Enzyme-Assisted Acidolysis of Menhaden and Seal Blubber Oils with γ-Linolenic Acid

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ABSTRACT: Oils containing both n-3 and n-6 fatty acids have important clinical and nutritional applications. Lipase-catalyzed acidolysis of seal blubber (SBO) and menhaden oils (MO) with γ-linolenic acid (GLA) was carried out in hexane. The process variables studied for lipase-catalyzed reaction were concentration of enzyme (100–700 units/g of oil), reaction temperature (30–60°C), reaction time (0–48 h), and mole ratio of GLA to triacylglycerols (TAG) (1:1 to 5:1). Two lipases chosen for acidolysis reaction were from *Pseudomonas* species (PS-30) and *Mucor miehei*. Lipase PS-30 was chosen over *Mucor* (also known as *Rhizomucor*) *miehei* to catalyze the acidolysis reaction owing to higher incorporation of GLA. For the acidolysis reaction, optimal conditions were a 3:1 mole ratio of GLA to TAG, reaction temperature of 40°C, reaction time of 24 h, and an enzyme concentration of 500 units/g of oil. Under these conditions, incorporation of GLA was 37.1% for SBO and 39.6% for MO.

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KEY WORDS: Enzyme-assisted acidolysis, GLA concentrate, γ-linolenic acid (GLA), menhaden oil, seal blubber oil.

The importance of marine oils in human nutrition and disease prevention was scientifically recognized three decades ago. Epidemiological studies in the early 1970s postulated that the low incidence of coronary heart disease among Greenland Eskimos might be related to their distinctive dietary habit and use of marine lipids rich in n-3 polyunsaturated fatty acids (PUFA) (1). Effects associated with n-3 PUFA, namely, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), but not docosapentaenoic acid (DPA; 22:5n-3), have since been well established. EPA and DHA are considered essential for normal growth and development (2) and may play an important role in the treatment and prevention of cardiovascular disease (3), hypertension (4,5), inflammation, autoimmune disorders (6), diabetes (7), and cancer (8). The beneficial effects of n-3 PUFA have been ascribed to their ability to lower serum cholesterol and triacylglycerol (TAG) levels and enhance their excretion. n-3 PUFA also increase membrane fluidity and, by conversion to eicosanoids, reduce thrombosis (9,10).

Another important group of PUFA is the n-6 family, which also includes γ-linolenic acid (GLA; 18:3n-6). GLA is an intermediate in the normal bioconversion of linoleic acid (LA; 18:2n-6) to arachidonic acid (AA; 20:4n-6) which is the eicosanoid precursor. The bioconversion includes a series of alternating desaturation and elongation steps. The desaturation of LA is catalyzed by the liver Δ-6 desaturase, the rate-limiting step. In healthy cells, the action of Δ-6 desaturase may be impaired by aging and high levels of alcohol or cholesterol. In the case of "sick" cells additional impairment may be caused by certain cancers and viral infections (11). GLA has been used in the treatment of rheumatoid arthritis, diabetic neuropathy, hypertension, premenstrual syndrome, asthma, atopic dermatitis, multiple sclerosis, migraine, and cancer (12,13). Therefore, many investigators have actively participated in research on GLA; this includes preparation of concentrates for dietetic and pharmaceutical purposes.

Until recently, n-3 PUFA-rich and GLA-rich oils have been used individually or as physical mixtures in feeding trials (14–16). Incorporation of GLA from borage oil into marine oils, by enzyme-catalyzed reactions, would provide unique specialty oils. Thus, the objectives of this study were to examine the ability of lipases from *Mucor* (also termed *Rhizomucor*) *miehei* and *Pseudomonas* species to catalyze the interesterification reaction of seal or menhaden oil with a GLA concentrate and to optimize reaction conditions.

MATERIALS AND METHODS

Materials. Borage oil was obtained from Bioriginal Food and Science Corporation (Saskatoon, Canada). Refined, bleached and deodorized (RBD) harp seal (*Phoca groenlandica*) oil and menhaden oil, devoid of any additives, were obtained from local sources in Newfoundland and Omega Protein Inc. (Reedville, VA), respectively. Lipozyme-IM (*M. miehei* lipase, immobilized on a macroporous anion-exchange resin) was provided by Novo Nordisk (Bagsvaerd, Denmark). Lipase PS-30 (*Pseudomonas* sp.) was obtained from Amano Enzyme USA (Lombard, IL). All other chemicals were American Chemical Society (ACS) grade or better.

Preparation of GLA concentrates from borage oil (BO) by urea complexation. GLA concentrate (up to 91%) was obtained from hydrolyzed borage oil by using the urea-fatty acid complexation procedure as described by Spurvey and Shahidi (17). The remaining fatty acids in the concentrate were mainly linoleic acid (7.6%).

Determination of lipase activity. Lipase activity was analyzed as described by Senanayake and Shahidi (18). Lipase activity was measured by assaying fatty acids produced from hydrolysis of triacylglycerols (TAG). All experiments were carried out in triplicate in screw-capped test tubes. Triolein was used as a substrate. It was emulsified at a concentration of 50 mM in 5% (wt/vol) gum arabic for 1 min using a Polytron (800 rpm; PT-3000; Brinkmann, Rexdale, Canada) homogenizer. The assay mixture contained 1 mL substrate-emulsion and the enzyme (10–100 mg). Reactions were

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carried out for up to 1 h in a shaking water bath at 35°C. The concentrations of released fatty acids increased linearly for more than 1 h. The released fatty acids were assayed colorimetrically as copper soaps using cupric acetate/pyridine reagent (19,20). The purity of the triolein was verified by thin-layer chromatography–flame ionization detection (TLC–FID); no mono- and diacylglycerols were detected.

The enzyme reaction in the emulsion system was stopped by adding 6 M HCl (1 mL) and isooctane (5 mL), followed by mixing for 1 min. Cupric acetate (1 mL, 5% wt/vol, pH 6.1) solution was then added and vortexed for 90 s; the absorbance of the upper isooctane layer was measured at 715 nm (21). One unit (U) of lipase activity was defined as the μmol quantities of fatty acids (equivalent to oleic acid) produced per min per gram of enzyme. Lipase activities of the *Mucor miehei* and *Pseudomonas* sp. were 13.6 and 11.9 U, respectively.

Acidolysis of seal blubber oil (SBO) and menhaden oil (MO) with GLA concentrate. In general, 300 mg of SBO or MO was mixed with a GLA concentrate (288–298 mg) at a mole ratio of 3:1 (GLA/TAG), in a screw-capped test tube. Then, lipase from *M. miehei* or *Pseudomonas* sp. (100–700 U/g oil), water (1% of the weight of substrates and enzyme), and hexane (2 mL) were added to the test tube. The mixture was stirred in a Gyrotory water bath shaker (Model G76, New Brunswick Scientific Co. Inc., New Brunswick, NJ) at 200 revolutions min^{-1} (rpm) under a blanket of nitrogen and at different temperatures (30, 40, 50, 60°C). Individual samples were removed at selected times (0–24 h) and analyzed.

Separation of acylglycerols and free fatty acids after interesterification. Once the reaction was complete, the enzymes were removed by filtration. Samples were placed in 250 mL conical flasks and quantities of 20 mL of a mixture of acetone/ethanol (1:1, vol/vol) were added to each. The reaction mixture was titrated with 0.5 M NaOH, to an end point indicated by phenolphthalein, in order to remove any remaining free fatty acids. 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 TAG was passed through a bed of anhydrous sodium sulfate. The TAG fraction was subsequently recovered following hexane removal at 40°C using a rotary evaporator.

Analysis of fatty acid composition of the acylglycerol fraction. Fatty acid composition was analyzed according to the method described by Senanayake and Shahidi (22). Oil samples were transmethylated with methanol in concentrated sulfuric acid to produce methyl esters. Quantities of about 20 mg of each oil were weighed into a 6 mL, Teflon-lined, screw-capped conical vial. Quantities of 2 mL of transmethylation reagent, freshly prepared using 6 mL of concentrated sulfuric acid made up to 100 mL with spectral-grade methanol and containing 15 mg of hydroquinone as an antioxidant, were added to the sample vial and mixed by vortexing. The mixture was incubated overnight at 60°C and subsequently cooled. Distilled water (1 mL) was added to the mixture. After mixing, the mixture was extracted three times with 1.5 mL of high-performance liquid chromatography-grade hexane. A few crystals of hydroquinone were added to each vial prior to extraction with hexane. Hexane layers were separated, placed in a clean test tube, and washed two times with 1.5 mL of distilled water. In the first wash, the aqueous layer was removed and in the second wash, the hexane layer was separated and evaporated under nitrogen. The fatty acid methyl esters (FAME) were dissolved in 1 mL of carbon disulfide and analyzed by gas chromatography. A Hewlett-Packard 5890 II gas chromatograph (Hewlett-Packard, Toronto, Canada) equipped with a flame-ionization detector and a split/splitless injector was used to analyze the FAME. An HP-Innowax column (30 m length, 0.25 mm diameter, 0.25 μm film thickness; Hewlett-Packard, Mississauga, Canada) was used for separation of FAME. Chromatographic parameters were set as follows: injector and detector temperatures, 250°C; oven temperature programming: held at 220°C for 10.25 min, then ramped at a rate of 30°C/min to 240°C, followed by a 9-min hold period. The FAME were identified by comparing their retention times with those of authentic standard mixtures and quantified using area normalization.

Positional distribution of structured lipids. Hydrolysis of modified SBO and MO by pancreatic lipase was carried out according to the method described by Christie (23). Tris-hydrochloric acid buffer (5 mL; 1.0 M, pH 8.0), 0.5 mL of calcium chloride (2.2%, wt/vol), and 1.25 mL of sodium taurocholate (0.05%, wt/vol) were added to 25 mg of oil in a glass test tube. The entire mixture was allowed to equilibrate at 40°C in a water bath for 1 min, and subsequently 5.0 mg of porcine pancreatic lipase (EC 3.1.1.3; Sigma) was added to it. The mixture was then placed in a Gyrotory water bath shaker at 200 rpm under nitrogen for 8 to 10 min at 40°C. Ethanol (5 mL) was added to stop the enzymatic hydrolysis, followed by addition of 5.0 mL of 6.0 M HCl. The hydrolytic products were extracted three times with 50 mL of diethyl ether, and the ether layers were removed and washed twice with distilled water and dried over anhydrous sodium sulfate. After removal of the solvent under vacuum at 30°C, the hydrolytic products were separated on silica gel thin-layer chromatographic plates (20 × 20 cm; 60 Å mean pore diameter, 2–25 μm mean particle size, 500 μm thickness, with dichlorofluorescein; Sigma, St. Louis, MO) impregnated with 5% (wt/vol) boric acid. The plates were developed using hexane/diethyl ether/acetic acid (70:30:1, by vol). After drying, the bands were located by viewing under short (254 nm)- and long (356 nm)-wavelength ultraviolet lights (Spectraline, Model ENF-240C; Spectronics Co., Westbury, NY). The bands were scraped off and their lipids extracted into chloroform/methanol (1:1, vol/vol) or diethyl ether and subsequently used for fatty acid analysis by the gas chromatographic procedure described by Senanayake and Shahidi (22).

Statistical analysis. All experiments and/or measurements were replicated three times. Mean ± standard deviations (SD) were reported for each case. SigmaStat (SPSS Science Corporation, Chicago, IL) was used to transform the data; then analysis of variance (ANOVA) and Tukey's studentized test were performed at a probability (P) level of $P < 0.05$ to evaluate the significance of differences among mean values.

RESULTS AND DISCUSSION

Two commercial lipases were screened by incubating 500 units of enzyme with a 3:1 mole ratio of GLA to TAG from either SBO or MO at 37°C for 24 h. The enzymes screened were nonspecific (toward acyl position within TAG) lipase from *Pseudomonas* sp. (PS-30) and *sn*-1,3-regiospecific lipase from *M. miehei* (Lipozyme-IM). A random and an *sn*-1,3-specific enzyme were chosen in order to determine the difference in GLA incorporation into the marine oils. These enzymes were selected based on an extensive literature review. Both enzymes catalyzed incorporation of GLA into SBO and MO to various extents (Fig. 1). Lipase PS-30 gave the highest degree of GLA incorporation into both oils (38% in SBO and 40% in MO). Thus, this lipase was chosen for subsequent experiments to determine optimal acidolysis conditions.

Figure 2 illustrates the effect of enzyme concentration on the incorporation of GLA into SBO and MO by lipase PS-30. The degree of incorporation increased with increasing enzyme load up to a level of 500 units/g of oil. Higher concentrations of enzyme did not increase the degree of incorporation significantly ($P > 0.05$), so there was no advantage to using more enzyme. Therefore, it was concluded that 500 units/g of oil was suitable to catalyze the synthesis of structured lipids. In this study, the water content in the reaction mixture was based on the reactant weight and was maintained at the 1% level. A possible explanation for the constant level of reaction rate with an increase in enzyme load may be that an insufficient amount of water was present in the reaction mixture, which led to a decrease in enzyme function because the enzyme was not able to assume its most effective three-dimensional configuration.

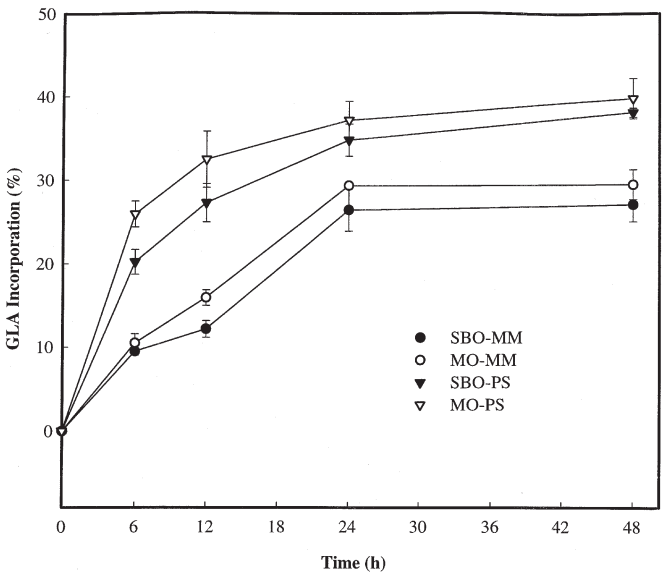

FIG. 1. Effect of reaction time on the acidolysis of seal blubber oil (SBO) and menhaden oil (MO) with lipases PS-30 (Amano Enzyme USA, Lombard, IL) from *Pseudomonas* species (PS) and Lipozyme-IM (Novo Nordisk, Bagsvaerd, Denmark) from *Mucor miehei* (MM). The reaction mixture contained 300 mg oil, 288–298 mg GLA (3:1 mole ratio of GLA/TAG, where GLA = γ-linolenic acid and TAG = triacylglycerol), enzyme concentration of 500 U/g, 1% water, and 2 mL hexane. Reactions were carried out at 37°C.

TABLE 1
Effect of Water Content on Acidolysis of Seal Blubber Oil or Menhaden Oil with γ-Linolenic Acid (GLA) Using Lipase PS-30[a]

Water (wt%) (weight of substrate and enzyme)	Degree of GLA incorporation	
	Seal blubber oil	Menhaden oil
0.5	16.5 ± 0.4	21.7 ± 0.4
1	22.0 ± 0.5	24.5 ± 0.5
2	21.6 ± 0.3	18.0 ± 0.2
3	17.9 ± 0.1	16.8 ± 0.3

[a]The reaction was performed with a 3:1 mole ratio (GLA/TAG) of substrates (300 mg oil/288–298 mg GLA) over a period of 24 h at 37°C with lipase PS-30 (300 U/g of oil). TAG, triacylglycerol; lipase PS-30 was obtained from Amano Enzyme USA (Lombard, IL).

In an enzymatic process, the content of water in the mixture affects the rate of reaction because a trace amount of water is necessary for the functioning of the enzyme and maintenance of its three-dimensional structure (24) and water thermodynamically favors hydrolysis of the TAG instead of acidolysis. Therefore, an experiment was performed to determine the optimal water content (Table 1). Based on the weight of substrate and enzyme, 1% water (1.08% including the moisture in the enzyme) provided the ideal environment for the acidolysis reaction. Higher percentages of water caused the percent incorporation of GLA to decrease. This result was in agreement with that of Cerdan *et al.* (25), who also reported that 1% water was best for production of TAG.

The substrate concentration was also investigated because large volumes of solvent decrease the concentration of reactants; 2 mL of solvent provided maximal acidolysis activity (Table 2). With a volume of 2 mL hexane in the reaction mixture, a degree of GLA incorporation of 23.8 and 26.5% was reached for SBO and MO, respectively. Increasing solvent volume decreased the GLA incorporation into oils. The decrease of GLA incorporation with an increase in solvent may be due to the effects of a reduction in the water concentration on the three-dimensional configuration and hence the activity of the enzyme.

Figure 3 shows the incorporation of GLA at various GLA/TAG mole ratios by lipase PS-30 at 37°C in 24 h. As the proportion of GLA increased, its percent incorporation increased steadily to approximately 30%. The steep part of the curve occurred from 1:1 to 3:1 mole ratio; from 3:1 to 5:1 mole ratio, results were not statistically different ($P > 0.05$) for both

TABLE 2
Effect of Solvent Volume on the Acidolysis of Seal Blubber Oil or Menhaden Oil with GLA Using Lipase PS-30[a]

Solvent volume (mL)	Degree of GLA incorporation	
	Seal blubber oil	Menhaden oil
1	23.5 ± 0.6	26.1 ± 0.3
2	23.8 ± 0.6	26.5 ± 0.8
3	22.1 ± 0.4	20.4 ± 0.2
4	19.9 ± 0.3	17.0 ± 0.5
5	18.9 ± 0.4	16.9 ± 0.3

[a]The reaction was performed with a 3:1 mole ratio (GLA/TAG) of substrates (300 mg oil/288–298 mg GLA) over a period of 24 h at 37°C with lipase PS-30 (300 U/g of oil). For abbreviations and manufacturer see Table 1.

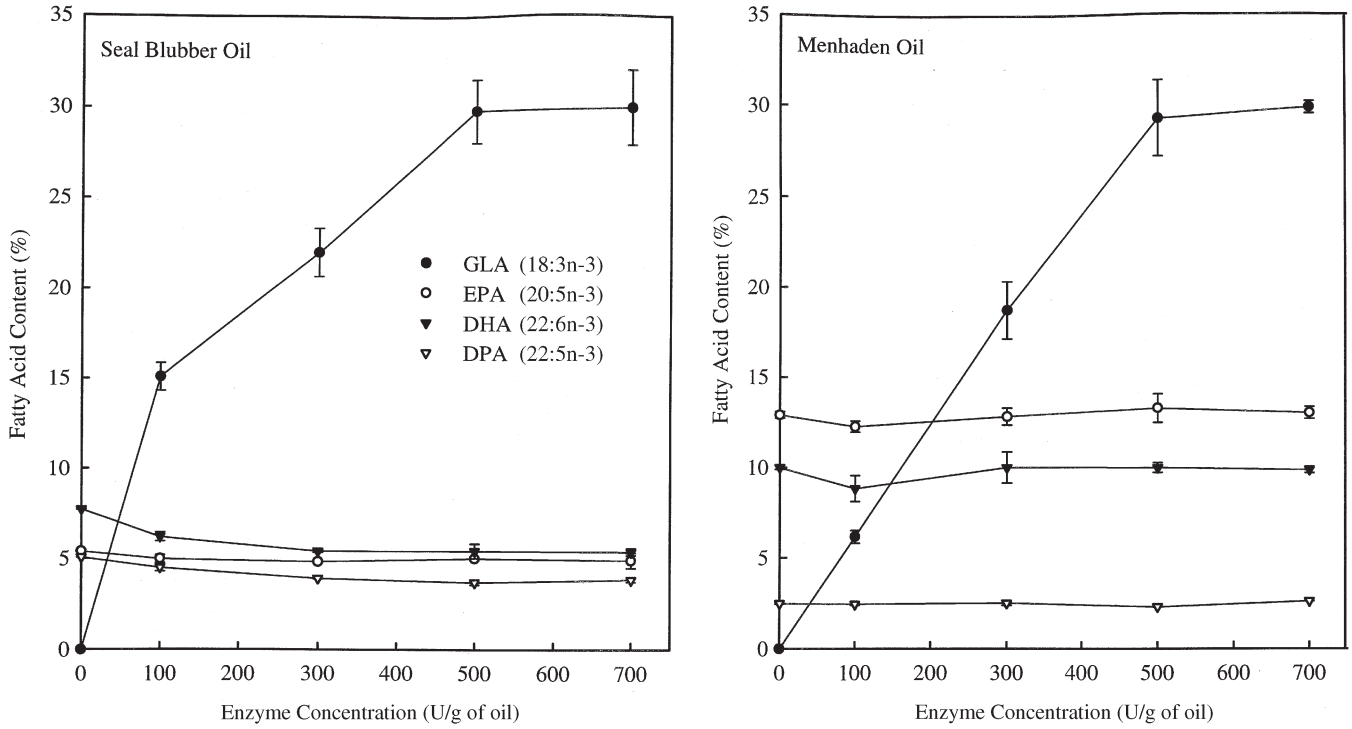

FIG. 2. Effect of varying enzyme concentration on the incorporation of GLA into SBO and MO with lipase PS-30 from *Pseudomonas* sp. The reaction mixture contained 300 mg oil, 288–298 mg GLA (3:1 mole ratio of GLA/TAG), an enzyme concentration of 100–700 U/g, 1% water, and 2 mL hexane. Reactions were carried out at 37°C for 24 h. Some standard deviations are within the domain of the symbols. For abbreviations and manufacturer see Figure 1.

substrates. For the MO sample, the mole ratios 3:1 and 5:1 were statistically different ($P < 0.05$) from one another. However, for economic reasons a mole ratio of 3:1 was chosen. This result agrees with that of Senanayake and Shahidi (26), who also found that the stoichiometric ratio of fatty acid to TAG of 3:1 was optimal for production of TAG, as there are three acyl groups per molecule of TAG. At higher mole ratios, incorporation of GLA into TAG may be sluggish because the structured lipid products may compete with the reactants (TAG and GLA) and slow the forward reaction (27). Mutua and Akoh (28) reported a decreased EPA incorporation into biosurfactant by IM-20 when the mole ratio of EPA to phospholipid exceeded 2:1. However, no decrease in GLA incorporation was observed at high mole ratios in this study. Osterberg *et al*. (29) postulated that TAG molecules possessing two or more GLA acyl groups are sterically hindered from entering the active site of lipases to act as acyl acceptors because of the "stiffness" of the molecules caused by the double bonds at the Δ-6 position. This may be a possible reason for the limitation of the percentage of GLA incorporation into oils at higher mole ratios.

Time course studies are useful in monitoring the progress of enzymatic reactions and assist in determining the shortest time necessary to obtain good yields and minimize production costs. Time course studies also help identify product formation and reactant disappearance with time and give an indication of when to stop the reaction, depending on the desired product. Figure 4 shows the time course of lipase-catalyzed acidolysis of GLA into SBO and MO by lipase PS-30. The percent GLA incorporated into SBO and MO increased to 27 and 32%, respectively, after 12 h, and essentially reached equilibrium at 24 h (35% GLA in SBO and 37% in MO), after which further incorporation was minimal. This result is in agreement with that of Akoh *et al.* (30) and Moussata and Akoh (31), who both reported that 24 h was the optimal reaction time for incorporation of EPA into evening primrose oil and oleic acid into melon seed oil, respectively.

The optimal performance temperature of enzymes depends on their source, the nature of the immobilization or chemical modification, and the pH of the reaction mixture (24). The temperature range tested was 30 to 60°C. Figure 5 illustrates the effect of temperature on incorporation of GLA into MO and SBO by lipase PS-30. The results show that maximal incorporation of GLA was achieved at 60°C, similar to that reported by Rahmatullah *et al.* (32). However, a temperature increase from 40 to 60°C produced only a minimal increase in the incorporation of GLA. Thus, 40°C was chosen as the best temperature because there was no significant difference between 40 and 50°C for any of the reactions. The 60°C temperature was rejected as optimal because at this temperature thermal degradation of PUFA may occur (32). Since the acidolysis reaction was carried out in sealed test tubes, the increase in temperature could not have evaporated the water from the reaction mixture, thus enhancing the extent of hydrolysis as a side reaction.

The foregoing results indicate that it is possible to incorporate GLA into marine oils through lipase-catalyzed acidolysis. Lipase PS-30 showed a better acidolysis activity than *M. miehei* lipase. The optimal reaction conditions were: enzyme concentration of 500 U/g of oil, a 3:1 mole ratio of GLA to

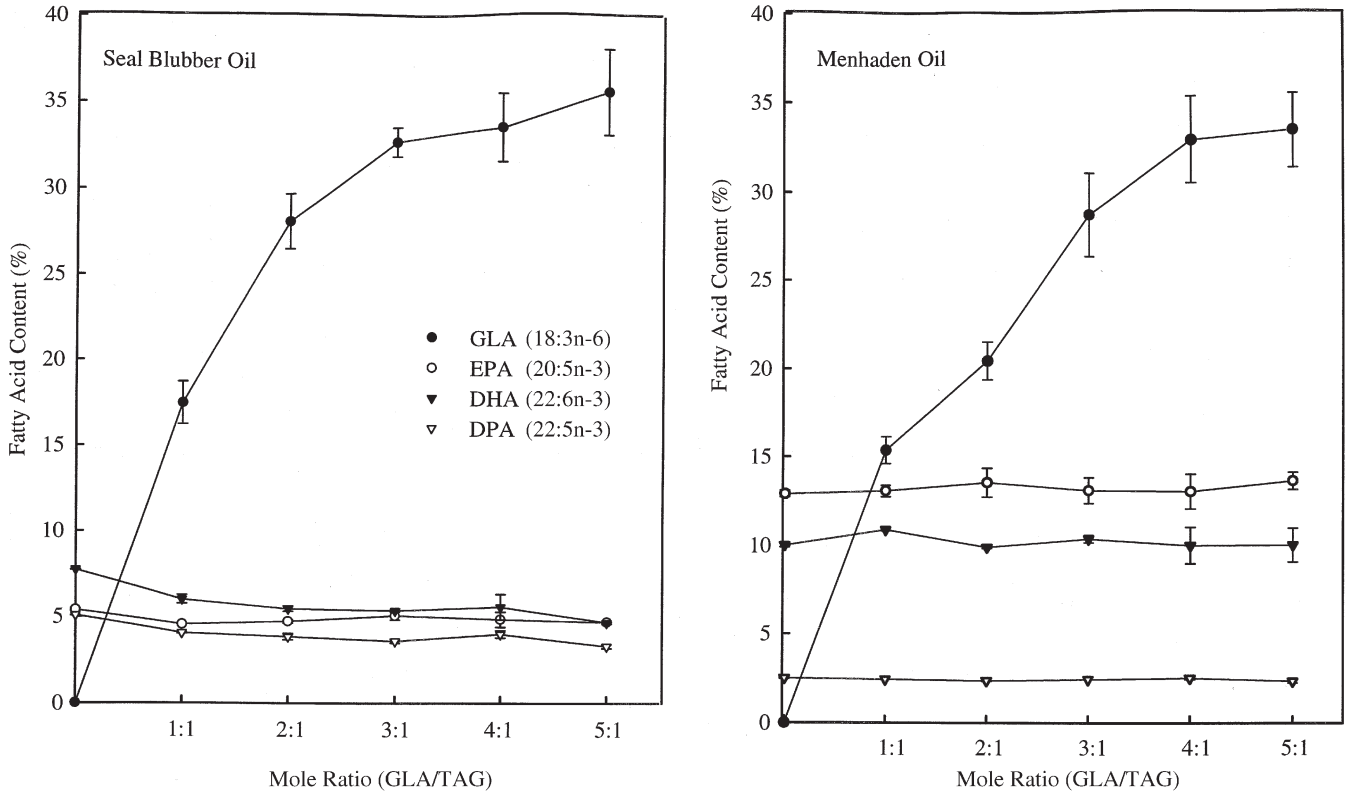

FIG. 3. Effect of varying the mole ratio (GLA/TAG) on the percent incorporation of GLA into SBO and MO with lipase PS-30 from *Pseudomonas* sp. Mole ratios of GLA to TAG were varied from 1:1 to 5:1.The reaction mixtures contained an enzyme concentration of 500 U/g, 1% water and 2 mL hexane. Reactions were carried out at 37°C for 24 h. Some standard deviations are within the domain of the symbols. For abbreviations and manufacturer see Figure 1.

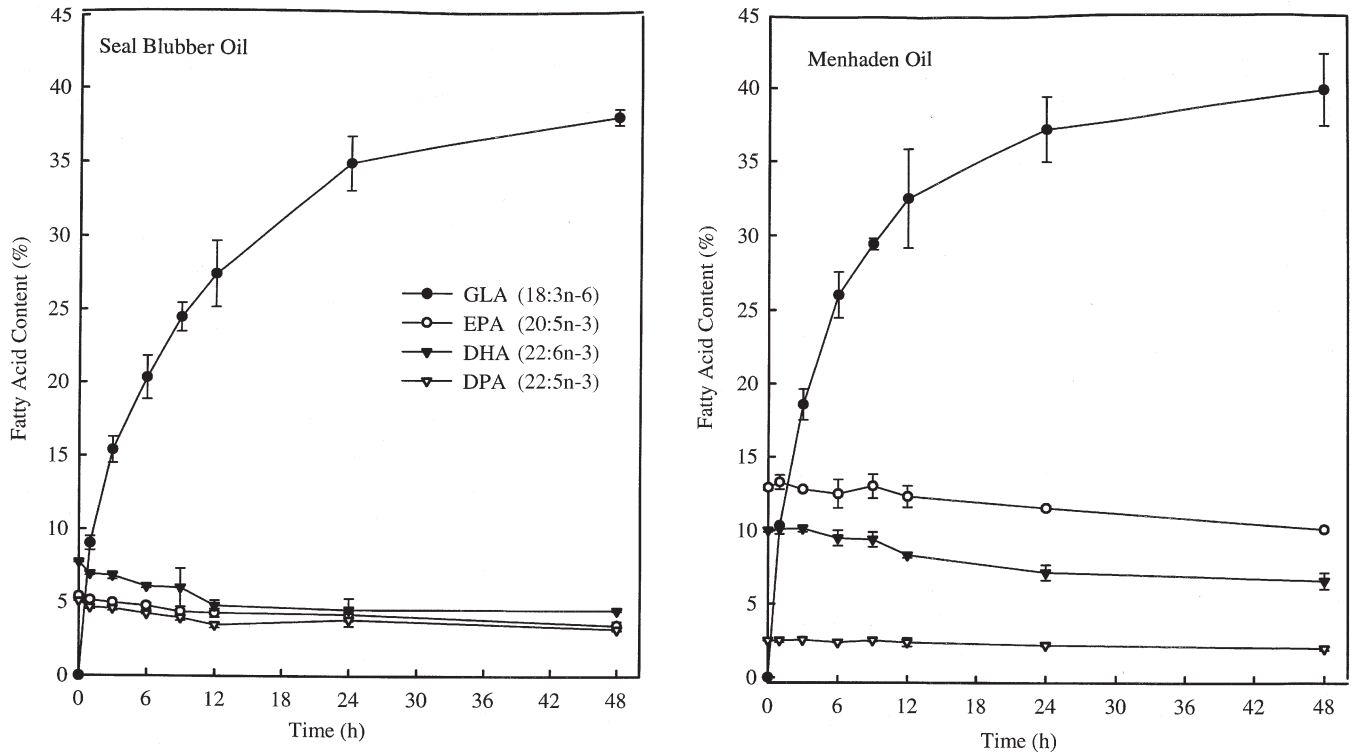

FIG. 4. The effect of varying the reaction time on the percent incorporation of GLA into SBO and MO with lipase PS-30 from *Pseudomonas* sp. The reaction mixture contained 300 mg oil, 288–298 mg GLA (3:1 mole ratio of GLA/TAG), an enzyme concentration of 500 U/g, 1% water, and 2 mL hexane. Reactions were carried out at 37°C. Some standard deviations are within the domain of the symbols. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; for other abbreviations and manufacturer see Figure 1.

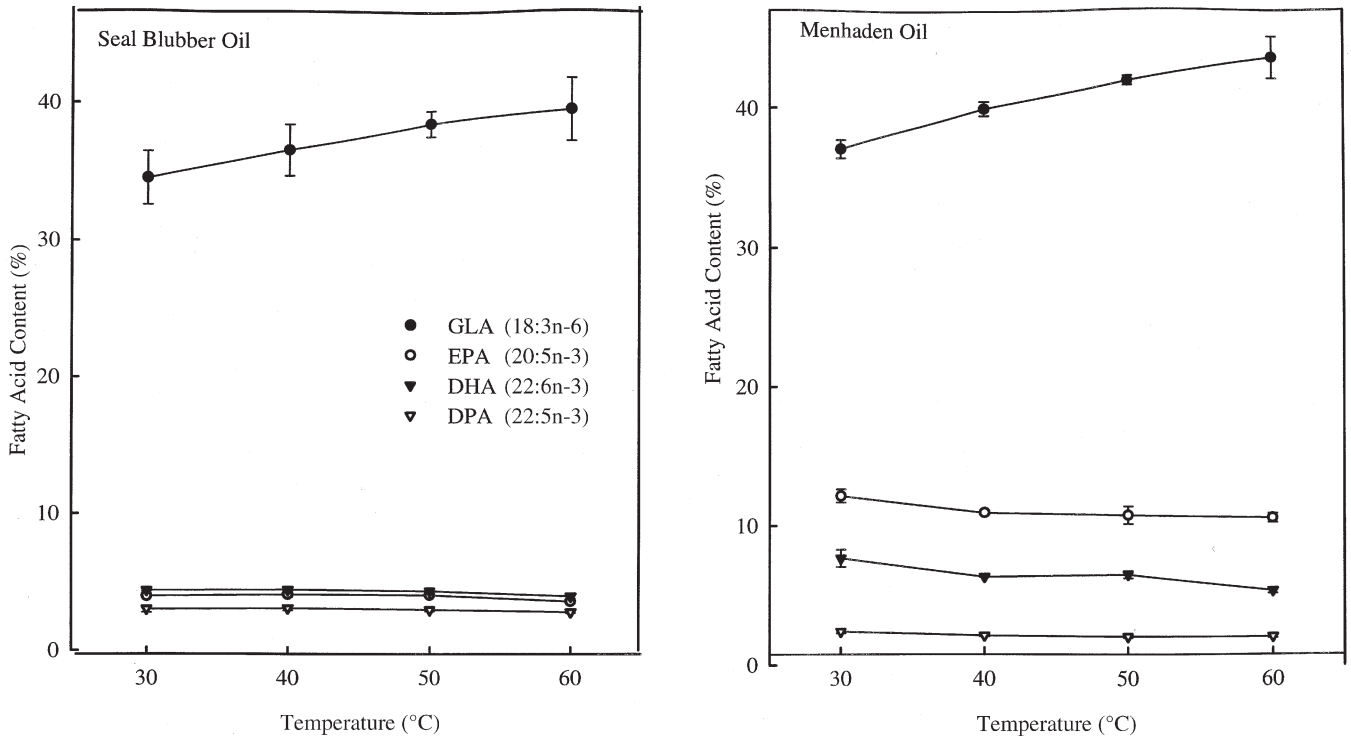

FIG. 5. The effect of varying the temperature on the percent incorporation of GLA into SBO and MO with lipase PS-30 from *Pseudomonas* sp. The reaction mixture contained 300 mg oil, 288–298 mg GLA (3:1 mole ratio of GLA/TAG), enzyme concentration of 500 U/g, 1% water, and 2 mL hexane. Reactions were carried out at 30–60°C for 24 h. Some standard deviations are within the domain of the symbols. For abbreviations see Figures 1 and 4; for manufacturer see Figure 1.

TAG for both SBO and MO, a reaction time of 24 h, and a reaction temperature of 40°C. Table 3 shows the fatty acid profile of unmodified and lipase-catalyzed modified SBO. The fatty acid profile of the modified SBO produced under optimal reaction conditions indicated that GLA, EPA, DPA, and DHA were present at 37.1, 3.80, 2.99, and 4.36%, respectively. The content of the n-3 PUFA decreased from 18.2 to 11.2% after the interesterification reaction. The ratio of n-3 to n-6 PUFA was 1:3.6. Similarly, the content of GLA, EPA, DPA, and DHA in the restructured MO was 39.6, 11.0, 2.07, and 6.56%, respectively, and the n-3 PUFA decreased by 1.3% after the interesterification reaction (Table 3). The corresponding mole ratio of n-3 to n-6 PUFA was 1:2.2. After modification by *Pseudomonas* sp. lipase, the contents of EPA and DHA in SBO decreased by 1.6 and 3.4%, respectively. Similarly, in modified MO, EPA and DHA contents decreased by 1.9 and 3.4%, respectively. This indicates that DHA was more readily replaced in these oils than EPA.

Since there has been no research conducted on the incorporation of GLA into marine oils, a direct comparison of the results is not possible. Nonetheless, considerable research has been conducted on the introduction of n-3 PUFA into borage, primrose, and vegetable oils. Thus, these results will be used for comparative purposes. Senanayake and Shahidi (18) modified the fatty acid composition of borage and evening primrose oils by incorporating EPA and DHA, using a nonspecific PS-30 lipase from *Pseudomonas* sp. They reported that incorporation of EPA + DHA into borage and evening primrose oils was 35.5 and 33.6%, respectively. At a substrate mole ratio of 1:0.5:0.5 (TAG/EPA/DHA), the corresponding n-3/n-6 PUFA ratio was 0.9:1 for borage oil and 0.63:1 for evening primrose oil. Akoh and Sista (33) and Akoh *et al.* (30) incorporated EPA into borage and evening primrose oil, respectively, by immobilized nonspecific SP435 lipase from *Candida antarctica* as the biocatalyst. The maximal percentage of EPA incorporation was 21.1% with an n-3/n-6 PUFA ratio of 1:2.4 for borage oil and 34.0% with a n-3/n-6 PUFA ratio of 1:1.6 for evening primrose oil.

Modified SBO and MO produced using lipase PS-30 (*Pseudomonas* sp.) were subjected to pancreatic lipase hydrolysis, and the results are shown in Table 4. Pancreatic lipase hydrolyzes fatty acids from the *sn*-1 and *sn*-3 positions of TAG while leaving the *sn*-2 position intact (26). This study is important since researchers have suggested improved absorption of fatty acids when they are esterified at the *sn*-2 position of TAG (34). Christensen *et al.* (35) also observed increased absorption of essential fatty acids at the *sn*-2 position of TAG molecules. Our results showed that lipase PS-30 incorporated 25.1 and 24.7% GLA, respectively, at the *sn*-2 position of TAG of SBO and MO. This indicates that the mid position of the TAG was involved in the acidolysis process when the nonspecific PS-30 (*Pseudomonas* sp.) lipase was used. In modified SBO, the *sn*-2 position had EPA and DHA contents of 3.0 and 2.0%, respectively. In modified MO, the *sn*-2 position contained 14.3% EPA and 6.1% DHA. Lipase PS-30 was also able to incorporate appreciable quantities of GLA (33.7% in SBO and 35.8% in MO) at *sn*-1,3 positions of TAG. The primary TAG positions (*sn*-1,3) of modified SBO had 8.2% EPA and 3.8% DHA. On the other hand, the *sn*-1,3 positions of

TABLE 3
Fatty Acid Composition (w/w%) of Refined-Bleached-Deodorized SBO and MO TAG Before and After Enzymatic Interesterification with GLA 1 (18:3n-6)[a]

Fatty acid (w/w%)	SBO-TAG		MO-TAG	
	Before modification[b]	After modification[b]	Before modification[b]	After modification[b]
12:0	ND	0.05 ± 0.01	ND	0.06 ± 0.00
14:0	3.36 ± 0.08	2.40 ± 0.04	8.18 ± 0.08	4.55 ± 0.08
14:1n-5	1.09 ± 0.04	0.72 ± 0.01	0.36 ± 0.01	0.20 ± 0.00
15:0	0.23 ± 0.00	0.13 ± 0.00	0.70 ± 0.02	0.35 ± 0.01
16:0	5.14 ± 0.03	3.04 ± 0.03	19.8 ± 0.24	8.78 ± 0.06
16:1n-7	14.5 ± 0.04	9.67 ± 0.00	11.5 ± 0.13	5.84 ± 0.01
17:0	0.92 ± 0.01	0.56 ± 0.01	1.68 ± 0.02	0.23 ± 0.01
17:1	0.55 ± 0.02	0.25 ± 0.00	1.43 ± 0.03	0.86 ± 0.02
18:0	1.02 ± 0.01	0.56 ± 0.00	3.83 ± 0.02	1.50 ± 0.01
18:1n-9	22.6 ± 0.06	14.1 ± 0.01	9.86 ± 0.11	4.24 ± 0.10
18:1n-11	4.88 ± 0.02	2.69 ± 0.01	3.71 ± 0.05	1.52 ± 0.01
18:2n-6	1.51 ± 0.02	3.80 ± 0.01	1.76 ± 0.09	3.81 ± 0.02
18:3n-6	0.59 ± 0.00	37.1 ± 0.16	0.43 ± 0.00	39.6 ± 0.07
18:3n-3	ND	0.33 ± 0.00	1.31 ± 0.02	0.66 ± 0.03
20:1n-9	17.3 ± 0.02	8.30 ± 0.06	1.62 ± 0.06	0.83 ± 0.10
20:4n-6	0.46 ± 0.01	0.32 ± 0.01	0.86 ± 0.02	0.70 ± 0.01
20:5n-3	5.40 ± 0.08	3.80 ± 0.01	12.9 ± 0.18	11.0 ± 0.10
22:1n-1	2.01 ± 0.04	0.56 ± 0.00	0.12 ± 0.04	0.30 ± 0.01
22:5n-3	5.07 ± 0.01	2.99 ± 0.01	2.48 ± 0.03	2.07 ± 0.01
22:6n-3	7.73 ± 0.02	4.36 ± 0.06	10.0 ± 0.11	6.56 ± 0.04
Total n-6	2.56	41.2	3.05	44.1
Total n-3	18.2	11.5	26.7	20.3
Ratio n-3/n-6	7.1:1	1:3.6	8.7:1	1:2.2

[a]Restructured SBO and MO were prepared under optimal conditions (enzyme concentration of 500 U/g of oil, 24 h, 40°C, 3:1 mole ratio, 2 mL hexane, 1% water).
[b]Mean ± SD. SBO, seal blubber oil; MO, menhaden oil; ND, not detected. For other abbreviations see Table 1.

TABLE 4
Positional Distribution of Structured Lipids Produced Using Lipase PS-30[a]

Fatty acid (w/w%)	SBO-TAG		MO-TAG	
	sn-1,3 positions	*sn*-2 position	*sn*-1,3 positions	*sn*-2 position
8:0	0.1 ± 0.03	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.03
10:0	0.1 ± 0.01	0.2 ± 0.03	0.1 ± 0.01	0.1 ± 0.01
12:0	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.04	0.1 ± 0.04
14:0	2.1 ± 0.15	3.0 ± 0.15	2.8 ± 0.31	4.9 ± 0.22
14:1n-5	0.9 ± 0.20	0.7 ± 0.09	0.2 ± 0.05	0.1 ± 0.04
15:0	0.1 ± 0.04	0.1 ± 0.02	0.3 ± 0.02	0.4 ± 0.01
16:0	2.4 ± 0.03	4.6 ± 0.36	5.4 ± 0.29	9.4 ± 0.27
16:1n-7	6.9 ± 0.27	9.5 ± 0.48	3.5 ± 0.61	5.1 ± 0.58
17:0	0.6 ± 0.12	0.7 ± 0.08	0.8 ± 0.11	0.2 ± 0.01
17:1	5.4 ± 0.48	3.8 ± 0.15	0.2 ± 0.03	4.8 ± 0.31
18:0	0.6 ± 0.07	1.0 ± 0.10	0.8 ± 0.04	1.2 ± 0.08
18:1n-9	8.2 ± 0.31	18.8 ± 1.10	2.2 ± 0.13	3.8 ± 0.52
18:1n-11	1.5 ± 0.19	2.4 ± 0.05	0.9 ± 0.02	1.1 ± 0.33
18:2n-6	6.6 ± 0.52	2.9 ± 0.18	2.0 ± 0.04	3.6 ± 0.09
18:3n-6	33.7 ± 1.02	25.1 ± 1.70	35.8 ± 0.83	24.7 ± 0.91
18:3n-3	0.4 ± 0.05	0.4 ± 0.09	0.5 ± 0.10	0.5 ± 0.07
20:0	0.1 ± 0.02	0.2 ± 0.03	0.1 ± 0.03	0.1 ± 0.06
20:1n-9	2.8 ± 0.11	4.5 ± 0.29	0.1 ± 0.01	0.8 ± 0.04
20:2n-6	0.2 ± 0.04	0.2 ± 0.08	0.2 ± 0.03	0.2 ± 0.06
20:3n-6	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.04	0.1 ± 0.03
20:4n-6	0.3 ± 0.01	0.3 ± 0.07	1.1 ± 0.06	1.0 ± 0.05
20:5n-3	8.2 ± 0.34	3.0 ± 0.11	13.8 ± 0.84	14.3 ± 0.61
22:0	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.02
22:1n-11	0.4 ± 0.05	0.4 ± 0.05	0.4 ± 0.05	0.6 ± 0.08
22:4n-6	0.1 ± 0.01	0.1 ± 0.02	0.2 ± 0.03	0.2 ± 0.01
22:5n-3	2.3 ± 0.09	1.3 ± 0.10	2.6 ± 0.18	2.6 ± 0.29
22:6n-3	3.8 ± 0.6	2.0 ± 0.06	7.3 ± 0.01	6.1 ± 0.12

[a]Restructured SBO and MO were prepared under optimal conditions (enzyme concentration of 500 U/g of oil, 24 h, 40°C, 3:1 mole ratio, 2 mL hexane, 1% water). The results are mean of triplicate determinations from different experiments. For abbreviations see Tables 1 and 3. For enzyme source see Table 1.

modified MO contained 13.8 and 7.3% EPA and DHA, respectively. Thus, structured lipids containing GLA, EPA, and DHA in the same TAG molecules were successfully produced and may have potential health benefits.

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