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Enrichment of polyunsaturated fatty acids from seal oil through urea adduction and the fatty acids change rules during the process

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Abstract

The seal oil is a good resource of ω-3 polyunsaturated fatty acids (PUFA). Besides being rich in EPA and DHA, it also contains a synergism factor-DPA. Urea adduction is a method that is widely used for the concentration of ω-3 PUFA. The aim of this work was to optimize the concentration conditions (urea/oil ratio, crystallization temperature, and time) for EPA, DPA, and DHA by response surface method and study the change rules of fatty acids during the process. The results showed that the optimal conditions for the enrichment was at 2.38:1 urea/FAEE ratio (w/w) upon crystallization at 15 °C for 2.5 hr. The concentration and recovery were 71.35 and 82.31%, respectively, under the optimal conditions. EPA was more easily to be entrapped into the urea than DPA and DHA. SFA (C14:0, C16:0) can be completely removed by urea adduction, while the urea adduction has limited effect on reducing the content of MUFA (C16:1, C18:1, and C20:1). It is necessary to take other methods to remove it.

Practical applications

Seal oil is rich in PUFAs (EPA, DPA, and DHA), which are helpful to human health. Urea adduction method is a good way to enrich PUFA from seal oil. The changes in FAEE profiles of the urea inclusion compounds and nonincluded FAEEs versus time is fundamental for a proper design, control, and optimization for the processing of functional seal oil.

1 | INTRODUCTION

The importance of long chain polyunsaturated fatty acids (PUFA) in human nutrition and disease prevention was scientifically recognized many years ago. Research have proved their prominent potential functions in preventing cardiovascular diseases, inflammation, cancer, and neurological disorders (Li et al., 2016; Liu et al., 2013; Simopoulos, 2002; Sun, Pigott, & Herwig, 2010). These health-promoting effects have been linked to the family of ω-3 PUFA, mainly to eicosapentenoic acid (EPA, 20:5 ω-3) and docosahexaenoic acid (DHA, 22:6 ω-3).The traditional source of ω-3 PUFA is fish oil. However, it has been reported that fish oil has negative aspects such as typically fishy smell, high content of cholesterol, and poor oxidative ability. Hence, it is of great significance to find new sources of ω-3 PUFA (Deng et al., 2011; Renyao et al., 2017; Shen et al., 2012).

The seal is one kind of marine mammals, which is widely distributed in countries near the polar region such as Canada, Norway, and Russia. Due to the cold living conditions, the fat layer of the seal is usually thicker than other mammals. Moreover, PUFA account for a reasonable part in the seal oil to keep the liquidity of the cell under the extremely cold environment. Therefore, seal is one of the new materials for the ω-3 PUFA products. Besides, the seal oil is also rich in docosapentaenoic acid (DPA), which is seldom found in fish oil. DPA is the intermediate of metabolism and biosynthesis of PUFA and researches have indicated that DPA has different pharmaceutical functions from DHA and EPA. Studies had showed that seal oil was more effective than fish oil in reducing the risk of heart disease and diabetes due to the function of DPA (Conquer, Cheryk, & Chan, 1999; Lin, Wu, & Yue, 2014; Mu, Jin, & Xie, 2016).

Some common procedures used to obtain PUFA concentrates are enzymatic purification, argentation silica gel chromatography, low temperature crystallization, supercritical fluid extraction, and urea complexation (Mu et al., 2016). However, only few are suitable for large-scale production (Ratnayake, Olsson, & Matthews, 2010; Senanayake & Shahidi, 2000; Shimada, Sugihara, & Tominaga, 2001). Urea complexation method has many advantages such as low cost, simple equipment, and mild conditions, which is an effective way to concentrate fatty

acids with different degrees of unsaturation. The tetragonal conformation of urea in the natural state is converted to the hexagonal crystalline structure by forming a spiral-shaped channel with an average diameter of approximately 5.5–5.8 Å, thus making it possible to entrap smaller-sized linear chain-length molecules (Eaton, Vasanthan, & Shin, 1996; Vasanthan, Shin, & Huang, 1997). With this method, saturated and monounsaturated fatty acids can be easily removed as urea complexation compounds while leaving PUFA in the nonurea complexation fraction (NUCF). The main variables controlling urea complexation are —(i) oil composition, (ii) oil : urea ratio, (iii) temperature of crystallization, and (iv) time of crystallization. The oil composition refers not only to degree of unsaturation but also the class of lipids, that is, triacylglycerols, free fatty acids, or esters. The high variety of fatty acids present in the oil leading to a heterogeneous composition of triglycerides hinders the concentration process (Morales-Medina, León, & Munio, 2016). Therefore, it is preferable to hydrolyze or esterify triacylglycerols to produce free fatty acids or fatty acids methyl/ethyl esters which can be more easily separated by physical approaches. Crexi, Monte, and Monte (2012) enriched the PUFA from free fatty acids of Carp oil by urea complexation method. They found an increase of 31.4% in unsaturated fatty acids content and a decrease of 75% in saturated fatty acids in the NUCF. Mu et al. (2016) concentrated the PUFA from free fatty acids of tuna oil through urea complexation method. A product containing 22.2% EPA, 10.5% DPA, and 42.3% DHA was obtained at 1:1.6 fatty acid/urea ratio (w/w) by crystallization at −8 °C for 16 hr. Shan, Li, and Zheng (2016) enriched EPA and DHA from fish oil ethyl ester by urea inclusion method. The results indicated that the optimum enrichment process was obtained at 1:1.5 oil ethyl ester/urea ratio (w/w), inclusion temperature of 75 °C, inclusion time of 1 hr, and crystallization temperature of 25 °C. The concentration of DHA and EPA in the final product reached 85%.

As reviewed before, some studies have been conducted aiming to qualitatively describe the influence of some variables on the concentration of PUFA, especially DHA and EPA in the fish oil. However, researches about urea complexation method applied to the enrichment of DHA, EPA, and DPA in the seal oil are rarely found (West, Burns, & Modafferi, 2011). Besides, most of the studies were focused on the composition of NUFA at fixed conditions without considering the regularity of different fatty acids changes during the process. Additionally, from an industrial point of view, the knowledge of the rules of fatty acids entrapped into the urea is fundamental for a proper design, control, and optimization. The aim of this work was to optimize the conditions for the enrichment of DHA, EPA, and DPA from seal oil ethyl ester by urea complexation method and study the change regularity of fatty acid composition during the process. We hope that our work will be helpful to the production and research of functional seal oil.

2 | MATERIALS AND METHODS

2.1 | Materials and chemicals

The seal oil ethyl ester was provided by OCEAN Co., Ltd. (Zhoushan, Zhejiang, China) with a total amount of DHA, EPA, and DPA of 21.85% (9.24% of DHA, 8.62% of EPA, and 3.99% of DPA). The fatty acids ethyl ester standard was purchased from ANPEL Laboratory Technologies, Inc. (Shanghai, China).

2.2 | PUFA enrichment by urea complexation technique

Urea complexation was performed according to the following procedure. The seal oil ethyl ester was mixed with different amounts of urea-saturated 95% aqueous ethanol and stirred at 60 °C with N_2 fluxing until homogenous solution was obtained. Then, the mixture was crystallized at required temperature and for required time. The urea adducts were separated as the urea complexation fraction (UCF, solid phase) and nonurea complexation fraction (NUCF, liquid phase) by filtration on a Buchner funnel under suction. Ethanol of NUCF was evaporated and an adequate amount of hot water was added to remove the urea residue. The upper layer was then extracted with n-hexane, and the solvent was removed at 45 °C by a vacuum rotary evaporator after drying over anhydrous sodium sulfate. The fatty acids from the UCF were recovered in the same manner. The samples were kept under a blanket of N_2 at −20 °C until analysis. The recoveries of the fatty acids were estimated as the weight proportion of amount of fatty acids in a fraction of those in the starting material (Gámez-Meza, Noriega-Rodriíguez, & Medina-Juárez, 2003; Liu, Zhang, & Hong, 2006; Wanasundara & Shahidi, 1999).

2.3 | Single-factor test design

Effect of urea/FAEE ratio (w/w), crystallization temperature, and crystallization time on the content of EPA + DPA + DHA and the recovery of them were investigated using single factor experiments. Variables and experimental levels for single-factor testing were designed with urea/oil (w/w) ratio of 1:1, 2:1, 3:1, 4:1, and 5:1, crystallization temperature of −20, −10, 0, 10, and 20 °C, crystallization time of 1, 2, 3, 4, and 5 hr.

2.4 | Optimization of an experiment design

Factors that influenced the results were used for optimum experimentation on the basis of single factor experiments. Concentration conditions of the seal oil were optimized using a response surface Box–Behnken central composite design taking the content of EPA + DPA + DHA and the recovery of them as the response value (Majumder & Wu, 2010).

2.5 | Change rules of fatty acid composition in the NUCF and UCF during urea adduction

PUFA in the seal oil ethyl ester was enriched at the optimal conditions (excepted crystallization time) obtained from the response surface methodology. The NUCF and UCF fractions were separated as described previously. The fatty acid composition of the NUCF and UCF fractions at different crystallization time (0.5, 1.0, 1.50, 2.0, 2.5, 3.0, and 4.0 hr) were analyzed by gas chromatography (GC).

TABLE 1 Fatty acid composition of the seal oil material and after urea adduction

Fatty acid	Material	After urea adduction	Fatty acid	Content (%)	After urea adduction
C14:0	6.45 ± 0.09	0.95 ± 0.02	C23:0	0.40 ± 0.02	ND[a]
C16:0	13.41 ± 0.04	1.29 ± 0.03	C22:2	ND[a]	0.80 ± 0.04
C16:1	14.18 ± 0.03	7.05 ± 0.02	C20:5 (EPA)	8.62 ± 0.05	28.97 ± 0.04
C17:1	0.99 ± 0.03	ND[a]	C24:1	0.75 ± 0.10	ND[a]
C18:0	2.67 ± 0.10	1.60 ± 0.05	C22:5 (DPA)	3.99 ± 0.07	11.08 ± 0.11
C18:1	24.21 ± 0.09	6.80 ± 0.04	C22:6 (DHA)	9.24 ± 0.34	31.30 ± 1.42
C18:2	1.58 ± 0.14	4.77 ± 0.10	∑SFA	23.84 ± 0.16	4.64 ± 0.07
C20:0	0.91 ± 0.10	ND[a]	∑MUFA	50.37 ± 1.05	19.25 ± 2.02
C20:1	10.24 ± 0.15	5.40 ± 0.03	∑PUFA	25.72 ± 1.02	76.12 ± 2.15
C20:4	2.29 ± 0.10	ND[a]	HUFA	21.85 ± 0.02	71.35 ± 1.72

Note. [a]Not detected.

2.6 | Fatty acid analysis

The fatty acid composition was analyzed on the GC (Agilent 7890B) equipped with a flame ionization detector and a HP-88column (30 m × 0.25 μm × 0.20 μm; Agilent). Ultra-high-purity nitrogen (99.99%) was used as the carrier gas at a constant flow rate of 30 mL/min. The split ratio was 100:1. The temperatures of the injector and the detector were 250 and 280 °C, respectively. The oven temperature was initially held at 70 °C for 2 min, followed by a 15 °C/min increase up to 150 °C for 3 min, then increased up to 220 at 3 °C/min, and held isothermally for 5 min. Identification of fatty acids was based on the retention time of the standards and the proportions were quantified by normalization of the relative area of the chromatogram.

2.7 | Statistical analyses

All tests were repeated three times. The data obtained were subjected to analysis of variance (ANOVA) and mean differences evaluated by Duncan's multiple range or the least square difference (LSD) test ($p < .05$). Statistical analysis was performed by the SPSS statistic program (Version 13.0) for Windows (SPSS Inc., Chicago, IL).

3 | RESULTS AND DISCUSSION

3.1 | Fatty acids composition and quality of the seal oil ethyl ester

The fatty acids composition of the seal oil is showed in Table 1. Among all fatty acids, C18:1 accounted for 24.21%, followed by C16:1 (14.18%) and C16:0(13.41%). The result was consistent with the conclusion of West and Burns (2011). The content of MUFA was 50.37%, which was larger than that of PUFA (26.32%) and SFA (23.24%). PUFA accounted for 26.32% of the total fatty acids, in which the overall proportion of EPA, DPA, and DHA was 21.85%. The fatty acids composition was similar to the result that Shahidi reported (Durnford, Shahidi, & Ackman, 2003). Peroxide value (PV) is an important index for evaluating oil quality. It is mainly related to the amount of hydrogen peroxide in the oil. The PV of the seal oil was 4.23 mEq/kg, which indicated the good quality of the oil. Iodine value (IV) is an index related to the unsaturated degree of the oil. It is usually expressed as the grams of iodine that can be absorbed or added in 100 g sample. The IV of the seal oil was 116.63 g I_2/100g and it was much lower than the 320 g I_2/100g that National Marine Fisheries Service (NMFS) (Deutch, Jørgensen, & Hansen, 2000) reported before. The oil detected by the NMFS was concentrated and the PUFA content in it was much higher than the raw oil leading to the higher IV in the NMFS report. Acid value (AV) was related to the content of FFA in the oil. High amounts of FFA cause the formation of soap that create emulsions and make the segregation of the products difficult (Abuzaytoun & Shahidi, 2006; Meher, Sagar, & Naik, 2006).

AV of the seal oil was 0.94 mg KOH/g, which was lower than the limit (<3 mg KOH/g). Saponification value (SV) is often used to estimate the molecular weight of the oil. SV of the seal oil was 170.73 mg KOH/g and it was higher than the carp oil that Crexi reported before (Crexi et al., 2012). The seal oil was rich in ω-3 PUFA such as EPA, DPA, and DHA, which were much helpful for human health. The characterization indexes of the seal oil indicated that the oil was in good quality. Hence, the seal oil is a good resource for the production of health food and pharmaceuticals.

3.2 | Urea complexation

3.2.1 | Effect of urea/FAEE ratio

Increasing the amount of urea caused more fatty acids to be entrapped in the UCF, resulting in an improved concentration of EPA, DPA, and DHA in the NUCF. Figure 1(a) showed the content and the recovery of EPA, DPA, and DHA under different urea/FAEE ratio (crystallization temperature and time was −10 °C and 3.0 hr, respectively). The maximum concentration (77.94%) and recovery (79.45%) of EPA, DPA, and DHA were obtained at the urea/oil ratio (w/w) of 3:1 and 2:1, respectively. Although the concentration reached the maximum when the ratio was 3:1, the recovery was much lower than that of other samples. Therefore, the ratio 3:1 was not suitable for the large-scale production.

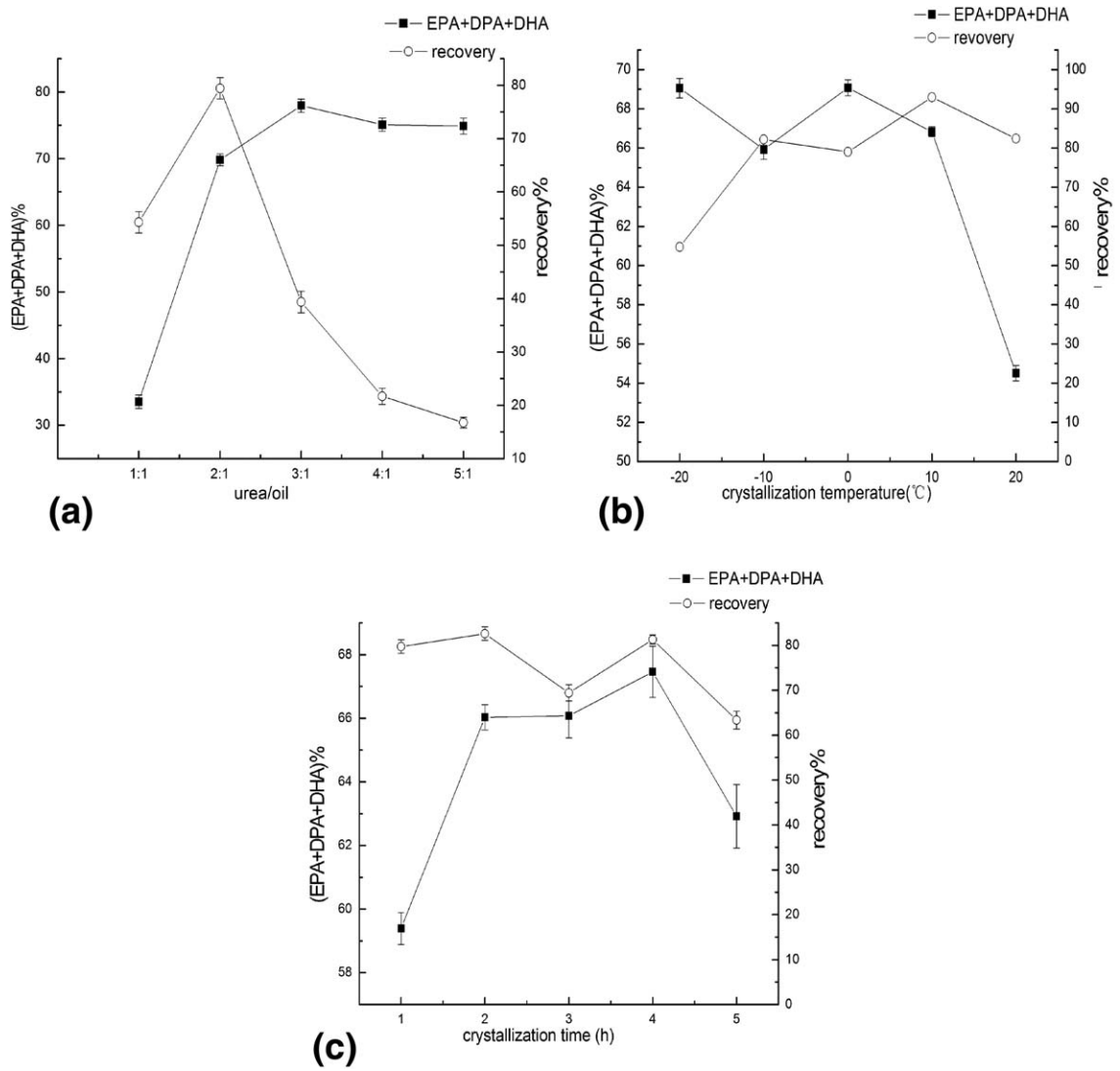

FIGURE 1 Effect of urea/FAEE (a), temperature (b) and time (c) on the content and recovery of EPA, DPA and DHA

As for the ratio of 2:1, the recovery was the highest and mass fraction also reached 63.82% at the same time. Based on this, the ratio 2:1 was chosen for the further research.

3.2.2 | Effect of crystallization temperature

The changes in the concentration and recovery of EPA, DPA, and DHA under different crystallization temperature were shown in Figure 1(b) (urea/FAEE and crystallization time was 2:1 and 3.0 hr, respectively). For the process was accompanied by exothermal activities, crystallization at lower temperatures facilitates highly enriched PUFA in the NUCF. The concentration increased over the decrease in temperature excepted for a minor decrease at −10°C and the concentration decreased quickly from 66.83 to 54.51% when the temperature increased from 10 to 20°C. The concentration kept over 65% without significant difference when the temperature was below 10°C. In the term of recovery, the opposite trend is present. The maximum recovery was found to be 92.96% at 10°C. Therefore, 10°C was chosen for the further research.

3.2.3 | Effect of crystallization time

Figure 1(c) showed the effect of crystallization time on the concentration and recovery of EPA, DPA, and DHA (urea/FAEE and crystallization temperature was 2:1 and −10°C, respectively). The concentration increased quickly before 2 hr and kept stable during 2–4 hr. There was an obvious decline after 4 hr. The recovery decreased over the crystallization time. The recovery reached its maximum value (82.60%) in 2 hr and the minimum value of it was found to be 63.35% in 5 hr. Although the maximum concentration was found to be 67.46% at 4 hr, the recovery of the 4 hr group was lower than the 2 hr group. Hence, 2 hr was chosen for the further research.

3.2.4 | Optimization of the concentration conditions by response surface methodology

On the basis of single factor experiment, the optimal concentration conditions of the seal oil were studied by RSM. The effects of urea/FAEE ratio (A), crystallization temperature (B), and crystallization time (C) on the concentration and recovery of EPA, DPA, and DHA were

TABLE 2 Analytical levels and results of response surface analysis

No.	A	B (°C)	C (h)	EPA + DPA + DHA (%)	Recovery (%)
1	1.50(−1)	10.00(0)	2.50(1)	55.1	98.27
2	1.50(−1)	15.00(1)	2.00(0)	55.38	103.00
3	2.00(0)	15.00(1)	2.50(1)	68.14	100.76
4	2.00(0)	10.00(0)	2.00(0)	61.54	87.69
5	2.00(0)	10.00(0)	2.00(0)	62.27	86.38
6	1.50(−1)	10.00(0)	1.50(−1)	53.29	103.16
7	2.00(0)	5.00(−1)	1.50(−1)	64.99	79.25
8	2.00(0)	15.00(1)	1.50(−1)	63.32	85.90
9	2.00(0)	10.00(0)	2.00(0)	64.43	80.53
10	2.00(0)	10.00(0)	2.00(0)	62.12	82.21
11	2.00(0)	5.00(−1)	2.50(1)	57.45	79.69
12	2.50(1)	10.00(0)	1.50(−1)	73.9	54.07
13	1.50(−1)	5.00(−1)	2.00(0)	52.28	102.81
14	2.50(1)	10.00(0)	2.50(1)	72.97	80.08
15	2.50(1)	5.00(−1)	2.00(0)	76.36	71.01
16	2.00(0)	10.00(0)	2.00(0)	65.78	81.22
17	2.50(1)	15.00(1)	2.00(0)	77.1	58.76

studied. A two-order polynomial mathematical model was established by using Box–Behnken design scheme. Analytical factors and levels for response surface methodology are shown in Table 2. The observed values for the mass fraction and recovery of EPA, DPA, and DHA at different combinations of independent variables are listed in Table 2. The range of the concentration and recovery of EPA, DPA, and DHA were

TABLE 3 ANOVA for regression equation of the mass fraction

Source	Sum of squares	*df*	Mean square	*F* value	*p* value
Model	961.07	9	106.79	30.36	<.0001
A–A	887.89	1	887.89	252.40	<.0001
B–B	20.67	1	20.67	5.88	.0458
C–C	0.42	1	0.42	0.12	.7389
AB	1.39	1	1.39	0.40	.5492
AC	1.88	1	1.88	0.53	.4888
BC	38.19	1	38.19	10.86	.0132
A^2	6.02	1	6.02	1.71	.2320
B^2	3.09	1	3.09	0.88	.3802
C^2	1.56	1	1.56	0.44	.5266
Residual	24.62	7	3.52		
Lack of fit	11.67	3	3.89	1.21	.4162
Pure error	12.95	4	3.24		
Cor total	985.70	16			

TABLE 4 ANOVA for regression equation of the recovery

Source	Sum of squares	*df*	Mean square	*F* value	*p* value
Model	3,114.09	9	346.01	9.57	.0035
A–A	2,567.15	1	2,567.15	71.03	<.0001
B–B	30.65	1	30.65	0.85	.3878
C–C	165.69	1	165.69	4.58	.0695
AB	38.61	1	38.61	1.07	.3357
AC	238.87	1	238.87	6.61	.0370
BC	51.92	1	51.92	1.44	.2697
A^2	5.19	1	5.19	0.14	.7160
B^2	8.17	1	8.17	0.23	.6490
C^2	8.26	1	8.26	0.23	.6472
Residual	252.99	7	36.14		
Lack of fit	211.49	3	70.50	4.25	.1305
Pure error	41.50	4	10.38		
Cor total	3,367.08	16			

53.29–77.10% and 54.07–103.16%, respectively. According to the results, the content of EPA, DPA, and DHA increased two- to threefold after the urea complexation technique. Besides, the higher content of EPA, DPA, and DHA corresponded to the lower recovery. Therefore, it is important to find a combination that improves the content without affecting the recovery.

Statistical testing of the regression equation was performed using an *F*-test. Analysis of variance (ANOVA) results are shown in Tables 3 and 4. The results demonstrated that the models for the mass fraction and the recovery were highly significant at 99% confidence level ($p < .01$). The regression equations for the concentration and the recovery varied with urea/oil ratio (A), crystallization temperature (B), and crystallization time (C) were derived as follows:

$$\begin{aligned}(\text{EPA}+\text{DPA}+\text{DHA})/\% = {} & 40.649 + 9.774A - 2.363B + 2.404C \\ & - 0.236AB - 2.74AC + 1.236BC \\ & + 4.784A^2 + 0.034B^2 - 2.436C^2\end{aligned}$$

$$\begin{aligned}\text{Recovery}/\% = {} & 270.981 - 67.464A - 1.119B - 89.542C \\ & - 1.243AB + 30.911AC + 1.441BC \\ & - 4.440A^2 + 0.056B^2 + 5.603C^2\end{aligned}$$

The lack of fit for the two models were not significant and the correction coefficient (R^2) were .975 and .925, respectively, which indicated that the regression equations were good with the experimental data and the linear relation between the independent variable and the response value was significant.

The significance of each coefficient was also determined using *F* values and *p* values. The results showed that urea/oil ratio had linear effect on both the concentration and the recovery ($p < .01$). The crystallization temperature had a significant ($p < .05$) linear effect on the concentration. The crystallization temperature and the crystallization time had quadratic effect ($p < .05$) on the mass fraction while the urea/

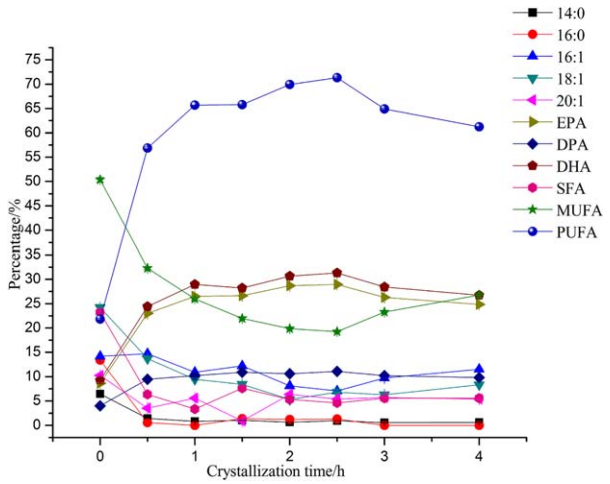

FIGURE 2 Content (%) change of major fatty acids in the NUCF over crystallization time

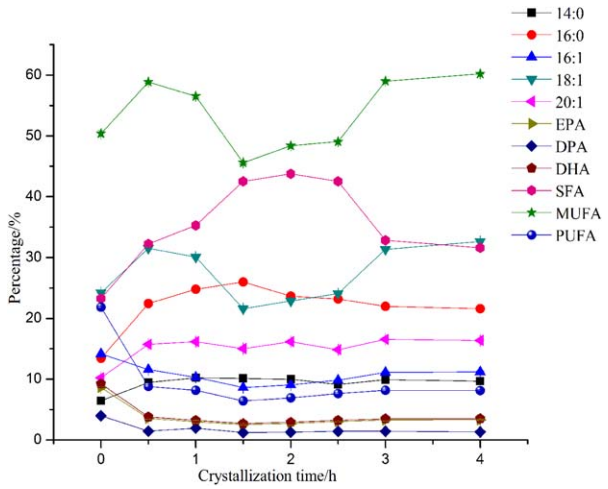

FIGURE 3 Content (%) change of major fatty acids in the UCF over crystallization time

oil ratio and the crystallization time had quadratic effect ($p < .05$) on the recovery.

3.2.5 | Validation of the predicative model

The suggested optimum conditions were at 2.38:1 urea/oil ratio (w/w) upon crystallization at 15 °C for 2.5 hr. The predicted concentration and recovery of EPA, DPA and DHA were 73.71 and 83.72%, respectively. To verify suitability of the model used for prediction of optimum response values, an optimization experiment was performed. Under optimal conditions, the concentration and recovery were 71.35 and 82.31%, which were close to the predicted value. The difference between real value and the predicted value was not significant ($p < .05$), which indicated that the model was adequate for the process.

The fatty acids composition of the seal oil after urea complexation was listed in Table 1. According to Table 1, the content of SFA in the seal oil before and after urea complexation was 23.24 and 4.64%, respectively. The SFA content decreased 5.01-folds after urea complexation. The content of MUFA in the seal oil before and after urea complexation was 50.37 and 19.25%, respectively. The MUFA content decreased 2.62-folds after urea complexation. This indicated that urea complexation can decrease the SFA and MUFA content in the seal oil. Moreover, the SFA was preferred to be adducted by urea comparing to the MUFA. The content of PUFA in the seal oil before and after urea complexation was 26.32 and 76.12%, respectively. The content of PUFA increased 2.89-folds after urea complexation. The content of EPA + DPA + DHA in the seal oil before and after urea complexation was 21.85 and 71.35%, respectively. The content of EPA + DPA + DHA increased 3.27-folds after urea complexation.

3.3 | Changes of fatty acid composition in the NUCF over time

Content change of major fatty acids in the NUCF over crystallization time is showed in Figure 2. The concentration of PUFA was significantly increased during the urea complexation process. It increased rapidly within 0–0.5 hr, from 26.32 to 61.38% and increased slowly during the 0.5–2.5 hr, from 61.38 to 76.12%, and then it gradually dropped during 2.5–4.0 hr. As for MUFA, the opposite trend was present. The initial concentration was 50.37% and it dropped to 19.25% at 2.5 hr. Then, a slight increase was presented. SFA in the raw material was 23.24% and it decreased significantly to 6.36% at 0.5 hr. Then, it kept at about 5%. The formation of the urea inclusion compounds depends on the degree of unsaturation of the fatty acids. The presence of double bonds in the carbon chain increases the volume of the molecule and reduces the probability of its urea complex (Hayes, 2006). It should also be noted that the content of MUFA accounted for about 25% in all groups, which indicated that urea adduction has limited effect on reducing the content of MUFA. The similar result was reported by Crexi et al. (2012). In his study, products of 50.15% PUFA were attained with a urea-to-fatty acid ratio of 4.5/1(w/w), at −10 °C for 24 hr. However, it was difficult to separate MUFA from PUFA through urea complexation method.

A similar trend of changes of EPA and DHA was presented in Figure 2. They increased quickly before 1.0 hr, and then kept relative stable during 1.0–4.0 hr. The maximum concentration of them was 28.97 and 31.10%, respectively obtained at 2.5 hr. As for DPA, it increased quickly from 3.99 to 9.45% during 0–0.5 hr. And then, it kept at about 10%. The content of C16:1, C18:1, and C20:1 was decreased after urea complexation. However, the change trends of them were not obvious as PUFA.

3.4 | Changes of fatty acid composition in the UCF over time

Content change of major fatty acids in the UCF over crystallization time is showed in Figure 3. Content of PUFA in the UCF was significantly decreased after urea complexation. The content decreased quickly from 26.32 to 8.81% during 0–0.5 hr. After 0.5 hr, it kept at about 8%. Content of SFA in UCF increased during 0–2.0 hr and then decreased. The content of SFA was from 31.68 to 43.76%. Trend of MUFA was complicated. But it was enriched in the UCF after urea complexation. The content of MUFA was from 45.57 to 60.19%, which

was the main ingredient in the UCF. It is recognized that urea complexation depends upon the configuration of fatty acids molecules rather than pure physical properties such as melting point or solubility (Ma, Field, & Clandinin, 2002; Strocchi & Bonaga, 1975). Long chain MUFA, especially those of C18 and C20, form complexes with urea more readily than those of shorter chain saturated fatty acid (C14 and C16) thus the amount of MUFA in UCF was the highest. Similar result was reported by Wanasundara and Shahidi (1999).

The original content of C14:0 in the seal oil was 6.45%. The content in the UCF increased during 0–1.0 hr and decreased gradually after 1.0 hr, excepted for a slight increase at 3.0 hr. The maximum value and the minimum was 10.21 and 9.14%, respectively, which were 1.58- and 1.39-folds of the original value. The content of C16:0 in the original seal oil was 13.41% and the content of C16:0 in the UCF was greatly improved to 22.45% at 0.5 hr. Then it increased during 0.5–2.0 hr and decreased after 2.0 hr. The maximum value and the minimum was 25.98 and 21.57%, respectively, which were 1.94- and 1.61-folds of the original value. As for C16:1, C18:1, and C20:1, content of C16:1 in UCF decreased after urea complexation, while C18:1 and C20:1 increased.

Comparing the content changes of SFA, MUFA, and PUFA in the UCF, the SFA was more easily to be adducted by urea. The chemical structures of PUFA are more complicated making them more difficult to be adducted by urea. As for MUFA, the content of them could be reduced by urea complexation but the decrease was limited. There was about 20% of MUFA in the seal oil after urea complexation, while the content of SFA was lower than 5%. Hence, it is necessary to take other ways to remove MUFA to obtain higher purity of ω-3 PUFA seal oil.

4 | CONCLUSIONS

The content of EPA, DPA, and DHA in the seal oil was 8.62, 3.99, and 9.24%, respectively. The total content of EPA, DPA, and DHA was 21.85%. The peroxide value (PV), iodine value (IV), acid value (AV), and saponification value (SV) of the seal oil was 4.23 mEq/kg, 116.63 g I_2/100g, 0.94 mg KOH/g, and 170.73 mg KOH/g, respectively, which indicated that the oil was in good quality. Therefore, the seal oil is a good resource of ω-3 PUFA.

The optimal conditions for the enrichment of EPA, DPA, and DHA by urea adduction were at 2.38:1 urea/FAEE ratio (w/w) upon crystallization at 15°C for 2.5 hr. The concentration and recovery of EPA, DPA, and DHA were 71.35 and 82.31% under the optimal conditions. The models for the concentration and the recovery of EPA, DPA, and DHA were highly significant at 99% confidence level ($p < .01$), which indicated that the models were adequate for the process.

PUFA content in the NUCF was greatly improved through urea complexation. DPA and DHA were more difficult to form clathrate compound than EPA since the complicated structure of them. The content of C14:0, C16:0 was greatly decreased through urea complexation. They contributed more of the decrease of SFA and MUFA in the NUCF.

MUFA (mainly C16:1, C18:1, and C20:1) accounted for nearly 20% in the seal oil recovered from the NUCF while the content of SFA was lower than 5%. Hence, it is necessary to take other methods to remove MUFA to obtain higher purity of ω-3 PUFA seal oil. Further studies will focus on the remove of MUFA and the separation the EPA, DPA, and DHA and techniques such as molecular distillation, rectification, and preparative chromatography will be applied.

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