Structured Lipids from High-Laurate Canola Oil and Long-Chain Omega-3 Fatty Acids

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ABSTRACT: The lipase-assisted acidolysis of high-laurate canola oil (HLCO; Laurical 25) with long-chain n-3 FA (DHA and EPA) was studied. Response surface methodology was used to obtain a maximal incorporation of DHA or EPA into HLCO. The studied process variables were the amount of enzyme (2–6%), reaction temperature (35–55°C), and incubation time (12–36 h). The amount of water added and the mole ratio of substrates (oil to DHA or EPA) were kept at 2% and 1:3, respectively. All experiments were conducted according to a face-centered cube design. Under optimal conditions (4.79% of enzyme; 46.1°C; 30.1 h), the incorporation of DHA into HLCO was 37.3%. The corresponding maximal incorporation of EPA (61.6%) into Laurical 25 was obtained using 4.6% enzyme, a reaction temperature of 39.9°C, and a reaction period of 26.2 h. Examination of the positional distribution of FA on the glycerol backbone of modified HLCO with DHA showed that the DHA was primarily located in the *sn*-1,3 positions of the TAG molecules. However, lauric acid also remained mainly in the *sn*-1,3 positions of the modified oil. For EPA-modified Laurical 25, lauric acid was present mainly in the *sn*-1,3 positions, whereas EPA was randomly distributed over the three positions.

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KEY WORDS: Acidolysis, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), high-laurate canola oil, lipase, medium-chain fatty acids, response surface methodology, structured lipids.

Structured lipids (SL) are TAG in which FA are placed in specific locations on the glycerol backbone using a chemical or enzymatic process. Much attention has been paid to SL because of their potential biological functions and nutritional perspectives. Designing SL with selected FA at specific locations of the TAG has attracted attention for medicinal applications. For example, for patients with maldigestion as well as those with cystic fibrosis, it may be desirable to develop a SL that contains PUFA at the *sn*-2 position with medium-chain FA (MCFA) at the *sn*-1,3 positions, because MCFA are quickly hydrolyzed by pancreatic lipase, absorbed into the intestine, and rapidly transported to the liver, where they are consumed as a rapid source of energy. The remaining PUFA in the middle position of the TAG molecule either accumulates as body fat or can be used in the synthesis of new TAG (1,2).

Epidemiological studies have indicated that dietary DHA may protect against cardiovascular disease (morbidity and mortality), including the risk of primary cardiac arrest and sudden cardiac death (3). Furthermore, Horrocks and Yeo (4) reported a strong link between fish consumption and a decrease in sudden death from myocardial infraction. The decline was about 50% with 200 mg per day of DHA from fish. DHA deficiency has been associated with depression and may be the underlying reason for the positive correlation between depression and myocardial infraction. Neurological disorders associated with decreased levels of DHA have been reported in schizophrenia, Alzheimer's disease, and depression, among others (5).

High-laurate canola oil (Laurical 25) is a genetically engineered oil containing a MCFA (lauric acid; C12:0). This oil was produced by Calgene Inc. (Davis, CA) to provide an alternative source of oils that are now produced from several palm kernel oil fractions (6). MCFA are saturated FA with carbon chain lengths ranging from 6 to 12, and are prepared mostly from tropical plant oils such as coconut and palm kernel oils. Medium-chain TAG (MCT) are a good source of MCFA for the production of SL. Pure MCT have a caloric value of 8.3 kCal/g. However, they do not supply EFA (7,8). MCT exhibit unique structural and physiological features. They are easily absorbed and metabolized (as quickly as glucose) and are rapidly cleared from the blood (9). They are different from other fats and oils because they can be absorbed *via* the portal system and do not require chylomicron formation to transfer from the blood stream to the cells.

Response surface methodology (RSM) is an optimization procedure that determines the optimal process conditions by combining particular experimental designs with modeling using first- or second-order polynomial equations in a sequential testing procedure. RSM examines a number of variables at a time, using special experimental designs to reduce the number of required determinations, and measures many effects by objective tests (10). The results of traditional one-variable-at-a-time designs do not indicate real changes in the environment because they disregard interactions among variables that are, in effect, concomitant. More complicated designs, such as RSM, can describe simultaneous influences more fully, can help in a more precise optimization of variables that affect the process, and can permit the concomitant solution of multivariate that specify the optimal product for a specific set of parameters through mathematical models.

Incorporation of DHA or EPA into canola oil rich in lauric acid (40%), mostly esterified to the *sn*-1,3 positions, would provide a

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novel specialty oil for specific nutritional and clinical needs. In this study, we explored the ability of different lipases to catalyze the acidolysis of high-laurate canola oil with DHA or EPA. The effects of enzyme load, incubation time, and reaction temperature on the extent of DHA or EPA incorporation into canola oil rich in lauric acid using RSM were also investigated.

MATERIALS AND METHODS

Materials. Two lipases from *Candida antarctica* (Novozym 435) and *Rhizomucor miehei* (Lipozyme RM IM) were acquired from Novozymes (Franklinton, NC). Other lipases, namely, *Pseudomonas* sp. (PS-30), *Aspergillus niger* (AP-12), and *C. rugosa* (AY-30), were obtained from Amano Enzyme (Troy, VA). FAME (GLC-461) were purchased from Nu-Check-Prep (Elysian, MN). Samples of high-laurate canola oil were products from Calgene's Oils Division (Davis, CA). Porcine pancreatic lipase (EC 3.11.3), sodium taurocholate, silica gel TLC plates (20 × 20 cm; 60 Å mean pore diameter, 2–25 μm mean particle size, 500 μm thickness, with dichlorofluorescein) were purchased from Sigma Chemical Co. (St. Louis, MO). All solvents used in these experiments were of analytical grade and were purchased from Fisher Scientific (Nepean, ON). DHA single-cell oil containing 40% DHA was obtained from Martek Bioscience Corporation (Columbia, MD). EPA samples, >99% pure, were made by Fuso Pharmaceutical Industries Ltd. (Osaka, Japan) and kindly provided by Dr. K. Miyashita.

Preparation of FFA from algal oil and concentration of DHA by urea complexation. FFA were prepared from algal oil according to the method described by Wanasundara and Shahidi (11) using a urea complexation process (>94%).

Experimental design for response surface analysis. The approximate conditions for DHA or EPA incorporation, enzyme load, reaction temperature, and reaction time were determined by changing one factor at a time while keeping the others constant. Thus, a proper range for each factor was determined for RSM. A three-factor and three-level face-centered cube design with 17 individual design points was then selected for this study (10–13). To avoid bias, 17 runs were performed in a totally random order. The independent variables or factors investigated were enzyme amount (wt%; X_1), reaction temperature (°C; X_2), and reaction time (h; X_3). The response or dependent variables studied were Y_1 (Laurical 25, DHA%) and Y_2 (Laurical 25, EPA%). Triplicate experiments were conducted at all design points.

The second-order polynomial model used for the optimization of DHA or EPA incorporation into the oil (Y) was:

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

where, β_o, β_i, β_{ii}, and β_{ij} are the regression coefficients for the intercept, linear, quadratic, and interaction terms, respectively, and X_i and X_j are the independent variables. Data were analyzed using the response surface regression procedure (14) and fitted to the second-order polynomial equation after logarithmic transformation (15). Response surfaces and contour plots were attained using the fitted model by keeping the least effective independent variable at a constant value while changing the other two variables. Confirmation experiments were performed using mixtures of variables at different levels (within the experimental range). The independent factors were coded for an experimental design. The center point for each independent variable level was given a code of zero. The highest and lowest levels of concern for each independent factor were coded plus or minus one, respectively, for this three-level design. The major benefit of the design is that it enables one to study one or more parameters at the same time in a single experimental design of workable size (16,17).

Acidolysis reaction. In general, high-laurate canola oil (Laurical 25, 100 mg each) was mixed with DHA or EPA at the respective mole ratio of 1:3 (oil/DHA or oil/EPA), in a screw-capped test tube, and lipase (2–6% by weight of substrates) and water (2% by weight of substrates and enzyme) were then added in hexane (3 mL). The mixture was incubated for different time periods (12 to 36 h) in an orbital shaker at 250 rpm with temperatures ranging from 35 to 55°C.

Separation of acylglycerols after acidolysis. After a given time period, a mixture of acetone and ethanol (20 mL; 1:1, vol/vol) was added to the reaction mixture to stop the reaction. To neutralize the FFA, the reaction mixture was titrated against a 0.5 M NaOH solution (using a phenolphathalein indicator) until the color of the solution turned pink. Hexane (25 mL) was added to the mixture to extract the acylglycerols. The mixture was thoroughly mixed and transferred into a separatory funnel. The two layers (aqueous and hexane) were allowed to separate, and the lower aqueous layer was discarded. The hexane layer was then passed through a bed of anhydrous sodium sulfate to remove any residual water. The hexane was evaporated using a rotary evaporator at 45°C and the acylglycerol fraction was recovered. A portion of the fraction (5–10 mg) was transferred to a special transmethylation vial.

Preparation of FAME. FA profiles of the products were determined following their conversion to methyl esters. The transmethylation reagent (2 mL) of freshly prepared 6% sulfuric acid in methanol containing 15 mg of hydroquinone as an antioxidant was added to the sample vial, followed by vortexing. The mixture was incubated overnight at 60°C and subsequently cooled to room temperature. Distilled water (1 mL) was added to the mixture and, after thorough mixing, a few crystals of hydroquinone were added to each vial to prevent further oxidation. The FAME were extracted three times, each with 1.5 mL of pesticide-grade hexane. The hexane layers were separated, combined, and transferred to a clean test tube and then washed two times, each with 1.5 mL of distilled water. The hexane (upper) layer was separated from the aqueous layer and evaporated under a stream of nitrogen. The FAME were then dissolved in 1 mL of carbon disulfide and used for subsequent GC analysis.

Analysis of FAME by GC. The FAME were analyzed using a Hewlett-Packard 5890 Series II gas chromatograph (Agilent, Palo Alto, CA) equipped with a SUPELCOWAX-10 column (30 m length, 0.25 mm diameter, 0.25 μm film thickness; Supelco Canada Ltd., Oakville, Ontario, Canada). The oven temperature was first set at 220°C for 10.25 min and then raised to 240°C at 30°C/min and held there for 15 min. The injector and FID temperatures were both set at 250°C. Ultra high purity helium was used as a carrier gas at a flow rate of 15 mL/min. The data were treated using Hewlett-Packard 3365 Series II ChemStation Software (Agilent). The FAME were identified by comparing their retention times with those of an authentic standard mixture (GLC-461; Nu-Check-Prep, Elysian, MN), and the results were presented as weight percentages.

Hydrolysis by pancreatic lipase. Hydrolysis of the modified oil by pancreatic lipase was performed according to the method described by Christie (18) with a slight modification. Tris-HCl buffer (5 mL; 1 M, pH 8), calcium chloride (0.5 mL; 2.2%, wt/vol), and sodium taurocholate (1.25 mL; 0.05%) were added to 25 mg of modified oil in a glass test tube. The entire mixture was allowed to stand at 40°C in a water bath for 1 min, followed by the addition of 5 mg of porcine pancreatic lipase (EC 3.11.3). The mixture was subsequently placed in a gyrotory water bath shaker at 250 rpm under nitrogen for 10–15 min at 40°C. Ethanol (5 mL) was added to the mixture to stop the enzymatic reaction, followed by the addition of 5 mL of 6 M HCl. The hydrolytic products were extracted three times with 50 mL of hexane, and the upper layer was removed and washed twice with distilled water and then passed through a bed of anhydrous sodium sulfate. The solvent containing hydrolytic products was evaporated under a stream of nitrogen. TLC plates (20 × 20 cm; 60 Å mean pore diameter, 2–25 μm mean particle size, 500 μm thickness, with dichlorofluorescein; Sigma) were evenly sprayed with 5% (wt/vol) boric acid and dried at 100°C for 1 h. The hydrolytic products were separated on silica gel TLC plates. The plates were developed using hexane/diethyl ether/acetic acid (70:30:1, by vol) for 40–50 min and then allowed to air dry. The bands were located by viewing them under a short- (254 nm) and a long- (365 nm) wavelength light (Spectroline Co., Westbury, NY). The bands were scraped off and their lipids extracted into methanol/chloroform (1:1, vol/vol). The FA profile of the lipids was analyzed by GC as described in the *Analysis of FAME by GC* section.

RESULTS AND DISCUSSION

The FA profile of high-laurate canola oil (Laurical 25) is shown in Table 1. The lauric acid content of Laurical 25 was 40.7%. This oil also contained other saturated FA such as stearic (21.9%), palmitic (2.93%), and myristic (3.82%) acids. The major unsaturated FA found in Laurical 25 before enzymatic modification was oleic acid (25.9%).

Lipase screening. Five lipases were screened for their ability to incorporate DHA or EPA into Laurical 25 (Table 2). These lipases catalyzed the incorporation of DHA into high-laurate canola oil to varying degrees. The degree of DHA incorporation into high-laurate canola oil with various lipases was in the following order: *Pseudomonas* sp. > *M. miehei* > *C. rugosa* > *C. Antarctica* > *A. niger.* The incorporation of DHA into high-laurate canola oil was effectively catalyzed by only three of the five lipases tested. However, there was no significant difference ($P > 0.05$) in the incorporation of DHA into high-laurate canola oil when lipases from *C. antarctica* and *C. rugosa* were used. We found that lipase from *Pseudomonas* sp. was most effective in its ability to incorporate EPA into high-laurate canola oil (Laurical 25) (Table 2). The order of incorporation of EPA into Laurical 25 was: *Pseudomonas* sp. > *C. rugosa* > *A. niger* > *M. miehei* > *C. antarctica. Pseudomonas* sp. gave the highest degree of incorporation of DHA (34.8%, after 24 h) or EPA (61.0%, after 24 h) into high-laurate canola oil. Because the acidolysis of DHA or EPA with Laurical 25 was best achieved with the *Pseudomonas* sp. lipase, this enzyme was selected for subsequent experiments.

Table 3 summarizes the results of studies on the positional distribution of FA in the unmodified and modified Laurical 25, respectively. Lauric acid was mostly esterified to the *sn*-1,3 positions in the unmodified oil. Lauric acid remained esterified mainly to the *sn*-1,3 positions of TAG molecules. Meanwhile, DHA was mostly incorporated into the *sn*-1,3 positions of the glycerol backbone of the DHA-modified high-laurate canola

TABLE 1
FA Composition (wt%) of High-Laurate Canola Oil (Laurical 25)[a] Before and After Enzymatic Modification

	Weight%		
FA	Unmodified	Modified with DHA	Modified with EPA
12:0	40.7 ± 1.24	14.4 ± 0.49	10.7 ± 0.49
14:0	3.82 ± 0.04	1.75 ± 0.04	1.25 ± 0.03
16:0	2.93 ± 0.06	1.64 ± 0.06	1.16 ± 0.01
18:0	21.9 ± 0.63	17.2 ± 0.53	10.3 ± 0.29
18:1n-9	25.9 ± 0.49	18.5 ± 0.19	9.08 ± 0.44
20:5n-3	—	—	62.5 ± 0.96
22:6n-3	—	42.0 ± 1.68	—
Others	4.66	5.51	5.01

[a]Values are mean values of triplicate determinations ± SD. Laurical 25 is a product of Calgene Inc. (Davis, CA).

TABLE 2
Effect of Enzyme Type on the Incorporation (%) of DHA or EPA into High-Laurate Canola Oil (Laurical 25)[a]

Source of enzyme	DHA incorporation (%)	EPA incorporation (%)
Candida antarctica	17.2 ± 0.10[b,c]	48.7 ± 2.3[a,b,c]
Mucor miehei	29.3 ± 2.54[d]	48.9 ± 1.55[b,a,c]
Pseudomonas sp.	34.8 ± 2.76[e]	61.0 ± 1.40[e]
Candida rugosa	17.5 ± 0.54[c,b]	55.2 ± 2.60[d]
Aspergillus niger	9.79 ± 3.19[a]	51.5 ± 0.45[c,a,b]

[a]Values are mean values of triplicate determinations ± SD. Values in each column with different roman superscript letters are different ($P < 0.05$). [a,b,c,d,e]The order of EPA or DHA incorporation into Laurical 25, from lowest to highest, was as follows: a < b < c < d < e. For the manufacturer of Laurical 25, see Table 1.

TABLE 3
Positional Distribution (*sn*-2 and *sn*-1 + *sn*-3) of FA in Modified High-Laurate Canola Oil (Laurical 25)[a]

	Unmodified		Modified with DHA		Modified with EPA	
FA	*sn*-2	*sn*-1 + *sn*-3	*sn*-2	*sn*-1 + *sn*-3	*sn*-2	*sn*-1 + *sn*-3
C12:0	28.5 ± 5.99 (23.3)	46.1 ± 8.21 (76.7)	7.07 ± 1.04 (16.4)	11.9 ± 3.61 (83.6)	7.39 ± 1.85 (16.4)	2.31 ± 0.19 (83.6)
C14:0	4.03 ± 0.44 (35.2)	4.33 ± 0.72 (64.8)	2.34 ± 0.59 (44.6)	1.84 ± 0.28 (55.6)	1.29 ± 0.35 (34.4)	0.25 ± 0.03 (65.6)
C16:0	4.36 ± 0.82 (49.6)	3.20 ± 1.01 (50.4)	2.45 ± 0.38 (49.8)	2.59 ± 0.79 (50.2)	1.56 ± 0.44 (44.8)	0.24 ± 0.05 (55.2)
C18: 0	26.6 ± 4.25 (40.4)	19.2 ± 4.98 (59.6)	12.0 ± 1.96 (23.3)	11.8 ± 3.38 (76.7)	9.91 ± 3.13 (32.1)	1.32 ± 0.34 (67.9)
C18:1n-9	28.8 ± 2.29 (37.0)	22.4 ± 5.59 (63.0)	12.3 ± 1.90 (22.2)	21.2 ± 1.96 (77.8)	7.28 ± 1.59 (26.7)	1.58 ± 0.32 (73.3)
C20:5n-3	—	—	—	—	63.4 ± 11.6 (33.8)	89.8 ± 0.94 (66.2)
C22:6n-3	—	—	11.8 ± 4.88 (9.37)	32.1 ± 7.90 (90.6)	—	—

[a]Values in parenthesis indicate percent FA distribution of total TAG present at the *sn*-1 + *sn*-3, and *sn*-2 positions. These values are (% FA at the *sn*-2 position/% FA in TAG × 3) × 100; for *sn*-1 + *sn*-3 = 100 – *sn*-2. For the manufacturer of Laurical 25, see Table 1.

oil. Examination of the positional distribution of FA on the glycerol backbone of modified high-laurate canola oil with EPA showed that EPA was randomly distributed over the three positions of the TAG molecules. Lauric acid remained esterified mainly to the *sn*-1 and *sn*-3 positions of the modified oil.

RSM. RSM is a statistical design that enables one to determine the optimal conditions for enzymatically assisted reactions by conducting a minimum number of experiments. Table 4 summarizes the experimental data for response variable Y_1 (DHA% incorporation into Laurical 25) or Y_2 (EPA% incorporation into

TABLE 4
Face-Centered Cube Design Arrangement and Response for the Analysis of High-Laurate Canola Oil (Laurical 25) with DHA or EPA[a]

	Independent variables			DHA	EPA
Run	Enzyme (%)	Temperature (°C)	Time (h)	incorporation[b] (%)	incorporation (%)
1	2[c] (–1)[d]	35(–1)	12(–1)	59.1	19.5
2	2 (–1)	35(–1)	36(+1)	57.7	22.2
3	2 (–1)	45(0)	24(0)	58.8	18.2
4	2 (–1)	55(+1)	12(–1)	56.2	18.9
5	2 (–1)	55(+1)	36(+1)	54.4	18.5
6	4(0)	35(–1)	24(0)	57.3	35.3
7	4(0)	45(0)	12(–1)	56.4	27.9
8	4(0)	45(0)	24(0)	61.0	34.8
9	4(0)	45(0)	24(0)	61.0	37.1
10	4(0)	45(0)	24(0)	61.0	36.1
11	4(0)	45(0)	36(+1)	61.9	38.9
12	4(0)	55(+1)	24(0)	66.8	35.6
13	6(+1)	35(–1)	12(–1)	57.1	28.1
14	6(+1)	35(–1)	36(+1)	60.6	32.5
15	6(+1)	45(0)	24(0)	62.0	35.5
16	6(+1)	55(+1)	12(–1)	59.2	31.7
17	6(+1)	55(+1)	36(+1)	57.7	32.7

[a]Nonrandomized.
[b]Average of triplicate determinations from different experiments.
[c]Uncoded variable levels.
[d]Coded variable levels. For the manufacturer of Laurical 25, see Table 1.

TABLE 5
Regression Coefficients of Predicted Quadratic Polynomial Model for Response *Y*

Coefficients[a]	Laurical 25, DHA (%) (Y_1)	Laurical 25, EPA (%) (Y_2)
β_0	−15.293	50.804
Linear		
β_1	17.365**	1.0104
β_2	−0.1903	−0.2634
β_3	1.0243	1.0405
Quadratic		
β_{11}	−2.1088**	−0.3323
β_{22}	0.0016	0.0032
β_{33}	−0.0131	−0.0179
Interactions		
β_{12}	0.0506	0.0337
β_{13}	0.0161	0.0271
β_{23}	−0.0068	−0.0056
β_{123}	—	—
R^2	0.94	0.49
CV%	9.06	5.28

[a]Coefficients refer to the general model. R2, coefficient of determination; **significant at $P \leq 0.001$.

Laurical 25). Multiple regression coefficients, obtained by employing a least-squares procedure to predict the second-order polynomial model for the incorporation of DHA or EPA into high-laurate canola oil, are summarized in Table 5. Testing of these coefficients with the *t*-test indicated that in modified high-laurate canola oil with DHA, linear and quadratic terms of the amount of enzyme (X_1) were highly significant ($P \leq 0.001$). Interactions of the amount of enzyme (X_1) and reaction temperature (X_2), reaction time (X_3) and temperature (X_2), and the amount of enzyme (X_1) and reaction time (X_3) were not significant ($P > 0.1$). In modified Laurical 25 with EPA, all linear, second-order, and interaction terms were insignificant ($P > 0.1$). Similarly, interactions of the amount of enzyme (X_1) and reaction temperature (X_2), or the amount of enzyme (X_1) and reaction time (X_3) were insignificant ($P > 0.1$).

The coefficient of determination for Y_1 (Laurical 25, DHA%) ($R^2 = 0.94$) indicated that the fitted model could explain 94% of the variation. The CV was 9%, thus indicating that this model was only 9% reproducible. The coefficient of determination for Y_2 (Laurical 25, EPA%) was 0.49.

A canonical analysis is a mathematical method used to locate the stationary point on the response surface and to determine whether it represents a maximum, minimum, or saddle point (16). Therefore, to characterize the nature of the stationary points, a canonical analysis was performed on the second-order polynomial models to examine the overall shape of the response surface curves. The canonical forms of the equations for Y_1 (Laurical 25, DHA%) or Y_2 (Laurical 25, EPA%) were:

$$Y_1 = 37.3 + 0.27W_1^2 - 1.95W_2^2 - 8.47W_3^2 \quad [2]$$

$$Y_2 = 61.6 + 0.41W_1^2 - 1.28W_2^2 - 2.71W_3^2 \quad [3]$$

TABLE 6
Canonical Analysis of the Response Surface for the Acidolysis of High-Laurate Canola Oil (Laurical 25) with DHA or EPA

Factor	Laurical 25, DHA (%)	Laurical 25, EPA (%)
Amount of enzyme (wt%, X_1)	4.79	4.61
Reaction temperature (°C, X_2)	46.1	39.9
Reaction time (h, X_3)	30.1	26.3
Stationary point	Saddle	Saddle
Predicted value[a]	37.3	61.6
Observed value[b]	41.9 ± 1.67	62.5 ± 0.96

[a]Predicted using the polynomial model.
[b]Mean of triplicate determinations from different experiments ± SD. For the manufacturer of Laurical 25, see Table 1.

where W_1, W_2, and W_3 are the axes of the response surface for the respective oils examined. The eigenvalue was positive for DHA or EPA incorporation into a high-laurate canola oil model, pointing out that the stationary points were saddle points.

Table 6 and Figures 1–4 show critical values for the three factors (enzyme load, reaction time, and temperature) examined. The stationary point for the degree of DHA incorporation (%) into high-laurate canola oil reached a maximum of 37.3% at a 4.79% enzyme concentration, at 46.1°C in 30.1 h. Similarly, the maximal incorporation of EPA (61.6%) into high-laurate canola oil was obtained when the enzyme amount, reaction temperature, and time were 4.6%, 39.9°C, and 26.2 h, respectively.

Among the five lipases tested, Lipase PS-30 from *Pseudomonas* sp. was the most effective for incorporating DHA or EPA into Laurical 25. RSM showed that in Laurical 25-based SL, the maximal incorporation of DHA (37.3%) was obtained at 4.79% enzyme, a reaction temperature of 46.1°C, and a reaction time of 30.1 h. The maximal incorporation of EPA into Laurical 25 (61.6%) was obtained when the enzyme amount, reaction temperature, and time were 4.61%, 39.9°C, and 26.3 h, respectively. The incorporation of EPA into Laurical 25 was 40%

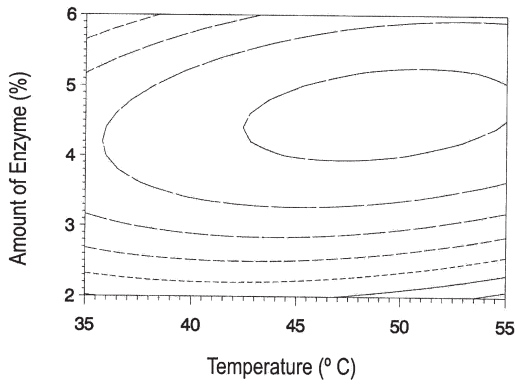

FIG. 1. 2-D contour plot demonstrating the effects of the enzyme amount and reaction temperature on the predicted incorporation of EPA (%) into Laurical 25 (high-laurate canola oil; Calgene Inc., Davis, CA). EPA incorporation (%) in the contour plot, defined by lines from the inside to the outside, was: 60.91, 60.25, 59.59, 58.92, 58.26, 57.59, and 56.93.

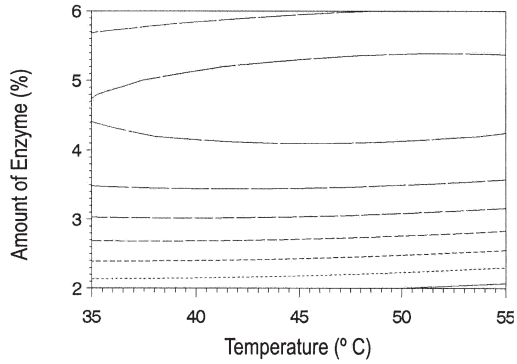

FIG. 2. 2-D contour plot demonstrating the effects of the enzyme amount and reaction temperature on the predicted incorporation of DHA (%) into Laurical 25 (see Fig. 1). DHA incorporation (%) in the contour plot, defined by lines from the inside to the outside, was: 35.49, 32.78, 30.07, 27.36, 24.65, 21.94, and 19.24.

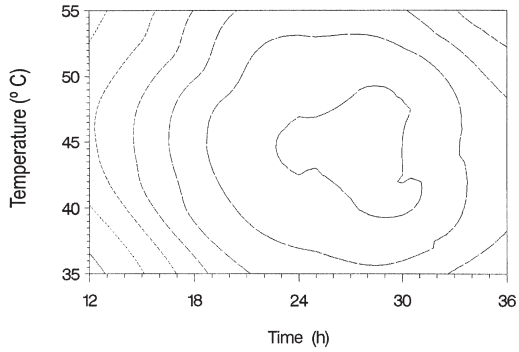

FIG. 3. 2-D contour plot demonstrating the effects of the incubation time and reaction temperature on the predicted incorporation of DHA (%) into Laurical 25 (see Fig. 1). DHA incorporation (%) in the contour plot, defined by lines from the inside to the outside, was: 33.45, 31.95, 30.45, 28.94, 27.44, 25.94, and 24.44.

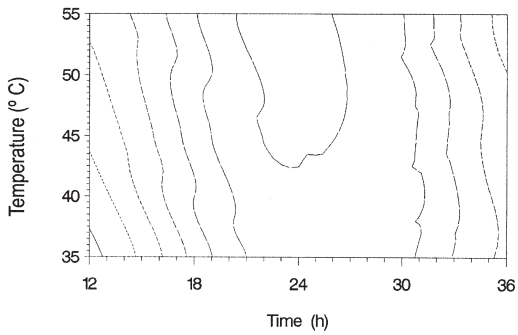

FIG. 4. 2-D contour plot demonstrating the effects of the incubation time and reaction temperature on the predicted incorporation of EPA (%) into Laurical 25 (see Fig. 1). EPA incorporation (%) in the contour plot, defined by lines from the inside to the outside, was: 61.15, 60.44, 59.73, 59.02, 58.30, 57.59, and 56.88.

higher than the incorporation of DHA, possibly because of the structural differences (chain length and number of double bonds) between the two molecules. A stereospecific analysis showed that lauric acid remained mainly esterified to the *sn*-1,3 positions of the modified Laurical 25 with DHA or EPA. DHA was incorporated mostly in the *sn*-1,3 positions, whereas EPA was randomly distributed over the three positions of the modified Laurical 25 with EPA (Table 3).

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