Research Article

D-Optimal Design Optimization for the Separation of Oleic Acid from Malaysian High Free Fatty Acid Crude Palm Oil Fatty Acids Mixture Using Urea Complex Fractionation

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Received: 19 November 2020; Revised: 18 January 2021; Accepted: 15 February 2021; Published online: 9 March 2021

Abstract
Oleic acid (OA) rich vegetable oils is important for the daily essential dietary oils intake but restrict to particular oil such as olive oil. However OA enrichment to other vegetable oil such as palm oil is always possible. OA can be obtained from cheap resources such as high free fatty acid crude palm oil (HFFA-CPO). OA concentrate from HFFA-CPO fatty acids mixture requires efficient and low cost technique. Urea complex crystallization fractionation is a classic method for fractionating saturated and monounsaturated fatty acids from polyunsaturated fatty acids of many vegetable oils. In this work, the separation and purification of oleic acid (OA) from unsaturated fatty acids mixture fraction (USFA) of HFFA-CPO fatty acids mixture by urea complex fractionation (UCF) was studied. The crystallization reaction conditions of urea inclusion for the non-urea complex fraction (NUCF) were optimized using the response surface methodology (RSM) and the optimal model was developed. The results showed high content of OA (88%) in urea complex fraction (UCF) with 86% yield at optimal conditions of urea-to-USFAs ratio of 4.62 : 1 (w/w), crystallization temperature at –10°C and crystallization time of 24 h. The results have demonstrated that urea complex crystallization fractionation method is a very effective with low cost, stable, obtainable, and comparatively ease to recover of OA from polyunsaturated fatty acids (PUFA) of an oil fatty acids mixture. Pure OA is plausible to be used back for the OA enrichment modification into palm oil for new dietary oil.

Keywords: Oleic acid isolation, Crude palm oil, Urea complex fractionation, Response surface methodology

1 Introduction

Oleic acid (OA) with the chemical formula of $C_{18}H_{34}O_2$ is a major component of the Mediterranean dietary oil. It presents different properties that can be useful both in the immunomodulation, treatment and prevention of different types of disorders such as cardiovascular or autoimmune diseases, metabolic disturbances, skin injury and cancer. OA or cis-9-octadecenoic acid ($C_{18:1}$) is a dominant common major fatty acid largely found in plant and/or animal oils. It is a monounsaturated fatty acid (MUFA) and a single double bond presence at C_9-C_{10} position. In naturally occurring plant and animal oils OA, the double bonds are in the cis configuration. This isomer configuration make the OA molecule bent or titled compared to its linear

Please cite this article as: M. Bahadi, N. Salih, and J. Salimon, "D-optimal design optimization for the separation of oleic acid from Malaysian high free fatty acid crude palm oil fatty acids mixture using urea complex fractionation," *Applied Science and Engineering Progress*, vol. 14, no. 2, pp. 175–186, Apr.–Jun. 2021, doi: 10.14416/j.asep.2021.03.004.

molecule trans isomer configuration. This also makes the molecules much less flexible than those of fully saturated fatty acids of the same carbon chain length, stearic acid. The bent shape of OA molecular structure makes unpacked rearrangement of the molecule that prevents easy crystallization at ambient temperature. This make OA acid is liquid at room temperature. So does other fatty acids with more than one double bonds such as linoleic and linolenic acids. On the hand, the saturated fatty acids that have a straight chains pack easily into a crystal lattice and this make them turn to solid at room temperature such as found in tallow, butter, cocoa butter and palm stearin. OA contributes in high percentages composition to essential dietary oil such as in olive oil which represents 70–80% of olive oil composition [1]. It constitutes at least one third of the total fatty acid intake [2] and attributes to a good general health, due to its high OA content [3]–[5] and minor phenolic compounds [6]. The benefit of OA was established in cholesterol levels reduction, atherogenesis risk [7]–[9], blood pressure and daily anti-hypertensive drug intake [10]. In addition, OA was proved to induce beneficial anti-inflammatory effects on autoimmune diseases [11], [12], protective effect on breast cancer and improvement of immune system function [13]–[17]. OA also can be used as starting raw materials in the pharmaceutical, cosmetics, ointments, lubricating oils, polyols, polyurethanes and bioplastic industries [18]. Other vegetable oils which are suitable for essential dietary oil used such as almond oil (69%) and canola oil (62%) rich in OA. However due to their high price, the alternative vegetable oils such as palm oil with 38–40% OA composition is potential candidate for dietary oil replacement especially in tropical countries. This is possible if the OA composition of palm oil is at far high as in olive oil. Moreover, OA rich palm oil fraction is yet not been produced industrially due to recent fractionation technology limitation and high cost incurred.

The palm oil tree (*Elaeis guineensis*) is capable of growing up to 20 m for more than 25 years of fruits profitable life cycle. Palm fruit bunches are produced along the year with high fruit yields per hectare. Each tree may produce about 10 t of fresh fruit bunches per hectare with an average of 3.9 t of crude palm oil (CPO) and 0.5 t palm kernel oil (PKO) per hectare [19]. Palm fruits contain high oil composition in the range of 45–50%. Palm oil trees are cultivated as cash crops in tropical and subtropical countries of Southeast Asia such as Malaysia, Indonesia and Africa [20]. Two types of oil obtained from palm fruits which can be extracted from the flesh of the fruit (mesocarp) known as crude palm oil (CPO) or the nut kernel known as crude palm kernel oil (CPKO). The Malaysian CPO for example, composes the major fatty acids include palmitic acid (43.4%) and oleic acid (38.4%) [21]. Therefore OA enrich version of palm oil is yet to be produced through green modification processes. These processes can be involved the separation of palm oil OA followed by recombine them with glycerol molecule to produce new version high oleic palm oils (HOPO) which is the potential alternative essential dietary oils.

Malaysian crude palm oil comprises lipids more than 80–85% triacylglycerols (TAG), 3 to 8% diacylglycerols (DAG), less than 3% monoacylglycerols (MAG) and 3 to 4% free fatty acids (FFA). However during rain and monsoon session, the content of FFA in CPO varied in the range of 9 to 15% and classified as high free fatty acids crude palm oil (HFFACPO) [22]. High level of FFA in CPO indicates its lower quality and thus has low market price. Furthermore the production cost increase for further refining processes for FFA removal. Therefore it is more economically to convert them into palm oil fatty acids, separate them and further use the particularly interest unsaturated fatty acids such as oleic acid for the production of HOPO.

Several methods have been used to separate or to concentrate a specific interested fatty acid such as OA from various naturally plant oils or their fatty acids mixtures. The commonly used methods are based on the fractional or molecular distillation [23], low temperature solvent crystallization and urea complexation [24]–[26], absorption column chromatography [27]–[29] and soap crystallization [30]. However only few methods are suitable for large-scale production based on their simplicity, efficiency and operation cost. Therefore, the isolation and purification of OA from polyunsaturated such as linoleic ($C_{18:2}$) and linolenic acids ($C_{18:3}$) rise of interest among the researchers. The most effective and economic method for separating OA from polyunsaturated fatty acids of Jatropha curcas has been reported by using urea complexation [31]. The advantage of urea complex fractionation method is due to the stability of the urea complexed. The urea complex method does not defense on the physical properties, but by

the structure of the fatty acid moieties either having single or multiple double bonds [32]. Despite their difference in linear and bend structure, the saturated fatty acids and OA easily complexation with urea. They crystallize after cooling at certain temperature and produce a solid fraction (urea complex fraction, UCF) while the polyunsaturated fatty acids stay as liquid fraction (non-urea complex fraction, NUCF). The UCF enriched with saturated fatty acids and OA while the liquid fraction is enriched with linoleic acid and linolenic acid.

An optimization method has been developed to isolate and concentrate linoleic acid (LA) of sunflower oil (compose of 10.5% saturated fatty acids (SFA) & 89.5% unsaturated fatty acids (USFA) by using urea complexation [24]. The optimal balance between the purity and the recovery of LA were high with 87.8%, and 83.4%. At the optimal condition, OA increase about 62% and LA increase about 48% respectively. The same technique however was not suitable to be used for the separation and isolation of USFA especially OA for palm oil due to its equally high percentages composition of SFAs (48.9%) and USFAs of 51.1% respectively. The separation between SFAs and USFAs mixture of PO was well established by low-temperature solvent crystallization (LTSC) techniques. By using the right solvent such as ethanol, saturated fatty acid easily crystalize to form solid fraction whereas the unsaturated fatty acids stay in liquid fraction and separated through filtration [22]. The purity and recovery were high with yields of 47.8% SFA and 52.2% of USFA respectively. At optimal condition the solid SFA fraction composes 90% palmitic acid, 6% stearic acid and 4% OA. The liquid USFA fraction contains the monounsaturated (OA) and polyunsaturated fatty acids (LA) and linolenic acid (LnA) mixture, where the OA can be further separated using urea complexation methods. The isolation between saturated and unsaturated fatty acids mixtures of their respective plant or vegetable oils commonly carried out by solvent crystallization due to their distinct solidification properties in various solvents. However it is difficult on the other hand to separate among the same class of saturated or unsaturated fatty acids. The oleic (has one double bond) and linoleic acids (has two double bonds) for example, is hardly to separate due to their tightly close physicochemical properties. In this study the separation and isolation of OA from a mixture of unsaturated fatty acids (USFAs) of crude palm oil which composes OA (76.3%), LA (18.3%) and LnA (0.2%) by using urea complexation method was developed and optimized. The effects of different process variables and conditions on the separation responses were determined and optimized using Response Surface Methodology (RSM) through the designing experiments D-optimal method.

2 Materials and Methods

2.1 *Materials*

Malaysian HFFA-CPO was obtained from a local refinery, Sime Darby, Sdn. Bhd, Carey Island, Selangor. The entire chemicals and solvents used in this study such as methanol 99.8%, urea 99%, hydrochloric acid 99.8%, petroleum ether, sodium chloride 99.8%, and anhydrous sodium sulfate 99% were purchased from Sigma-Aldrich. All the chemicals were analytical reagents and used without additional purification.

2.2 *Gas-chromatography analysis*

The composition of fatty acids was determined by using GC-FID (Shimadzu, Series GC-17A) equipped with BPX 70 polarized capillary column (30 m × 0.25 mm × 0.25 μm thinning thickness; SGE). Fatty acid methyl ester (FAME) was prepared from HFFA-CPO using base-catalyzed transesterification was carried out according to Salimon *et al.* [33]. For the FAME acid-catalyzed transesterification was prepared according to Ichihara and Fukubayashi [34]. The injector and detector temperature were set at 250°C and 280°C respectively. The column temperature was maintained at 120°C for 1 min and then increased to 250°C at a rate of 3°C/min and maintained at the final temperature for 15 min. Nitrogen gas was used as gas carrier at a flow rate of 0.40 mL/min with a total flow rate of 13 mL/min. The sample in the solvent was injected using a split injector mode with a separation ratio of 29 : 1. The fatty acid composition was identified based on the retention time of Merck authentic standards FAME solution analyzed under the same condition.

2.3 *High-performance liquid chromatography analysis*

The triacylglycerol composition was determined by using HPLC model 3000 DIONEX equipped with

evaporative light scattering (ELS) detector and an auto-injection. The separation of triacylglycerols (TAGs) was carried out with commercially-packed C_{18} column 5 µm × 120Å (4.6 × 250 mm) at room temperature. The parameters of HPLC were set according to Salimon *et al.* [33]. The mobile phase comprises a mixture of acetone: acetonitrile (63.5% : 36.5%), fixed at a flow rate of 1 mL/min. Sample preparation entailed the dilution of 0.1 mL sample with 1.5 mL acetone to form acetonitrile (63.5 : 36.5) mixture. The HPLC was immersed in the mixture and auto-injected with an overall operation time of 40 min.

2.4 *Optimizing conditions for the separation and purification of oleic acid*

Mixture of palm fatty acids were hydrolyzed from high free fatty acids CPO (HFFACPO). The saturated fatty acids (SFA) and unsaturated fatty acids (USFAs) were separated by using low-temperature solvent crystallization (LTSC) techniques according Japir *et al.* [22]. USFAs fraction was used to concentrate OA from polyunsaturated fatty acids. OA was separated and purified using urea complex fractionation method according to Salimon *et al.* [35]. The separation process involved mixing 10 g of USFAs with urea in 99% methanol, which was subsequently, heated at 60°C and stirring until a homogeneous and clear mixture was obtained. The ratio of urea-to-USFAs was varied using different quantities of urea (Tables 1 and 2). The urea-unsaturated fatty acid adduct was then left to crystallize at room temperature. The mixture was later subjected to colder temperatures maintained for different durations to promote crystallization. The formed crystals (urea-unsaturated fatty acid adducts/urea complexing fraction (UCF) which includes oleic acid) were separated from the liquid (non-urea complexing fraction (NUCF), which includes the polyunsaturated fatty acids) by rapid filtration. The UCF was then diluted with an equal volume of water and acidified to pH 2–3 using 6 N HCl. An equal volume of petroleum ether was added to the dissolved UCF to extract OA. The upper layer containing the enlightened OA was decanted from the aqueous residual layer containing urea. The petroleum ether layer was washed off with 5% NaCl solution to eliminate any residual urea and then dehydrated over anhydrous Na_2SO_4 at 65°C by means of a rotary evaporator.

2.5 *Experimental design and statistical analysis*

In the current study, the software Design-expert version 6.0.10 (Stat Ease, USA) was applied to performing the D-optimal design. A three-factor D-optimal design was employed to study the responses after urea complex fractionation that affected the yield of OA (Y_1 %) and concentration of OA (Y_2 %). The independent variables were denoted as X_1 for urea-to-USFAs ratio (w/w), X_2 for crystallization temperature (°C) 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 were equivalent with the range setting of each parameter: 1–5 g/g for X_1, –10 ±10°C for X_2 and 8–24 h for X_3. To predict the responses, a quadratic or linear model was assumed for the optimization process as indicated in Equation (1).

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

Where 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 Minitab software version 10 (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 R2 and the analysis of variance (ANOVA). Execution of the experimental design with D-optimal generated 18 runs which are required to estimate the coefficients of all models by using a quadratic polynomial regression model as presented in Table 2.

3 Results and Discussion

The D-optimal method for designing experiments was used to optimize urea complex crystallization fractionation for OA separation from a mixture of unsaturated fatty acids fraction (USFA) of HFFA-CPO. The effects of different process conditions on the separation responses were determined and optimized using Response Surface Methodology (RSM). The three-factorial D-optimal design was used to determine the responses of OA yield (Y_1) and concentration of OA (Y_2) in urea complex fraction. An initial screening step was performed to select the main response factors and their values. The independent variables were urea-to-USFAs ratio (g/g), crystallization

temperature (°C) and crystallization time (h), which were denoted by X_1, X_2 and X_3, respectively. Each variable was evaluated by varying their values within a minimum (–1) and maximum (+1) value, as presented in Table 1.

Table 1: Parameters and levels for D-optimal design for the separation and purification of OA

Independent Variables	Factor X_i	Variable Levels		
		–1	0	+1
Urea-to-USFs ratio (g/g)	X_1	1	3	5
Crystallization temperature (°C)	X_2	–10	0	10
Crystallization time (h)	X_3	8	16	24

3.1 *Response surface methodology (RSM)*

RSM is a statistical tool for designing experiments and constructing experimental models that incorporate several interactive parameters. RSM is also used to assess the interaction between selected multiple independent variables (factors) and to define the requisite optimum conditions for generating a specific preferred response. Typically, RSM approaches also reduce the amount of required experimental runs. The final product of a given process parameter is optimized in order to increase its significance in applications. In this study, the experimental values obtained for the yield and concentration of OA in the urea complex fraction for eighteen design points are specified in Table 2.

Table 2: Experimental runs from D-optimal design and the respective responses

Run	Variables Levels			Responses, Y	
	X_1 (g/g)	X_2 (°C)	X_3 (h)	Y_1 (Yield %)	Y_2 (OA %)
1	1	0	16	47	77.8
2	1	–10	8	48.5	81.6
3	5	10	8	61.2	79.58
4	5	–10	16	80.5	82.64
5	1	10	8	41.55	77.08
6	5	10	24	78.5	83.12
7	5	0	24	82.25	80.98
8	3	10	16	76.5	85.86
9	3	0	8	65	84.41
10	5	–10	24	86.5	87.27
11	1	10	8	46	76.08
12	1	–10	8	52.5	80.6
13	3	–5	20	76	84.63
14	1	–10	24	48.27	82.25
15	1	10	24	55.27	81.11
16	5	–10	8	62	80.43
17	5	10	8	56.2	77.59
18	1	10	24	52.8	81.87

3.2 *D-optimal design model fitting*

In this study, D-optimal design was used instead of the standard classical designs due to its advantages. First, the design affords greater flexibility to select the number of experimental runs and the type of response surface model. Standard factorial or fractional factorial designs require too many runs for the number of resources or time allowed for the experiment, while D-optimal designs allow parameters to be estimated without bias and with minimum-variance. Hence, practically, D-optimal experiments can reduce experimentation costs [36]. The quadratic regression coefficients derived with the use of least squares method to predict quadratic polynomial models for the yield % OA (Y_1) and the concentration % of OA are given in Tables 3 and 4, respectively. Close scrutiny of these coefficients with a t test shows that the linear and quadratic terms of urea-to-USFAs ratio (X_1) and linear term of crystallization time (X_3) for the yield % of OA (Y_1) are highly significant ($p < 0.01$). For the concentration % of OA (Y_2), the linear term of the crystallization time (X_3) was highly significant at $p < 0.01$, the linear term of crystallization temperature (X_2) was significant at $p < 0.05$, the quadratic term of urea-to-USFAs ratio (X_1) was highly significant at $p < 0.01$, and the quadratic of the crystallization temperature (X_2) was significant at $p < 0.05$. Among the three interactions, the urea-to-USFAs ratio (X_1) and the crystallization time (X_3) for the yield % of OA (Y_1) were determined to be highly significant ($p < 0.01$), while the other variables were not. Equations (2) and (3) show that the yield % of OA (Y_1) and the concentration % of OA (Y_2) in UCF have a complex relationship with independent variables (X_1, X_2, and X_3), which involves both first -and second- order polynomials.

$$Y_1 = + 73.74 + 11.77\ X_1 - 1.60\ X_2 + 6.61\ X_3 - 13.37\ X_1^2 + 3.55\ X_2^2 - 2.95 X_3^2 - 1.21 X_{12} + 4.45 X_{13} + 1.45 X_{23} \quad (2)$$

$$Y_2 = + 84.03 + 0.84\ X_1 - 1.30\ X_2 + 1.87\ X_3 - 6.23\ X_1^2 + 2.68\ X_2^2 + 0.91\ X_3^2 + 0.026\ X_{12} + 0.41\ X_{13} + 0.39\ X_{23} \quad (3)$$

3.3 *Fitted model diagnostic checking*

The results of the ANOVA analysis for the fitted models

are summarized in Table 5. The two models were examined using an F-test and a P-test. Examination of the two models (Y_1 and Y_2) indicate a non-significant lack-of-fit at ($p > 0.05$) and very small pure error with values of 8.36 and 0.82%, respectively. The regression models for the yield and concentration % of OA showed high significance ($p < 0.01$) with satisfactory regression coefficients (R^2) of 0.9712 and 0.8899, respectively, as shown in Tables 3 and 4. These results indicate that the generated models effectively elucidated the data variation by representing the actual relationships among all the reaction parameters.

Table 3: Regression coefficients of the predicted quadratic polynomial model for response variables (yield % of OA) (Y_1)

Source	Coefficients (ß) (Y_1)	F-value	*p*-value	Notability
Intercept Linear	73.74	29.94	< 0.0001	***
X_1	11.77	153.26	< 0.0001	***
X_2	–1.60	2.55	0.1489	
X_3	6.61	43.55	0.0002	***
Quadratic				
X_1^2	–13.37	23.73	0.0012	***
X_2^2	3.55	2.06	0.1887	
X_3^2	–2.95	1.42	0.2672	
R^2	0.9712			
Interaction				
X_1X_2	–1.21	1.38	0.2741	
X_1X_3	4.45	18.72	0.0025	***
X_2X_3	1.45	1.83	0.2136	

Notes: ** $p < 0.05$; *** $p < 0.01$

3.4 *Analysis of response surface and optimization conditions*

The 3-D response surfaces and contour graphs were used to show the effect of the interaction between the variables, urea-to-USFAs ratio and crystallization temperatures (X_1X_2), urea-to-USFAs ratio and crystallization time (X_1X_3), crystallization temperatures and crystallization time (X_2X_3). The response surfaces for the yield and concentration of OA are shown in Figures 1–3, respectively. Figure 1(a) and (b) shows the effect of urea-to-USFAs ratio and crystallization temperature (X_1X_2) on the separation of OA. The increase of yield and concentration of OA is evident in the urea complex fraction as the temperature decreased from 10 to −10°C. Similarly, ratio of urea-to-USFAs showed a positive correlation with the yield and concentration of OA.

Table 4: Regression coefficients of the predicted quadratic polynomial model for response variables (concentration % of OA) (Y_2)

Source	Coefficients (ß) (Y_2)	F-value	*p*-value	Notability
Intercept Linear	84.03	7.18	0.0054	***
X_1	0.84	4.58	0.0647	
X_2	–1.30	9.85	0.0138	**
X_3	1.87	20.50	0.0019	***
Quadratic				
X_{11}	–6.23	30.30	0.0006	***
X_{22}	2.68	6.92	0.0302	**
X_{33}	0.91	0.79	0.3987	
Interaction				
X_{12}	0.026	3.862E-003	0.9520	
X_{13}	0.41	0.93	0.3637	
X_{23}	0.39	0.77	0.4047	
R^2	0.8899			

Notes: ** $p < 0.05$; *** $p < 0.01$

The effect of the interaction between urea-to-USFAs ratio and crystallization time (X_1X_3) was shown in Figure 2(a) and (b). Increasing urea-to-USFAs ratio and crystallization time possibly increased to higher

Table 5: Analysis of variance (ANOVA) for the responses (yield and concentration % of OA)

	Source	Df	Sum of Squares	Mean Square	F-value	P-value	Notability
Y_1	Model	9	3461.70	384.63	29.94	0.0001	Significant
	Residual	8	102.77	12.85			
	lack-of-fit	4	69.32	17.33	2.07	0.2489	Not significant
	Pure error	4	33.45	8.36			
Y_2	Model	9	141.41	15.71	7.18	0.0054	Significant
	Residual	8	17.50	2.19			
	lack-of-fit	4	14.23	3.56	4.35	0.0916	Not significant
	Pure error	4	3.27	0.82			

Notes: Df = Degree of freedom

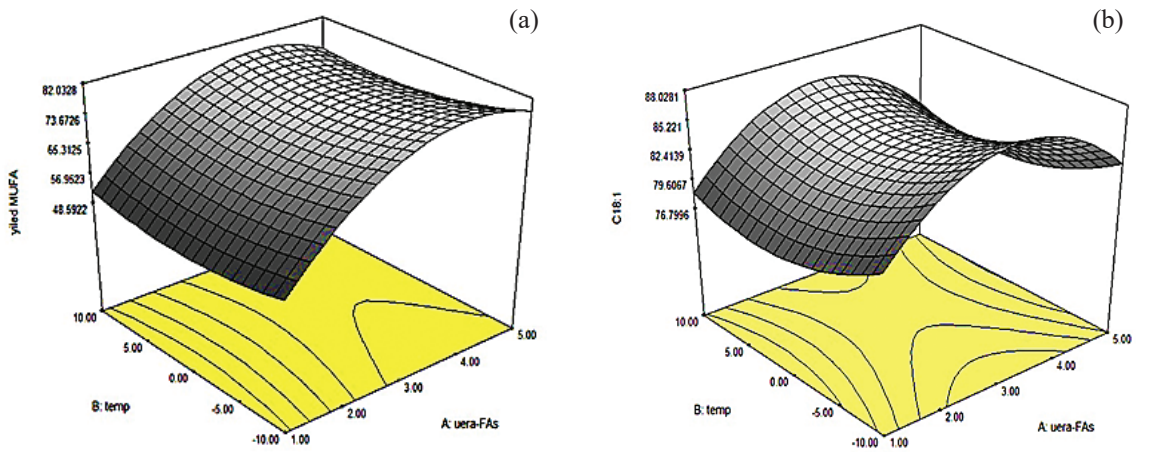

Figure 1: Response surfaces for the effect urea-to-USFAs ratio (X_1, w/w) and crystallization temperatures (X_2, °C) on the yield of OA (Y_1) (a) and concentration of OA (Y_2) (b).

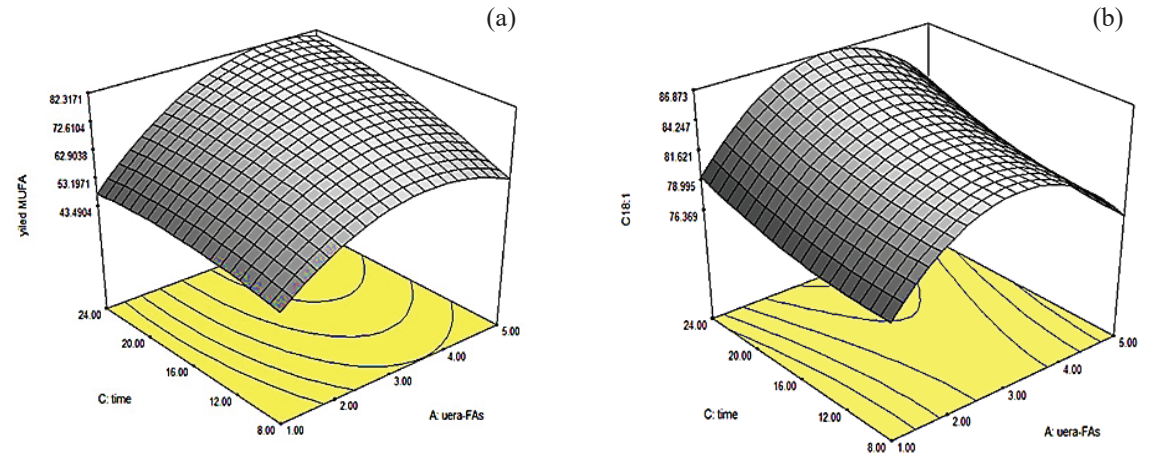

Figure 2: Response surfaces for the effect urea-to-USFAs ratio (X_1, w/w) and crystallization time (X_3, h) on the yield of OA (Y_1) (a) and concentration of OA (Y_2) (b).

OA yield and concentration in the urea complex fraction. The interaction between crystallization time and crystallization temperature (X_2X_3) is a major factor that controls the separation of OA, as shown in Figure 3(a) and (b). It is discernible from the response surfaces that the yield and concentration of OA increased in the urea complex fraction with rise in crystallization time from 8 to 24 h and decrease in crystallization temperature from 10 to –10°C. Thus, performing urea complex fractionation using high amount of urea with cooling would provide the preferred high yield and concentration of OA. The observed values were practically close to the predicted values, as shown in Figures 4 and 5. Therefore, at this point, the optimization tool, as part of the next level of analysis, can now be explored.

3.5 *Validation of model and confirmation of experiment*

After performing ANOVA, numerical optimization was used to obtain the optimum conditions for separate OA. A set of range were selected for all controlling parameters (urea-to-USFAs ratio, crystallization temperature and crystallization time) to attain the ultimate objective of maximum yield and concentration of OA. The higher desirability of the function increases the accuracy of the model. Based on the selected criteria,

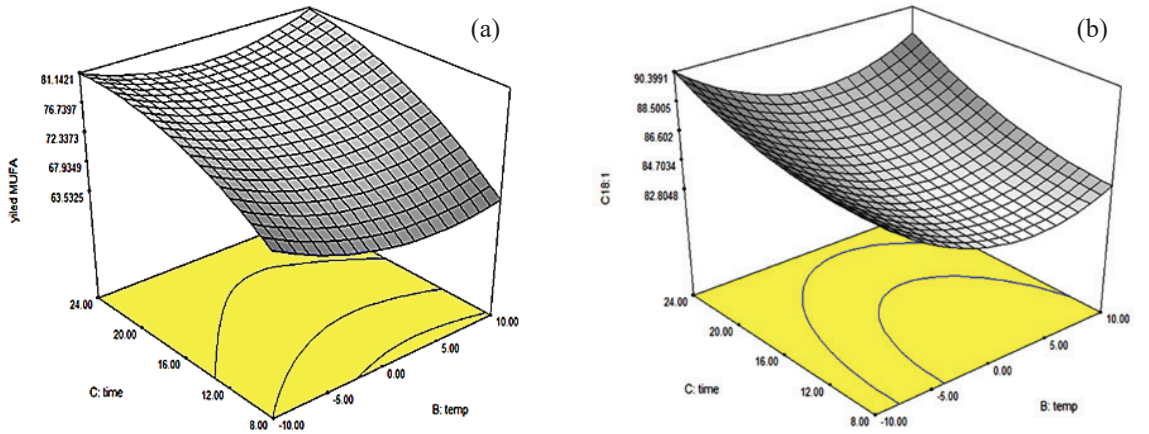

Figure 3: Response surfaces for the effect crystallization temperatures (X_2, °C) and crystallization time (X_3, h) on the yield of OA (Y_1) (a) and concentration of OA (Y_2) (b).

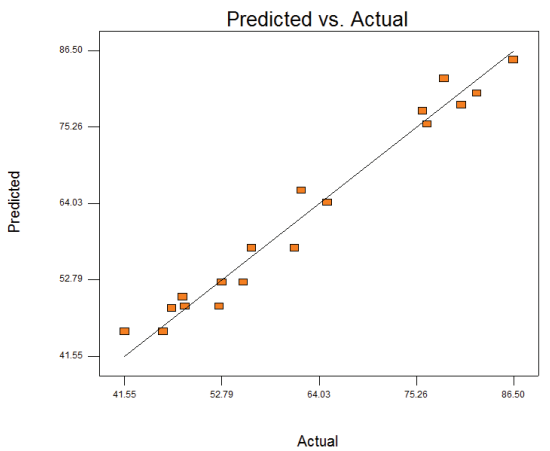

Figure 4: Regression plot of predicated value vs. actual data of yield of OA (Y_1).

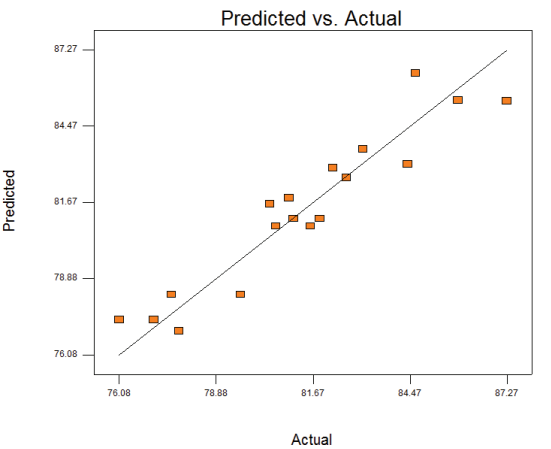

Figure 5: Regression plot of predicated value vs. actual data of concentration of OA (Y_2).

the predicted models showed a desirability function of 0.999. The estimated parameters from numerical optimization are shown in Figure 6. Using D-optimal design, the optimum conditions predicted were a urea-to-USFAs ratio (w/w) of 4.62 : 1, crystallization temperature of −10°C and crystallization time of 24 h. Under these conditions, the yield and concentration values of OA were 86.4427% and 87.2436%, respectively. As shown in Table 6, to validate the predicted model, the experiments were carried out in triplicate. This produced an average 86.3 ± 0.6% yield and 86.9 ± 0.7% concentration of OA. These results are in good agreement with the data generated from the model. The results also show relatively high OA recovery of 86.4%. In summary, this study confirms the reliability of D-optimal design as a simple and valuable approach to assessing the optimum conditions for urea complex fractionation, specifically in the separation of oleic acid from a mixture of unsaturated fatty acids CPO fatty acid.

3.6 *Fatty acids composition*

Table 7 summarize the fatty acids composition of PFAM after hydrolysis, USFAs after LTSC, and OA after UCF. The original mixture of PFAM after hydrolysis was composed of 47.7% SFA, 42.2% MUFA ($C_{18:1}$) and 10.1% PUFA. The percentages of OA increased from 42.2% to 76.3% after separation

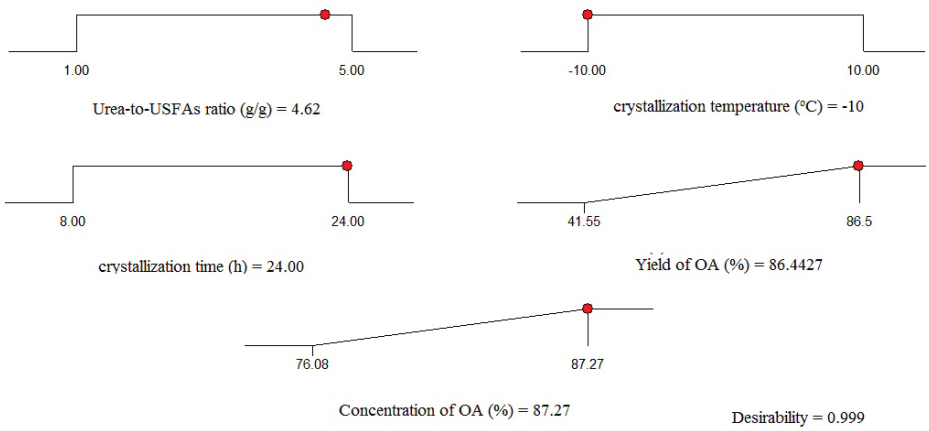

Figure 6: OA as derived from the RSM predicted model using optimal conditions.

Table 6: Validation test result of an OA optimum conditions

	No.	Independent Variables			Responses		
		X_1	X_2	X_3	OA Yield (%)	OA Concentration (%)	OA Recovery (%)
Actual	1	4.62 : 1	−10	24	86.5	87.7	85.4
	2	4.62 : 1	−10	24	85.6	85.9	86.3
	3	4.62 : 1	−10	24	86.7	87.3	87.5
Actual average predicted		4.62 : 1	−10	24	86.3 ± 0.6	86.9 ± 0.7	86.4 ± 0.5
		4.62 : 1	−10	24	86.4427	87.2436	87.1

Notes: X_1 = Urea-to- USFAs (g/g); X_2 = crystallization temp. (°C); X_3 = crystallization time (h)

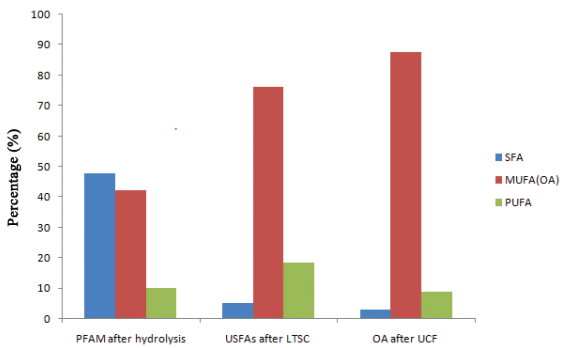

Figure 7: Fatty acids composition of PFAM, USFAs and OA.

using low-temperature solvent crystallization (LTSC). Furthermore, the purity of OA after separation using UCF under optimum conditions reached 87.7%, as shown in Figure 7. The percentage of OA composition has further increase after using LTSC and followed by UCF separation methods. The overall OA concentration has been increased from 42.2% to 88% with considerably high recovery percentage of 86%.

Table 7: Fatty acids relative composition (%) of PFAM, USFAs and OA after separation

Fatty Acids	Relative Composition (%)		
	PFAM after Hydrolysis	USFAs after LTSC	OA after UCF
Lauric acid $C_{12:0}$	0.2 ± 0.2	0.1 ± 0.5	-
Myristic acid $C_{14:0}$	0.9 ± 0.3	0.7 ± 0.3	-
Palmitic acid $C_{16:0}$	42.3 ± 0.7	4.1 ± 0.1	3.2 ± 0.3
Stearic acid $C_{18:0}$	4.3 ± 0. 5	0.3 ± 0.4	-
Oleic acid $C_{18:1}$	42.2 ± 0.3	76.3 ± 0.2	87.7 ± 0.5
Linoleic acid $C_{18:2}$	9.9 ± 0.4	18.3 ± 0.3	9.1 ± 0.1
Linolenic acid $C_{18:3}$	0.2 ±0.6	0.2 ± 0.1	-
SFA %	47.7	5.2	3.2
MUFA (OA) %	42.2	76.3	87.7
PUFA %	10.1	18.5	9.1

Notes: LTSC = Low-temperature solvent crystallization; SFA = Saturated fatty acids; PUFA = Polyunsaturated fatty acids; PFAM = Palm fatty acids mixture

The results showed that OA has been successfully separated from polyunsaturated fatty acids with high yields from the urea complex fraction. Thus, it can be inferred that OA has a high propensity to form urea adducts than polyunsaturated fatty acids [35]. The results confirm that the experimental conditions are suitable for the recovery of high yield and concentration of OA. However, it is challenging to have total removal all the polyunsaturated fatty acids in order to recover OA of 100% purity in the concentrate. This was earlier asserted by Swern *et al.* [37] and Rubin *et al.* [38], who both reported that complete removal of polyunsaturated fatty acids by urea complexation may not be attainable as some of the polyunsaturated fatty acids do not complicate with urea in the course of crystallization. OA obtained from the study with high yields, concentration and recovery make the urea complexation method is favorable separation method to OA attain from many vegetable oils fatty acids mixtures. Therefore OA is plausible to be used for the OA enrichment into palm oil to produce HOPO via various modification methods such as transesterification between OA with PO.

4 Conclusions

The separation and purification of OA from unsaturated fatty acids fraction of HFFA-CPO by urea complex fractionation, UCF method was performed following the design of an experiment based on the D-optimal approach. The effects of the interactions between independent variables on the separation of OA were investigated and the consistency between the predicted data from the model and the experimental data were confirmed. The design offers greater flexibility to runs the experiments that allow parameters to be estimated without bias and with minimum-variance and reduce experiments cost. At the optimal condition the UCF contain 88% of oleic acid with 86% recovery. The study shows that OA easily recover from low cost type palm oil with high recovery. Therefore OA is plausible to be used for OA enrichment into palm oil to produce HOPO via various modification methods such as transesterification between OA with PO to produce dietary oil as good as olive oil.

Acknowledgements

We would like to thank Universiti Kebangsaan Malaysia for the project funding under research grant no. GUP-2017-008 and for research facilities provided.

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