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Concentration of omega 3-polyunsaturated fatty acids of seal blubber oil by urea complexation: optimization of reaction conditions

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Abstract

Production of omega-3 fatty acid concentrates from seal blubber oil (SBO) was optimized. In this process, the content of total ω3-fatty acids, Y_1; eicosapentaenoic acid (EPA), Y_2; and docosahexaenoic acid (DHA), Y_3 in the final product was maximized. A three-factor central composite rotatable design (CCRD) was used to study the effect of urea-to-fatty acid ratio (X_1), crystallization time (X_2), and crystallization temperature (X_3). Second-order polynomial regression models for Y_1, Y_2 and Y_3 were employed to generate response surfaces. Under optimum conditions the maximum amount of total ω3 fatty acids (88.2%) from SBO was obtained at a urea-to-fatty acid ratio of 4.5, a crystallization time of 24 h, and a crystallization temperature of −10°C.

Keywords: Seal blubber oil; Omega-3 polyunsaturated fatty acids; Urea complexation; Process optimization

1. Introduction

The importance of marine oils in human nutrition and disease prevention was scientifically recognized three decades ago. Epidemiological studies in the early 1970s postulated that the low incidence of coronary heart disease of Greenland Eskimos might be related to their distinctive dietary habit and use of marine lipids rich in polyunsaturated fatty acids (PUFA). The beneficial effects of PUFA have been ascribed to their ability to lower serum triacylglycerol (TAG) and cholesterol levels and enhance their excretion, to increase membrane fluidity and by conversion to eicosanoids to reduce thrombosis (Kinsella, 1986; Simopoulos, 1997). The ω3-PUFA are considered essential for normal growth and development throughout the life cycle. Therefore, consumption of appropriate amounts of ω3 fatty acids needs to be considered.

It has been suggested that PUFA concentrates devoid of more saturated fatty acids are much better than marine oils themselves since they allow keeping the daily intake of total lipids as low as possible (Haagsma et al, 1982). With the growing public awareness of the nutritional benefits of consuming PUFA concentrates, the market for these products is expected to grow in the future.

The simplest and most efficient technique for obtaining ω3-PUFA concentrates in the form of free fatty acids is urea complexation. This is a well established technique for elimination of saturated and monounsaturated fatty acids (Iverson & Weik, 1967; Strocchi & Bonaga, 1975). Initially the TAG of the oil are split into their constituent fatty acids by alkaline hydrolysis using alcoholic KOH or NaOH and these free fatty acids are then mixed with an ethanolic 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 with ω3-PUFA.

Urea complexation has the advantage that complexed crystals are extremely stable, and filtration does not necessarily have to be carried out at the very low temperatures which solvent crystallization of fatty acids would require (Anon., 1986). This method is also

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favoured by many researchers because complexation 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 (Wanasundara, 1996).

Experimental design is a systematic approach that enables several variables to be studied simultaneously, allowing the acquisition of a considerable amount of data from the minimum number of experiments and at the lowest cost. Experimental designs also allow prediction of the effects or changes that occur in any of the variables found to be critical for giving the user a competitive edge. Good design strategies can reduce time, cost, wastage and rework during production.

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 affect desired responses, response surface methodology (RSM; Thompson, 1982) is an effective tool for optimizing the process (Hunter, 1959). This method has been successfully adapted in many optimization studies (Hill & Hunter, 1966; Lee & Hoseney, 1982; Shieh et al., 1995; Wanasundara & Shahidi, 1996a). The central composite rotatable design (CCRD) is the preferred experimental design for fitting polynomial models to analyze response surfaces of multi-factor combinations. The design is considered rotatable because the variance of the predicted response, Y, at the point X is a function only of the distance of the point from the design centre irrespective, of the direction. This implies that the variance contours of predicted responses are concentric circles. Also rotatable design has the property that the variance of predicted response does not change when the design is rotated around the centre point (Montgomery, 1984). Thus, CCRD with RSM is a very effective tool for reducing the number of combinations required without compromising the validity of the results in studies where a large number of independent variables are included. If the proposed model is adequate, as revealed by the diagnostic checking provided by analysis of variance (ANOVA) and residual plots, contour plots can be usefully employed to study response surface and locate the optimum. The method of process optimization by RSM is a faster and more economical method for gathering research results than classical one-variable-at-a-time or full-factorial experimentation (Lee & Hoseney, 1982).

In this study, urea complexation of seal blubber oil (SBO) was carried out to concentrate ω3 fatty acids of the oil. Factors (variables) such as urea-to-fatty acid ratio (w/w, X_1), crystallization time (h, X_2) and crystallization temperature (°C, X_3) were studied collectively in order to optimize the conditions to obtain a maximum concentration of ω3-PUFA.

2. Materials and methods

2.1. Materials

Fresh, blubbers of harp seal (*Phoca groenlandica*) were obtained from local sources in Newfoundland. The extraction, refining, bleaching and deodorization of the oil were carried out as described elsewhere (Shahidi et al., 1994; Wanasundara & Shahidi, 1996b). Fatty acid methyl esters were purchased from either Supelco (Oakville, ON) or Nu-Check (Elysian, MN) companies. All other chemicals used in this study were of the American Chemical Society (ACS) grade or better.

2.2. Preparation of free fatty acids from seal blubber oil (SBO)

Preparation of free fatty acids from RBD-SBO was carried out according to the scheme given in Fig. 1. Seal blubber oil (25 g, treated with 200 ppm butylated hydroxytoluene; BHT) was saponified by refluxing for 1 h at the boiling temperature of the mixture (62 ± 2°C) under a blanket of nitrogen using a mixture of KOH (5.75 g), water (11 ml) and 95% (v/v) aqueous ethanol (66 ml). To the saponified mixture, distilled water (50 ml) was added and the unsaponifiable matter was extracted

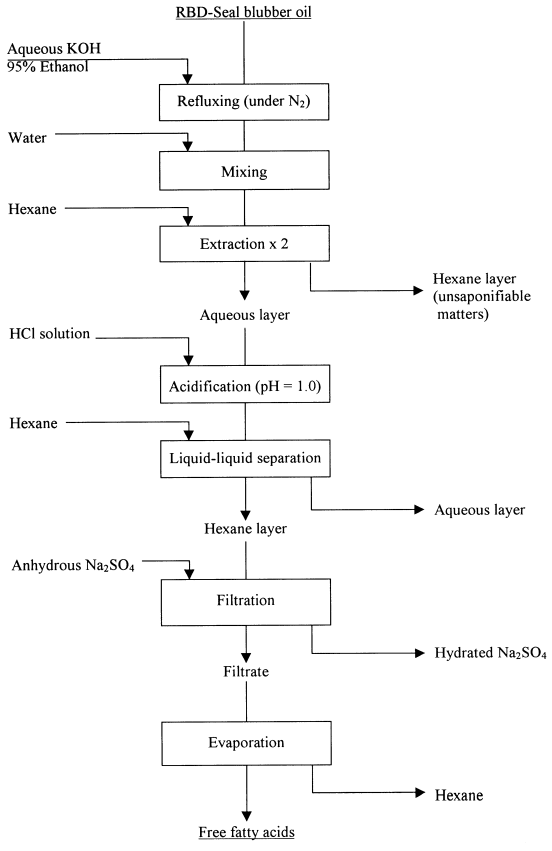

Fig. 1. Flowsheet for preparation of free fatty acids from refined-bleached and deodorized (RBD) seal blubber oil (SBO).

into hexane (2×100 ml) and discarded. The aqueous layer containing saponifiable matter was acidified (pH = 1.0) with 3N HCl. The mixture was transferred to a separatory funnel and the liberated fatty acids were extracted into 50 ml of hexane. The hexane layer, containing free fatty acids, was then dried over anhydrous sodium sulphate and the solvent removed at 40°C to recover free fatty acids which were then stored at −60°C until use.

2.3. Preparation of ω3 fatty acid concentrates from seal blubber oil (SBO) by urea complexation

The separation of ω3 fatty acids from the hydrolyzed fatty acid mixture of SBO was carried out by urea-fatty acid adduct formation according to the scheme given in Fig. 2. Free fatty acids (10 g) were mixed with urea (20%, w/v) in 95% aqueous ethanol and heated at 60°C with stirring until the whole mixture turned into a clear homogeneous solution. The ratio of urea-to-fatty acids was changed by using different amounts of urea. Initially, the urea–fatty acid adduct was allowed to crystallize at room temperature but colder temperatures (−24, −18, −9, 0 and +6°C) were maintained later for different periods for further crystallization. The crystals formed (urea-fatty acid adducts are also referred to as the urea complexing fraction; UCF) were separated from the liquid (non-urea complexing fraction, NUCF) by filtration under suction using a Büchner funnel lined with a thin layer of glass wool. The NUCF (filtrate) was diluted with an equal volume of water and acidified to pH 4–5 with 6N HCl; an equal volume of hexane was subsequently added and the mixture was stirred thoroughly for 1 h, then transferred to a separatory funnel. The hexane layer, containing liberated fatty acids, was separated from the aqueous layer containing urea. The hexane layer was washed with distilled water to remove any remaining urea and then dried over anhydrous sodium sulphate and the solvent was then removed at 40°C using a rotary evaporator. Fatty acids from UCF were recovered after addition of water/6N HCl and hexane in a similar manner (Fig. 2). The two fractions (NUCF and UCF) were weighed separately and percentage recovery of each was calculated. The fatty acid compositions of the two fractions were determined using a gas chromatographic procedure described elsewhere (Wanasundara & Shahidi, 1996b). Details of the experimental design to determine optimum conditions (urea-to-fatty acid ratio, crystallization temperature and crystallization time) for concentration of ω3 fatty acid by urea complexation are explained below.

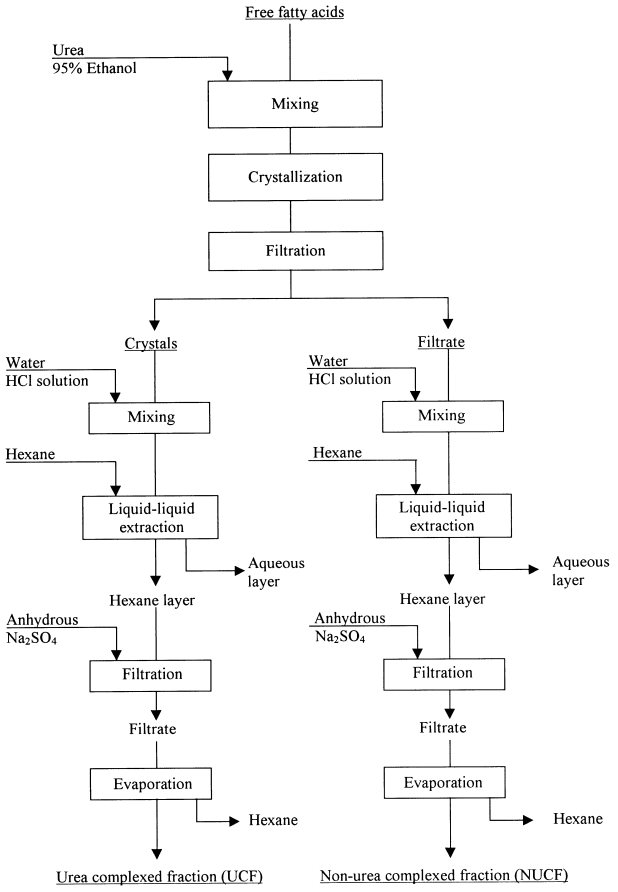

Fig. 2. Flowsheet for preparation of ω3 fatty acid concentrates by urea complexation.

2.4. Optimization procedure for production of ω3 fatty acid concentrates via urea complexation of seal blubber oil (SBO)

A three-factor central composite rotatable design (CCRD) (Box, 1954; Cornell, 1992) was employed to study the responses, such as concentration of total ω3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Y variables) by urea complexation of SBO. The urea-to-fatty acid ratio (X_1), crystallization time (X_2) and crystallization temperature (X_3) were independent variables studied to optimize Y variables. Duplicate reactions were carried out at all design points except at the centre point (0,0,0) where five replications were performed to allow the estimation of the 'pure error'. All experiments were carried out in a randomized order to minimize the effect of unexplained variability in the observed responses due to extraneous factors. Coded (x) and uncoded (X) variables of CCRD and treatment combinations were used for optimization of ω3 concentrates production.

A quadratic polynomial regression model was assumed for predicting individual Y variables. The model proposed for each response of $\mathbf{Y}$ was:

$$\boldsymbol{Y} = \beta_o + \sum_{i=1}^{3} \beta_i \boldsymbol{x}_i + \sum_{i=1}^{3} \beta_{ii} \boldsymbol{x}_i^2 + \sum \sum_{i<j=1}^{3} \beta_{ij} \boldsymbol{x}_i \boldsymbol{x}_j$$

where β_o, β_i, β_{ii} and β_{ij} are intercept, linear, quadratic and interaction regression coefficient terms, respectively,

and $\mathbf{x}_i$ and $\mathbf{x}_j$ are independent variables. The Statistical Analytical System (SAS, 1997) was used for multiple regression analysis, analysis of variance (ANOVA), canonical analysis and analysis of ridge maximum of data in the response surface regression (RSREG) procedure. 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 a constant value and changing the levels of the other two variables.

2.5. Determination of cholesterol in non-urea complexed fractions (NUCF)

The cholesterol content of NUCF of SBO was determined according to Rudel & Morris (1973) using *O*-phthalaldehyde reagent (Sigma). Samples (0.07–0.08 g of oil) were weighed into 15 ml screw-capped tubes and mixed thoroughly with 0.3 ml of 33% (w/v) KOH and 3.0 ml of 95% (v/v) aqueous ethanol. Tubes were then heated to 60°C in a water bath for 15 min, cooled, then treated with 10 ml of hexane and 3 ml of distilled water and mixed thoroughly using a vortex mixer. The whole mixture was allowed to separate into two layers. Aliquots (1 ml) of upper hexane layer were pipetted into clean tubes and the solvent was then evaporated under a stream of nitrogen. Two ml of the 0.05% (w/v) *O*-phthalaldehyde reagent in glacial acetic acid and 1 ml of concentrated sulphuric acid were carefully added to the tubes and then mixed thoroughly. After 10 min, the absorbance of the solutions was read at 550 nm. A standard curve was prepared using a cholesterol standard (Sigma). Cholesterol content of the oil was expressed as mg/100 g oil.

3. Results and discussion

Experimental values obtained for responses; PUFA, total ω3 fatty acids, EPA, docosapentaenoic acid (DPA) and DHA in NUCF of SBO as well as percentage recovery of NUCF for nineteen design points are given in Table 1. Table 2 shows values of the above-mentioned responses for UCF of SBO. Among the major ω3-PUFA, DHA was found almost exclusively in the NUCF of some treatment conditions. Although a major portion of EPA was recovered in the NUCF, a small proportion invariably complexed with urea and was detected in UCF. The amount of EPA in the UCF was considerable (6.69–6.77%) with a urea-to-fatty acid ratio of 3.5, crystallization time of 18 h and a crystallization temperature of −9°C. Therefore, these results demonstrate that EPA has more tendency to form urea adducts than DHA. Haagsma et al. (1982) and Ratnayake et al. (1988) have reported similar results for urea complexation experiments carried out for cod liver and menhaden oils, respectively. The highest DPA

Table 1
Central composite design arrangement and responses for non-urea-complexed fraction of seal blubber oil (SBO)

Run	Variable levels			Responses, Y (non-urea complexed fraction, NUCF)					
	Urea/FAs[a] (X_1)	Time[b] (X_2)	Temperature[c] (X_3)	Yield[d] (%)	PUFA (%)	EPA (%)	DPA (%)	DHA (%)	Total ω3 (%)
1	2	12	−18	28.5	69.2	22.1	12.7	24.4	63.4
2	5	12	−18	22.9	87.3	18.1	2.72	56.8	86.7
3	2	24	−18	27.2	68.8	21.9	12.6	24.4	62.9
4	5	24	−18	20.0	89.5	17.7	2.10	59.5	88.4
5	2	12	0	34.6	57.5	18.3	10.6	19.9	52.3
6	5	12	0	20.9	87.1	21.6	5.18	50.7	84.6
7	2	24	0	35.2	59.6	18.9	11.0	20.7	54.2
8	5	24	0	20.3	87.4	20.6	4.90	52.3	85.1
9	1	18	−9	52.8	33.7	10.4	6.46	11.2	30.1
10	6	18	−9	20.0	87.8	20.3	3.59	54.2	85.9
11	3.5	8	−9	19.6	86.7	11.4	0.53	70.3	86.4
12	3.5	28	−9	20.8	87.7	11.6	0.64	67.3	87.3
13	3.5	18	−24	18.2	80.8	11.0	1.36	57.1	80.8
14	3.5	18	6	24.6	80.8	24.9	2.07	43.2	77.2
15	3.5	18	−9	19.3	88.4	10.9	2.35	66.8	88.3
16	3.5	18	−9	20.4	85.3	10.9	3.31	60.8	88.5
17	3.5	18	−9	20.7	85.9	7.82	3.11	65.9	84.1
18	3.5	18	−9	19.3	87.6	7.68	2.20	66.4	85.9
19	3.5	18	−9	20.0	87.7	7.61	2.96	65.9	86.0

PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.
[a] Urea-to-fatty acid ratio (w/w);
[b] crystallization time (h);
[c] crystallization temperature (°C);
[d] percentage recovery of NUCF.

Table 2
Central composite design arrangement and responses for urea-complexed fraction of seal blubber oil (SBO)

Run	Variable levels			Responses, Y (urea complexed fraction, UCF)					
	Urea/FAs[a] (X_1)	Time[b] (X_2)	Temperature[c] (X_3)	PUFA (%)	MUFA[d] (%)	EPA (%)	DPA (%)	DHA (%)	Total ω3 (%)
1	2	12	−18	4.95	73.6	1.23	1.21	0.90	3.66
2	5	12	−18	19.0	63.3	6.03	4.17	5.00	16.2
3	2	24	−18	5.33	73.5	1.36	1.32	0.99	4.00
4	5	24	−18	19.3	63.0	6.28	4.43	4.74	16.4
5	2	12	0	6.87	70.4	1.86	1.36	1.97	5.59
6	5	12	0	16.3	65.3	5.59	3.79	3.54	13.7
7	2	24	0	35.55	71.3	1.79	1.28	0.60	4.15
8	5	24	0	15.1	68.5	5.61	3.89	0.13	10.4
9	1	18	−9	7.04	64.7	2.68	1.68	0.78	5.66
10	6	18	−9	17.2	64.6	5.85	3.92	3.90	14.5
11	3.5	8	−9	20.2	62.4	6.86	4.36	5.52	17.7
12	3.5	28	−9	19.5	62.7	6.57	3.94	5.07	16.6
13	3.5	18	−24	14.5	69.8	6.50	4.27	0.27	12.1
14	3.5	18	6	11.7	69.3	4.46	3.93	0.35	9.31
15	3.5	18	−9	19.3	62.8	6.77	3.92	5.27	17.0
16	3.5	18	−9	19.1	62.7	6.69	3.73	5.31	16.7
17	3.5	18	−9	19.2	62.5	6.71	3.80	5.29	16.8
18	3.5	18	−9	18.2	63.8	6.72	3.92	4.19	15.7
19	3.5	18	−9	18.2	63.7	6.77	3.97	4.23	15.8

See Table 1 for explanation of abbreviations.
[a] Urea-to-fatty acid ratio (w/w);
[b] crystallization time (h);
[c] crystallization temperature (°C).
[d] monounsaturated fatty acids.

content (12.6%) was achieved with a urea-to-fatty acid ratio of 2, a crystallization time of 12 h and a crystallization temperature of −18°C (Table 1). The content of DPA in the original SBO was 4.66%, but by urea complexation a 3-fold increase in the DPA content may be achieved. Urea complexation of SBO resulted in an increase in the total PUFA content up to 89.5% in the NUCF. It is difficult to remove all saturated fatty acids to obtain 100% PUFA in the concentrate. Ratnayake et al. (1988) have also reported that complete removal of saturated fatty acids by urea complexation may be impossible since some of the saturated fatty acids do not complex with urea during crystallization. Long chain monounsaturated fatty acids (MUFA), especially those of the C20 and C22, form complexes with urea more readily than those of shorter chain saturated fatty acids (C14 and C16) thus the amount of MUFA in UCF was increased up to 73% in some treatment conditions. Enrichment of total ω3 fatty acids in the concentrate and overall recovery varied inversely with increasing urea-to-fatty acid ratio as well as decreasing crystallization temperatures. Therefore, these experimental variables should be carefully controlled in order to achieve a maximum content of total ω3 fatty acids in the concentrate with a reasonable recovery.

The amount of cholesterol in the NUCF of SBO under different treatment conditions is given in Table 3. Although it was assumed that the content of cholesterol in the NUCF may increase due to its large molecular size, the use of crystallization temperatures below 0°C, lowered the content of cholesterol. The melting point of cholesterol is 148.5°C, so at low temperatures it may solidify with urea (without complexing) and separate along with the UCF. Original SBO contained 106 mg cholesterol/100 g oil. When crystallization temperatures were set at −9, −18 and −24°C, the amount of cholesterol in NUCF was reduced down to 88–95, 68–72 and 60 mg/100 g oil, respectively.

3.1. Optimization of process conditions to maximize contents of total ω3 fatty acids, EPA and DHA of seal blubber oil (SBO) concentrate

Optimization of process conditions such as urea-to-fatty acid ratio (X_1), crystallization time (X_2) and crystallization temperature (X_3) to maximize contents of total ω3 fatty acids, EPA and DHA in the prepared concentrate was carried out. The response surface methodology (RSM) was employed for this purpose.

3.2. Diagnostic checking of fitted models

Multiple regression coefficients obtained by employing a least squares technique to predict quadratic polynomial models for contents of total ω3 fatty acids (Y_1), EPA (Y_2) and DHA (Y_3) are summarized in Table 4.

Table 3
Cholesterol content of non-urea-complexed fraction (NUCF) of seal blubber oil at different treatment conditions[a]

Treatment			Cholesterol content (mg/100 g oil)
Urea-to-fatty acid ratio (w/w)	Time[b] (h)	Temperature[c] (°C)	
2	12	−18	72.1 ± 6.0
5	12	−18	71.6 ± 4.0
2	24	−18	70.3 ± 5.2
5	24	−18	68.4 ± 3.9
2	12	0	129 ± 4.5
5	12	0	109 ± 2.1
2	24	0	119 ± 8.7
5	24	0	116 ± 4.5
1	18	−9	90.1 ± 3.6
6	18	−9	90.4 ± 7.2
3.5	8	−9	95.9 ± 3.2
3.5	28	−9	88.2 ± 3.1
3.5	18	−24	60.3 ± 4.2
3.5	18	6	128 ± 8.0
3.5	18	−9	95.2 ± 4.5
	RBD-seal blubber oil		106 ± 22.0

[a] Mean ± SD ($n = 3$);
[b] crystallization time (h);
[c] crystallization temperature (°C).

Table 4
Regression coefficients of predicted quadratic polynomial model for response variables (total ω3, EPA and DHA contents) in urea complexation experiment of seal blubber oil (SBO)

Variables	Coefficients (β)		
	Total ω3 (%) (Y_1)	EPA (%) (Y_2)	DHA (%) (Y_3)
Intercept	−18.98212	38.05204**	−61.545841**
Linear			
X_1	44.17949***	−7.015524	52.360500***
X_2	0.267651	−1.509582	0.886283
X_3	−1.450864***	0.649557	−1.914253
Quadratic			
X_{11}	−4.721517***	1.315469**	−6.248160***
X_{22}	−0.005895	0.044117	−0.029410
X_{33}	−0.037687***	0.050363***	−0.095827***
Interaction			
X_{12}	0.009264	−0.024361	0.048181
X_{13}	0.134343	0.121981	−0.046639
X_{23}	0.002613	0.000218	−0.000734
X_{123}	—	—	—
R^2	0.99	0.71	0.93

X_1 = urea-to-fatty acid ration; X_2 = crystallization time; X_3 = crystallization temperature.
**$p < 0.05$; ***$p < 0.01$.

Examination of these coefficients with the t-test indicated that linear and quadratic terms of urea-to-fatty acid ratio and crystallization temperature were highly significant ($p < 0.01$) but crystallization time was not for total ω3 fatty acids content in the concentrate. For the content of EPA in the concentrate, none of the linear terms of independent variables was significant ($p < 0.05$) but quadratic terms of urea-to-fatty acid ratio and crystallization temperature were significant at $p < 0.05$ and $p < 0.01$, respectively. The coefficients obtained for DHA content of the concentrate showed that the linear term of urea-to-fatty acid ratio was highly significant ($p < 0.01$), whereas crystallization time and temperature were not. However, quadratic terms of urea-to-fatty acid ratio and crystallization temperature were significant at $p < 0.01$. Therefore, these results suggest that linear and/or quadratic effect of urea-to-fatty acid ratio and crystallization temperature are the primary determining factors for the amounts of total ω3 fatty acids, EPA and DHA in the prepared concentrate of SBO. No statistically significant ($p > 0.05$) interactions existed between any two of the three factors. The contribution of linear and quadratic terms to the models was 0.66 and 0.32 for the total ω3, 0.09 and 0.58 for the EPA and 0.47 and 0.46 for the DHA, respectively. The coefficients of independent variables (urea-to-fatty acid ratio; X_1, crystallization time; X_2 and crystallization temperature; X_3) determined for the quadratic polynomial models (Table 4) for total ω3 fatty acids (Y_1), EPA (Y_2) and DHA (Y_3) of the prepared concentrate are given below:

$$Y_1 = -18.982 + 44.179X_1 + 0.268X_2 - 1.451X_3 - 4.72152X_1^2 - 0.00589X_2^2 - 0.03769X_3^2 + 0.00926X_1X_2 + 0.13434X_1X_3 + 0.00261X_2X_3$$

$$Y_2 = 38.052 - 7.016X_1 - 1.511X_2 + 0.65X_3 + 1.31547X_1^2 + 0.04412X_2^2 + 0.05036X_3^2 - 0.02436X_1X_2 + 0.12198X_1X_3 + 0.00022X_2X_3$$

$$Y_3 = -61.546 + 52.361X_1 + 0.886X_2 - 1.914X_3 - 6.24816X_1^2 - 0.02941X_2^2 - 0.09583X_3^2 + 0.04818X_1X_2 - 0.04664X_1X_3 - 0.00073X_2X_3$$

The models predicted for Y_1, Y_2 and Y_3 were adequate as indicated by error analysis that showed non-significant ($p > 0.05$) lack-of-fit. The regression models for data on total ω3 fatty acids and DHA were highly significant ($p < 0.01$) with satisfactory coefficients of determinations (R^2) 0.99 and 0.93, respectively. However, the coefficient of determination for data on EPA was 0.71. The models indicated that urea-to-fatty acid ratio (X_1) was a significant variable with the most linear effect on both total ω3 fatty acids and DHA contents in the

concentrate as it had the largest linear coefficients of 44.179 and 52.361, respectively (Table 4).

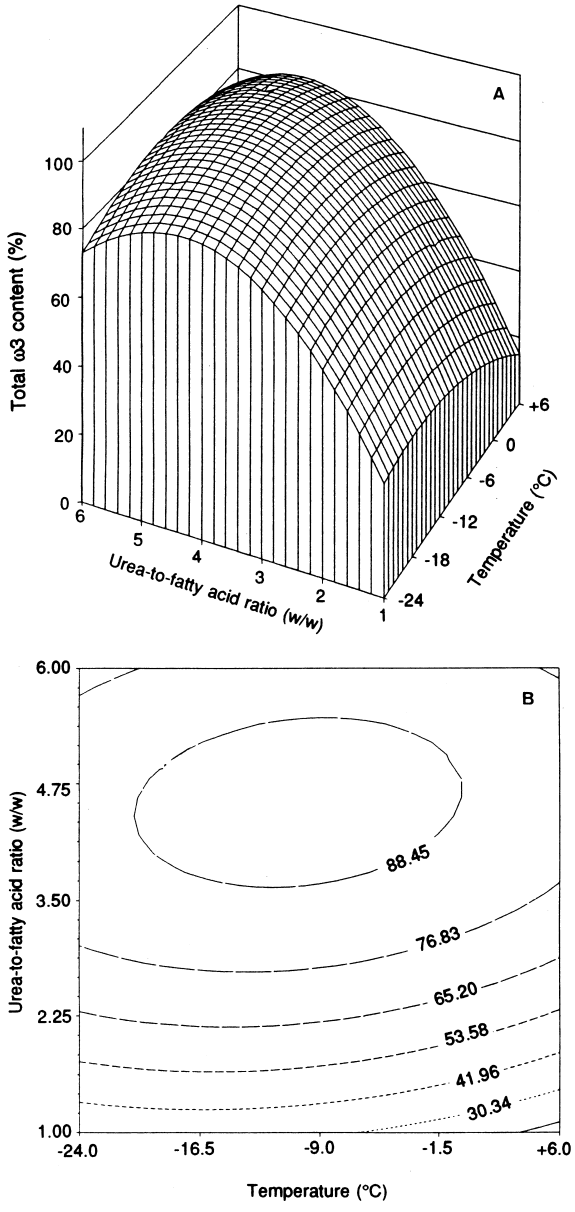

Fig. 3. (A) Response surface and (B) contour plots for the effect of urea-to-fatty acid ratio and crystallization temperature on total ω3 fatty acid content of the prepared concentrate of seal blubber oil (SBO).

3.3. Response surface plotting and optimization based on canonical analysis

Variables giving linear and quadratic terms with the largest absolute coefficients in the fitted models (Table 4) were chosen as the axes (urea-to-fatty acids ratio and crystallization temperature) 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 contents of total ω3 fatty acids, EPA and DHA, in the concentrates are given in Figs. 3–5, respectively.

Canonical analysis was performed on the predicted quadratic polynomial models to examine the overall shape 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 (Mason et al., 1989). Results of canonical analysis of the response surfaces are given in Table 5. The stationary point for total ω3 fatty acids content of the prepared concentrates by urea complexation predicted a maximum of 92.3% at a urea-to-fatty acid ratio of 4.5, crystallization time of 24 h and crystallization temperature of −10°C. The contour plot derived from the results of canonical analysis showed ellipsoidal contours at the maximum point (Fig. 3).

In a contour plot, curves of equal response values are drawn on a plane whose coordinates represent the levels of the independent variables (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. The contour plots show the combination of levels of urea-to-fatty acid ratio and crystallization temperature that can afford the same amount of total ω3 fatty acids in the concentrate.

At the maximum level of total ω3 fatty acids of the concentrate, DHA comprised a major proportion of it; its content was predicted to increase up to 70.1% (calculated from the predicted equation for DHA [Y_3])

Table 5
Predicted and observed values for response variables (total ω3, EPA and DHA contents) in urea complexation experiment of seal blubber oil (SBO)

Response variables	Critical values of independent variables			Stationary point	Predicted value	Observed value[a]
	Urea-to-fatty acid ratio (w/w)	Crystallization time (h)	Crystallization temperature (°C)			
Total ω3 fatty acids (%)	4.5	24	−10	Maximum	92.3	88.2 ± 3.44
EPA (%)	3.3	18	−10	Minimum	9.36	—
DHA (%)	4.3	19	−11	Maximum	70.1	67.6 ± 2.54

See Table 1 for description of abbreviations.
[a] Mean ± SD ($n = 3$).

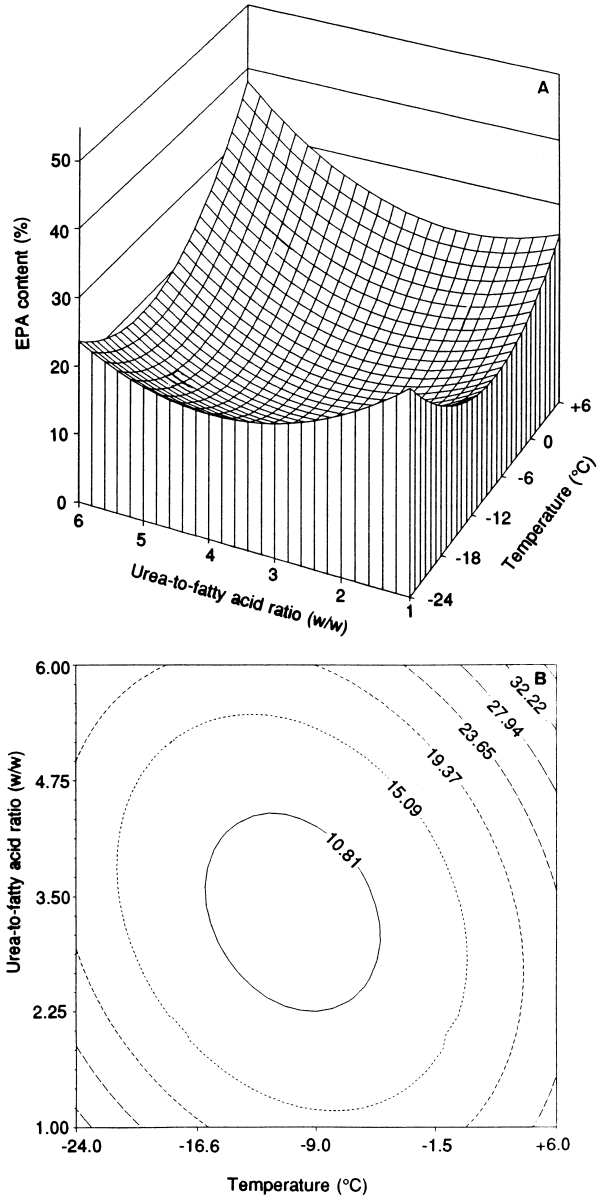

Fig. 4. (A) Response surface and (B) contour plots for the effect of urea-to-fatty acid ratio and crystallization temperature on EPA content of the prepared concentrate of seal blubber oil (SBO).

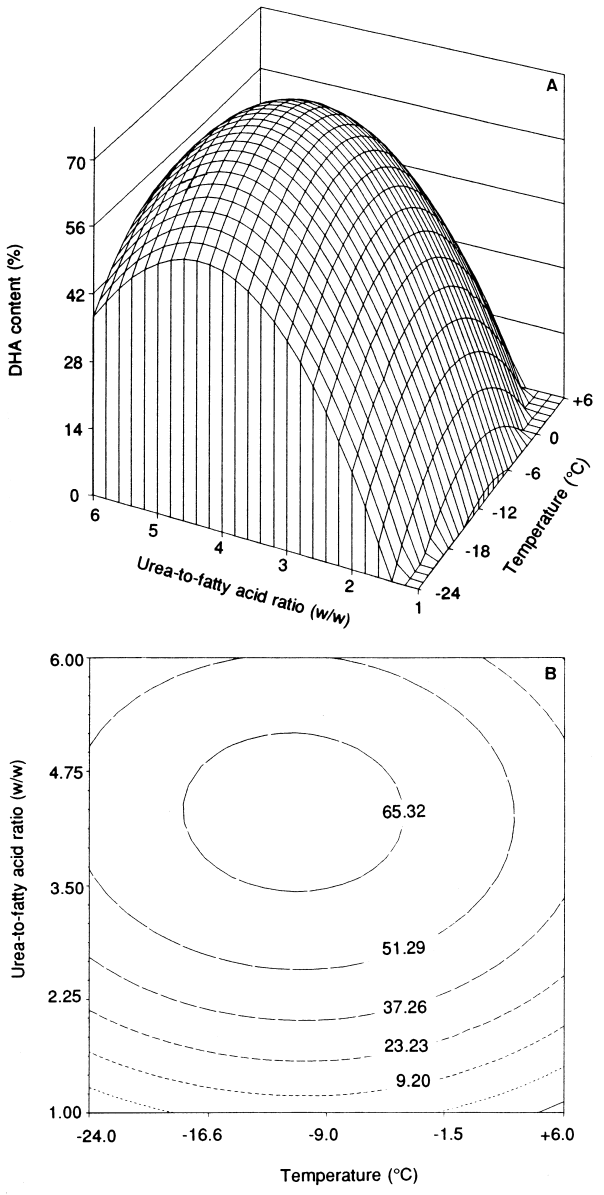

Fig. 5. (A) Response surface and (B) contour plots for the effect of urea-to-fatty acid ratio and crystallization temperature on DHA content of the prepared concentrate of seal blubber oil (SBO).

(Table 5 and Fig. 5), but the content of EPA in the concentrate was predicted to decrease with the increasing DHA content (Table 5 and Fig. 4). Therefore, in order to obtain a high content of EPA in the concentrate, process variables should be changed but the DHA content may be reduced accordingly. The contour plot for EPA content shows an increase with increasing urea-to-fatty acid ratio and crystallization temperature. At the same time, the content of DHA in the concentrate should also be considered using the contour plot approach. Therefore, if a high DHA and a low EPA content in the concentrate was desired, the urea-to-fatty acid ratio of 4.3, crystallization time of 19 h and crystallization temperature of −11°C may be suitable. However, if a higher content of EPA was needed in the concentrate, the levels of these three variables has to be changed and it would result in a lower content of DHA.

The adequacy of the models predicted was examined by performing independent experiments at the optimal conditions for both the total ω3 fatty acids and the DHA contents. Verification results revealed that the predicted values from these models were reasonably close to the observed values (Table 5). Therefore, using urea-to-fatty acid ratio of 4.5, crystallization temperature of −10°C and crystallization time of 24 h, the amount of total 3 fatty acids can be increased up to 88.2% with a recovery (yield) of 21.5% of the weight of the original SBO by urea complexation.

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