J Am Oil Chem Soc (2008) 85:677–684
DOI 10.1007/s11746-008-1245-7

ORIGINAL PAPER

Optimizing Conditions for the Purification of Linoleic Acid from Sunflower Oil by Urea Complex Fractionation

Mingyi Wu · Hui Ding · Song Wang · Shimin Xu

Received: 11 December 2007 / Revised: 5 May 2008 / Accepted: 5 May 2008 / Published online: 31 May 2008

Abstract The separation and purification of linoleic acid (LA) from sunflower seed oil by urea complex fractionation was studied. Crystallization reaction conditions of urea inclusion were optimized using the response surface method, and the optimal model was developed. Using the linear weighting method of the fitting model for optimization, the optimal balance between the purity and the recovery of LA was obtained. Under optimal conditions, the purity of LA was 87.8%, and the recovery was 83.4% at a urea-to-fatty acids ratio (w/w) of 0.94, 95% ethanol-to-urea (v/w) of 5.00, a crystallization temperature of 18.0 °C, and a crystallization time of 5.0 h. Verification results revealed that the predicted values from these models were reasonably close to the experimentally observed values.

Keywords Sunflower seed oil · Fatty acid · Linoleic acid · Urea complex fractionation · Process optimization

Introduction

Linoleic acid (LA; 18:2(n-6); an $\omega-6$ fatty acid) is an essential fatty acid (EFA), whose absence in the diet is responsible for the development of a wide variety of abnormalities such as diabetic neuropathy, rheumatoid arthritis, and cardiovascular, reproductive and autoimmune disorders [1, 2]. Conjugated linoleic acid (CLA) can be viewed as the derivative or second growth metabolite of LA [3]. It has received much attention among chemists, nutritionists and pharmacologists, and became a new research focus of fatty acid chemistry, since Ha et al. [4] reported that CLA had anti-carcinogenesis properties. It is unfortunate that there is little occurrence in the natural oils of plant seeds [5], which only exists in small amount in butter and meat products of ruminant animals such as cattle, goats, etc. However, butter and meat are high caloric foods. Therefore, consumption of appropriate amounts of CLA needs to be considered. Using the natural oils of plant seeds such as sunflower oil as raw material, realization of direct-conversion synthesis of CLA from LA greatly reduces the cost for the development of high additional value product such as food additives, etc.

The simple and effective technique for obtaining polyunsaturated fatty acids (PUFA) concentrates in the form of free fatty acids (FFA) is urea complex fractionation. This is a well-established technique for the elimination of saturated and monounsaturated fatty acids [6, 7]. Initially the triacylglycerols (TAG) of the oil are split into their constituent fatty acids by alkaline hydrolysis using alcoholic KOH or NaOH and these FFA are then mixed with an ethanol solution of urea for complex formation. The saturated and monounsaturated fatty acids easily complex with urea and crystallize out on cooling and may subsequently be removed by filtration. The liquid or non-urea complexed fraction (NUCF) is enriched in PUFA.

Urea complex fractionation has the advantage that complexed crystals are extremely stable, and filtration does not necessarily need to be carried out at very low temperatures which solvent crystallization of fatty acids is always required [8]. This method is also favored because

M. Wu · S. Wang · S. Xu
School of Chemical Engineering and Technology,
Tianjin University, 300072 Tianjin, People's Republic of China

H. Ding · S. Xu (✉)
National Engineering Research Center for Distillation
Technology, 300072 Tianjin, People's Republic of China
e-mail: mingyiwu_tju@yahoo.com

inclusion fractionation depends upon the configuration of the fatty acid moieties due to the presence of multiple double bonds, rather than pure physical properties such as melting point or solubility [9]. Urea inclusion fractionation has the potential value as a large-scale and robust pre-fractionation step because of its low temperature and environmentally friendly operating conditions, and its use of inexpensive renewable materials (urea and ethanol or methanol as solvent) [10].

The results of one-factor-at-a-time experiments do not reflect actual changes in the environment as they ignore interactions between factors which are present simultaneously. When many factors and interactions give desired responses, response surface methodology (RSM) [11] is an effective tool for optimizing the process [12]. The method of process optimization by RSM is a faster and more economical method than the classical one-variable-at-a-time or full-factorial experimentation for gathering research results [13], and has been successfully adapted in many optimization studies [13–16].

In this work, urea complex fractionation of sunflower oil was carried out to purify LA from the FFA derived from the oil. Factors (variables) such as urea-to-fatty aid ratio (w/w, X_1), ethanol-to-urea ratio (v/w, X_2), crystallization time (h, X_3) and crystallization temperature (°C, X_4) were studied collectively in order to optimize the conditions to obtain an optimal balance between the purity and the recovery of LA, i.e. the purity and recovery of LA should be more 80% to serve as an effective feedstock to make CLAs and to be required for efficient economics.

Experimental Procedures

Materials

Sunflower oil was obtained from a Shanghai oil processor (Shanghai, People's Republic of China). All solvents and chemicals used were of analytical grade. Fatty methyl esters standards (including methyl oleate and methyl linoleate) were purchased from Sigma Chemical Co. (USA). The standards were greater than 98% chemical purity according to the manufacturer.

Procedures

Free fatty acids were formed from sunflower oil (100 g) by saponifying with 10% KOH aqueous ethanol (200 mL, 95%) at reflux for 1.5 h, then releasing FFA by acidification to pH = 3.0 with 6 N HCl and by treatment with saturated NaCl solution (200 mL) and petroleum ether (400 mL × 3) in sequence. The recovery of FFA was 95%. FFA (40 g) were mixed with urea in 95% aqueous ethanol and heated at 60 °C with stirring until the mixture was turned into a clear homogeneous solution. The ratio of urea-to-fatty acids was changed by using different amounts of urea, and the ratio of urea-to-ethanol was changed by using different amounts of 95% aqueous ethanol (see Tables 1, 2). Initially, the urea-fatty acid adduct was allowed to crystallize at room temperature but colder temperatures were maintained later for different periods for further crystallization. The crystals formed (urea-fatty acid adducts, also referred to as the urea complexing fraction; UCF) were separated from the liquid (non-urea complexing fraction, NUCF) by fast filtration. The liquid (NUCF) was diluted with an equal volume of water and acidified to pH 2–3 with 6 N HCl; an equal volume of petroleum ether was subsequently added and the FFA were extracted. The top phase, containing liberated fatty acids, was separated from the aqueous layer containing urea. The petroleum ether layer was washed with 5% NaCl solution to remove any remaining urea and then dried over anhydrous Na_2SO_4 and the solvent was then removed at 65 °C using a rotary evaporator. Fatty acids from the UCF were recovered after addition of water/6 N HCl and petroleum ether in a similar manner.

Fatty Acids Analysis

Free fatty acids were transformed into the corresponding methyl esters with 12% boron trifluoride in methanol [17]. An autosystem XL gas chromatograph (PerkinElmer Co., America), equipped with a flame ionization detector and a Totalchrom integrator analyzed the composition of FFA. The column used was a fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; PE-225, PerkinElmer Co., USA). The oven temperature was held at 70 °C for 1 min, increased to 180 °C at 10 °C/min then to 200 °C at 2 °C/min, and was held at 200 °C for 10 min. The injector and detector were held at 250 and 300 °C, respectively. Nitrogen was used as the carrier gas at 20 cm/s

Table 1 Independent variables and their levels for central composite design

Independent variables	Code	Variable levels				
		−2	−1	0	+1	+2
The urea-to-fatty acid ratio (w/w) (g/g)	X_1	0.5	1.5	2.5	3.5	4.5
95% Ethanol-to-urea ratio (v/w) (mL/g)	X_2	1	2	3	4	5
Crystallization temperature (°C)	X_3	−20	−10	0	10	20
Crystallization time (h)	X_4	3	10	17	24	31

Table 2 Central composite design arrangement and responses for non-urea-complexed fraction of sunflower oil

Run	Variable levels (X)				Responses, Y (non-urea-complexed fraction, NUCF)		
	Urea/FFA[a] (X_1)	Ethanol/urea[b] (X_2)	Temperature[c] (X_3)	Time[d] (X_4)	Yield[e] (%)	Y_1[f] (%)	Y_2[g] (%)
1	1.5	2	−10	10	60.7 ± 1.1	83.8 ± 2.41	60.4 ± 3.5
2	3.5	2	−10	10	10.0 ± 3.3	96.3 ± 1.30	14.3 ± 3.1
3	1.5	4	−10	10	40.0 ± 1.5	89.8 ± 2.12	53.9 ± 3.3
4	3.5	4	−10	10	9.71 ± 5.1	94.4 ± 1.19	15.2 ± 4.1
5	1.5	2	10	10	36.5 ± 2.1	88.1 ± 2.31	52.3 ± 2.1
6	3.5	2	10	10	16.4 ± 3.5	95.2 ± 1.12	25.5 ± 3.4
7	1.5	4	10	10	48.9 ± 5.3	93.2 ± 1.18	65.2 ± 1.4
8	3.5	4	10	10	17.2 ± 3.1	96.2 ± 1.30	27.1 ± 3.6
9	1.5	2	−10	24	39.0 ± 2.2	90.7 ± 2.14	57.8 ± 2.3
10	3.5	2	−10	24	9.33 ± 5.3	96.5 ± 1.12	14.7 ± 5.2
11	1.5	4	−10	24	40.2 ± 1.2	91.5 ± 1.13	60.0 ± 1.5
12	3.5	4	−10	24	10.5 ± 4.1	96.7 ± 1.41	14.2 ± 4.6
13	1.5	2	10	24	36.8 ± 3.2	87.9 ± 3.10	52.7 ± 3.3
14	3.5	2	10	24	20.6 ± 3.6	95.8 ± 2.12	29.4 ± 3.0
15	1.5	4	10	24	44.4 ± 2.3	90.2 ± 2.11	65.2 ± 2.7
16	3.5	4	10	24	7.00 ± 5.6	93.0 ± 2.14	10.6 ± 3.1
17	2.5	3	0	17	18.0 ± 3.1	92.5 ± 1.10	27.1 ± 3.3
18	2.5	3	0	17	19.3 ± 4.8	94.3 ± 1.40	29.7 ± 2.1
19	2.5	3	0	17	19.7 ± 2.4	94.3 ± 1.16	30.7 ± 2.4
20	2.5	3	0	17	19.0 ± 5.8	91.5 ± 1.17	28.6 ± 1.7
21	0.5	3	0	17	77.1 ± 1.0	76.5 ± 1.21	88.6 ± 1.9
22	4.5	3	0	17	16.4 ± 4.7	96.9 ± 1.15	23.7 ± 5.1
23	2.5	1	0	17	16.2 ± 4.1	87.4 ± 2.37	23.0 ± 6.2
24	2.5	5	0	17	23.8 ± 3.7	95.1 ± 1.17	36.9 ± 1.1
25	2.5	3	−20	17	14.8 ± 4.3	97.2 ± 1.14	23.5 ± 5.9
26	2.5	3	20	17	26.0 ± 1.3	95.9 ± 1.46	40.6 ± 4.1
27	2.5	3	0	3	15.7 ± 4.1	98.2 ± 1.57	25.1 ± 6.0
28	2.5	3	0	31	19.0 ± 2.1	94.0 ± 1.64	29.1 ± 3.7
29	2.5	3	0	17	19.5 ± 3.1	94.8 ± 1.47	30.2 ± 3.3
30	2.5	3	0	17	17.3 ± 1.8	95.4 ± 1.38	24.5 ± 1.9

[a] Urea-to-fatty acid ratio (w/w)

[b] 95% Ethanol-to-urea ratio (v/w)

[c] Crystallization temperature (°C)

[d] Crystallization time (h)

[e] Percentage recovery of NUCF

[f] The mass % of LA among the FFA recovered from the NUCF

[g] The recovery of LA in the NUCF

flow rate and the split ratio of the injector was 20:1. The injection volume was 1 μL. Fatty acid methyl esters were identified by comparing their relative retention times with those of known standards. Standard curves were generated from known concentrations of pure methyl ester standards of oleic acid and LA ranging from 5 to 20 mg/mL and their peak heights, and Origin 7.0 (OriginLab Co., USA) was used to generate calibrations.

Experimental Design and Statistical Analysis

A four-factor central composite design [18, 19] was employed to study the responses, namely after urea inclusion fractionation the purity of LA [Y_1 in % by wt, see Eq. (1)] and recovery of LA [Y_2 in % by wt, see Eq. (2)]. An initial screening step was carried out to select the major response factors and their values [20]. The independent variables were

X_1, X_2, X_3 and X_4 representing the urea-to-fatty acid ratio (w/w), 95% ethanol-to-urea ratio (v/w), crystallization temperature (°C), and crystallization time (h), respectively. The settings for the independent variables were as follows (low and high values): urea-to-fatty acid ratio of 0.5 and 4.5; 95% ethanol-to-urea ratio of 1 and 5; crystallization temperature of −20 and 20, crystallization time of 3 and 31. Each variable to be optimized was coded at four levels: −2, −1, 0, +1 and +2. Six replicates at the center (0, 0, 0, 0) of the design were performed to allow the estimation of the pure error. The central composite design is shown in Table 1. All experiments were carried out in randomized order to minimize the effect of extraneous factors on the observed responses.

$$Y_1 = \varphi_{\mathrm{NUCF}} \times 100 \tag{1}$$

$$Y_2 = \frac{\varphi_{\mathrm{NUCF}}}{\varphi_{\mathrm{FFA}}} \times 100 \tag{2}$$

where φ_{NUCF} and φ_{FFA} are defined as the mass % of LA among the FFA derived from the NUCF phase and the mass % of LA from sunflower oil, respectively.

A quadratic polynomial regression model was assumed for predicting individual Y variables. The model proposed for each response of Y was:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum \sum_{i<j=1}^{4} \beta_{ij} x_i x_j \tag{3}$$

where $\beta_0, \beta_i, \beta_{ii}$ and β_{ij} are constant, linear, square and interaction regression coefficient terms, respectively, and x_i and x_j are independent variables. The Minitab software version 14 (Minitab Inc., USA) was used for multiple regression analysis, analysis of variance (ANOVA), and analysis of ridge maximum of data in the response surface regression (RSREG) procedure. The goodness of fit of the model was evaluated by the coefficient of determination R^2 and the analysis of variance (ANOVA). Response surfaces and contour plots were developed using the fitted quadratic polynomial equations obtained from RSREG analysis and holding the independent variables with the least effect on the response at two constant values and changing the levels of the other two variables.

Results and Discussion

Experimental values obtained for response: the purity of LA, the recovery of LA in the NUCF as well as percentage recovery of NUCF for thirty design points are given in Table 2. The results show that LA had been purified in the filtrate, while monounsaturated fatty acid (oleic acid, OA) was enriched in the crystal phase. Thus these results demonstrate that OA has more tendency to form urea adducts than LA. Hayes et al. [10, 21] have reported similar results for urea complex fractionation experiments carried out for low erucic acid rapeseed oil, canola, and vegetable and fish oil. In certain experimental conditions the mass % of LA among the FFA derived from the NUCF phase was relatively high, and some even greater than 97% (Table 2). This showed that the experimental conditions should be suitable for the preparation of high purity LA. However, it is difficult to completely remove all the saturated fatty acids to obtain 100% purity of unsaturated fatty acids in the concentrate. Ratnayake et al. [22] and Wanasundara [9] have reported that complete removal of saturated fatty acids by urea complexation may be impossible since some of the saturated fatty acids do not bind with urea during crystallization.

Model Fitting

The quadratic regression coefficient obtained by employing a least squares method technique to predict quadratic polynomial models for purity of LA (Y_1) and the percentage recovery of LA (Y_2) are given in Table 3. Examination of these coefficients with a t test shows that for the purity of LA in the concentrate (Y_1) the linear and square terms of urea-to-fatty acid ratio (X_1) were highly significant ($p < 0.01$), and the linear terms of the ethanol-to-urea ratio (X_2) was significant ($p < 0.05$), while for the recovery of LA (Y_2) the linear terms of crystallization temperature (X_3) were highly significant at $p < 0.01$; among six interactions, the urea-to-fatty acid ratio (X_1) and the ethanol-to-urea ratio (X_2) for the content of LA (Y_1) were significant, while the urea-to-fatty acid ratio (X_1) and the crystallization temperature (X_3) for the recovery of LA (Y_2) in the concentrate were significant at $p < 0.05$, and others between any two of the four factors were not. The coefficients of independent variables (urea-to-fatty acid ratio; X_1, ethanol-to-urea ratio; X_2, crystallization temperature; X_3 and crystallization time; X_4) determined for the quadratic polynomial models (Table 3) for the purity of LA (Y_1) and percentage recovery of LA (Y_2) are given below:

$$\begin{aligned} Y_1 = {} & 52.68 + 16.93X_1 + 9.344X_2 + 0.350X_3 \\ & - 0.012X_4 - 1.807X_1^2 - 0.670X_2^2 + 0.007X_3^2 \\ & + 0.011X_4^2 - 1.106X_1X_2 - 0.046X_1X_3 - 0.049X_1X_4 \\ & + 0.003X_2X_3 - 0.087X_2X_4 - 0.015X_3X_4 \end{aligned} \tag{4}$$

$$\begin{aligned} Y_2 = {} & 101.58 - 48.33X_1 + 2.050X_2 - 0.355X_3 \\ & - 0.028X_4 + 7.405X_1^2 + 0.855X_2^2 + 0.014X_3^2 \\ & + 0.003X_4^2 - 1.806X_1X_2 + 0.221X_1X_3 - 0.071X_1X_4 \\ & + 0.103X_2X_3 - 0.038X_2X_4 - 0.009X_3X_4 \end{aligned} \tag{5}$$

Table 3 Regression coefficients of the predicted quadratic polynomial model for response variables (the percentage content and percentage recovery of LA) in urea inclusion fractionation experiment of sunflower oil

Variables	Coefficients (β)							
	The mass % of LA (Y_1)				Percentage recovery of LA (%) (Y_2)			
	Coefficients (β)	T	p	Notability	Coefficients (β)	T	p	Notability
Constant	52.68	108.77	0.000	***	101.58	15.24	0.000	***
Linear								
X_1	16.93	8.768	0.000	***	−48.33	−22.95	0.000	***
X_2	9.344	2.551	0.023	**	2.049	2.577	0.022	**
X_3	0.350	−0.624	0.796		−0.355	4.445	0.001	***
X_4	−0.012	−0.303	0.766		0.282	0.806	0.434	
Square								
X_{11}	−1.807	−4.532	0.000	***	7.405	10.14	0.000	***
X_{22}	−0.670	−1.680	0.115		0.855	1.171	0.261	
X_{33}	0.007	1.643	0.123		0.014	1.890	0.080	
X_{44}	0.011	1.361	0.195		0.003	0.195	0.848	
Interaction								
X_{12}	−1.106	−2.119	0.050	**	−1.806	−1.889	0.080	
X_{13}	−0.045	−0.874	0.397		0.221	2.307	0.037	**
X_{14}	−0.049	−0.658	0.521		−0.071	−0.516	0.614	
X_{23}	0.003	0.060	0.953		0.103	1.078	0.299	
X_{24}	−0.086	−1.161	0.265		−0.038	−0.281	0.783	
X_{34}	−0.015	−2.023	0.063		−0.009	−0.686	0.504	
R^2	0.90				0.98			

** $p < 0.05$; *** $p < 0.01$. T: F test value

See Table 2 for a description of the abbreviations

Diagnostic Checking of the Fitted Models

ANOVAs for the fitted models are summarized in Tables 4 and 5. Examinations of the two models with an F test and t test indicate a non-significant lack-of-fit at $p > 0.05$ and pure error were very small (1.49 and 5.82%, respectively). The regression models for data on the purity and recovery of LA were highly significant ($p < 0.01$) with satisfactory regression coefficients (R^2) of 0.90 and 0.98, respectively (Table 3). These indicate that the generated models adequately explained the data variation and represented the actual relationships among the reaction parameters.

Response Surface Plotting and Optimization in the Linear Weighting Method

Equations (4) and (5) showed that the purity and recovery of LA have a complex relationship with independent variables that encompass both first- and second-order polynomials. RSM is one of the best ways of evaluating the relationships between responses, variables and interactions that exist. Significant interaction variables in the fitted models (Table 3) were chosen as the axes (urea-to-fatty acids ratio X_1 and ethanol-to-urea ratio X_2, urea-to-fatty acids ratio X_1 and crystallization temperature X_3) for the response surface plots. The relationships between independent and dependent variables are shown in the three-dimensional representation as response surfaces. The response surfaces for the purity of LA (Y_1) and the recovery of LA (Y_2) in the concentrates were given in Figs. 1 and 2, respectively.

In a contour plot, curves of equal response values are drawn on a plane whose coordinates represent the levels of the independent factors. Each contour represents a specific value for the height of the surface above the plane defined for combination of the levels of the factors. Therefore, different surface height values enable one to focus attention on the levels of the factors at which changes in the surface height occur [23]. The contour plots (Figs. 1b, 2b) show the combination of levels of the urea-to-fatty ratio and the ethanol-to-urea ratio that can afford the same level of the purity of LA, and that of levels of urea-to-fatty ratio and crystallization temperature that can afford the same amount for the recovery of LA.

Canonical analysis was performed on the predicted quadratic polynomial models to examine the overall shape

Table 4 Analysis of variance, showing the effect of the variables as linear, square and interactions on the response Y_1 (the content of LA) of the central composite design

Source	*df*	Sum of squares	Mean squares	*F* ratio	*p*
Blocks	1	1.980	1.980	0.45	0.511
Regression	14	549.694	439.264	9.00	0.000
Linear	4	364.342	91.085	20.88	0.000
Square	4	136.804	34.201	7.84	0.002
Interaction	6	48.549	8.091	1.86	0.160
Residual error	14	61.059	4.361		
Lack-of-fit	10	55.089	5.509	3.69	0.110
Pure error	4	5.970	1.492		
Total	29	612.734			

Table 5 Analysis of variance, showing the effect of the variables as linear, square and interactions on the response Y_2 (percentage recovery of LA of NUCF) of the central composite design

Source	*df*	Sum of squares	Mean square	*F* ratio	*p*
Blocks	1	47.2	47.17	3.22	0.094
Regression	14	9,796.1	699.72	47.82	0.000
Linear	4	8,103.6	2,025.89	138.44	0.000
Square	4	1,533.5	383.38	26.20	0.000
Interaction	6	159.0	26.51	1.81	0.169
Residual error	14	204.9	14.63		
Lack-of-fit	10	181.5	18.15	3.10	0.143
Pure error	4	23.4	5.85		
Total	29	10,048.2			

of the response surface curves and used to characterize the nature of the stationary points. Canonical analysis is a mathematical approach used to locate the stationary point of the response surface and to determine whether it represents a maximum, minimum or saddle point [23, 24]. Table 6 displays the results of canonical analysis of the response surfaces. The stationary point for LA purity of the prepared concentrates by urea complex fractionation predicted a maximum of 97.04% at a urea-to-fatty acid ratio (w/w) of 3.5, 95% ethanol-to-urea (v/w) of 3.14, crystallization temperature of 1.7 °C, and crystallization time of 14.7 h. The contour plot derived from the results of canonical analysis showed ellipsoidal contour at the maximum point (Fig. 1). Under this condition, experimental value was 95.2 ± 1.86% ($n = 3$). The similarity of estimated value and real value proved the validity of Eq. (4) and the existence of the maximum value.

However, the recovery of LA was low and just 41.2% (observed value at the maximum point of Y_1) too low efficient economics; therefore the purity and recovery of LA need to be weighed. There is a multiple-objective optimization approach in mathematics [25]. Multi-objective functions are Y_1 and Y_2 [Eqs. (4), (5)] to achieve local maximum values.

Subject to

$$0.5 \le X_1 \le 4.5; \quad 1 \le X_2 \le 5; \quad -20 \le X_3 \le 20; \quad 3 \le X_4 \le 31$$

In the linear weighing method based on expert evaluation [25], weight coefficients of Eqs. (4) and (5) were 0.2 and 0.8 (initial screened values), respectively. It was mainly because the recovery of LA should be as high as possible, which offered more feed materials to prepare CLAs. The Minitab software version 14 (Minitab Inc. USA) was used to solve maximum values of above-mentioned problem. One reasonable maximum point was *X* (0.94, 5.00, 18.0, 5.0), and maximum values of Y_1 and Y_2 were 89.14% and 84.08%, respectively.

Table 6 shows the optimized solutions and the observed values. Verification results revealed that the predicted values from these models were reasonably close to the observed values. This solved the problem of multiple-objective optimization and validated the utility and accuracy of the method. Compositions of FFA in two phases under optimum conditions are summarized in Table 7. The

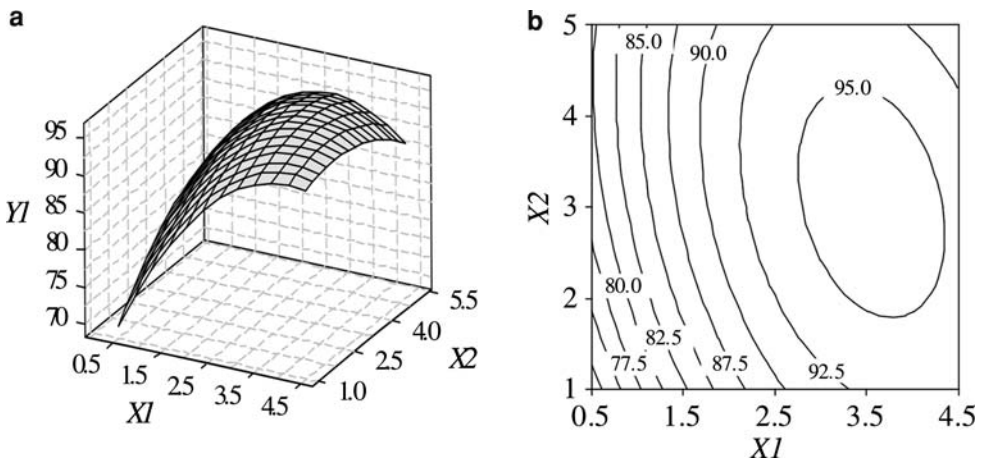

Fig. 1 **a** Response surface and **b** contour plots for the effect of the urea-to-fatty acid ratio (X_1, w/w) and 95% ethanol-to-urea ratio (X_2, v/w) on the content of LA (Y_1, %) in the NUCF

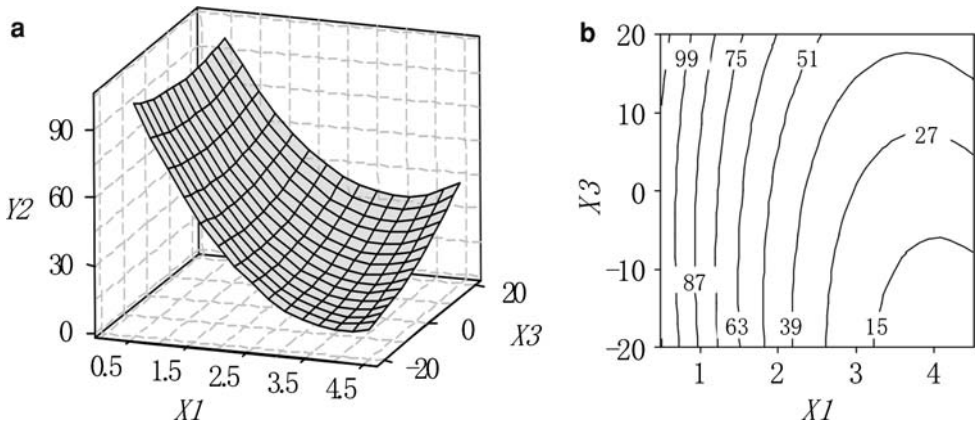

Fig. 2 **a** Response surface and **b** contour plots for the effect of urea-to-fatty acid ratio (X_1, w/w) and crystallization temperature (X_3, °C) on the recovery of LA (Y_2, %) in the concentration

Table 6 Predicted and observed values for response variables (percentage linoleic acid content Y_1 and percentage recovery of linoleic acid Y_2) in urea inclusion fractionation experiment of sunflower oil

Critical values of independent variables				Response variables	Predicted value	Observed value[a]
Urea-to-fatty acid ratio (w/w)	Ethanol-to-urea ratio (v/w)	Crystallization temperature (°C)	Crystallization time (h)			
3.50	3.14	1.70	14.7	Y_1 (%)	97.04	95.2 ± 1.86
				Y_2 (%)	–[b]	41.2 ± 1.21
0.94	5.00	18.0	5.0	Y_1 (%)	89.14	87.8 ± 1.84
				Y_2 (%)	84.08	83.4 ± 2.14

[a] Mean SD ($n = 3$)

[b] Not predicted value

Table 7 Compositions of FFA in two phases (UCF and NUCF) under the optimization conditions ($n = 3$)

Composition	Urea complexed fraction (UCF)	Non-urea complexed fraction (NUCF)	FFA of sunflower oil
Palmitic acid; C16:0 (%)	12.9 ± 1.24	0.2 ± 0.12	6.2 ± 0.32
Stearic acid; C18:0 (%)	13.2 ± 1.74	0.0 ± 0.03	4.3 ± 0.72
Oleic acid; C18:1 (%)	45.2 ± 2.14	10.4 ± 2.31	27.9 ± 1.22
Linoleic acid; C18:2 (%)	26.1 ± 1.41	87.8 ± 1.84	59.4 ± 1.41
Linolenic acid; C18:3 (%)	0.7 ± 0.28	1.7 ± 0.85	0.7 ± 0.76
Others (%)	1.9 ± 0.71	0.0 ± 0.10	1.5 ± 0.12

purity of LA was up to 87.8 ± 1.84%, and the recovery was 83.4 ± 2.44% ($n = 3$).

The model of separation of LA was developed on the basis of the analysis of RSM. The urea-to-fatty acid ratio was the most important parameter for the purity and recovery of LA. The process may be help produce highly pure LA and/or CLA from an economic point of view, as well as being a promising measure for further utilization of agriculture products.

Acknowledgments Financial support from the government of Tianjin, People's Republic of China (no. 07JCZDJC02600), is gratefully acknowledged. Thanks also given to J. J. Zeng for his valuable suggestions and Dr. X. Han for his comments on this manuscript.

References

1. Kinsella JE (1986) Food components with potential therapeutic benefits: the n-3 polyunsaturated fatty acids of fish oils. Food Technol 40:89–97
2. Simopoulos AP (1997) Essential fatty acids in health and chronic disease. Food Rev Int 13:623–631
3. Gurr M (1995) A *trans* fatty acid that is good to eat? Conjugated linoleic acid. Lipids Technol 11:133–135
4. Ha YL, Grimm NK, Pariza MW (1987) Anticarcinogens from fried ground beef heat-altered derivatives of linoleic acid. Carcinogenesis 8:1881–1887
5. Hopkins CY, Chisholm MJ (1968) A survey of the conjugated fatty acids of seed oils. J Am Oil Chem Soc 45:176–182
6. Iverson JL, Weik RW (1967) Correlation of fatty acid structure with preferential order of urea complex formation. J Assoc Off Anal Chem 50:1111–1118

7. Strocchi A, Bonaga G (1975) Correlation between urea inclusion compounds and conformational structure of unsaturated C-18 fatty acid methyl esters. Chem Phys Lipids 15:87–94
8. Anonymous (1986) Fish oil production and potential in Atlantic Canada. Project report no. 115. Fisheries Development Branch, Halifax, NS
9. Wanasundara UN (1996) Marine oils: Stabilization, structural characterization and omega-3 fatty acid concentration. Ph.D. Thesis, Memorial University of Newfoundland, Canada
10. Hayes DG (2006) Effect of temperature programming on the performance of urea inclusion compound-based free fatty acid fractionation. J Am Oil Chem Soc 83:253–259
11. Thompson DR (1982) Production optimization. J Food Proc Preserv 6:1–13
12. Hunter JS (1959) Determination of optimum operating conditions by experimental methods. Ind Qual Control 158:6–11
13. Lee CC, Hoseney RC (1982) Optimization of the fat-emulsifier system and the gum-egg white-water system for a laboratory-scale single stage cake mix. Cereal Chem 59:392–396
14. Shieh CJ, Akoh CC, Koehler PE (1995) Four-factor response surface optimization of the enzymatic modification of triolein to structured lipids. J Am Oil Chem Soc 72:619–623
15. Wanasundara PKJPD, Shahidi F (1996) Optimization of hexa-metaphosphate-assisted extraction of flaxseed proteins using response surface methodology. Food Chem 61:604–607
16. Jiang ST, Shao P, Pan LJ, Zhao YY (2006) Molecular distillation for recovering tocopherol and fatty acid methyl esters from rapeseed oil deodorizer distillate. Biosys Eng 93:383–391
17. AOCS (1998) Official methods and recommended practices of American Oil Chemists' Society, 5th edn. American Oil Chemists' Society, Champaign
18. Box GEP (1954) The exploration and exploitation of response surfaces, some general considerations and examples. Biometrics 10:16–60
19. Cornell JA (1992) How to apply response surface methodology. ASQC basic reference in quality control. ASQC, Milwaukee
20. Wu M, Ding H et al (2007) Separation and Purification of linoleic acid from sunflower oil by urea complexation. ICSST
21. Hayes DG, Bengtsson YC, Van Alstine JM, Setterwall FN (1998) Urea complexation for the rapid, ecologically responsible fractionation of fatty acid from seed oil. J Am Oil Chem Soc 75:1403–1409
22. Ratnayake WMN, Olsson B, Matthews D, Ackman RG (1988) Preparation of omega-3 PUFA concentrates from fish oils via urea complexation. Fat Sic Technol 90:381–386
23. Wanasundara NU, Shahidi F (1999) Concentration of omega 3-polyunsaturated fatty acids of seal blubber oil by urea complexation: optimization of reaction conditions. Food Chem 65:41–49
24. Mason RL, Gunst RF, Hess JL (1989) Statistical design and analysis of experiments with applications to engineering and science. Wiley, New York
25. Xie KX, Han J, Lin YL (2004) Optimize methodology, 2nd edn. Tianjin university press, Tianjin, pp 208–241