Enzyme-Assisted Acidolysis of Borage (*Borago officinalis* L.) and Evening Primrose (*Oenothera biennis* L.) Oils: Incorporation of Omega-3 Polyunsaturated Fatty Acids

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Lipase-catalyzed acidolysis of borage (*Borago officinalis* L.) and evening primrose (*Oenothera biennis* L.) oils with long-chain ω3 polyunsaturated fatty acids (PUFA), namely, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, was carried out in hexane, and the products were analyzed using gas chromatography. The most effective lipase for incorporation of ω3 PUFA into these oils was *Pseudomonas* sp. as compared to lipases from *Mucor miehei* and *Candida antarctica*. Response surface methodology was used to obtain a maximum yield of EPA+DHA incorporation while using the minimum amount of enzyme possible. The process variables studied were the amount of enzyme (150−350 units), reaction temperature (30−60 °C), and reaction time (6−30 h). All experiments were carried out according to a face-centered cube design. Under optimum conditions, incorporation of EPA+DHA was 35.5% in borage oil and 33.6% in evening primrose oil. The modified borage and evening primrose oils containing γ-linolenic acid, EPA, and DHA were successfully produced and may have potential health benefits.

Keywords: *Acidolysis; borage oil; Candida antarctica; evening primrose oil; Mucor miehei; optimization; Pseudomonas; response surface methodology*

INTRODUCTION

Borage (*Borago officinalis* L.) and evening primrose (*Oenothera biennis* L.) have been the subject of increasing agricultural interest because of the potential market for their seed oils, which contain γ-linolenic acid (GLA; 18:3ω6). Borage seeds contain 28−38% oil, of which 17−25% is GLA, whereas evening primrose contains 17−25% oil, of which 8−10% is GLA (Beaubaire and Simon, 1987). GLA is an essential ω6 polyunsaturated fatty acid (PUFA) and must be provided in food because it cannot be easily synthesized within the body (Horrobin, 1992). GLA has also been used to treat rheumatoid arthritis, diabetic neuropathy, hypertension, premenstrual syndrome, asthma, atopic dermatitis, multiple sclerosis, migraine, and cancer, but the results so far are inconclusive (Horrobin, 1992, 1994; Gurr, 1997).

The long-chain ω3 PUFA, especially eicosapentaenoic acid (EPA; 20:5ω3) and docosahexaenoic acid (DHA; 22:6ω3), may be obtained directly through consumption of seafoods. EPA and DHA are synthesized mainly by both uni- and multicellular marine plants. They are eventually transferred through the food chain and are incorporated into lipids of aquatic species such as fish and marine mammals (Ward, 1995).

Over 1 million metric tons of marine oils are produced annually worldwide (Bimbo, 1990), which are used as food ingredients. Marine oils are also used in shortenings that are used in breads, cakes, and cookies and in the production of food emulsifiers. In addition to the direct use of marine oils in foods, concentrates of EPA and DHA may now be employed as encapsulated products or dietary supplements (Bimbo, 1990; Shahidi, 1998).

The beneficial effects of long-chain ω3 PUFA that are characteristic of marine lipids, especially EPA and DHA, have now been well established. EPA and DHA are considered to be essential for normal growth and development (Simopoulos, 1990) and may play an important role in the prevention and treatment of cardiovascular disease (Bruckner, 1992), hypertension (Meland et al., 1989; Deferne and Leeds, 1992), inflammatory and immune disorders (Boissonneault and Hayek, 1992), diabetes (Bhathena, 1992), and cancer (Carroll, 1990). As a structural component of brain and retina, DHA appears to be linked to proper tissue function and needs to be supplied in sufficient amounts during tissue development (Salem et al., 1986). The PUFA composition of cell membranes is highly dependent on the dietary intake. Therefore, consumption of appropriate amounts and proportions of ω6 and ω3 fatty acids needs to be considered.

Lipases have been employed for the production of structured lipids by various transesterification reactions as they catalyze the incorporation of particular fatty acids at specific positions of the triacylglycerol molecules. In addition, enzyme-assisted reactions usually occur under mild conditions with little or no side product formation (Akoh, 1995). However, a major obstacle to the widespread use of lipases for production of bulk products is cost of the enzymes. Accordingly, research has been conducted with the aim of optimizing process conditions to address the economical issues. An effective statistical tool for this purpose is response surface methodology (RSM). This technique determines optimum conditions by combining special experimental designs with modeling by first- and second-order poly-

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nomial equations in a sequential testing procedure. RSM defines the effect of the independent variables, alone or in combinations, on the process as it has been applied to several reaction systems in organic solvents (Haas et al., 1993; Shieh et al., 1995; Huang and Akoh, 1996). Recently, the ω3 PUFA, especially EPA and DHA, have been incorporated into groundnut oil (Sridhar and Lakshminarayana, 1992), melon seed oil (Huang et al., 1994), vegetable oils (Huang and Akoh, 1994), trilinolein (Akoh et al., 1995), borage oil (Akoh and Sista, 1995; Ju et al., 1998; Akoh and Moussata, 1998), and evening primrose oil (Akoh et al., 1996).

The objectives of this study were to determine the ability of different lipases to catalyze the acidolysis reaction of borage or evening primrose oil with EPA and DHA and to optimize the reaction conditions for incorporation of EPA+DHA in these oils.

MATERIALS AND METHODS

Materials. Borage oil was obtained from Bioriginal Food and Science Corp. (Saskatoon, SK), and evening primrose oil was provided by Efamol, Inc. (Kentville, NS). Algal oil containing DHA (47.4%) was obtained from Martek Biosciences Corp. (Columbia, MD). EPA concentrate (93% EPA) was provided by Dr. T. Ohshima (Tokyo, Japan). Novozyme 435 from *Candida antarctica* (immobilized on a macroporous acrylic resin) and Lipozyme-IM (*Mucor miehei*; immobilized on a macroporous anion-exchange resin) were provided by Novo Nordisk (Bagsvaerd, Denmark). Lipase PS-30 (*Pseudomonas* sp.) was obtained from Amano Enzyme USA Co., Ltd. (Lombard, IL). HPLC grade hexane was procured from Fisher Scientific Chemical Co. (Nepeon, ON).

Preparation of DHA Concentrate from Algal Oil by Urea Complexation. DHA concentrate (up to 97%) was obtained from hydrolyzed algal oil by using the urea−fatty acid complexation procedure as described by Wanasundara and Shahidi (1999).

Determination of Lipase Activity. Lipase activity was measured by assaying fatty acids produced from the hydrolysis of triacylglycerols. All experiments were carried out in screw-capped test tubes in triplicate. Triolein was used as the substrate. It was emulsified at a concentration of 50 mM in 5% (w/v) gum arabic for 1 min using a Polytron homogenizer (Model PT-3000; Brinkmann, Littau-Switzerland) at 8000 rpm. The assay mixture contained 1 mL of substrate emulsion and the enzyme (10−100 mg). Reactions were carried out for up to 1 h in a shaking water bath at 35 °C. Fatty acid release varied linearly with time for >1 h. The released fatty acids were assayed colorimetrically as copper soaps using cupric acetate−pyridine reagent (Lowry and Tinsley, 1976; Kwon and Rhee, 1986). The purity of triolein was verified by thin-layer chromatography−flame ionization detection (TLC−FID); no mono- or diacylglycerols were present.

The enzyme reaction in the emulsion system was stopped by adding 6 N HCl (1 mL) and isooctane (5 mL) followed by mixing for 1 min. Cupric acetate (1 mL, 5% w/v, pH 6.1) solution was then added to the mixture and stirred for 90 s with a vortex mixer; the absorbance of the upper isooctane layer was read at 715 nm (Arribére et al., 1994). One unit of lipase activity was defined as nanomoles of fatty acids (oleic acid equivalents) produced per minute per gram of enzyme. Lipase activities of *C. antarctica*, *M. miehei*, and *Pseudomonas* sp. were 554, 13613, and 11936 units, respectively.

Acidolysis. In general, borage (300 mg) or evening primrose oil (297 mg) was mixed with EPA (53.5 mg) and DHA (58.2 mg), at a mole ratio of 1:0.5:0.5, in a screw-capped test tube, and then lipase (150−350 enzyme activity units) and water (2 wt % of substrates and enzyme) were added in hexane (3 mL). The mixture was stirred in an orbital shaker at 250 rpm and at different temperatures (30, 35, 45, and 60 °C). Individual sample vials were removed and analyzed at different time periods (6−30 h).

Table 1. Independent Variables and Experimental Design Levels Used for Face-Centered Cube Design

independent variable	symbol	coded-variable levels −1	0	+1
amount of enzyme[a] (units)	X_1	150	250	350
reaction temp (°C)	X_2	30	45	60
reaction time (h)	X_3	6	18	30

[a] Lipase PS-30 from *Pseudomonas* sp. was used as the biocatalyst.

Analysis of Products. The enzymes were removed by passing the reaction mixture through a bed of anhydrous sodium sulfate. Samples were placed in 250-mL conical flasks, and 20 mL of a mixture of acetone/ethanol (1:1, v/v) was added. The reaction mixture was titrated with 0.5 N NaOH to a phenolphthalein endpoint. The mixture was transferred to 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 sulfate. The acylglycerol fraction was subsequently recovered following hexane removal at 45 °C using a rotary evaporator. The fatty acid composition of the acylglycerols was analyzed by gas chromatography as described elsewhere by Wanasundara and Shahidi (1995).

The products [triacylglycerols (TAG), diacylglycerols (DAG), and monoacylglycerols (MAG)] obtained under optimum reaction conditions were quantified by TLC−FID using benzene/chloroform/acetic acid (70:30:4, v/v/v) as the developing solvent (Angelo and James, 1993). The reaction products were also fractionated on TLC plates (20 × 20 cm; silica gel, 60 Å mean pore diameter, 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) UV lights (Spectroline, Model ENF-240C, Spectronics Co., New York). The bands were scraped off and their lipids extracted into diethyl ether and subsequently used for fatty acid analysis according to the gas chromatographic procedure of Wanasundara and Shahidi (1995).

Experimental Design. A three-factor and three-level face-centered cube design with 17 individual design points was adopted in this study (Mason et al., 1989; Gao and Mazza, 1996). The independent variables (X_i) and their levels are presented in Table 1. 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 EPA+DHA incorporation (%). To avoid bias, 17 runs were performed in a totally random order. Duplicate experiments were carried out at all design points.

The second-order polynomial model predicted for optimization of EPA+DHA incorporation into borage or evening primrose oil (eq 1) was

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

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. The statistical analysis system (SAS Institute Inc., 1990) was used for multiple regression analysis, analysis of variance (ANOVA), and canonical analysis. Response surfaces and contour plots were developed using the fitted quadratic polynomial equations obtained from response surface regression (RSREG) analysis and holding the independent variable with the least effect on the response at a constant value and changing the levels of the other two variables. Verification experiments were carried out using combinations of variables at different levels (within the experimental range).

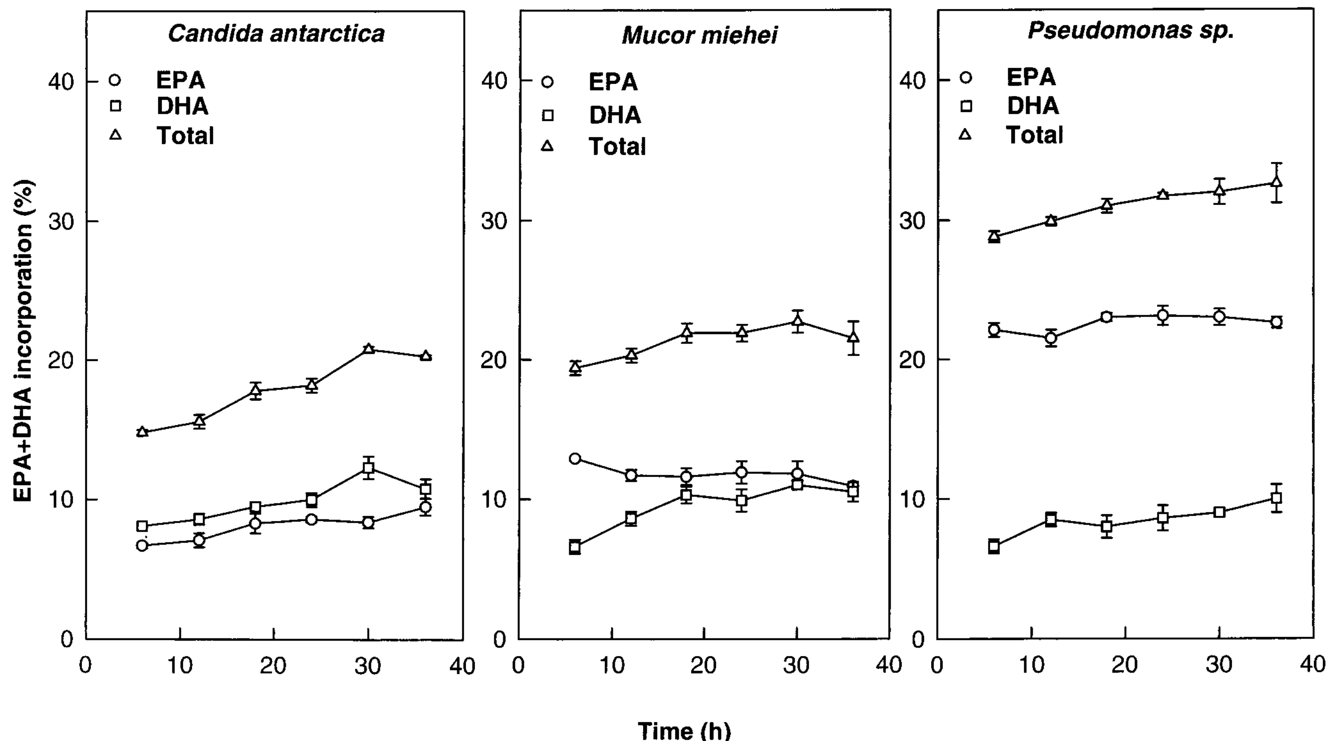

Figure 1. Incorporation of EPA and DHA into borage oil by different microbial lipases.

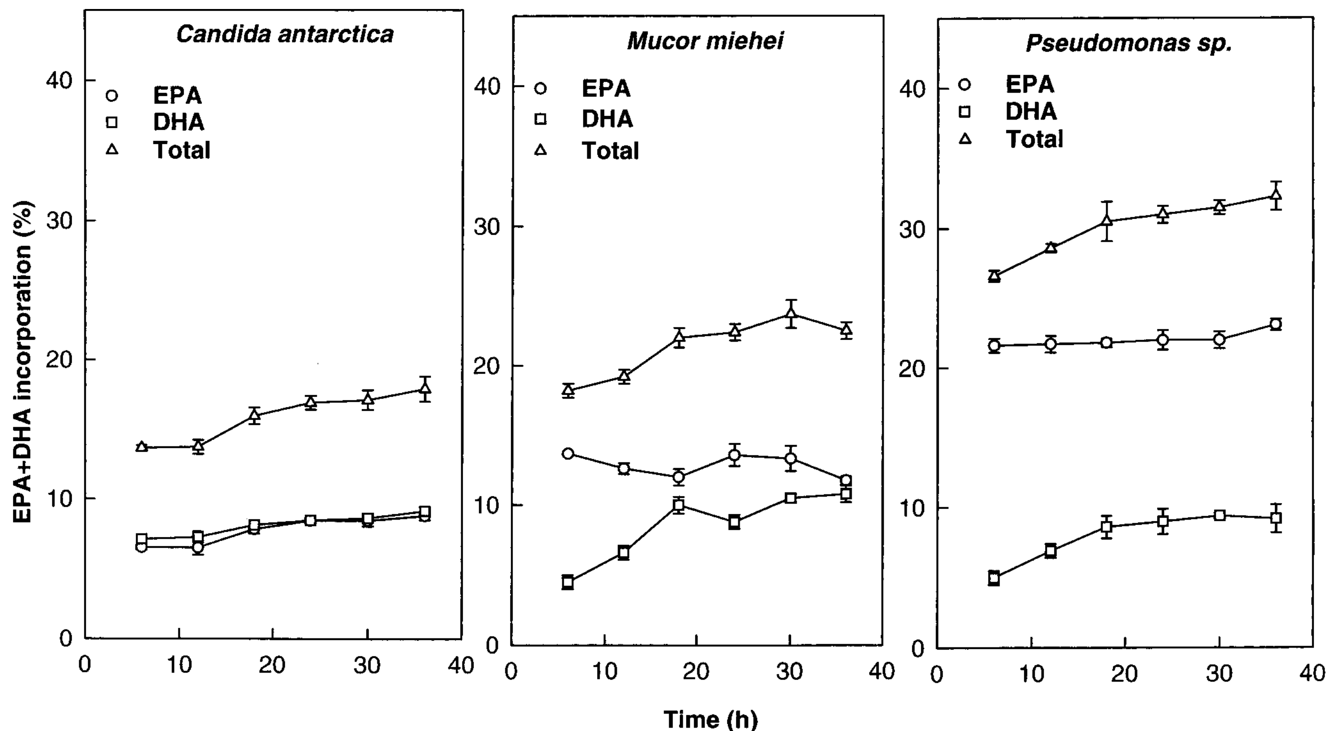

Figure 2. Incorporation of EPA and DHA into evening primrose oil by different microbial lipases.

RESULTS AND DISCUSSION

Screening of Microbial Lipases. Three commercially available lipases from different sources were screened by incubating 350 units of enzyme with a 1:0.5:0.5 mole ratio of borage or evening primrose oil, EPA, and DHA at 35 °C for 36 h. The enzymes screened were nonspecific *C. antarctica*, sn-1,3 specific *M. miehei*, and nonspecific *Pseudomonas* sp. All microbial lipases tested were able to catalyze the incorporation of EPA+DHA in these oils to various extents (Figures 1 and 2). Among the lipases tested, *Pseudomonas* sp. gave the highest degree of EPA+DHA incorporation in both oils (32.6 and 32.3% after 36 h, in borage and evening primrose oils, respectively) followed by *M. miehei* and *C. antarctica*. Thus, the most effective lipase was selected for subsequent experiments to determine optimal acidolysis conditions.

Previously, Akoh and Moussata (1998) used two immobilized lipases, nonspecific SP435 from *C. antarctica* and sn-1,3 specific IM60 from *Rhizomucor miehei*,

as biocatalysts for the restructuring of borage oil to incorporate EPA and capric acid (10:0) with free fatty acids as acyl donors. They obtained a higher incorporation of EPA (10.2%) and capric acid (26.3%) using IM60 lipase, compared to 8.8 and 15.5%, respectively, when SP435 lipase was used.

The ability of immobilized lipases IM60 from *M. miehei* and SP435 from *C. antarctica* to modify the fatty acid composition of selected vegetable oils (canola, peanut, and soybean oils) by incorporation of ω3 polyunsaturated fatty acids into the vegetable oils was studied by Huang and Akoh (1994). These authors used free acid and ethyl esters of EPA and DHA as acyl donors. Using free EPA as acyl donor, IM60 gave higher incorporation of EPA than SP435. However, when ethyl esters of EPA and DHA were used as acyl donors, SP435 gave higher incorporation of EPA and DHA than IM60.

Optimization of Enzymatic Acidolysis of Borage and Evening Primrose Oils. The most effective lipase for acidolysis of borage and evening primrose oils was *Pseudomonas* sp. at a level of 350 units and 35 °C. However, many other factors may affect the product yield (incorporation of EPA and DHA) of lipase-catalyzed acidolysis of acylglycerols. These include temperature of the reaction medium, reaction time, and substrate and enzyme concentrations, among others. Therefore, it is necessary to study these factors collectively to find the optimum reaction conditions to obtain the maximum incorporation of EPA+DHA by the most effective enzyme *Pseudomonas* sp. For this study, reaction parameters such as the amount of enzyme (X_1), reaction temperature (X_2), and reaction time (X_3) were selected for optimization. The substrate mole ratio of 1:0.5:0.5 (oil/EPA/DHA) was kept constant because the incorporation of EPA+DHA was satisfactory at this mole ratio. Therefore, this ratio was selected for optimization by response surface methodology (RSM). Enzyme concentration and reaction time are major factors affecting the economy of preparation of EPA- and DHA-enriched oils via lipase-catalyzed acidolysis. Furthermore, the temperature of the reaction medium and also the reaction time can be considered important as they influence the oxidative status of prepared oils.

The results of classical one-variable-at-a-time experiments do not reflect actual changes in the environment as they ignore interactions between factors which are in effect simultaneous. More sophisticated designs such as RSM can describe concomitant effects more fully and help in more accurate optimization of factors that affect the process and allow simultaneous solution of multivariate equations that specify the optimum yield for a specific set of factors through mathematical models.

In this study, a face-centered cube design was employed (Table 2), and the actual levels of variables used in each experimental run are shown in Table 1. The independent variable levels are coded for an experimental design (Table 1). The center 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 +1 or −1, respectively, for this three-level design (Table 1). The main advantage of this class of design is that it enables one to study one or more variables simultaneously in a single experimental design of feasible size (Montgomery, 1991).

The data obtained for EPA+DHA incorporation (percent) were used for statistical analysis to optimize the process variables: amount of enzyme, reaction temperature, and reaction time. Table 2 shows experimental data observed for response variables Y_1 and Y_2 (percent EPA+DHA incorporation), for borage and evening primrose oils, respectively. Multiple regression coefficients obtained by employing a least-squares technique to predict a quadratic polynomial model for the EPA+DHA incorporation (percent) in borage and evening primrose oils are summarized in Table 3. In both oils, all linear terms had the greatest effect on EPA+DHA incorporation as they were highly significant ($p \leq 0.01$). In borage oil, all quadratic terms and some interaction effects were also significant ($p \leq 0.05$). In evening primrose oil, the quadratic terms of reaction temperature and time were significant. There was also a significant interaction ($p \leq 0.05$) between the amount of enzyme (X_1) and reaction time (X_3). The coefficients of determination (R^2) for Y_1 and Y_2 were 0.99 and 0.98, respectively. This indicated that most of the variation observed for design points could be explained by the fitted model. The linear, quadratic, and interaction terms for Y_1 (borage oil)

Table 2. Face-Centered Cube Design Arrangement and Responses[a]

	independent variables[b]			response[c]	
design point	X_1	X_2	X_3	Y_1	Y_2
1	−1	−1	−1	21.7	23.2
2	−1	−1	+1	28.9	28.8
3	−1	0	0	30.1	30.2
4	−1	+1	−1	20.5	23.1
5	−1	+1	+1	27.9	28.3
6	0	−1	0	32.0	30.2
7	0	0	−1	29.8	29.5
8	0	0	0	35.3	33.3
9	0	0	0	34.0	33.7
10	0	0	0	33.8	34.6
11	0	0	+1	36.4	33.6
12	0	+1	0	29.3	28.1
13	+1	−1	−1	29.5	29.0
14	+1	−1	+1	31.7	30.3
15	+1	0	0	34.8	33.8
16	+1	+1	−1	25.3	25.4
17	+1	+1	+1	27.7	28.6

[a] Nonrandomized. [b] Coded symbols and levels of independent variables refer to Table 1. [c] Average of duplicate determinations from different experiments. Y_1 (% EPA+DHA) = borage oil. Y_2 (% EPA+DHA) = evening primrose oil.

Table 3. Regression Coefficients of Predicted Quadratic Polynomial Models for Responses

coefficient[a]	borage oil,[c] % EPA+DHA (Y_1)	evening primrose oil,[c] % EPA+DHA (Y_2)
β_0	−34.3671***	−21.7043***
linear		
β_1	0.1731***	0.0945***
β_2	1.6482***	1.5779***
β_3	0.8601***	0.6489***
quadratic		
β_{11}	−0.0002***	−0.0001
β_{22}	−0.0179***	−0.0172***
β_{33}	−0.0110***	−0.0103**
interactions		
β_{12}	−0.0005**	−0.0004
β_{13}	0.0010***	−0.0007**
β_{23}	0.0003	−0.0010
β_{123}		
R^2[b]	0.99	0.98
CV%[b]	2.25	2.47

[a] Coefficients refer to the general model. [b] R^2 = coefficient of determination; CV% = coefficient of variation. [c] **, significant at 5% level; ***, significant at 1% level.

Table 4. ANOVA for Second-Order Polynomial Model Fitted to Response Surfaces

source	DF[a]	sum of squares	mean square	F value
borage oil				
lack of fit[b]	5	1.85	0.37	0.56[c]
pure error	2	1.32	0.66	
total error	7	3.18	0.45	
evening primrose oil				
lack of fit[b]	5	2.87	0.57	1.29[c]
pure error	2	0.89	0.44	
total error	7	3.76	0.54	

[a] Degrees of freedom. [b] Lack of fit sum of squares (SS) = total SS − pure error SS. [c] Nonsignificant.

Table 5. ANOVA of the Regression Parameters for the Response Surface Models

regression	DF[a]	sum of squares	R^2[b]	F value[c]
borage oil				
linear	3	123.33	0.38	90.60***
quadratic	3	178.73	0.55	131.30***
cross-product	3	17.02	0.05	12.50***
total	9	319.10	0.99	78.14***
evening primrose oil				
linear	3	62.26	0.32	38.63***
quadratic	3	123.11	0.62	76.38***
cross-product	3	8.00	0.04	4.97**
total	9	193.37	0.98	39.99***

[a] Degrees of freedom [b] R^2 = coefficient of determination. [c] ***, significant at 1% level; **, significant at 5% level.

contributed 38.0, 55.0, and 5.0% to the model, respectively. Contributions of linear, quadratic, and interaction terms of Y_2 (evening primrose oil) to the model were 32.0, 62.0, and 4.0%, respectively (Table 5). Coefficient of variation (CV) for both models of <5% indicated that the models were reproducible (Table 3).

The predicted second-order polynomial models for EPA+DHA incorporation in borage (eq 2) and evening primrose oils (eq 3) were

$$Y_1 = -34.3671 + 0.1731X_1 + 1.6482X_2 + 0.8601X_3 - 0.0002X_1^2 - 0.0179X_2^2 - 0.0110X_3^2 - 0.0005X_1X_2 + 0.0003X_2X_3 + 0.0010X_1X_3 \quad (2)$$

$$Y_2 = -21.7043 + 0.0945X_1 + 1.5779X_2 + 0.6489X_3 - 0.0001X_1^2 - 0.0172X_2^2 - 0.0103X_3^2 - 0.0004X_1X_2 - 0.0010X_2X_3 - 0.0007X_1X_3 \quad (3)$$

The analysis of variance (ANOVA) for the second-order polynomial model fitted to response surfaces is given in Table 4. The results of the error analysis indicated that the lack of fit was insignificant ($p > 0.05$) in both oils, and therefore the fitted models were appropriate for the description of the response surfaces (Table 4). The ANOVA for the three response variables (Table 5) indicated that the models developed for borage and evening primrose oils appeared to be adequate. On the basis of statistical analysis (Table 6), amount of enzyme, reaction temperature, and reaction time were all significant variables because they affected EPA+DHA incorporation in borage and evening primrose oils significantly ($p \leq 0.01$).

Figures 3 and 4 depict the nature of the response surfaces for borage and evening primrose oils, respectively. Contour plots (Figures 3 and 4) were also generated using the data obtained by canonical analysis.

Table 6. ANOVA of the Factors Studied for the Response Surface Models

independent variable	DF[a]	sum of squares	mean square	F value[b]
borage oil				
amount of enzyme (X_1)	4	70.03	17.51	38.59***
reaction temperature (X_2)	4	65.38	16.35	36.03***
reaction time (X_3)	4	85.85	21.46	47.30***
evening primrose oil				
amount of enzyme (X_1)	4	28.79	7.19	13.39***
reaction temperature (X_2)	4	49.78	12.45	23.16***
reaction time (X_3)	4	48.75	12.18	22.68***

[a] Degrees of freedom. [b] ***, significant at 1% level.

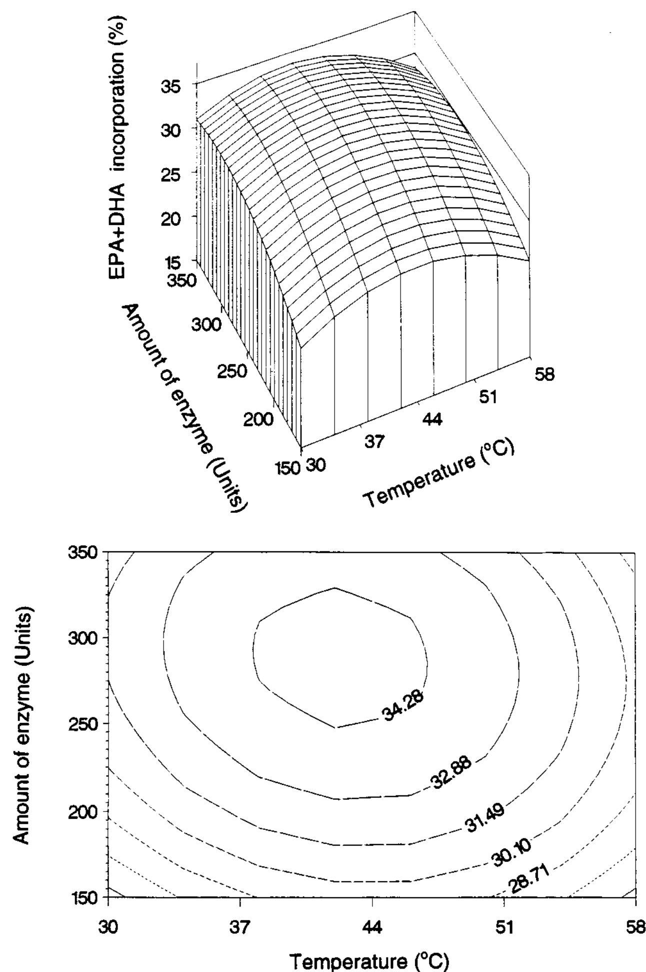

Figure 3. Three-dimensional response surface and two-dimensional contour plot illustrating the effects of amount of enzyme and reaction temperature on the predicted EPA+DHA incorporation (percent) in borage oil.

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 forms of the equations for borage (eq 4) and evening primrose (eq 5) oils were

$$Y_1 = 35.85 - 1.19W_1^2 - 2.56W_2^2 - 4.12W_3^2 \quad (4)$$

$$Y_2 = 34.30 - 0.77W_1^2 - 1.71W_2^2 - 3.91W_3^2 \quad (5)$$

where W_1, W_2, and W_3 are the axes of the response surfaces. It is evident that all eigenvalues were negative (eqs 4 and 5), thus indicating that the stationary point

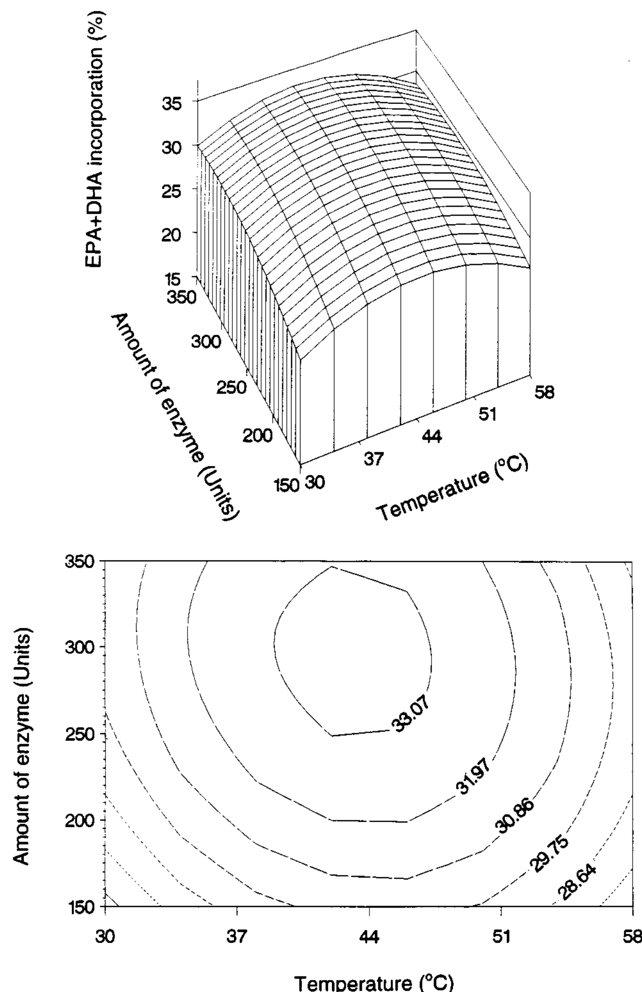

Figure 4. Three-dimensional response surface and two-dimensional contour plot illustrating the effects of amount of enzyme and reaction temperature on the predicted EPA+DHA incorporation (percent) in evening primrose oil.

was a maximum for both Y_1 and Y_2. Results of canonical analysis of the response surface are given in Table 7. The stationary point for EPA+DHA incorporation in borage oil predicted a maximum value of 36.0% at 278 units of enzyme, reaction temperature of 42 °C, and reaction time of 26 h. In evening primrose oil, the maximum EPA+DHA incorporation of 34.3% was predicted at 299 units of enzyme, 43 °C, and 24 h.

Results of independent experiments carried out to examine the adequacy of the predicted results by the models for both oils showed very close values for responses (Table 7). These verification experiments revealed that the predicted values from the models were reasonable and reproducible. Therefore, incorporation of, respectively, up to 35.5 ± 2.6 and 33.6 ± 1.8% EPA+DHA into borage and evening primrose oils via acidolysis with EPA and DHA by *Pseudomonas* lipase is possible. The fatty acid profile of restructured borage oil produced under optimum reaction conditions showed that the contents of GLA, EPA, and DHA were 17.1, 25.9, and 9.6%, respectively. The content of GLA was decreased from 24.4 to 17.1% after the acidolysis reaction. The ratio of ω3 PUFA to ω6 PUFA was 0.90. Similarly, the contents of GLA, EPA, and DHA in restructured evening primrose oil were 7.6, 25.7, and 7.9%, respectively, and the GLA content decreased by 1.5% after the acidolysis reaction. The corresponding ratio of ω3 PUFA to ω6 PUFA was 0.63. Ju et al. (1998) first selectively hydrolyzed borage oil using immobilized *Candida rugosa* and then used the product and ω3 PUFA as substrates to produce structured lipids using immobilized Lipozyme IM60 from *M. miehei* as the biocatalyst. For the acidolysis reaction of acylglycerols with ω3 PUFA, the total content of ω3 and ω6 PUFA in acylglycerols was 72.8% after 18 h of reaction. The contents of GLA, EPA, and DHA were 26.5, 19.8, and 18.1%, respectively, with a corresponding $\omega3/\omega6$ ratio changing from 0 to 1.09 after modification.

Previously, Akoh and Sista (1995) modified the fatty acid composition of borage oil using EPA ethyl ester, with an immobilized nonspecific SP435 lipase from *C. antarctica* as a biocatalyst. The highest incorporation (31%) was obtained with 20% SP435 lipase. At a substrate mole ratio of 1:3, the corresponding ratio of ω3 to ω6 PUFA was 0.64. Under similar conditions, Akoh et al. (1996) were able to effectively increase the ω3 PUFA (up to 43%) and lower the ω6 PUFA (by 32%) of evening primrose oil with a corresponding increase in the $\omega3/\omega6$ ratio from 0.01 to 0.60. Sridhar and Lakshminarayana (1992) reported that EPA and DHA can be incorporated into groundnut oil by interesterification reaction with an sn-1,3 specific lipase from *M. miehei*. The incorporations of EPA and DHA in groundnut oil were 9.5 and 8.0%, respectively.

In another study, the restructured borage and evening primrose oils, produced under optimum reaction conditions, were quantified by TLC−FID. The results showed that the content of TAG (89−91%) was much higher than that of the DAG (8.5−9.0%) and MAG (0.3−1.8%) (Tables 8 and 9). Because free fatty acids were removed by NaOH after the acidolysis reaction, they were not detected by TLC−FID. The products were also separated by TLC, and the fatty acid compositions of the isolated bands were analyzed by gas chromatography (Tables 8 and 9). The results showed that EPA (26%) was mainly located in TAG fractions of restructured borage and evening primrose oils. The contents of GLA and DHA in TAG fractions were 7.6−17.1 and 7.9−9.6%, respectively. The contents of GLA and DHA in DAG fractions were 9.0−22.7 and 11%, respectively. However, the content of EPA in DAG fractions was negligible. The contents of GLA, EPA, and DHA in the MAG fraction were 9.4−19.6, 4.2−7.3, and 8.4−9.8%, respectively.

It is anticipated that production of a TAG rich in GLA, EPA, and DHA would provide the maximum health benefits. Long-chain PUFA, with a balance between ω3

Table 7. Canonical Analysis of the Response Surfaces

response variable	critical values of independent variables: amount of enzyme[a] (units)	reaction temp (°C)	reaction time (h)	stationary point	EPA+DHA (%): predicted value[b]	obsd value[c]
Y_1[d]	278	42	26	max	36.0	35.5 ± 2.6
Y_2[d]	299	43	24	max	34.3	33.6 ± 1.8

[a] Lipase PS-30 from *Pseudomonas* sp. was used as the biocatalyst. [b] Predicted using the polynomial model. [c] Mean ± SD of triplicate determinations from different experiments. [d] Y_1 = borage oil; Y_2 = evening primrose oil.

Table 8. Fatty Acid Profile of Acylglycerol Components of Restructured Borage Oil Separated after Acidolysis by *Pseudomonas* Species Lipase[a]

fatty acid	lipid component (%)		
	TAG[b] 91.2 ± 0.68	DAG 8.5 ± 0.64	MAG 0.3 ± 0.07
10:0	0.05 ± 0.01	0.05 ± 0.01	ND[c]
12:0	0.04 ± 0.02	0.06 ± 0.05	ND[c]
14:0	0.04 ± 0.01	0.06 ± 0.02	0.37 ± 0.02
16:0	5.06 ± 0.56	6.92 ± 0.22	6.84 ± 0.27
16:1	0.38 ± 0.32	0.15 ± 0.04	ND[c]
18:0	2.05 ± 0.01	2.60 ± 0.56	3.52 ± 0.50
18:1	10.87 ± 0.09	14.61 ± 0.10	11.72 ± 0.35
18:2ω6	22.47 ± 0.33	34.28 ± 0.52	27.65 ± 0.87
18:3ω6	17.09 ± 0.19	22.66 ± 0.20	19.55 ± 0.90
18:3ω3	0.04 ± 0.02	0.17 ± 0.03	0.91 ± 0.07
20:0	0.14 ± 0.01	ND[c]	ND[c]
20:1	2.61 ± 0.02	3.43 ± 0.12	2.55 ± 0.52
20:2	0.13 ± 0.02	0.17 ± 0.04	ND[c]
20:4	0.56 ± 0.01	ND[c]	ND[c]
20:5ω3	25.9 ± 0.20	0.50 ± 0.07	4.23 ± 0.55
22:1	1.24 ± 0.20	2.09 ± 0.13	2.66 ± 0.62
24:1	0.83 ± 0.01	1.15 ± 0.25	4.11 ± 0.33
22:6ω3	9.6 ± 0.02	11.1 ± 0.89	9.83 ± 0.51

[a] Restructured borage oil was prepared under optimum reaction conditions (278 enzyme units, 42 °C, 26 h). [b] TAG, triacylglycerol; DAG, diacylglycerol; MAG, monoacylglycerol. [c] Not detected.

Table 9. Fatty Acid Profile of Acylglycerol Components of Restructured Evening Primrose Oil Separated after Acidolysis by *Pseudomonas* Species Lipase[a]

fatty acid	lipid component (%)		
	TAG[b] (89.2 ± 0.51)	DAG (9.0 ± 0.95)	MAG (1.8 ± 0.64)
10:0	0.04 ± 0.04	0.50 ± 0.02	0.14 ± 0.02
12:0	0.04 ± 0.01	0.07 ± 0.03	1.51 ± 0.03
14:0	0.02 ± 0.01	0.05 ± 0.01	0.23 ± 0.03
16:0	3.25 ± 0.06	4.62 ± 0.46	4.46 ± 0.50
16:1	0.07 ± 0.02	0.06 ± 0.02	ND[c]
18:0	1.15 ± 0.02	1.32 ± 0.23	1.32 ± 0.04
18:1	6.74 ± 0.05	8.96 ± 0.53	7.44 ± 0.07
18:2ω6	45.67 ± 0.10	62.07 ± 1.21	54.63 ± 0.55
18:3ω6	7.63 ± 0.01	8.96 ± 0.46	9.38 ± 0.26
18:3ω3	0.13 ± 0.05	0.16 ± 0.02	ND[c]
20:0	0.21 ± 0.06	0.28 ± 0.08	ND[c]
20:1	0.29 ± 0.02	0.21 ± 0.06	ND[c]
20:4	0.58 ± 0.02	ND[c]	ND[c]
20:5ω3	25.7 ± 0.90	0.59 ± 0.06	7.27 ± 0.28
22:1	0.33 ± 0.06	0.16 ± 0.21	0.65 ± 0.30
22:6ω3	7.91 ± 0.50	11.26 ± 0.53	8.40 ± 0.76

[a] Restructured evening primrose oil was prepared under optimum reaction conditions (299 enzyme units, 43 °C, 24 h). [b] TAG, triacylglycerol; DAG, diacylglycerol; MAG, monoacylglycerol. [c] Not detected.

and ω6 components, are required by the body as their deficiency has been associated with a number of clinical disorders (Meland et al., 1989; Simopoulos, 1990; Carroll, 1990; Bruckner, 1992; Deferne and Leeds, 1992; Bhathena, 1992; Horrobin, 1992, 1994; Gurr, 1997). Thus, structured lipids obtained enzymatically from borage or evening primrose oil may be useful in certain nutritional applications.

CONCLUSIONS

Enzyme-catalyzed acidolysis of borage and evening primrose oils with EPA and DHA by lipases from *C. antarctica*, *M. miehei*, and *Pseudomonas* sp. was carried out. The highest incorporation of EPA+DHA was obtained when lipase from *Pseudomonas* sp., as compared to *C. antarctica* and *M. miehei*, was used for the acidolysis reaction. Optimization of reaction parameters, namely, the amount of enzyme, reaction temperature, and reaction time, gave a maxima of 35.5 and 33.6% EPA+DHA incorporation in borage and evening primrose oils, respectively. Conditions for optimum incorporation were closely related for both oils and were achieved at 278−299 units of enzyme at 42−43 °C and 24−26 h.

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