ELSEVIER

Food Chemistry 77 (2002) 115–123

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Lipase-catalyzed incorporation of docosahexaenoic acid (DHA) into borage oil: optimization using response surface methodology

S.P.J. Namal Senanayake, Fereidoon Shahidi*

Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9

Received 5 June 2001; accepted 6 August 2001

Abstract

The ability of immobilized lipase Novozym 435, from *Candida antarctica,* to modify the fatty acid composition of borage oil (*Borago officinalis* L.) in hexane, by incorporation of docosahexaenoic acid (DHA), was studied. Response surface methodology (RSM) was used to evaluate the effects of variables, namely the amount of enzyme (100–200 U), reaction temperature (30–60 °C) and reaction time (18–30 h), on the yield (%) of DHA incorporation. Optimization of the acidolysis reaction was attempted in order to obtain a maximum yield of DHA incorporation while using the minimum amount of enzyme possible. Computer-generated contour plot interpretation revealed that an enzyme concentration of 165 units, after 25 h of reaction at 50 °C, gave optimum incorporation of DHA, up to 34.1%. Analysis of variance (ANOVA) showed that 94% ($R^2 = 0.94$) of the observed variation was explained by the polynomial model. A low coefficient of variation (2.92) showed that the reproducibility of the model was satisfactory. Lack of fit analysis revealed a non-significant value for the model equation, indicating that the regression equation was adequate for predicting the degree of DHA incorporation under any combination of values of the variables. The positional distribution of DHA in modified borage oil, produced under optimum reaction conditions, was determined using pancreatic lipase hydrolysis. The results showed that DHA was fairly evenly distributed at the *sn*-2 and *sn*-1 + *sn*-3 positions of the structured triacylglycerols.

Keywords: Borage oil; *Candida antarctica*; Docosahexaenoic acid; Novozym 435; Response surface methodology

1. Introduction

Borage oil (*Borago officinalis* L.) is a rich commercial source of γ-linolenic acid (GLA; 18:3ω6). GLA is an essential ω6 polyunsaturated fatty acid (PUFA) and must be provided in the food because it cannot always be easily manufactured within the body (Horrobin, 1992). GLA is important for a healthy skin, helping to keep it smooth and supple, to protect it from injury and infections, and to regulate body temperature and water loss. Individuals with atopic eczema (a skin disorder) are thought to have a deficiency that interferes with the production of other ω6 PUFA from linoleic acid. GLA applied to skin or taken orally, has often helped to relieve symptoms of this disorder (Horrobin, 1993). This fatty acid also seems to provide some benefit in pre-menstrual syndrome, diabetes and old age, where the same metabolic effect is suspected. It has also been used to treat rheumatoid arthritis, asthma, multiple sclerosis, migraine and cancer, but the results so far are inconclusive (Horrobin, 1992, 1994).

The beneficial effects of PUFA have been ascribed to their ability to lower serum triacylglycerol and cholesterol levels and enhance their excretion, to increase membrane fluidity, and, by conversion to eicosanoids, to reduce thrombosis (Kinsella, 1986). The ω3 PUFA, which include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are considered essential for normal growth and development (Simopoulos, 1990) and may play an important role in the prevention and treatment of cardiovascular diseases (Bruckner, 1992), hypertension (Deferne & Leeds, 1992; Meland, Fugelli, Lerum, Rønneberg, & Sandvik, 1989), inflammatory and immune disorders (Boissonneault & Hayek, 1992), diabetes (Bhathena, 1992) and cancer (Carroll, 1990). A significant amount of DHA is found in human milk

* Corresponding author. Tel.: +1-709-737-8552; fax: +1-709-737-4000.

E-mail address: fshahidi@mun.ca (F. Shahidi).

0308-8146/02/$ - see front matter
PII: S0308-8146(01)00311-9

(Horrobin, 1995). High levels of DHA are also found in the human brain and retina (Salem, Kim, & Yergey, 1986). Therefore, DHA in breast-milk or infant formula meets the requirements of the developing human brain and eye (Carlson, 1995). The PUFA composition of cell membranes is highly dependent on their dietary intake. Therefore, consumption of appropriate amounts and proportions of ω6 and ω3 fatty acids needs to be considered.

Modification of lipids by lipase-catalyzed reactions has been reported in a large number of publications in the past few years. The use of lipases is becoming increasingly important in a number of applications. Lipases can be used as biocatalysts for hydrolysis, esterification, interesterification and modification of fats and oils (Gandhi, 1997). Lipases may catalyze the incorporation of fatty acids into triacylglycerols. Application of lipases in the modification of lipids may offer some advantages over conventional chemical catalysts, such as synthesis of novel products, and incorporation of desirable fatty acids at specific positions of the lipid to improve functionality, absorption, metabolism, nutrition and for clinical use (Akoh, 1995). In addition, enzyme-mediated reaction has become the preferred method for small scale modified lipid production because reaction conditions tend to be milder and less side reactions may occur (Senanayake & Shahidi, 2001). Lipase-catalyzed incorporation of long-chain ω3 PUFA into borage oil has recently been reported (Akoh & Sista, 1995; Akoh & Moussata, 1998; Ju, Huang, & Fang, 1998; Senanayake & Shahidi, 1999a,1999b, 2000b, 2001).

Response surface methodology (RSM) has been a popular and effective statistical technique for investigation of complex processes. Hill and Hunter (1966) first reported the origin of RSM and its application in food research. RSM consists of a group of mathematical and statistical procedures that can be used to study relationships between one or more responses and a number of independent variables. RSM defines the effect of the independent variables, alone or in combination, on the process. In addition to analyzing the effects of independent variables, this experimental methodology generates a mathematical model that accurately describes the overall process. It has been successfully applied to optimize enzymatic reactions in organic solvents (Haas, Cichowicz, Phillips, & Moreau, 1993; Huang & Akoh, 1996; Shieh, Akoh, & Koehler, 1995). In this study, RSM was used to evaluate the effect of several variables on the acidolysis reaction of borage oil with DHA. Thus, optimization of process conditions for incorporation of DHA in borage oil by immobilized lipase from *Candida antarctica* was undertaken. The amount of enzyme, reaction temperature and reaction time were the variables investigated.

2. Materials and methods

2.1. Materials

Borage oil was obtained from Bioriginal Food and Science Corporation (Saskatoon, SK). Algal oil, containing 47.4% DHA, was obtained from Martek Biosciences Corporation (Columbia, MD). Novo Nordisk (Bagsvaerd, Denmark) generously provided Novozym 435 from *Candida antarctica* (immobilized on a macroporous resin). HPLC grade hexane was obtained from Fisher Scientific (Nepean, ON).

2.2. Experimental design

Before RSM was applied, approximate conditions for DHA incorporation, namely the amount of enzyme, reaction temperature and reaction time, were determined by varying one factor at a time while keeping the others constant (Fig. 1). An appropriate range for each factor was determined for RSM.

A three-factor and three-level face-centred cube design with 17 individual design points was adopted in this study (Gao & Mazza, 1996; Mason, Gunst, & Hess, 1989). The independent variables (X_i) and their levels are presented in Table 1. To avoid bias, 17 runs were performed in a totally random order. The independent variables, or factors studied, were the amount of enzyme (units; X_1), reaction temperature (°C; X_2) and reaction time (h; X_3); (Table 1). Response or dependent variable (Y) studied was DHA incorporation (%). Duplicate experiments were carried out at all design points.

The second order polynomial model predicted for optimization of DHA incorporation in borage oil (Y) was:

$$Y = \beta_o + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum \sum_{i<j=1}^{3} \beta_{ij} X_i X_j$$

where, β_o, β_i, β_{ii} and β_{ij} are regression coefficients for intercept, linear, quadratic and interaction terms, respectively, and X_i and X_j are independent variables. Data were analyzed using the response surface regression (RSREG) procedure (SAS Institute Inc., 1990) and fitted to the second order polynomial equation after logarithmic transformation (Thompson, 1982). Response surfaces and contour plots were obtained using the fitted model, by keeping the least effective independent variable at a constant value while changing the other two variables. Verification experiments were carried out using combinations of variables at different levels (within the experimental range).

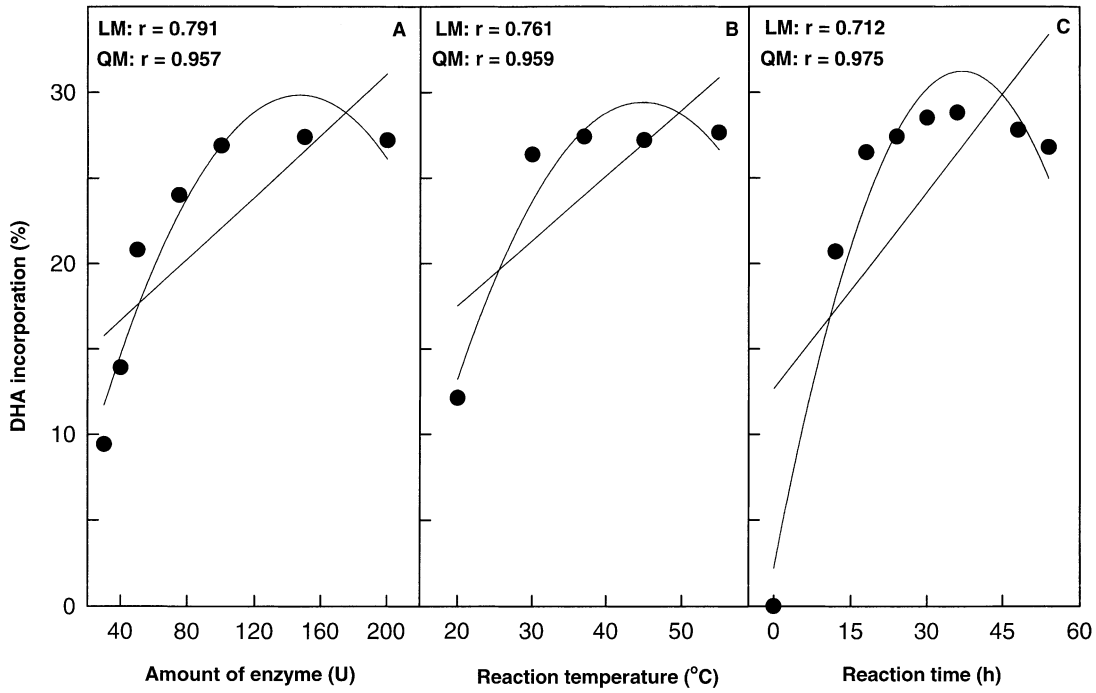

Fig. 1. Effect of the amount of enzyme (a), incubation temperature (b) and reaction time (c) on DHA incorporation in borage oil. LM and QM denote linear and quadratic models, respectively.

Table 1
Variables (factors) used for face-centred cube design

Independent variables	Symbols	Coded-variable levels		
		−1	0	+1
Amount of enzyme (units)	X_1	100	150	200
Reaction temperature (°C)	X_2	30	45	60
Reaction time (h)	X_3	18	24	30

2.3. Preparation of DHA concentrate from algal oil

DHA concentrate was obtained from fatty acids of algal oil by the urea-fatty acid adduct formation method, as described elsewhere (Senanayake & Shahidi, 2000a).

2.4. Determination of lipase activity

Lipase activity was measured by assaying fatty acids produced from the hydrolysis of triacylglycerols as described by Senanayake and Shahidi (1999b). One unit (U) of lipase activity was defined as nanomoles of fatty acids (oleic acid equivalents) produced per gram of enzyme. Lipase activity of *Candida antarctica* was 554 U.

2.5. Acidolysis

In general, borage oil (300 mg) was mixed with DHA concentrate (120 mg; 97.1% DHA) in screw-capped test tubes, then immobilized Novozym 435 lipase and water (2% by weight of substrate and enzyme) were added in hexane (3 ml). The mixture was incubated in an orbital shaker at 250 rpm and at different temperatures (30, 45 and 60 °C). They were sampled and analyzed at 18, 24 and 30 h. All reactions were performed in duplicate.

2.6. Analysis of products

The enzyme was removed by passing reaction media through a bed of anhydrous sodium sulphate. The samples were placed in 250 ml conical flasks and 20 ml of a mixture of acetone–ethanol (1:1, v/v) were added. The reaction mixture was titrated against a 0.5 N NaOH solution (using a phenolphthalein indicator) in order to neutralize free fatty acids. The mixture was transferred into a separatory funnel and thoroughly mixed with 25 ml of hexane. The lower aqueous layer was separated and discarded. The upper hexane layer, containing acylglycerols, was passed through a bed of anhydrous sodium sulphate. The acylglycerol fraction was subsequently recovered, following hexane removal at 45 °C using a rotary evaporator. The fatty acid compositions of acylglycerols were determined using gas chromatography, as described by Senanayake and Shahidi (2000a).

2.7. Pancreatic lipase hydrolysis

Hydrolysis of DHA-enriched borage oil by pancreatic lipase was carried out according to the method descri-

bed by Christie (1982). Tris–HCl buffer (5 ml; 1.0 M, pH 8.0), 0.5 ml of calcium chloride (2.2%, w/v) and 1.25 ml of sodium taurocholate (0.05%, w/v) were added to 25 mg of oil in a glass test tube. The whole mixture was allowed to equilibrate at 40 °C in a water bath for 1.0 min, and subsequently, 5.0 mg of porcine pancreatic lipase (EC. 3.1.1.3; Sigma) were added to it. The mixture was then placed in a Gyrotory water bath shaker (Model G76, New Brunswick Scientific Co. Inc., New Brunswick, NJ) at 250 rpm under nitrogen for 1 h at 40 °C. Ethanol (5 ml) was added to stop the enzymatic hydrolysis followed by addition of 5.0 ml of 6.0 N HCl. The hydrolytic products were extracted three times with 50 ml of diethyl ether and the ether layer was washed twice with distilled water and dried over anhydrous sodium sulphate. After removal of solvent under nitrogen, the hydrolytic products were separated on silica gel thin-layer chromatography (TLC) plates (20×20 cm, 60Å mean pore diameter, 2–25 μm mean particle size, 500 μm thickness, with dichlorofluorescein, Sigma), impregnated with 5% (w/v) boric acid. The plates were developed using hexane/diethyl ether/acetic acid (70:30:1, v/v/v). After drying, the bands were located by viewing under short (254 nm) and long (365 nm) wavelength UV lights (Spectroline, Model ENF-240C, Spectronics Co., Westbury, NY). The bands were scraped off and their lipids extracted into diethyl ether and subsequently used for fatty acid analysis.

3. Results and discussion

3.1. Selection of factor levels of amount of enzyme, reaction temperature and time

Fig. 1a shows the effect of amount of enzyme on DHA incorporation (%) in borage oil. The response behaved as a second order function of the independent variable (amount of enzyme) as the second order model had a higher correlation coefficient than that of the linear model. As the amount of enzyme increased, the DHA incorporation increased. The incorporation of DHA was maximum at the 100–200 enzyme unit range. The design points selected for optimization of DHA-enriched oil production were 100, 150 and 200 enzyme activity units. The effects of incubation temperature and reaction time on DHA incorporation in borage oil were second order functions (Fig. 1b c). The DHA incorporation at 20 °C was low, but increased with increasing temperatures up to 50–60 °C. Therefore, variable levels of 30, 45 and 60 °C were chosen as the lower, middle and upper points, respectively. Results for the time course study showed that, as the reaction time increased, DHA incorporation increased, reaching a maximum, and then declining with increasing reaction time. The three design points selected for time course were 18, 24 and 30 h.

3.2. Response surface analysis

RSM is an optimization technique that determines optimum process conditions by combining special experimental designs with modelling by first or second order polynomial equations in a sequential testing procedure. RSM tests several variables at a time, uses special experimental designs to cut the number of required determinations, and measures several effects by objective tests. In this study, a face-centred cube design was employed (Table 2); the actual levels of variables used in each experimental run are shown in Table 1. This design was chosen over alternatives, such as a rotatable design, because it uses only three levels of each factor, whereas other central composite designs would require five levels of each (Mason et al., 1989). Having three levels instead of five is considered desirable because it reduces the preparation time. Three replicates were taken at the design centre (0,0,0) so that total number of observations was $n = 8 + 6 + 3 = 17$. The independent variable levels are coded for an experimental design (Table 1). The centre point for each independent variable level is given a code of zero. The highest and lowest levels of interest for each independent variable are coded plus or minus one, respectively, for this three level design (Table 1). The main advantage of the design is that it enables one to study one or more variables simultaneously in a single experimental design of practicable size (Montgomery, 1991; Senanayake & Shahidi, 1999b).

Table 2
Face-centred cube design arrangement and responses[a]

Run	Independent variables[b]			Response (Y)[c]
	Amount of enzyme	Temperature	Time	
1	−1	−1	−1	25.7
2	−1	−1	+1	29.6
3	−1	−1	−1	28.0
4	−1	+1	+1	30.9
5	+1	−1	−1	28.6
6	+1	−1	+1	29.7
7	+1	+1	−1	30.8
8	+1	+1	+1	31.4
9	−1	0	0	32.4
10	+1	0	0	32.5
11	0	−1	0	32.2
12	0	+1	0	32.1
13	0	0	−1	29.2
14	0	0	+1	32.4
15	0	0	0	34.1
16	0	0	0	34.7
17	0	0	0	34.5

[a] Non-randomized.
[b] Coded symbols and levels of independent variables refer to Table 1.
[c] Averages of duplicate determinations from different experiments.

The data obtained for DHA incorporation (%) from the 17 experimental points were used for statistical analysis to optimize the process variables; amount of enzyme, reaction temperature and reaction time. In this study, the substrate mole ratio of 1:1 (oil/DHA) was kept constant because the incorporation of DHA was satisfactory at this mole ratio. Table 2 shows experimental data for response variable Y (% DHA incorporation). Multiple regression coefficients, obtained by employing a least squares technique to predict a quadratic polynomial model for the DHA incorporation (%) in borage oil, are summarized in Table 3. Examination of these coefficients with the t-test indicated that, in borage oil, linear and quadratic terms of reaction time were highly significant ($P \leqslant 0.01$). However, linear and quadratic terms of amount of enzyme and reaction temperature were not significant ($P \geqslant 0.01$). There were no significant interaction ($P \geqslant 0.01$) between any variables tested. Therefore, these results suggest that linear and/or quadratic effects of reaction time may be the primary determining factors affecting DHA incorporation into borage oil. The contributions of linear and quadratic terms to the model were 25 and 65%, respectively. The coefficient of determination ($R^2 = 0.94$) implies that 94% of the variations could be explained by the fitted model. A coefficient of variation (CV) of less than 5% indicated that the model was reproducible (Table 3).

The coefficients of independent variables, amount of enzyme (X_1), reaction temperature (X_2) and reaction time (X_3), determined for the quadratic polynomial model for DHA incorporation into borage oil (Y) were as follows:

Table 3
Regression coefficients of predicted quadratic polynomial model for response (Y)

Coefficients[a]	% DHA Incorporation (Y)	Standard error of Y
β_0	−43.5149[b]	9.4071
Linear		
β_1	0.1668	0.0741
β_2	0.5628	0.2470
β_3	3.9774[b]	0.7749
Quadratic		
β_{11}	−0.0004	0.0002
β_{22}	−0.00523	0.0025
β_{33}	−0.07020[b]	0.0154
Interactions		
β_{12}	0.00005	0.0004
β_{13}	−0.0021	0.0010
β_{23}	−0.0020	0.0036
β_{123}	–	–
R^2	0.94	
CV%	2.92	

[a] Coefficients refer to the general model. R^2 = coefficient of determination; CV% = coefficient of variation.
[b] Significant 1% level.

$$Y = -43.515 + 0.167X_1 + 0.563X_2 + 3.98X_3 - 0.0004X_1^2 - 0.0052X_2^2 - 0.07X_3^2 + 0.00005X_1X_2 - 0.002X_2X_3 - 0.0021X_1X_3 \quad (1)$$

The plot of experimental values of DHA incorporation (%), versus those calculated from the above equation, indicated a very good fit for borage oil (Fig. 2) with a correlation coefficient (r) of 0.964. The analysis of variance (ANOVA), for second order polynomial model fitted to the response surface, is given in Table 4. The $F_{0.05}$ value for lack of fit (11.96) did not exceed the tabulated value of 19.3 (5,2 d.f.), indicating that lack of fit was not significant and therefore the fitted model was appropriate for the description of the response surface. The ANOVA, for the three response variables (Table 5), indicated that the model developed for the extent of DHA incorporation into borage oil was adequate. From statistical analysis (Table 6), reaction time was the most important factor because it affected DHA incorporation significantly ($P \leqslant 0.01$). Within the experimental range, enzyme concentration and reaction temperature had no significant effects ($P \geqslant 0.01$) on DHA incorporation.

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 (Montgomery, 1991). Thus, to determine the nature of the stationary point, canonical analysis was carried out on the second order polynomial model. The canonical form of the equation demonstrating the nature of the response surface, was:

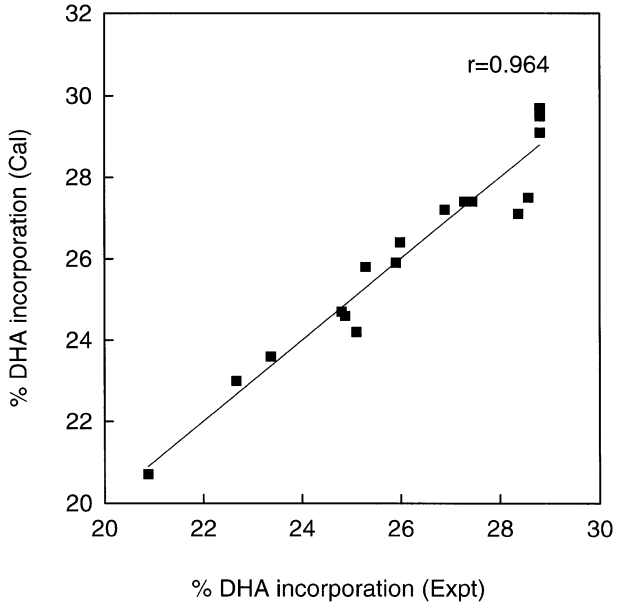

Fig. 2. Correlation of calculated versus experimental docosahexaenoic acid (DHA) incorporation (%) values for borage oil.

$$Y = 34.11 - 0.81W_1^2 - 1.18W_2^2 - 2.59W_3^2 \quad (2)$$

where W_1, W_2 and W_3 are the axes of the response surface. It is evident that all the eigenvalues, i.e. −0.81, −1.18 and −2.59, were negative, indicating that the stationary point was, in fact, a maximum (Montgomery, 1991).

The linear, quadratic and cross-product terms in the second order polynomial were used to generate a three-dimensional response surface graph and a two-dimensional contour plot (Fig. 3) of DHA incorporation into borage oil. While this three-dimensional graph can assist the researcher to determine the direction to take to increase a desired response and graphically show the nature of the fitted surface as a maximum, minimum or a saddle point, it is difficult to determine the levels of variables to give a specific DHA incorporation (%) from such a graph. This can be more readily achieved from a contour plot of the same variables, an example of which is shown in 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. Each contour represents a specific value for the height of the surface, above the plane defined for combination of the levels of the independent variables. Therefore, different surface height values enable one to focus attention on the levels of the independent variables at which changes in the surface height occur. The contour plot illustrates the combination of levels of enzyme, reaction temperature and reaction time that can afford the same amount of DHA incorporation (Fig. 3).

Critical values for the three variables were within the experimental region (Table 7). The stationary point for the extent of DHA incorporation (%) into borage oil, by acidolysis reaction, predicted a maximum of 34.1%

Table 4
Analysis of variance (ANOVA) for second order polynomial model fitted to response surface

Source	d.f.[a]	Sum of squares	Mean square	*F*-value
Lack of fit[b]	5	5.58	1.12	11.96[c]
Pure error	2	0.19	0.09	
Total error	7	5.77	0.82	

[a] Degrees of freedom.
[b] Lack of fit sum of squares (SS) = Total SS − Pure error SS.
[c] Non-significant.

Table 5
Analysis of variance (ANOVA) of the regression parameters for the response surface model[a]

Regression	d.f.[b]	Sum of squares	R^2	*F*-value
Linear	3	23.26	0.25	9.41[c]
Quadratic	3	61.56	0.65	24.89[c]
Cross product	3	3.54	0.04	1.43
Total	9	88.36	0.94	11.91[c]

[a] R^2 = coefficient of determination.
[b] Degrees of freedom.
[c] Significant at 1% level.

Table 6
Analysis of variance (ANOVA) of the factors studied for the response surface model

Independent variables	d.f.[a]	Sum of squares	Mean square	*F*-value
Amount of enzyme (X_1)	4	9.42	2.36	2.86
Reaction temperature (X_2)	4	9.48	2.37	2.88
Reaction time (X_3)	4	34.34	8.58	10.42[b]

[a] Degrees of freedom.
[b] Significant at 1% level.

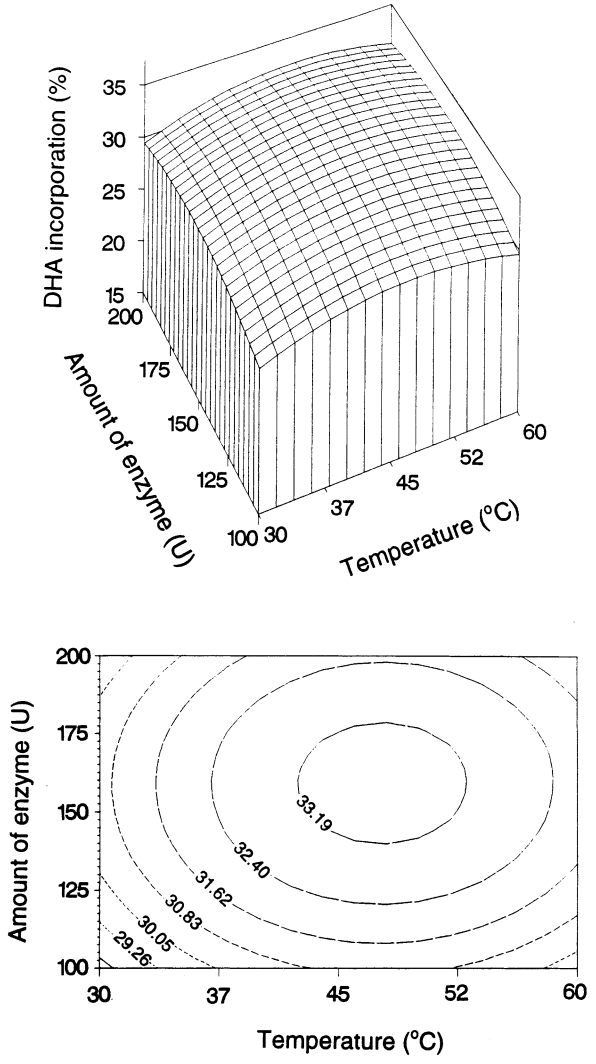

Fig. 3. A three-dimensional response surface and a two-dimensional contour plot illustrating optimal conditions for the docosahexaenoic acid (DHA) incorporation (%) in borage oil when time was fixed at 25 h.

at 165 units of *Candida antarctica* enzyme, reaction temperature of 50 °C and reaction time of 25 h (Table 7). The contour plot derived from the result of canonical analysis showed ellipsoidal contours at the maximum point (Fig. 3). Results of independent experiments, carried out to examine the adequacy of the predicted values by the model, showed a value very close to that observed for response Y (Table 7). This verification result revealed that the predicted value from the model was reasonable and reproducible. Therefore, acidolysis of borage oil with DHA by *Candida antarctica* lipase can increase the incorporation of DHA up to 35.6%, with a process yield of 80.5%.

Previously, Akoh and Sista (1995) modified the fatty acid composition of borage oil using the ethyl ester of EPA, with an immobilized nonspecific lipase from *Candida antarctica* as a biocatalyst. The highest incorporation of 31% was obtained with 20% *Candida antarctica* lipase. Recently, capric acid (10:0) and EPA have been incorporated into borage oil using two immobilized lipases from *Candida antarctica* and *Rhizomucor miehei* as biocatalysts (Akoh & Moussata, 1998). Higher incorporations of EPA (10.2%) and 10:0 (26.3%) were obtained with *Rhizomucor miehei* lipase, compared with 8.8 and 15.5%, respectively, with *Candida antarctica* lipase. In our studies, we have shown that an immobilized lipase from *Candida antarctica* effectively incorporated DHA into borage oil. Table 8 shows the fatty acid profile of borage oil before and after modification with DHA by *Candida antarctica* lipase. The major fatty acids found in borage oil before enzymatic incorporation were 18:2ω6 (38.4%) and 18:3ω6 (24.4%). The concentrations of these acids were comparable with those reported by Akoh and Sista (1995). After enzymatic incorporation, 18:2ω6 and 18:3ω6 decreased by 11.7 and 7.4%, respectively. The amount of DHA incorporated into borage oil was 35.6%. Ju et al. (1998) incorporated EPA and DHA into acylglycerols of borage oil. They have selectively hydrolysed borage oil using immobilized lipase from *Candida rugosa* and then used this product with EPA and DHA for the *Mucor miehei* -catalyzed acidolysis reaction. After modification, the contents of GLA, EPA and DHA in acylglycerols were 26.5, 19.8 and 18.1%, respectively.

The DHA-enriched borage oil, which was produced under optimum reaction conditions (165 enzyme units, 50 °C and 25 h), was separated and quantified by thin layer chromatography-flame ionisation detection (TLC-FID). The results showed that the relative content of triacylglycerols (TAG; 88.5%) was much higher than that of diacylglycerols (DAG; 8.8%) and monoacylglycerols (MAG; 2.6%). No free fatty acids were found, since the NaOH removed these after the acidolysis reaction.

Table 7
Canonical analysis of response surface

Factor	Coded	Uncoded
Amount of enzyme (X_1)	0.305	165
Reaction temperature (X_2)	0.304	50
Reaction time (X_3)	0.182	25
Stationary point	Maximum	
Predicted value[a]	34.1	
Observed value[b]	35.6±1.7	

[a] Predicted using the polynomial model.
[b] Mean±S.D. of triplicate determinations from different experiments.

Table 8
The fatty acid profile of borage oil before and after DHA incorporation by *Candida antarctica* lipase

Fatty acid	Unmodified borage oil	Modified borage oil
10:0	0.08±0.01	0.16±0.02
12:0	0.07±0.02	0.16±0.01
14:0	0.06±0.01	0.08±0.02
16:0	9.81±0.12	6.81±0.67
18:0	3.12±0.26	2.49±0.25
18:1	15.2±0.74	11.1±0.87
18:2ω6	38.4±0.89	26.7±1.02
18:3ω6	24.4±0.90	17.0±1.58
18:3ω3	0.17±0.02	0.18±0.08
20:0	0.20±0.07	ND[a]
20:1	4.09±0.25	3.00±0.23
22:1	2.49±0.10	1.75±0.14
24:1	1.52±0.20	ND[a]
22:6ω3	ND[a]	35.6±1.68

[a] Not detected.

Table 9
Positional distribution of fatty acids in DHA-enriched borage oil (%)

Fatty acids	TAG[a]	Position	
		sn-2	*sn*-1 + *sn*-3
8:0	0.04±0.01	ND[b]	0.05±0.03
10:0	0.15±0.02	0.07±0.02	0.20±0.02
12:0	0.16±0.03	0.10±0.05	0.18±0.08
14:0	0.09±0.01	0.08±0.04	0.11±0.03
14:1	0.04±0.01	ND[b]	0.06±0.02
16:0	4.33±0.57	3.52±0.52	5.33±0.05
16:1	0.19±0.03	0.17±0.05	0.23±0.10
18:0	2.09±0.15	2.92±0.07	2.38±0.20
18:1	12.3±0.50	11.7±0.82	10.3±0.40
18:2ω6	24.7±0.90	22.8±1.53	24.9±0.39
18:3ω6	16.1±0.26	18.4±0.78	15.2±0.65
18:3ω3	0.19±0.09	0.17±0.06	0.27±0.07
20:0	ND[b]	0.22±0.05	0.11±0.03
20:1	2.12±0.20	2.70±0.10	1.79±0.03
20:2	0.13±0.07	0.12±0.09	0.11±0.04
22:1	1.24±0.08	1.90±0.09	0.79±0.07
22:6ω3	35.3±1.50	33.5±0.65	35.3±0.76
24:1	0.47±0.09	0.11±0.06	0.79±0.07

[a] TAG, triacylglycerols.
[b] Not detected.

3.3. Pancreatic lipase study

The DHA-enriched borage oil was subjected to pancreatic lipase hydrolysis and the fatty acid compositions of the *sn*-2 and *sn*-1 + *sn*-3 positions are shown in Table 9. Stereospecific analysis of TAG and hydrolysis products provides some useful information about the mechanism of gastric digestion. Pancreatic lipase is often used for such analysis. This enzyme is used to hydrolyse the fatty acids esterified to the *sn*-1 and *sn*-3 positions of TAG, yielding 2-MAG. The fatty acid composition of 2-MAG accurately reflects that of the position *sn*-2 in the original TAG. The results of our study showed that DHA was fairly evenly distributed at *sn*-2 and *sn*-1,3 positions of TAG molecules of modified borage oil. The positional specificity of *Candida antarctica* depends on the type of reactants. In some reactions, this enzyme functions as a nonspecific lipase whereas in other reactions it shows *sn*-1,3 positional specificity (Novo Nordisk Biochem. North America Inc., 1999). The results showed that, under assay conditions employed in this work, *Candida antarctica* acts as a nonspecific lipase. Redden, Lin, Fahey, and Horrobin (1995) determined the positional distribution of fatty acids in unmodified borage oil and found that GLA was concentrated at the *sn*-2 and *sn*-3 positions of TAG. A similar observation was previously reported by Lawson and Hughes (1988). In DHA-enriched borage oil, however, GLA was mainly associated with the *sn*-2 position (18.4%) of TAG.

Acknowledgements

We are grateful to the Natural Sciences and Engineering Research Council (NSERC) of Canada for partial financial support. The supply of algal oil from Martek Biosciences Corporation (Columbia, MD) and borage oil from Bioriginal Food and Science Corporation (Saskatoon, SK) is gratefully acknowledged.

References

Akoh, C. C. (1995). Structured lipids-enzymatic approach. *INFORM*, *6*, 1055–1061.

Akoh, C. C., & Moussata, C. O. (1998). Lipase-catalyzed modification of borage oil: incorporation of capric and eicosapentaenoic acids to form structured lipids. *Journal of the American Oil Chemists' Society*, *75*, 697–701.

Akoh, C. C., & Sista, R. V. (1995). zymatic modification of borage oil: incorporation of eicosapentaenoic acid. *Journal of Food Lipids*, *2*, 231–238.

Bhathena, S. J. (1992). Fatty acids and diabetes. In C. K. Chow (Ed.), *Fatty acids in foods and their health implications* (pp. 823–855). New York: Marcel Dekker.

Boissonneault, G. A., & Hayek, M. G. (1992). Dietary fat, immunity, and inflammatory disease. In C. K. Chow (Ed.), *Fatty acids in foods and their health implications* (pp. 707–734). New York: Marcel Dekker.

Bruckner, G. (1995). Fatty acids and cardiovascular disease. In C. K. Chow (Ed.), *Fatty acids in foods and their health implications* (pp. 735–752). New York: Marcel Dekker.

Carlson, S. E. (1995). The role of PUFA in infant nutrition. *INFORM*, *6*, 940–946.

Carroll, K. K. (1990). Experimental and epidemiological evidence on marine lipids and carcinogenesis. In R. S. Lees, & M. Karel (Eds.), *Omega-3 fatty acids in health and disease* (pp. 99–114). New York: Marcel Dekker.

Christie, W. W. (1982). Structural analysis of lipids by means of enzymatic hydrolysis. In W. W. Christie (Ed.), *Lipid analysis* (pp. 155–166). NewYork: Pergamon press.

Deferne, J. L., & Leeds, A.R (1992). The antihypertensive effects of dietary supplementation with a 6-desaturated essential fatty acid concentrate as compared with sunflower seed oil. *Journal of Human Hypertens*, *6*, 113–119.

Gandhi, N. N. (1997). Applications of lipase. *Journal of the American oil chemists society*, *74*(6), 621–634.

Gao, L., & Mazza, G. (1996). Extraction of anthocyanin pigments from purple sunflower hulls. *Journal of Food Science*, *61*, 600–603.

Haas, M. J., Cichowicz, D. J., Phillips, J., & Moreau, R. (1993). The hydrolysis of phosphatidylcholine by an immobilised lipase: optimization of hydrolysis in organic solvents. *Journal of the American oil chemists' society*, *70*, 111–117.

Hill, W. J., & Hunter, W.G (1966). A review of response surface methodology: a literature survey. *Technometrics*, *8*, 571–575.

Horrobin, D. F. (1992). Clinical applications of n-6 essential fatty acids: atopic eczema and inflammation, diabetic neuropathy and retinopathy, breast pain and viral infections. In A. Sinclair, & R. Gibson (Eds.), *Essential fatty acids and eicosanoids* (pp. 367–372). Champaign, IL: American Oil Chemists' Society.

Horrobin, D. F. (1993). Medical uses of essential fatty acids. In A. T. Diplock, J. M. C. Gutteridge, & V. K. S. Shukla (Eds.), *Antioxidants, free radicals and polyunsaturated fatty acids in biology and medicine* (pp. 181–198). Lystrup, Denmark: International food science centre.

Horrobin, D. F. (1994). Unsaturated lipids: a new approach to the treatment of cancer. In C. Galli, A. P. Simopoulos, & E. Tremoli (Eds.), *Effects of fatty acids and lipids in health and disease* (pp. 77–80). Basel, Karger: World Review of Nutrition and Dietetics.

Horrobin, D. F. (1995). Medical roles of metabolites of precursor EFA. *INFORM*, *6*, 428–435.

Huang, K., & Akoh, C. C. (1996). Optimization and scale-up of enzymatic synthesis of structured lipids using RSM. *Journal of Food Science*, *61*, 137–141.

Ju, Y., Huang, F., & Fang, C. (1998). The incorporation of n-3 polyunsaturated fatty acids into acylglycerols of borage oil via lipase-catalyzed reactions. *Journal of the American Oil Chemists' Society*, *75*, 961–965.

Kinsella, J. E. (1986). Food components with potential therapeutic benefits: the n-3 polyunsaturated fatty acids of fish oils. *Food Technology*, *40*, 89–97.

Lawson, L. D., & Hughes, B. G. (1988). Triacylglycerol structure of plant and fungal oils containing γ-linolenic acid. *Lipids*, *23*, 313–317.

Mason, R. L., Gunst, R. F., & Hess, J. L. (1989). Response-surface designs. In *Statistical design and analysis of experiments* (pp. 204–233). New York: John Wiley & Sons.

Meland, E., Fugelli, P., Lerum, E., Rønneberg, R., & Sandvik, L. (1989). Effect of fish oil on blood pressure and blood lipids in men with mild to moderate hypertension. *Scandinavian Journal of Prim Health Care*, *7*, 131–135.

Montgomery, D. C. (1991). Response surface methods and designs. In *Design and analysis of experiments* (3rd ed.; pp. 521–568). New York: John Wiley & Sons.

Novo Nordisk Biochem North America, Inc. (1999). Product sheet B 606c-GB. Franklinton, NC.

Redden, P. R., Lin, X., Fahey, J., & Horrobin, D. F. (1995). Stereospecific analysis of the major triacylglycerol species containing γ-linolenic acid in evening primrose oil and borage oil. *Journal of Chromatography*, *704*, 99–111.

Salem, N., Kim, H., & Yergey, J. A. (1986). Docosahexaenoic acid: membrane function and metabolism. In A. P. Simopoulos, R. R. Kifer, & R. E. Martin (Eds.), *Health effects of polyunsaturated fatty acids in seafoods* (pp. 263–317). New York: Academic Press.

SAS Institute, Inc. (1990). *Statistical analytical systems user's guide (Vol. 2)*. Cary, NC: SAS Institute Inc.

Senanayake, S. P. J. N., & Shahidi, F. (1999a). Enzymatic incorporation of docosahexaenoic acid into borage oil. *Journal of the American Oil Chemists' Society*, *76*, 1009–1015.

Senanayake, S. P. J. N., & Shahidi, F. (1999b). Enzyme-assisted acidolysis of borage (*Borago officinalis* L.) and evening primrose (*Oenothera biennis* L.) oils: incorporation of omega-3 polyunsaturated fatty acids. *Journal of Agricultural and Food Chemistry*, *47*, 3105–3112.

Senanayake, S. P. J. N., & Shahidi, F. (2000a). Concentration of docosahexaenoic acid (DHA) from algal oil *via* urea complexation. *Journal of Food Lipids*, *7*, 51–61.

Senanayake, S. P. J. N., & Shahidi, F. (2000b). Structured lipids containing long-chain omega-3 polyunsaturated fatty acids. In F. Shahidi (Ed.), *Seafood in health and nutrition. transformation in fisheries and aquaculture: global perspectives* (pp. 29–44). St John's, Canada: ScienceTech publishing company.

Senanayake, S. P. J. N., & Shahidi, F. (2001). Modified oils containing highly unsaturated fatty acids and their stability. In F. Shahidi, & J. W. Finley (Eds.), *Omega-3 fatty acids: chemistry, nutrition and health effects*, *ACS Symposium Series 788* (pp. 162–173). Washington, DC: American chemical society.

Shieh, C. J., Akoh, C. C., & Koehler, P. E. (1995). Four factor response surface optimization of the enzymatic modification of triolein to structured lipids using capric acid. *Journal of the American Oil Chemists' Society*, *72*, 619–623.

Simopoulos, A. P. (1990). Omega-3 fatty acids in growth and development. In R. S. Lees, & M. Karel (Eds.), *Omega-3 fatty acids in health and disease* (pp. 115–1506). New York: Marcel Dekker.

Thompson, D. (1982). Response surface experimentation. *Journal of Food Processing and Preservation*, *6*, 155–188.