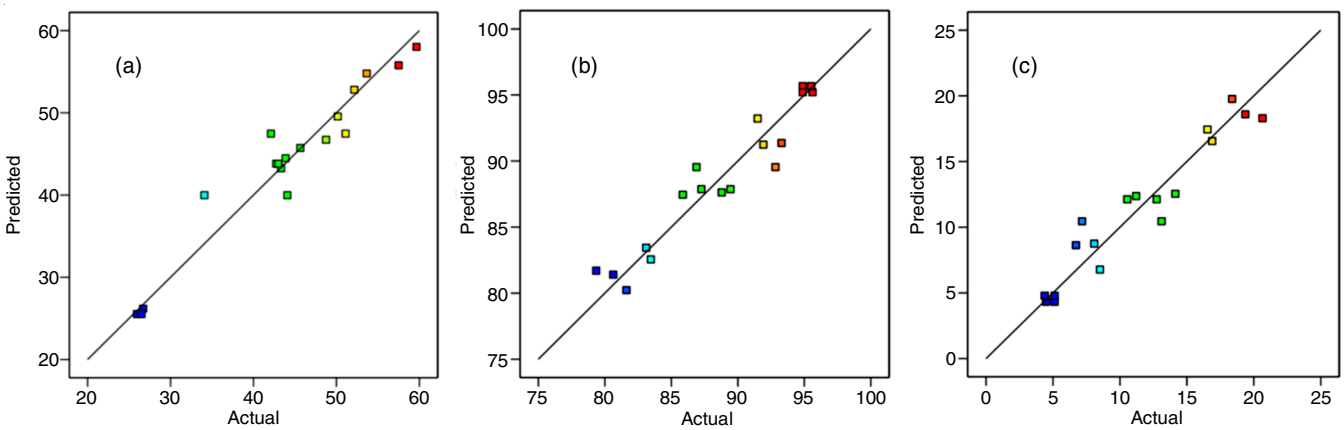

Fig. 2. Regression plot of predicated values *versus* actual data of USFA yields (a), USFA percentages (b) and SFA percentages (c)

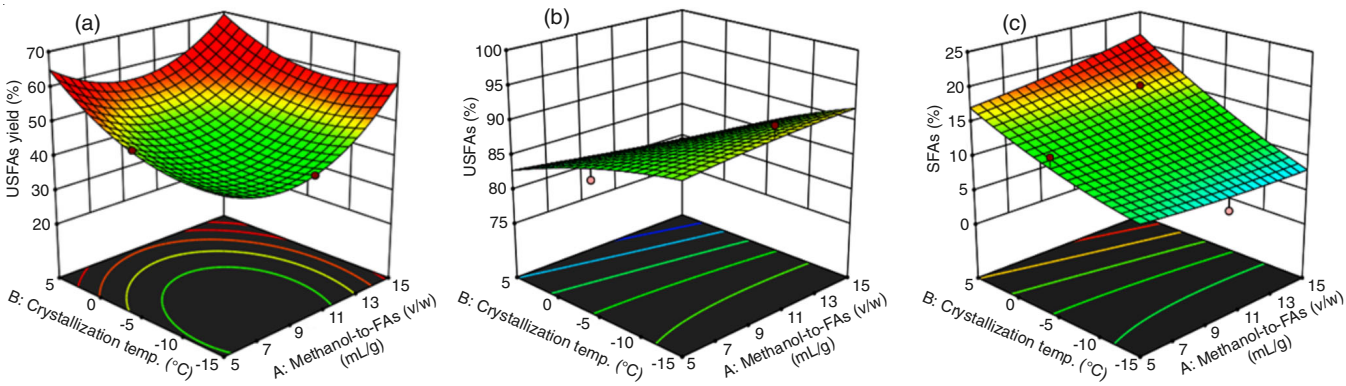

Fig. 3. Effect of the methanol-to-CPFAMs ratio and crystallization temperatures on the USFA yields (a), USFAs percentages (b) and SFA percentages (c)

of SFAs (Fig. 3c) in the non-crystallized fraction as the temperature went down from 5 ºC to −15 ºC. On a similar way, the ratio of methanol-to-CPFAMs shows a positive correlation with the yield and percentages but it highlights a negative effect on the percentage of saturated fatty acids as it decreased with higher proportion of methanol-to-fatty acid in a gradual manner (Fig. 3c).

The effect of interaction between methanol-to-CPFAMs ratio and crystallization time is given in Fig. 4. The higher methanol-to-CPFAMs ratio and crystallization time showed a higher yield and percentages of USFAs in the non-crystallized fraction. SFAs content in the non-crystallized fraction was also enriched under these conditions. It seems that there was a linear relationship established between the ratio of methanol and crystallization time. However, as the parameters continued to increase, the SFA percentages dropped. It is evident from Fig. 4c that the crystallization time exerts a significant influence on the concentration of SFA in the product. These results could also be implicited to the idea that there will be more crystal growth within longer reaction time. It is interesting to note that this marks a positive outcome as the formation of more crystallized fraction lowers the concentration of saturated fatty acids in the product through time [26,27].

The key factor dominating the USFA separation could possibly be from the interaction established between crystallization time and crystallization temperature. Brought together, crystallization time and crystallization temperature led to high yield and percentages of USFAs as can be seen in Fig. 5. It is encouraging to relate these response surfaces with a classic study by Kolb and Brown [28], suggesting that longer reaction time and lower temperature relatively helps ease the low

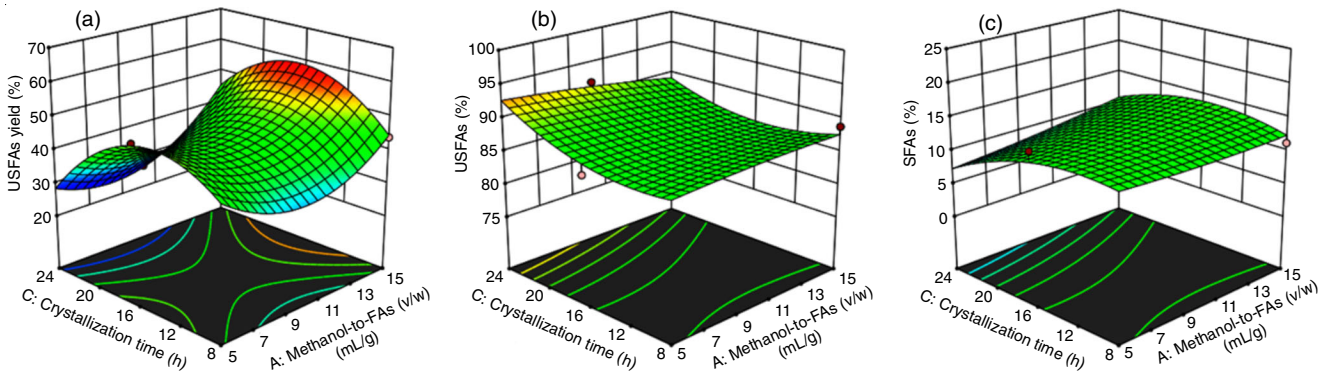

Fig. 4. Effect of the methanol-to-CPFAMs ratio and crystallization time on USFA yields (a), USFA percentages (b) and SFA percentages (c)

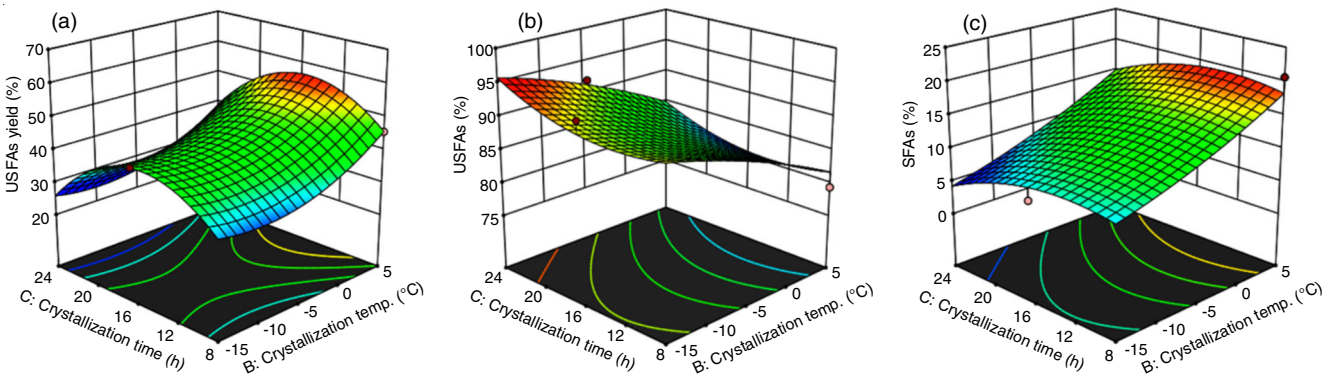

Fig. 5. Effect of crystallization temperature and crystallization time on USFAs yields (a), USFA percentages (b) and SFA percentages (c)

temperature solvent fractionation. With that, between the studied ranges of this paper, 8 to 24 h of crystallization time and from −15 ºC to 5 ºC of crystallization temperature have been proven efficient to separate USFA.

Model validation and experimental confirmation: After ANOVA is performed, numerical optimization was required to seek for the optimum condition to isolate the USFAs. A set of the range was selected for all controlling parameters (methanol-to-CPFAMs ratio, crystallization temperature and crystallization time) to get the final goal of maximum yield and percentage of unsaturated fatty acids while bringing the percentage of SFA to the minimum. The function's higher desirability led to the model′s better accuracy. From the criteria selected, the predicted models have a desirability function that is the same as 0.872. The estimated parameters from the numerical optimization are shown in Fig. 6. The optimum condition forecast through the D-optimal design generated methanol-to-CPFAMs ratio (v/w) of 15:1, the crystallization temperature of −15 ºC and 20.8 h of crystallization time. Given these conditions, the yield of USFA was 59.19 % with 93.32 % USFA percentages and 6.67 % SFA percentages. As given in Table-8, three replications of experiments were done to make the accuracy of the

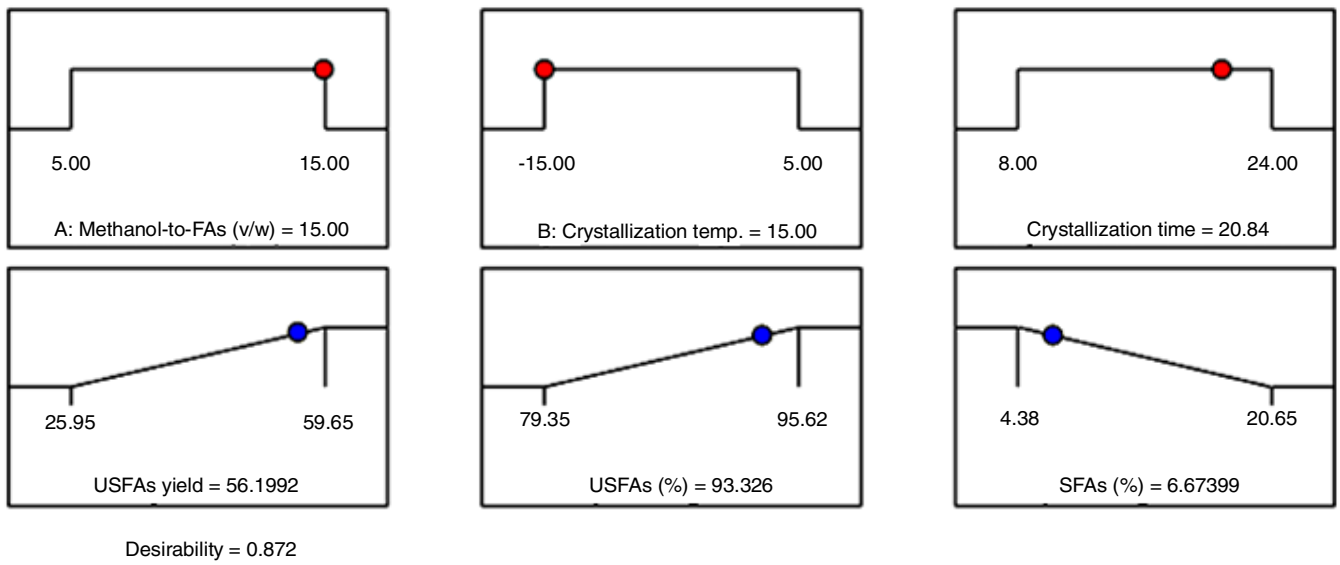

Fig. 6. Unsaturated fatty acids, as derived from the RSM predicted model using optimal conditions

TABLE-8
VALIDATION TEST RESULT OF THE OPTIMUM CONDITION

No		Independent variables			Responses		
		X_1	X_2	X_3	Y_1, USFA yields (%)	Y_2, USFA (%)	Y_3, SFA (%)
Actual	1	150:10	–15	20.8	54.3	95.3	4.6
	2	150:10	–15	20.8	55.5	95.1	4.9
	3	150:10	–15	20.8	55.9	94.8	5.2
Actual average		150:10	–15	20.8	55.2 ± 0.8	95 ± 0.3	4.9 ± 0.3
Predicted		150:10	–15	20.8	56.19	93.32	6.67

Notes: X_1: Methanol-to-CPFAMs ratio (ml:g); X_2: crystallization temperature (°C); X_3: crystallization time (h)

predicted model which produced an average of 55.2 ± 0.8 % yield of USFA, 95 ± 0.3 % USFA percentages and 4.9 ± 0.3 SFA percentages valid. These results seem to be consistent with the data obtained from the model. Thus, this study agrees that the D-optimal design is reliable as a simple and useful approach of assessing the best conditions for low-temperature crystallization, or specifically in isolating the unsaturated fatty acids from crude palm oil.

Unsaturated fatty acids composition: After esterification, the fatty acid products were GC-analyzed. The chromatograms are given in Fig. 7. There is a great distinction between fatty acids composition pre- and post-crystallization under optimized condition (Table-9). The most prominent difference can be seen in the composition of palmitic acid (C16:0) and oleic acid (C18:1). Palmitic acid serves to be the major component of saturated fatty acids in crude palm oil that decreased rapidly to 4.1 % and oleic acid contents went up to 76.3 %. The maximum percentage of USFAs was 94.8 %. The total amount of SFAs, of those include lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) had dropped from 47.7 to 5.2 %. In addition, what corroborates this finding further is the higher iodine value from 54.0 ± 0.15 mg/g to 94.4 ± 0.2 mg/g. Moreover, the iodine value had verified this conclusion. It is increased from 54.0 ± 0.1 mg/g to 94.4 ± 0.2 mg/g. Hence, it could be hypothesized that low-temperature methanol crystallization stands out as a perfect technique to separate USFA from CPFAMs.

TABLE-9
FATTY ACIDS COMPOSITION (%) of CPFAMs BEFORE METHANOL CRYSTALLIZATION AND USFAs AFTER METHANOL CRYSTALLIZATION

Fatty acid composition	CPFAMs %	USFA after separation (%)
Lauric acid ($C_{12:0}$)	0.2	0.1
Myristic acid ($C_{14:0}$)	0.9	0.8
Palmitic acid ($C_{16:0}$)	42.3	4.1
Stearic acid ($C_{18:0}$)	4.3	0.2
Σ Saturated fatty acid	47.7	5.2
Oleic acid ($C_{18:1}$)	42.2	76.3
Linoleic acid ($C_{18:2}$)	9.9	18.3
Linolenic acid ($_{C18:3}$)	0.2	0.2
Σ Unsaturated fatty acid	52.3	94.8
Iodine value (mg/g)	54.0 ± 0.1	94.4 ± 0.2

CPFAMs: Crude palm fatty acid mixture; USFA: Unsaturated fatty acids

Conclusion

The separation and purification of unsaturated fatty acids (USFAs) from crude palm fatty acids mixture (CPFAMs) by low-temperature methanol crystallization was performed following the design of experiment with D-optimal approach. The interaction effect between the independent variables on the USFAs purification is examined and the results from ANOVA had shown consistency between the predictions from the model and the experimental data. It was found that 15:1 (mL/g) methanol-to-CPFAMs ratio, the crystallization temperature of −15 ºC and crystallization time of 20.8 h served to be the optimal conditions causing maximum yield and percentages of unsaturated fatty acids, also minimum saturated fatty acid percentages. Under these conditions, the model forecast a yield of 56.19 % with 93.32 %, and this can be compared with the real experimental data of 55.2 ± 0.8 % USFAs and 95 ± 0.3 % purity on average, as GC analysis had verified. The relevance of using D-optimal design through the response surface methodology in getting the optimal condition for low-temperature methanol crystallization has been proven systematically. The findings offer the much needed support to validate the model that predicts the separation and purification of unsaturated fatty acids successfully from CPFAMs.

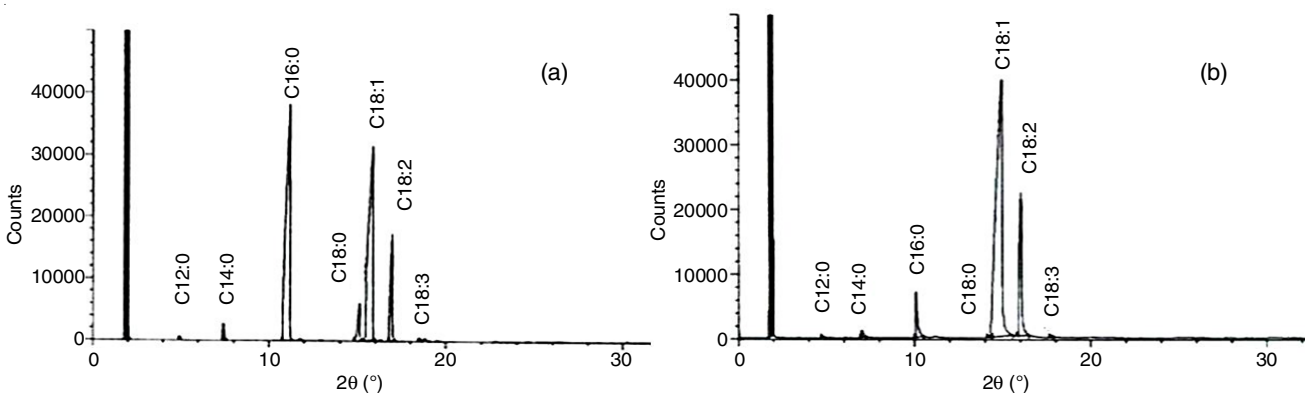

Fig. 7. GC chromatogram of FAs composition in CPFAMs before methanol crystallization (a) and USFAs after methanol crystallization (b)

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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