Study on the Concentration of Unsaturated Fatty Acid Methyl Esters by Urea Complexation

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Summary: This study was done to obtain concentrated unsaturated fatty acid methyl esters (FAME) by urea complexation from soybean derived FAME. Effects of urea-to-FAME ratio, 95% ethanol-to-FAME ratio, crystallization temperature and time on the purification of unsaturated FAME were investigated through single factor experiments. Optimum conditions to obtain maximum FAME yield of NUCF with the purity of unsaturated FAME greater than 98% were established using Box-Behnken design (BBD) method and response surface methodology (RSM). Under optimal conditions, the FAME yield was 58.08%, and the purity of unsaturated FAME was 98% at a urea-to-FAME ratio of 1.23, 95% ethanol-to-FAME ratio of 7 and crystallization temperature of 0ºC. Verification results revealed that the predicted values were reasonably close to experimentally observed values of 56.93% and 98.01%.

Keywords: Urea complexation, Unsaturated FAME, Single factor influence, Optimization, Purity, Recovery.

Introduction

Unsaturated fatty acid methyl esters (FAME), like methyl oleate (C18-1), methyl linoleate (C18-2) and methyl linolenate (C18-3) are widely used in the production of epoxy-plasticizer, dimer acids, low melting-point biodiesel and so on. Compared with traditional plasticizer like Dioctyl-Phthalate (DOP), epoxy-plasticizer is non-toxic, biodegradable and of enhanced property. Dimer acids can be further used to produce a variety of resins through polymerization. Low melting-point biodiesel is a good alternative for oil fuels. All these applications make unsaturated FAME a very important chemical material in industry.

Since unsaturated FAME often co-exist with saturated FAME in the mixture derived from natural oils, it is necessary to separate unsaturated FAME from the mixture for industrial usage.

Several methods have been developed for the separation and purification of unsaturated FAME [1, 2] including low-temperature crystallization from various solvents [3], urea complexation [4], silver complex [5], molecular distillation and supercritical fluid extraction. The energy consumption for low-temperature crystallization is quite large, the silver nitrate employed for silver-complex is very expensive, and the initial capital investment required for molecular distillation and supercritical fluid extraction is huge. Compared to the above four methods, urea complexation is the most cost-effective

one as it can be operated under mild conditions and the reagents (e.g. ethanol, water and urea) and equipment used are very common and cheap [6, 7]. By far, urea complexation is the most commonly used primary fractionation method for the enrichment of unsaturated FAME.

Urea complex [8] consists of spiral, hydrogen-bonded networks of urea molecules surrounding narrow, linear hydrocarbon chains (e.g. saturated FAME), producing a stable solid phase. Branched, bulky, double bond-containing, less linearly shaped and small size molecules are less likely to form urea complex [9]. Saturated FAME can be easily complexed by urea due to its linear shape. Unsaturated FAME, since it contains double bonds, is less likely to form urea complex compared with saturated FAME, leading to its enrichment in non-urea complexed fraction (NUCF).

In this study, urea complexation of soybean derived FAME (mainly C16 and C18 methyl esters) was carried out to concentrate unsaturated FAME (C18-1, C18-2 and C18-3). Factors affecting FAME fractionation, including urea-to-FAME ratio (w/w), 95% ethanol-to-FAME ratio (v/w), crystallization temperature and crystallization time were investigated through the single factor experiments. Conditions to obtain a maximum yield of product with a desirable purity of unsaturated FAME were optimized using

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Box-Behnken Design (BBD) method and response surface methodology (RSM).

Results and Discussion

Part 1 Single Factor Influence Discussion

Effect of Crystallization Time on the Concentration of Unsaturated FAME

As shown in Fig. 1, the purity of unsaturated FAME and the recovery of non-urea complexed FAME increased slightly (around 1%) as crystallization time prolonged from 2h to 3h, then remained unchanged after crystallization time reached 6h.Saturated FAME like C16-0 and C18-0 content showed a slight decreasing tendency. Unsaturated C18-1 and C18-2 contents increased slightly (around 0.5%) after a crystallization time of 6h and then remained almost constant. These results indicated that 6h was enough for the crystallization process to reach equilibrium as all the variables kept stable after crystallization time reached 6h. Thus a crystallization time of 6h was kept constant for the rest studies.
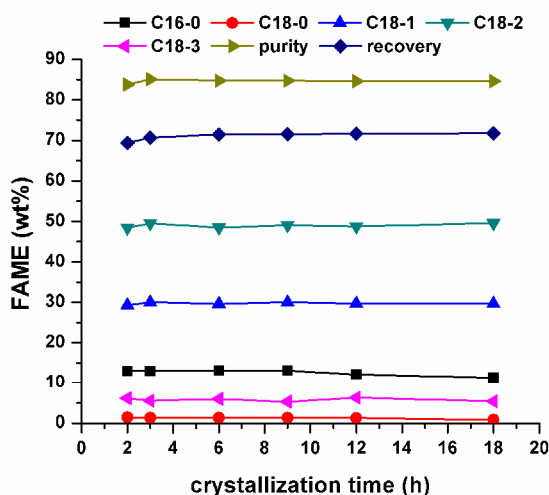

Fig. 1:   Effect of crystallization time at processing conditions of a 0.5:1 (w/w) urea-to-FAME ratio, a 3.5:1 (v/w) 95% ethanol-to-urea ratio and 15ºC crystallization temperature.

Effect of Urea-to-FAME Ratio on the Concentration of Unsaturated FAME

There was a great increase in the content of unsaturated C18-2 (from 42% to67%), as more and

more saturated FAMEs (C16-0 and C18-0) were complexed by urea and removed from NUCF when increasing the ratio of urea to FAME from 0.5:1 to 1.5:1 (Fig. 2a). Similar to C18-2, the content of C18-3 also increased gradually from 5.65% to 7.57%. While the trend for C18-1 is somewhat different. It increased slowly at first, and then started to decline after reaching a maximum of 31%. The reason was that mono-unsaturated FAME (C18-1) would form complex with urea readily after saturated FAME had been complexed to a certain extent.C16-0 content decreased rapidly from 20% to 1% with the increase of urea-to-FAME ratio from0.5:1 to 1.25:1, then the curve started to become flatten when further increasing the amount of urea. For C18-0, the most readily complexed component, its content dropped from 3.64% to nearly 0%when the ratio of urea to FAME reached 1.5:1 from 0.5:1.
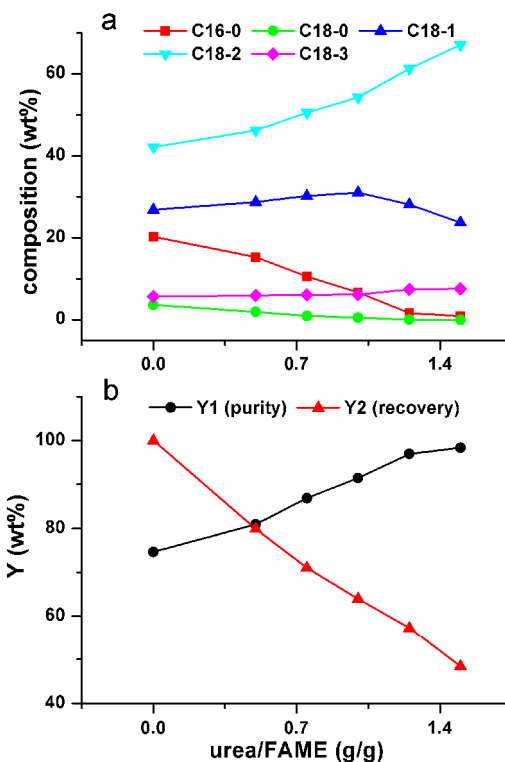

Fig. 2:   Effect of urea-to-FAME ratio at processing conditions of a 7:1 ratio of 95% ethanol to FAME, 20ºC crystallization temperature and 6h crystallization time. Fig. 2a represents the composition of NUCF. Fig. 2b represents the purity of unsaturated FAME and the recovery of NUCF.

Fig. 2b shows that the purity of unsaturated FAME in NUCF increased greatly to a value of more than 98%, while the recovery of FAME in NUCF decreased dramatically from 80% to 48% as the ratio of urea to FAME rose from 0.5:1 to 1.5:1. This is not difficult to explain. The formation of urea inclusion compounds depends on the degree of FAME unsaturation. The presence of double bonds in the carbon chain increases the volume of molecule and reduces the probability to form urea complex. With the increase of urea-to-FAME ratio, more saturated FAME will be complexed. Thus unsaturated FAME will be enriched in NUCF, resulting in the rise of its purity. Meanwhile, the recovery of non-urea complexed FAME will decrease.

Effect of 95% Ethanol-to-FAME Ratio on the Concentration of Unsaturated FAME

The ratio of 95% ethanol to FAME had a small effect on the composition of FAME in NUCF as we can see from Fig. 3a. There was no obvious change in the content of individual FAME.

The recovery of FAME in NUCF showed a general upward trend from 76% to 84% (Fig. 3b). However, a decline of about 2%was observed in the purity of unsaturated FAME when increasing the ratio of 95% ethanol to FAME from 3.5:1 to 7:1. This was attributed to more FAME dissolving in 95% ethanol (serving as solvent) and more saturated FAME remaining in NUCF.

Effect of Crystallization Temperature on the Concentration of Unsaturated FAME

Fig. 4a demonstrates that crystallization temperature had a small effect on the composition of FAME under the temperature range we studied. There was no obvious change in the content of individual FAME.

A slight improvement of approximately 2% was found for the purity of unsaturated FAME when crystallization temperature was lowered from 30ºC to 5ºC (Fig. 4b). On the contrary, the recovery of FAME in NUCF reduced from 75% to 69% when lowering crystallization temperature from 30ºC to 5ºC. These results indicated that the formation of urea complex became easier with the decrease of crystallization temperature. At lower temperatures, larger proportions of FAME formed complex with urea and fewer saturated FAME remained in NUCF.
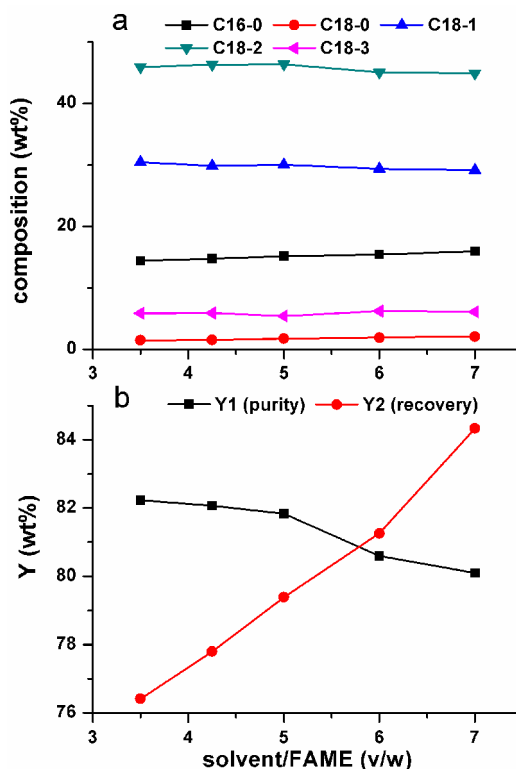

Fig. 3: Effect of 95% ethanol-to-FAME ratio at processing conditions of a 0.5:1 ratio of urea to FAME, 20ºC crystallization temperature and 6h crystallization time. Fig. 3a represents the composition of NUCF. Fig.3b represents the purity of unsaturated FAME and the recovery of NUCF.

Summary of part 1

Urea-to-FAME ratio was the primary determining factor for the separation of unsaturated FAME by urea complexation when comparing with 95% ethanol-to-FAME ratio and crystallization temperature as we can see from the discussion above. The influence of 95% ethanol-to-FAME ratio and crystallization temperature was small under the range we studied. A crystallization time of 6h was enough for the process to reach equilibrium, so it was kept constant and not chosen as an independent variable for the RSM study.

Part 2   RSM analysis and optimization of conditions

Experimental values obtained for responses Y_1 and Y_2 are given in Table-1. The results showed that the percentage of unsaturated FAME increased considerably compared with the initial FAME mixture, some even greater than 98%. In high-urea samples, the

elimination of saturated FAME was more complete, while the recovery was much lower than low-urea conditions.
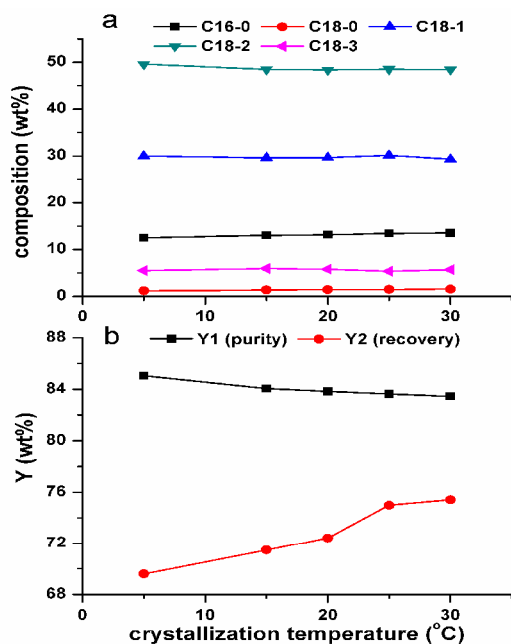

Fig. 4: Effect of crystallization temperature at processing conditions of a 0.5:1 (w/w) urea-to-FAME ratio, a 3.5:1 (v/w) 95% ethanol-to-urea ratio and 6h crystallization time. Fig. 4a represents the composition of NUCF. Fig. 4b represents the purity of unsaturated FAME and the recovery of NUCF.

Model Fitting and Diagnostic Checking

The purity of unsaturated FAME (Y_1) and recovery of FAME in NUCF (Y_2) were fitted in modified quadratic regression model and linear regression model respectively. Examinations of the two models with F test indicated that the lack of fit was not significant ($p>0.05$) relative to the pure error. Regression models for data on responses Y_1 and Y_2 were highly significant ($p<0.01$) with satisfactory R^2 of 0.9976 and 0.9936, respectively. These indicated that the generated models were very adequate. Final equations in terms of actual values are given:

For the purity of unsaturated FAME (Y_1), all the linear terms of three variables, the square term of urea-to-FAME ratio and the interaction between urea-to-FAME ratio and crystallization temperature were highly significant ($p<0.01$). The square term of crystallization temperature and the other two interactions were significant ($p<0.05$). Highly significant terms for the recovery of FAME in NUCF (Y_2) were linear urea-to-FAME ratio and 95% ethanol-to-FAME ratio, significant term was linear crystallization temperature. Analysis of variance (ANOVA) for the two responses was summarized in Table-2 and 3.

Response Surface Plotting and Optimization of Conditions

The response surfaces for the purity of unsaturated FAME (Y_1) and the recovery of FAME in NUCF (Y_2) are shown in Fig. 5-8.

$$Y_1 = 72.25 + 40.3875X_1 - 0.70375X_2 - 0.04258X_3 + 0.55X_1X_2 + 0.09366X_1X_3 - 0.01683X_2X_3 - 15.67X_1^2 - 1.922 \times 10^{-3}X_3^2$$

$$Y_2 = 78.6769 - 32.17X_1 + 2.68813X_2 + 0.06275X_3$$

Table-1: Box-Behnken design arrangement and responses for non-urea complexed fraction.

Run	Variables			Responses	
	Urea / FAME (X_1)	95% ethanol/FAME (X_2)	Crystallization temperature (X_3)	Purity (Y_1)	Recovery (Y_2)
1	1	3	0	96.68	54.89
2	1	5	15	95.39	60.97
3	1	5	15	95.14	61.35
4	0.5	5	30	82.74	77.58
5	1	5	15	95.05	60.27
6	1	5	15	95.68	61.63
7	1	7	30	91.78	66.60
8	0.5	3	15	85.74	72.55
9	1.5	7	15	97.86	50.22
10	1.5	5	30	96.97	47.10
11	1	5	15	95.50	60.90
12	1	3	30	94.58	55.55
13	1.5	5	0	97.90	45.33
14	0.5	5	0	86.48	75.55
15	0.5	7	15	83.18	83.37
16	1.5	3	15	98.22	37.72
17	1	7	0	95.90	63.53

Table-2:   Analysis of variance (ANOVA) for the purity of unsaturated FAME (Y_1).

Source	Sum of squares	df	Mean squares	F value	P value	
Model	439.51	8	54.94	412.08	<0.0001	***
X_1	348.61	1	348.61	2614.88	<0.0001	***
X_2	5.28	1	5.28	39.61	0.0002	***
X_3	14.82	1	14.82	111.19	<0.0001	***
X_1X_2	1.21	1	1.21	9.08	0.0167	**
X_1X_3	1.97	1	1.97	14.81	0.0049	***
X_2X_3	1.02	1	1.02	7.65	0.0244	**
X_1^2	64.80	1	64.80	486.04	<0.0001	***
X_3^2	0.79	1	0.79	5.92	0.0409	**
Residual	1.07	8	0.13			
Lack-of-fit	0.80	4	0.20	2.99	0.1567	Not significant
Pure error	0.27	4	0.067			

*** $p < 0.01$, highly significant; ** $p < 0.05$, significant.

Table-3:   Analysis of variance (ANOVA) for the recovery of FAME in NUCF (Y_2).

Source	Sum of squares	Df	Mean squares	F value	P value	
Model	2308.14	3	769.38	673.32	<0.0001	***
X_1	2069.82	1	2069.82	1811.39	<0.0001	***
X_2	231.23	1	231.23	202.36	<0.0001	***
X_3	7.09	1	7.09	6.20	0.0271	**
Residual	14.85	13	1.14			
Lack-of-fit	13.79	9	1.53	5.78	0.0532	Not significant
Pure error	1.06	4	0.27			

Fig. 5 indicates that there was an improvement in the purity of unsaturated FAME (Y_1) when increasing the amount of urea or decreasing the amount of solvent as more saturated FAME would complex with urea and remove from NUCF. While the recovery of FAME in NUCF (Y_2) showed an opposite trend under the same conditions (Fig. 7). In addition, urea-to-FAME ratio exhibited more significant influence on the unsaturated FAME purity and recovery compared with 95% ethanol-to-FAME ratio.

Fig. 6 shows that high concentration of unsaturated FAME could be obtained by using small amount of solvent at low temperature. However, this could also lead to a reduction in the FAME yield of NUCF as more unsaturated FAME especially C18-1 would be lost into UCF (Fig.8). And 95% ethanol-to-FAME ratio was the factor with more significant influence when comparing with crystallization temperature. To summarize, urea-to-FAME ratio was the most significant parameter for the purification of unsaturated FAME. All these results were in good agreement with the single factor influence discussed above.
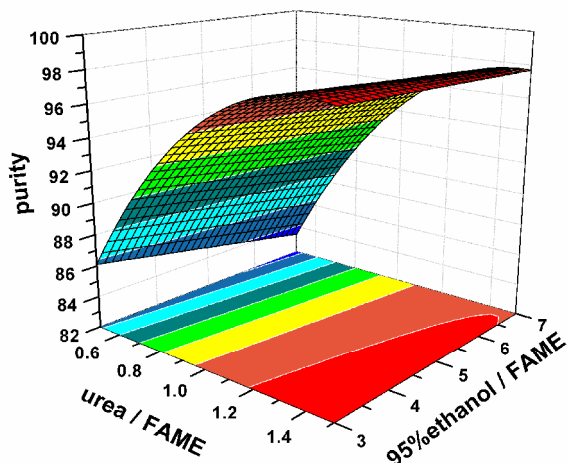

Fig. 5:   Response surface for the effect of urea-to-FAME ratio and 95% ethanol-to-FAME ratio on unsaturated FAME purity (crystallization temperature = 15ºC).
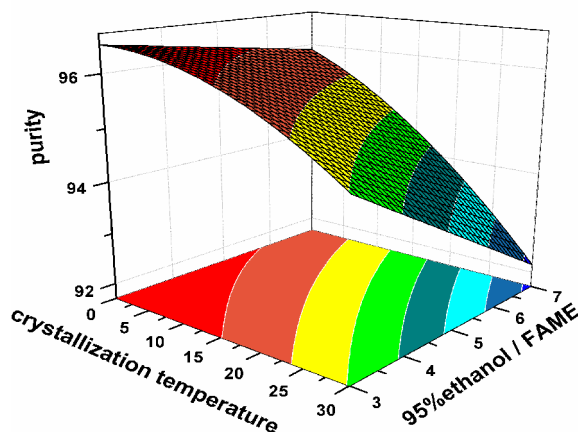

Fig. 6:   Response surface for the effect of 95% ethanol-to-FAME ratio and crystallization temperature on unsaturated FAME purity (urea-to-FAME ratio = 1).
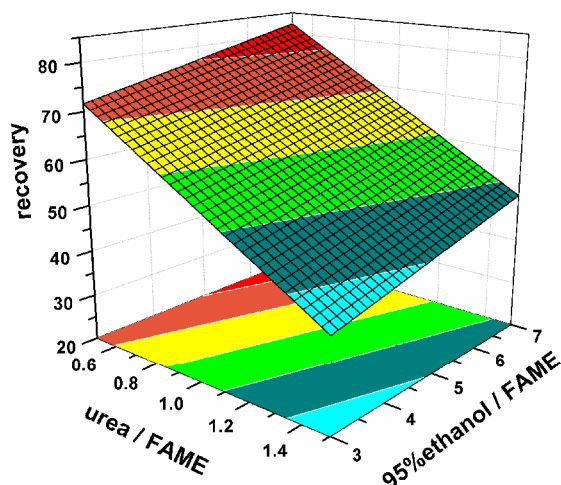
Fig. 7: Response surface for the effect of urea-to-FAME ratio and 95% ethanol-to-FAME ratio on the recovery of FAME (crystallization temperature =15ºC).
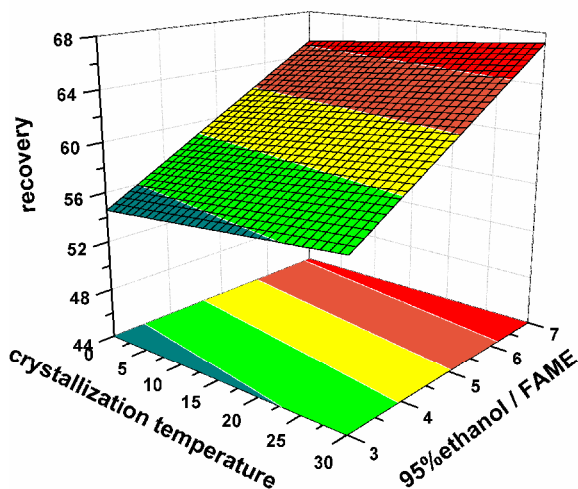

Fig. 8: Response surface for the effect of 95% ethanol-to-FAME ratio and crystallization temperature on the recovery of FAME (urea-to-FAME ratio = 1).

Optimal conditions of the experiment to obtain maximum yield meanwhile to maintain the purity of unsaturated FAME greater than 98% (As raw materials for plasticizer, the unsaturated FAME content must be larger than 98%) were determined by ridge analysis. The generated conditions were as follows: urea-to-FAME ratio of 1.23, 95% ethanol-to-FAME ratio of 7, crystallization temperature at 0ºC. The predicted values for the recovery of FAME in NUCF and the purity of unsaturated FAME were 58.08% and 98% respectively. Performing a verification experiment gave the result of 56.93% and 98.01%. The observed values were reasonably close to the predicted values, which proved the validity and accuracy of our predicted models.

Experimental

Materials

Soybean derived FAME was obtained from JIA AO Technology Co. Ltd. (Zhejiang, China) and used without further purification (Table-4).Urea (>99%) and 95% ethanol were purchased from GUANGFU Fine Chemicals (Tianjin, China). FAME standards were purchased from Sigma-Aldrich. All other chemicals and reagents were of analytical grade.

Concentration of Unsaturated FAME by Urea Complexation

Soybean derived FAME (40g) was mixed with 95% ethanol and urea [10] in a three-neck flask with a reflux and heated at 75ºC (±1ºC) with continuous stirring for 45min. The ratios of urea-to-FAME and ethanol-to-FAME were changed by using different amounts of urea and 95% ethanol. Then the resulting mixture was cooled to a specific temperature in the range of 5-30ºC for a specific time in the range of 2-18h. The crystals formed (urea complexed fraction, UCF) were separated from the liquid (non-urea complexed fraction, NUCF) by filtration under suction with a funnel. Ethanol was removed from the NUCF by a rotary evaporator at 40ºC. Then the filtrate was washed with 75-80ºC distilled water to remove residue ethanol and urea. Finally the residue water in the NUCF was removed with a rotary evaporator at 90ºC under vacuum for 1h and the FAME recovered was weighed to calculate yield.

Table-4: Composition of soybean derived FAME.

Component	Methyl palmitate (C16-0)	Methyl stearate (C18-0)	Methyl oleate (C18-1)	Methyl linoleate (C18-2)	Methyl linolenate (C18-3)	Others[a]
Content (wt%)	20.25	3.64	26.85	42.12	5.65	1.49

[a]Others are components of shorter carbon chains including methyl esters of C12 and C14.

Experimental Design and Statistical Analysis

A three-factor Box-Behnken design [11] was employed to study the responses, namely the purity of unsaturated FAME in NUCF[Y_1 in % by wt, see Eq.(1)] and the recovery of FAME in NUCF[Y_2 in % by wt, see Eq.(2)].Urea-to-FAME ratio (X_1), 95% ethanol-to-FAME ratio (X_2), and crystallization temperature (X_3) were chosen as independent variables (coded at three levels). The coded values for urea-to-FAME ratio (w/w, g/g) were: -1 (0.5), 0 (1), 1 (1.5), for 95% ethanol-to-FAME ratio (v/w, ml/g): -1 (3), 0 (5), 1 (7), and for crystallization temperature: -1 (0ºC), 0 (15ºC),1 (30ºC). The total design consisted of 17 experiment points including 5 duplications of the center point. All experiments were carried out randomly in order to minimize the effect of unexplained variability in the observed responses.

$$Y_1 = \varphi_1 + \varphi_2 + \varphi_3 \quad (1)$$

$$Y_2 = \left({}^{m_2}/_{m_1} \right) \times 100 \quad (2)$$

where φ_1, φ_2, φ_3 represent the mass percentage of C18-1, C18-2 and C18-3 in non-urea complexed FAME, m_2 and m_1 are the weight (g) of non-urea complexed FAME and the total weight (g) of the added FAME.

Experimental data was fitted to linear or quadratic regression models. When any of these models were found to be insignificant to represent the data, minor modification was made on the models for better representation of data. Then analysis of variance (ANOVA) was applied to evaluate the models' goodness of fit [12-14]. Response surfaces were developed using the fitted equations. And the optimal conditions for response values were established by ridge analysis [15-17]. Design Expert 7.0 software was used for all the above analysis.

FAME Composition Analysis

The composition of FAME mixtures was determined using an auto-system gas chromatography (PerkinElmer Co., America) equipped with a flame-ionization detector (FID) and a PE-WAX capillary column (30m × 0.53mm × 1um). Injection and detection temperatures were set at 260ºC and 260ºC respectively. Flow rates of hydrogen and air for the detector were 40ml/min and 400ml/min. Nitrogen was used as the carrier gas with a column flow rate of 1ml/min. The oven temperature was programmed at 140ºC, followed by a 20ºC/min increase up to 200ºC, then to 230ºC at 10ºC/min, and was held at 230ºC for 2min. Fatty acid methyl esters were identified in reference to the retention times of its standards performed at the same conditions, and quantified by normalization of areas.

Conclusions

Urea complex formation is an effective way to concentrate unsaturated FAME. Urea-to-FAME ratio is the primary determining factor for the purification of unsaturated FAME compared with 95% ethanol-to-FAME ratio and crystallization temperature. A crystallization time of 6h is enough for the process to reach equilibrium under the scale we studied. Through optimization, a maximum yield of 56.93% with a purity of 98% unsaturated FAME can be reached at crystallization temperature of 0ºC, 95% ethanol-to-FAME ratio of 7 and urea-to-FAME ratio of 1.23.

Acknowledgments

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