

Optimisation of Urea Complexation by Box-Behnken Design (Pengoptimuman Pengkompleksan Urea Menggunakan Reka Bentuk Box-Behnken)

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ABSTRACT

Urea complexation is a method favoured by researchers to separate fatty acids based on molecular structure. In this study, effects of urea amount, crystallisation temperature and crystallisation time on the final products of urea complexation were examined. Box-Behnken Design (BBD) was employed to study the significance of these factors and the optimum conditions for the technique were predicted and verified. Results showed that urea-to-polyunsaturated fatty acid (PUFA) mole ratio and crystallisation temperature were two significant variables for enrichment of PUFA in non-urea complexing fraction (NUCF). In a 17-point experimental design, percentage of saturated fatty acid (SFA) was reduced almost entirely from initial 4.49%, monounsaturated fatty acid (MUFA) reduced from 57.02% to 41.32%, while PUFA increased from 33.49% to 53.87% in NUCF. Optimum condition for maximum PUFA and minimum SFA and MUFA contents was predicted at urea-to-PUFA mole ratio of 20, crystallisation temperature of -20°C and crystallisation time of 29.67 h.

Keywords: Box-Behnken design (BBD); optimisation; polyunsaturated fatty acid (PUFA); urea complexation

ABSTRAK

Pengkompleksan urea merupakan kaedah yang sering digunakan oleh para penyelidik untuk memisahkan campuran asid lemak berdasarkan struktur molekul. Dalam kajian ini, kesan amaun urea, suhu penghabluran dan tempoh penghabluran terhadap hasil akhir pengkompleksan urea telah dikaji. Reka bentuk Box-Behnken (BBD) telah digunakan untuk mengkaji signifikansi faktor-faktor ini. Keadaan optimum faktor-faktor ini telah diramal dan disahkan. Keputusan menunjukkan nisbah mol urea-kepada-asid lemak poli tak tepu (PUFA) serta suhu penghabluran merupakan dua pembolehubah yang signifikan bagi peningkatan kandungan PUFA di dalam fraksi bukan pengkompleks urea (NUCF). Dalam suatu reka bentuk eksperimen 17 titik, hampir kesemua asid lemak tepu (SFA) telah disingkirkan daripada peratusan asal 4.49%, asid lemak mono tak tepu (MUFA) telah dikurangkan daripada 57.02% kepada 41.32%, manakala PUFA telah ditingkatkan daripada 33.49% kepada 53.87% di dalam NUCF. Keadaan optimum bagi pengasingan PUFA yang maksimum dan kandungan SFA dan MUFA yang minimum telah diramalkan pada nisbah mol urea-kepada-PUFA sebanyak 20 mol, suhu penghabluran -20°C dan tempoh penghabluran selama 29.67 jam.

Kata kunci: Asid lemak poli tak tepu (PUFA); pengkompleksan urea; pengoptimuman; reka bentuk Box-Behnken (BBD)

INTRODUCTION

The importance of polyunsaturated fatty acids (PUFA) in human nutrition and their effects has been widely reported and reviewed (Albertazzi & Coupland 2002; Diniz et al. 2004; Nageswari et al. 1999; Roynette et al. 2004). Most of these researches focused on the omega-3 and omega-6 fatty acids as these fatty acids are not synthesised by human body and have to be acquired through diet. These PUFA, which include linoleic and linolenic acids are classified as essential fatty acids (EFA) and play roles as precursors to hormone-like compounds known as eicosanoids. Eicosanoids are involved in many important biological processes in the human body (Shahidi & Wanasundara 1998). Linoleic acid is widely present in most vegetable oils, especially corn and sunflower oils, while major sources of linolenic acid are soybean and canola oils and leafy green vegetables (Hu 2001).

Among various methods to produce PUFA concentrates, the urea complexation is considered the simplest and most efficient technique for obtaining omega-3 PUFA concentrates in the form of free fatty acids (FFA). The process is considered simple, quick, inexpensive, robust, and environmental friendly (Hayes et al. 1998; Wanasundara & Shahidi 1999). This technique is widely used to separate mixtures of fatty acids according to their degree of unsaturation, or to separate straight-chained from branched acids. The other methods to fractionate PUFA are chromatographic methods, distillation method, enzymatic methods, low-temperature crystallisation and supercritical fluid extraction. However, these methods are slow, inefficient, expensive and often difficult to scale up (Hayes et al. 1998; Shahidi & Wanasundara 1998).

In the present study, urea complexation was used to increase the content of PUFA (particularly linoleic and

linolenic acids) from a mixture of FFA. Box-Behnken Design (BBD) was used to evaluate the effect of three factors, i.e. urea-to-PUFA-mole ratio (X_1), crystallisation temperature (X_2), and crystallisation time (X_3) on urea complexation. Fatty acids present in urea complexed fraction (UCF) and non-urea complexing fraction (NUCF) were analysed. The focus was on enriching PUFA, mainly C18:2 and C18:3 in vegetable oil. Box-Behnken Design was chosen as the experimental model as this design is deemed rotatable but requires fewer design points, design points are within the original factor ranges.

MATERIALS AND METHODS

The FFA mixture used in this research was hydrolysed from canola-sunflower oil blend that was produced by a local edible oil manufacturer. Fatty acid methyl ester (FAME) standards were purchased from Sigma. All chemicals used were of analytical grade.

Saponification of oil was done according to methods by Syed Rahmatullah et al. (1994) and Senanayake and Shahidi (1999) with modifications. The oil was saponified with 1 N potassium hydroxide in aqueous ethanol 95% (v/v) and the unsaponifiable matters were extracted by n-hexane and discarded. Distilled water was added to the soap and then acidified with 6 N hydrochloric acid to release the FFA. These FFA were extracted three times, each by using 50 mL n-hexane. The combined solvent was washed with distilled water and dried over anhydrous sodium sulphate. Solvent was removed by rotary evaporator at 45°C and the FFA converted to FAME for GC analysis according to PORIM Method p3.4 (1995) (Siew et al. 1995). Fatty acid profile was analysed with Shimadzu GC-17A with a BPX70 column (30 m × 0.25 mm × 0.25 µm). Injection and detection (FID) temperatures were set at 260°C and 280°C, respectively and nitrogen was used as the carrier gas with flow rate of 0.3 mL/min. The split ratio was 1:39.

BBD was used to create an experimental design with 17 design points. Three variables evaluated were urea-to-PUFA mole ratio, X_1 , crystallisation temperature, X_2 , and crystallisation time, X_3 . Table 1 shows a 17-trial experimental design with the three variables tested at three coded level, i.e. low (−1), middle (0) and high (+1). The coded values for urea-to-PUFA mole ratio (actual values in brackets) were: −1 (10.0), 0 (15.0), +1 (20), for crystallisation temperature: −1 (−20°C), 0 (5°C), +1 (30°C), and for crystallisation time: −1 (6 hr), 0 (27 hr), +1 (48 hr). All experiments were carried out randomly to minimise the effect of unexplained variability in the observed responses due to extraneous factors (Wanasundara & Shahidi 1999).

Urea complexation was performed by dissolving 250 to 500 mmol of urea in 100 mL of ethanol by heating. After a clear, homogeneous solution had formed, 75 mmol of fatty acid was added into the solution with continuous stirring. The mixture was allowed to cool to

room temperature and then stored at different temperature (−20 to 30°C) for different duration (6 to 48 h) for further crystallisation according to the experimental design.

All the samples were taken out from storage according to the experimental duration and filtered through sintered glass. The liquid NUCF were added an equal amount of distilled water and acidified with 6 N hydrochloric acid to between pH 4 and 5. It was then extracted 3 times with 30 mL of n-hexane. The combined n-hexane was then washed 3 times to remove remaining urea in the n-hexane and dried over anhydrous sodium sulphate.

UCF of all samples were also collected and dissolved in distilled water. n-Hexane was used to extract the fatty acids in UCF. Solvent in all fatty acid samples (NUCF and UCF) were removed by rotary evaporator at 45°C and the fatty acid recovered were weighed and transformed into FAME for gas chromatography analyses.

Design-Expert version 6.0.10 was used to analyse the data of urea complexation by fitting all the data to suitable regression models.

RESULTS AND DISCUSSION

The initial fatty acid mixture hydrolysed from the canola-sunflower oil blend was composed of 4.49% of palmitic (C16:0), 57.02% of oleic (C18:1), 27.65% of linoleic (C18:2) and 5.84% of linolenic (C18:3) acids. Other minor fatty acids made up the remaining composition of the fatty acid mixture. Average molecular weight of the fatty acids was 279.7 as obtained from saponification test of the original oil blend. Out of 75 mmol of FFA used in the samples, one-third or 25 mmol was PUFA (C18:2 and C18:3).

Table 1 shows fatty acid composition in the UCF. Compared to the starting material, saturated fatty acid (SFA) percentage was significantly higher while PUFA was lower in all the samples. Highest percentage of SFA (27.2%) was observed for sample treated with 10 urea/PUFA ratio (mol/mol) at 5°C for 48 hours. This sample also had the lowest percentage of PUFA (8.84%) incorporated into urea complex. Samples loaded with highest amount of urea had the lowest percentages of SFA in UCF. Inclusion of more PUFA into UCF reduced the percentage of SFA in these samples.

Table 1 shows data obtained from the experiment on fatty acid composition in the NUCF of all the samples. Results showed that the percentage of PUFA has increased to as much as 53.87% from 33.49% while SFA has been reduced considerably compared to the initial FFA mixture. In high-urea samples, the elimination of SFA was near completion in NUCF. However, total removal of SFA by urea complexation may be impossible because some of the SFA do not complex with urea during crystallisation (Liu et al. 2006; Shahidi & Wanasundara 1998).

The results obtained from the experiment were analysed with linear or quadratic regression models. When any of these models were found to be insignificant

TABLE 1. Box-Behnken Design arrangement and responses for urea complexing fraction (UCF) and non-urea complexing fraction (NUCF)

Run no.	Urea/PUFA ^a (X ₁)	Temp ^b (X ₂)	Time ^c (X ₃)	Responses, Y																
				Urea complexing fraction (UCF)							Non-urea complexing fraction (NUCF)									
				C16:0 (%)	C18:0 (%)	Y ₁ , SFA (C16:0 + C18:0) (%)	C18:2 (%)	C18:3 (%)	Y ₂ , MUFA C18:1 (%)	Y ₃ , PUFA (C18:2 + C18:3) (%)	Y ₄ , SFA C16:0 (%)	Y ₅ , MUFA C18:1 (%)	C18:2 (%)	C18:3 (%)	Y ₆ , PUFA (C18:2 + C18:3) (%)					
1	10	30	27	14.23	8.30	22.53	10.74	2.06	56.69	12.80	1.40	53.94	32.80	7.04	39.84					
2	15	5	27	10.84	5.19	16.03	9.79	1.87	66.38	11.66	0.32	47.97	39.07	8.23	47.30					
3	20	-20	27	8.94	3.82	12.76	11.11	2.08	68.80	13.19	n.d.	41.32	44.42	9.45	53.87					
4	15	5	27	11.36	5.39	16.75	9.28	1.71	66.18	10.99	0.35	48.14	38.03	8.01	46.04					
5	20	5	6	9.77	4.13	13.90	10.50	1.96	68.72	12.46	0.76	43.59	41.16	8.81	49.97					
6	10	-20	27	12.36	6.23	18.59	10.07	1.93	61.72	12.00	0.69	49.76	36.25	7.66	43.91					
7	10	5	6	13.78	7.29	21.07	10.13	1.95	58.88	12.08	0.88	53.39	34.50	7.24	41.74					
8	15	5	27	10.71	5.14	15.85	10.54	1.98	66.07	12.52	0.31	47.52	39.26	8.38	47.64					
9	10	5	48	17.26	9.94	27.20	7.42	1.42	55.15	8.84	1.36	53.64	33.82	7.06	40.88					
10	20	5	48	9.67	4.47	14.14	9.12	1.64	69.16	10.76	n.d.	43.94	42.69	8.91	51.60					
11	15	30	6	12.45	5.93	18.38	9.92	1.85	62.64	11.77	0.48	50.34	37.04	7.68	44.72					
12	15	-20	48	11.29	5.03	16.32	8.57	1.56	66.43	10.13	n.d.	46.92	40.14	8.44	48.58					
13	20	30	27	9.64	4.52	14.16	12.00	2.25	66.38	14.25	0.38	45.72	40.49	8.72	49.21					
14	15	5	27	10.44	4.59	15.03	12.36	2.39	65.52	14.75	0.33	47.46	39.42	8.46	47.88					
15	15	5	27	10.64	4.87	15.51	9.58	1.79	67.47	11.37	n.d.	45.88	41.06	8.88	49.94					
16	15	-20	6	10.73	4.96	15.69	9.61	1.78	66.90	11.39	0.22	46.66	39.65	8.39	48.04					
17	15	30	48	14.16	7.08	21.24	7.53	1.38	60.15	8.91	0.62	51.70	35.86	7.40	43.26					

C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; n.d., not detected

^a urea-to-polyunsaturated fatty acid (PUFA) ratio (mol/mol);^b crystallisation temperature (°C);^c crystallisation time (h);

to represent the data, minor modification was made on the models for better representation of data. Responses studied were SFA, monounsaturated fatty acid (MUFA), and PUFA percentages in both NUCF and UCF. All responses were fitted in quadratic regression model, except data for PUFA in NUCF and PUFA in UCF that were fitted in linear regression model and modified quadratic regression model respectively. Table 2 shows regression coefficients of predicted models for all the responses.

The lack of fit F-value for all the responses showed that the lack of fit is not significant ($p>0.05$) relative to the pure error. This indicates that all the models predicted for the responses were adequate. Regression models for data on responses Y_1 , Y_2 , Y_4 , Y_5 , and Y_6 were highly significant ($p<0.01$) with satisfactory R^2 . However R^2 for Y_3 was lower although the model was significant. Table 3 summarizes the analysis of variance (ANOVA) of all the responses of this study.

For the content of SFA in UCF, linear terms of urea-to-PUFA ratio, crystallization temperature and quadratic term of crystallization time were highly significant ($p<0.01$) and linear term of crystallization time and interaction between urea-to-PUFA ratio and crystallization time were significant ($p<0.05$). Highly significant ($p<0.01$) terms of MUFA content in UCF were linear, while urea-to-PUFA ratio was quadratic and of crystallization time was linear. Quadratic term of crystallization time was also significant ($p<0.05$) for MUFA content in UCF. As for PUFA content

in UCF, linear term of crystallization time was significant ($p<0.05$) while its quadratic term was highly significant ($p<0.01$).

In NUCF, linear terms of urea-to-PUFA ratio and crystallization temperature, quadratic term of crystallization time, and interaction between urea-to-PUFA ratio and crystallization time were highly significant ($p<0.01$). Linear terms of urea-to-PUFA ratio and crystallization temperature and quadratic term of crystallization time were highly significant ($p<0.01$) for MUFA content in NUCF, while linear term of crystallization time was also significant at $p<0.01$. Lastly, linear terms of urea-to-PUFA ratio and crystallization temperature were highly significant ($p<0.01$). In all but one of all the responses, linear terms of urea-to-PUFA ratio and crystallization temperature were found to be highly significant.

These results suggest that linear effect of urea ratio and crystallization temperatures are the primary determining factors for fatty acid separation by urea complexation. Liu et al. (2006) also concluded that these two variables had a very large effect on the results of their urea complexation study. Crystallization time was found to be the insignificant factor ($P>0.05$). This finding is in agreement with the results reported by other researchers that also concluded that crystallization time was not significant for the concentration of omega-3 fatty acids (Liu et al 2006; Wanasundara & Shahidi 1999). Final equation in terms of actual factors are:

TABLE 2. Regression coefficients of predicted models for response variables in urea complexation experiment of canola-sunflower fatty acids

Variables	Coefficients					
	SFA in UCF (%) (Y_1)	MUFA in UCF (%) (Y_2)	PUFA in UCF (%) (Y_3)	SFA in NUCF (%) (Y_4)	MUFA in NUCF (%) (Y_5)	PUFA in NUCF (%) (Y_6)
Intercept	+34.89935	+36.52991	+14.54847	+3.89897	+67.76821	+34.27416
Linear						
X_1	-1.86431***	+3.12523***	-0.64870	-0.46204***	-1.49230***	+0.88150***
X_2	+0.11223***	-0.13059***	+0.00256400	+0.015637***	+0.065969***	-0.08685***
X_3	+0.010207**	-0.016689	+0.17462**	+0.029010	-0.27826**	-0.018869
Quadratic						
X_{11}	+0.046920	-0.080080***	+0.025740	+0.015510***	+0.015420	-
X_{22}	+0.00000480000	-0.00151920	+2.53600	-0.0000516000	-1.51200	-
X_{33}	+0.00469501***	-0.00304875**	-0.00423243***	+0.000227324	+0.00364059***	-
Interaction						
X_{12}	-0.00508000	+0.00542000	-	-0.000660000	+0.000440000	-
X_{13}	-0.014024**	+0.00992857	-	-0.00295238***	+0.00733333	-
X_{23}	+0.00106190	-0.000961905	-	+0.000171429	+0.000523810	-
X_{123}	-	-	-	-	-	-
R^2	0.9652	0.9692	0.7225	0.9656	0.9811	0.8854

X_1 = urea-to-PUFA-mole ratio; X_2 = crystallisation temperature; X_3 = crystallisation time

** $P<0.05$, *** $P<0.01$.

TABLE 3. Analysis of variance (ANOVA) of all the responses

	Responses	Degree of Freedom	Sum of Squares	Mean Square	F-Value	Prob>F
Y ₁	Model	9	217.95	24.22	21.58	0.0003
	Residual	7	7.86	1.12		
	Lack of fit	3	6.23	2.08	5.10	0.0748
	Pure error	4	1.63	0.41		
Y ₂	Model	9	291.47	32.39	24.46	0.0002
	Residual	7	9.27	1.32		
	Lack of fit	3	7.22	2.41	4.70	0.0845
	Pure error	4	2.05	0.51		
Y ₃	Model	6	29.40	4.90	4.34	0.0205
	Residual	10	11.29	1.13		
	Lack of fit	6	2.26	0.38	0.17	0.9728
	Pure error	4	9.03	2.26		
Y ₄	Model	9	2.91	0.32	21.82	0.0003
	Residual	7	0.10	0.015		
	Lack of fit	3	0.017	5.708 × 10 ⁻³	0.26	0.8491
	Pure error	4	0.087	0.022		
Y ₅	Model	9	191.76	21.31	40.47	< 0.0001
	Residual	7	3.69	0.53		
	Lack of fit	3	0.48	0.16	0.20	0.8902
	Pure error	4	3.20	0.80		
Y ₆	Model	3	194.38	64.79	33.48	< 0.0001
	Residual	13	25.16	1.94		
	Lack of fit	9	17.21	1.91	0.96	0.5616
	Pure error	4	7.95	1.99		

$$Y_1 = 34.89935 - 1.86431X_1 + 0.11223X_2 + 0.010207X_3 + 0.046920X_1^2 + 0.00000480000X_2^2 + 0.00469501X_3^2 - 0.00508000X_1X_2 - 0.014024X_1X_3 + 0.00106190X_2X_3$$

$$Y_2 = 36.52991 + 3.12523X_1 - 13.059X_2 - 0.016689X_3 - 0.080080X_1^2 - 0.00151920X_2^2 + 0.00304875X_3^2 + 0.00542000X_1X_2 + 0.00992857X_1X_3 - 0.000961905X_2X_3$$

$$Y_3 = 14.54847 - 0.64870X_1 + 0.00256400X_2 + 0.17462X_3 + 0.025740X_1^2 + 2.53600X_2^2 + 0.00423243X_3^2$$

$$Y_4 = 3.89897 - 0.46204X_1 + 0.015637X_2 + 0.029010X_3 + 0.015510X_1^2 + 0.0000516000X_2^2 + 0.000227324X_3^2 - 0.000660000X_1X_2 - 0.00295238X_1X_3 + 0.000171429X_2X_3$$

$$Y_5 = 67.76821 - 1.49230X_1 + 0.065969X_2 - 0.27826X_3 + 0.015420X_1^2 - 1.51200X_2^2 + 0.00364059X_3^2 - 0.000440000X_1X_2 - 0.007333333X_1X_3 + 0.000523810X_2X_3$$

$$Y_6 = 34.27416 + 0.88150X_1 - 0.08685X_2 - 0.018869X_3$$

Figures 1 to 6 are the Design-Expert plots for all the responses. In the solid UCF, performing the technique using low amount of urea without cooling would give the desired

high percentage of SFA as shown in Figure 1. MUFA (Figure 2) and PUFA (Figure 3) concentrations were also lower at this condition. However, by increasing the amount of urea and reducing crystallization temperature, more MUFA and PUFA would form urea adduct, reducing recoverable quantity of these unsaturated fatty acids in NUCF.

DESIGN-EXPERT Plot

SFA UCF
X = A: Urea to PUFA Ratio
Y = B: Crystallisation Temperature

Actual Factor

C: Crystallisation Time = 27.00

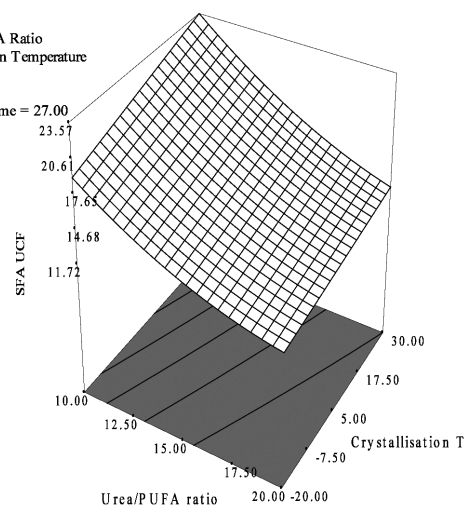


FIGURE 1. Response surface for the effect of urea-to-PUFA ratio and crystallisation temperature on SFA in UCF. Crystallisation time = 27 h

DESIGN-EXPERT Plot

MUFA UCF

X = A: Urea to PUFA Ratio

Y = B: Crystallisation Temperature

Actual Factor

C: Crystallisation Time = 27.00

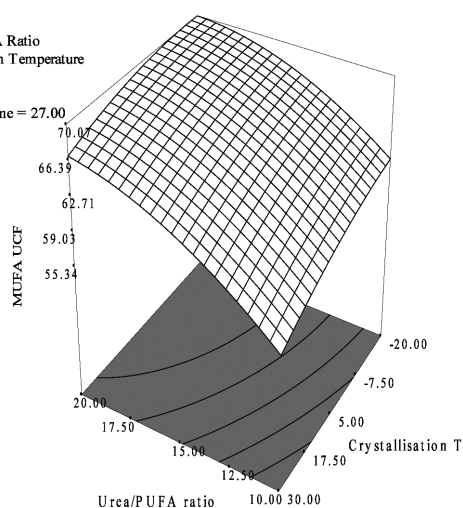


FIGURE 2. Response surface for the effect of urea-to-PUFA ratio and crystallisation temperature on MUFA in UCF. Crystallisation time = 27 h.

DESIGN-EXPERT Plot

SFA NUCF

X = A: Urea to PUFA Ratio

Y = B: Crystallisation Temperature

Actual Factor

C: Crystallisation Time = 27.00

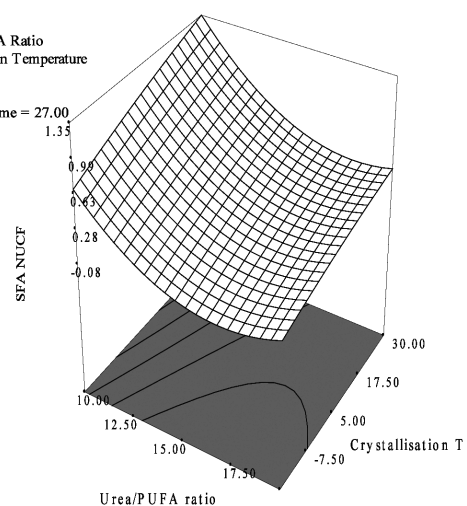


FIGURE 4. Response surface for the effect of urea-to-PUFA ratio and crystallisation temperature on SFA in NUCF. Crystallisation time = 27 h.

DESIGN-EXPERT Plot

PUFA UCF

X = A: Urea to PUFA Ratio

Y = B: Crystallisation Temperature

Actual Factor

C: Crystallisation Time = 27.00

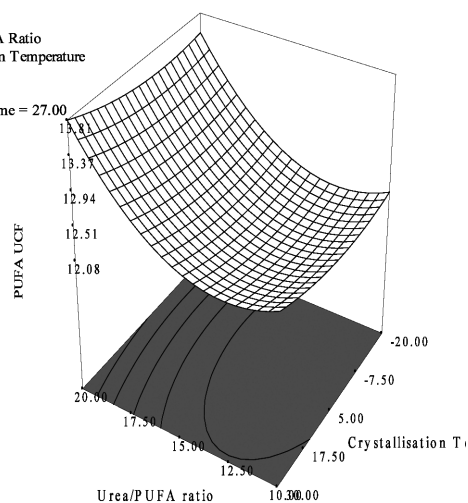


FIGURE 3. Response surface for the effect of urea-to-PUFA ratio and crystallisation temperature on PUFA in UCF. Crystallisation time = 27 h.

DESIGN-EXPERT Plot

MUFA NUCF

X = A: Urea to PUFA Ratio

Y = B: Crystallisation Temperature

Actual Factor

C: Crystallisation Time = 27.00

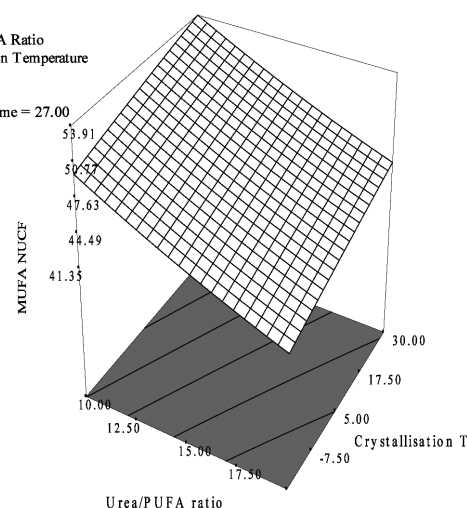


FIGURE 5. Response surface for the effect of urea-to-PUFA ratio and crystallisation temperature on MUFA in NUCF. Crystallisation time = 27 h.

As shown in Figures 4 and 5, the increasing amount of urea and the decreasing crystallisation temperature led to reduction of percentage of SFA and MUFA in liquid NUCF. This condition would also enrich the content of PUFA in the liquid fraction (Figure 6). The relationships between the parameters and fatty acids percentage were linear or almost linear. High concentration of PUFA could be obtained by using high amount of urea at low temperature. However, this could also reduce the yield of PUFA in the final product as more PUFA would be lost into urea adducts. Experimental variables should be carefully controlled in order to recover a maximum content of PUFA of interest with reasonable yield (Shahidi & Wanasundara 1998).

Straight-chained molecules such as SFA were readily forming stable adduct with urea. SFA form complexes more readily than MUFA do, and MUFA form inclusion compounds more readily than PUFA do. Similar complexation tendency pattern was also obtained by Hayes et al. (1998) and Wu et al. (2008). Although addition of more urea could reduce SFA percentage in NUCF to a minimum level, it also results in indiscriminate fatty acid complexation and thus reducing the amount of MUFA and PUFA. A lower urea to FA ratio was able to prevent indiscriminate FFA complexation (Hayes et al. (1998). Lower crystallisation temperature may facilitate formation of more stable urea adducts, that would reduce SFA in NUCF. Longer period of crystallisation time would

DESIGN-EXPERT Plot

PUFA NUCF

X = A: Urea to PUFA Ratio

Y = B: Crystallisation Temperature

Actual Factor

C: Crystallisation Time = 27.00

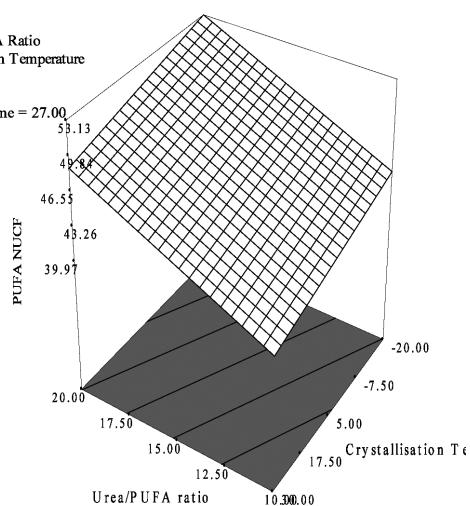


FIGURE 6. Response surface for the effect of urea-to-PUFA ratio and crystallisation temperature on PUFA in NUCF. Crystallisation time = 27 h.

allow the crystals for further stabilisation. The parameters would have to be set at a reasonable level to achieve an acceptable yield of product with desired purity. Higher purity of PUFA will always give lower yield of NUCF.

Optimum conditions of the experiment to obtain maximum concentration of PUFA and minimum concentration of both SFA and MUFA were predicted at urea-to-PUFA mole ratio of 20, crystallisation temperature of -20°C and 29.67 h of crystallisation time. At this condition, the final NUCF was predicted to contain 0% of

SFA (C16:0), 41.52% of MUFA (C18:1) and 53.08% of PUFA (C18:2 and C18:3). Performing a verification experiment gave the result of 38.36% of C18:1, 47.49% of C18:2, and 10.34% of C18:3 (total C18:2 and C18:3 is 57.83%) with the NUCF yield of 41.53%. The observed value was reasonably close to the predicted value as shown in Figures 7-12.

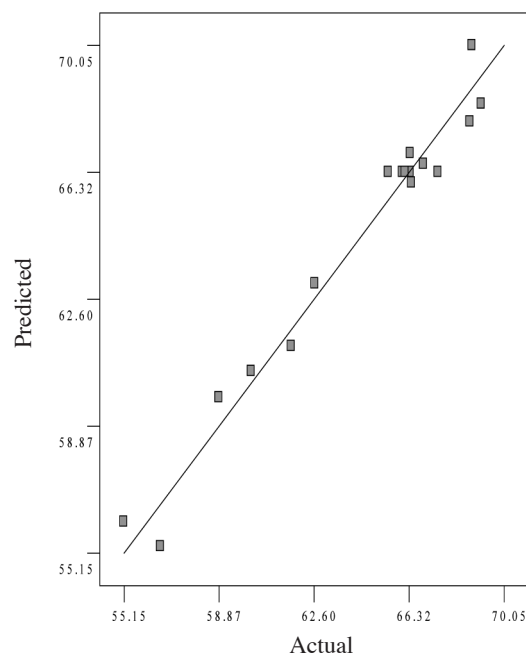


FIGURE 8. Predicted vs. actual plot of Y_2

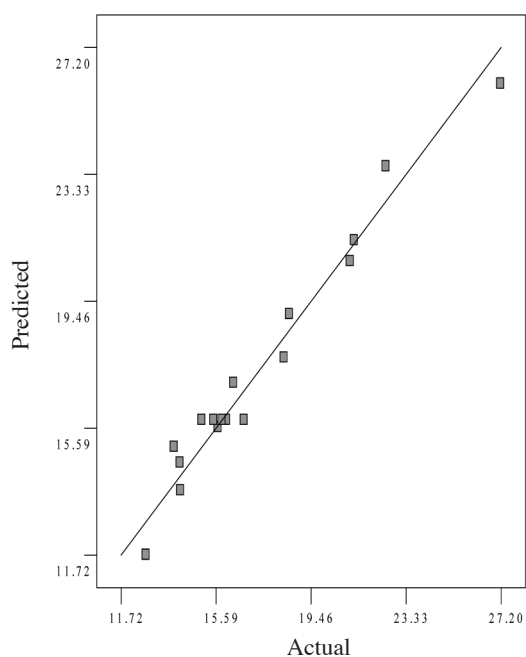


FIGURE 7. Predicted vs. actual plot of Y_1

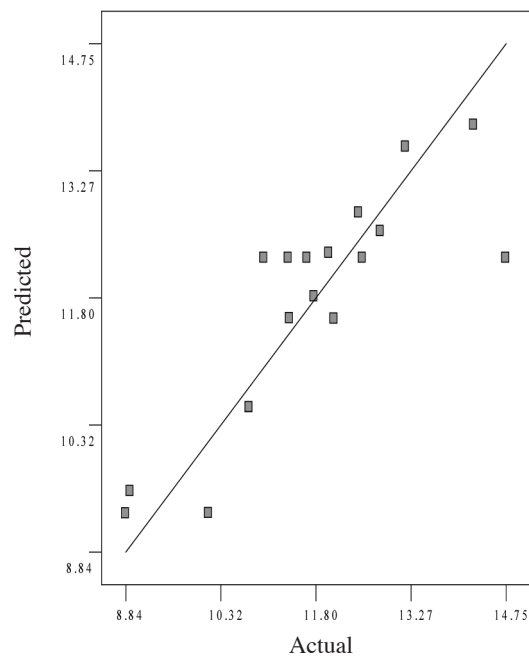
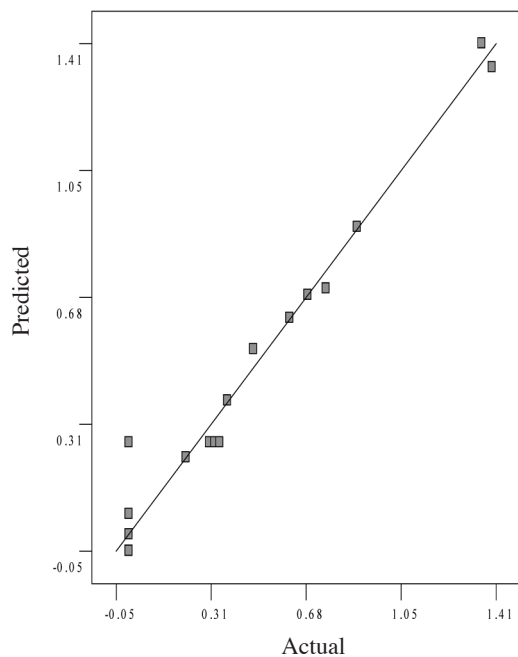
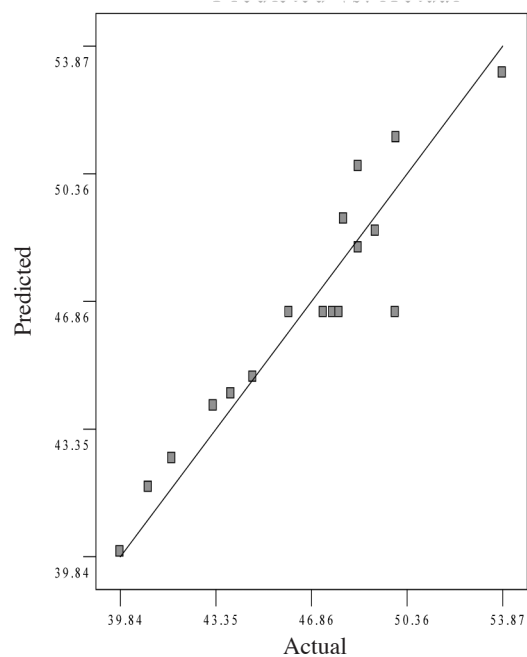
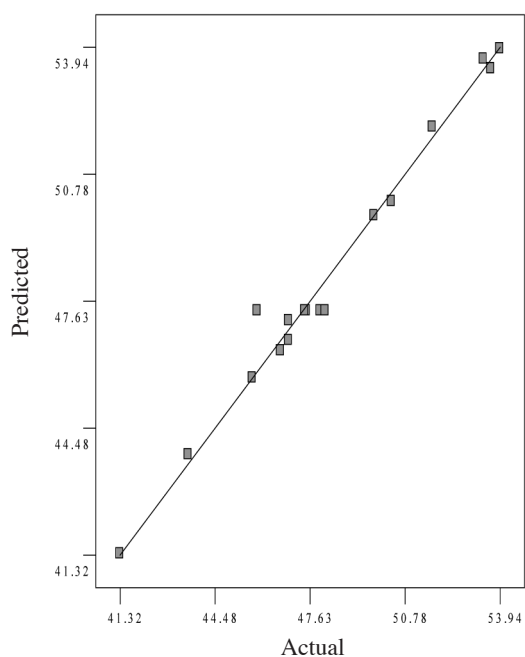


FIGURE 9. Predicted vs. actual plot of Y_3

FIGURE 10. Predicted vs. actual plot of Y_4 FIGURE 12. Predicted vs. actual plot of Y_6 FIGURE 11. Predicted vs. actual plot of Y_5

CONCLUSION

Urea complexation is a common method used to separate a mixture of fatty acids based on their molecular structure. Straight-chained molecules are more readily forming urea adducts compared to branched or bend molecules such as linoleic acid. Factors that influence the fatty acid composition of the products include urea amount, crystallisation temperature, and crystallisation time. All of these factors have to be controlled to yield a reasonable

amount of product with a desirable purity of fatty acid of interest.

ACKNOWLEDGMENTS

This study was carried out with the support of Universiti Kebangsaan Malaysia via UKM-GUP-NBT-08-27-113 research grant and also the Ministry of Science, Technology & Innovation (MOSTI), Malaysia via 05-01-02-SF0199 grant and scholarship.

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Received: 14 April 2009

Accepted: 10 December 2009