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## Synthesis of Structured Lipids via Acidolysis of Docosahexaenoic Acid Single Cell Oil (DHASCO) with Capric Acid

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Screening of five commercially available lipases for the incorporation of capric acid (CA) into docosahexaenoic acid single cell oil (DHASCO) indicated that lipase PS-30 from *Pseudomonas* sp. was most effective. Of the various reaction parameters examined, namely, the mole ratio of substrates, enzyme amount, time of incubation, reaction temperature, and amount of added water, for CA incorporation into DHASCO, the optimum conditions were a mole ratio of 1:3 (DHASCO/CA) at a temperature of 45 °C, and a reaction time of 24 h in the presence of 4% enzyme and 2% water content. Examination of the positional distribution of fatty acids on the glycerol backbone of the modified DHASCO with CA showed that CA was present mainly in the sn-1,3 positions of the triacylglycerol (TAG) molecules. Meanwhile, DHA was favorably present in the sn-2 position, but also located in the sn-1 and sn-3 positions. The oxidative stability of the modified DHASCO in comparison with the original DHASCO, as indicated in the conjugated diene values, showed that the unmodified oil remained relatively unchanged during storage for 72 h, but DHASCO-based structured lipid was oxidized to a much higher level than the original oil. The modified oil also attained a considerably higher thiobarbituric acid reactive substances value than the original oil over the entire storage period. However, when the oil was subjected to the same process steps in the absence of any enzyme, there was no significant difference ( $p > 0.05$ ) in its oxidative stability when compared with enzymatically modified DHASCO. Therefore, removal of antioxidants during the process is primarily responsible for the compromised stability of the modified oil.

**KEYWORDS:** Acidolysis; lipase; structured lipids; medium-chain fatty acids; capric acid (C10:0); docosahexaenoic acid single cell oil (DHASCO); oxidative stability; conjugated dienes; thiobarbituric acid reactive substances (TBARS); positional distribution

### INTRODUCTION

The concept of structured lipids (SL) for nutritional and medical use was first introduced in 1987 by Babayan (*1*). SL are triacylglycerols (TAG) modified to alter the fatty acid composition and/or their location in the glycerol backbone via chemical or enzymatic means. The triacylglycerols in SL consist of a glycerol moiety esterified to a mixture of short-, medium-, and long-chain fatty acids for functional purposes (*2*). Lipids can be restructured to meet essential fatty acid requirements or to incorporate specific fatty acids of interest. SL may offer the most efficient means of delivering target fatty acids for nutritive or therapeutic purposes as well as to alleviate specific disease and metabolic conditions. Structured lipids can also be produced to obtain TAG with modified physical and/or chemical features, including melting point and iodine and saponification values (*1*). Depending on the type of substrates available, chemical or

enzymatic reactions can be used to synthesize SL, including direct esterification (reaction of fatty acids and glycerol), acidolysis (transfer of acyl group between an acid and ester), and alcoholysis (exchange of alkoxy group between an alcohol and an ester) (*3*). Chemically catalyzed interesterification, using an alkali such as sodium methoxide, is cheap and easy to scale-up. However, such reactions lack specificity and offer little or no control over the positional distribution of fatty acids in the final product (*4*). In addition, the reactions carried out under harsh conditions such as high temperatures (80–90 °C) may produce side products, which are difficult to eliminate. An alternative to the chemical synthesis of SL is enzymatic processes using a variety of lipases. Potential advantages of lipase-assisted interesterification are enzyme regioselectivity, chemoselectivity, and fatty acid chain length specificity. Lipases can be used in the design of SL for specific food or medical uses on a case-by-case basis (*5, 6*). The products so obtained have a defined structure because of incorporating desired fatty acids at a specific position and such products have enhanced

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functionality and desirable attributes. Under mild experimental conditions employed in enzymatic modification, there is little or no side product formation with reduced energy consumption and heat damage to reactants, and the end products are easily recovered.

Docosahexaenoic acid single cell oil (DHASCO) is an important source of commercial DHA, which is derived from *Cryptocodinium cohnii* microalgae (7). DHASCO contains ~40% (w/w) DHA, but no eicosapentaenoic acid (EPA) or other long-chain polyunsaturated fatty acids (LCPUFA) are present in the oil (7). DHASCO represents a well-defined source of DHA for infant formulas and food supplementation. Medium-chain fatty acids (MCFA) comprise 6–12 carbon saturated fatty acids, and these are obtained primarily from hydrolysis of tropical plant oils such as those of coconut and palm kernel (4, 5). Medium-chain triacylglycerols (MCT) exhibit unique structural and physiological characteristics; they are different from other fats and oils by being absorbed via the portal vein (5). MCT do not require chylomicron formation to transfer from the bloodstream to the cells and have a more rapid  $\beta$ -oxidation to form acetyl CoA end products, which are further oxidized to yield CO<sub>2</sub> in the Krebs cycle (3). Many researchers have successfully incorporated MCFA (caprylic or capric acids) into PUFA-rich fish oil via lipase-assisted acidolysis (8–13) and into borage and evening primrose oils, which contain  $\gamma$ -linolenic acid (GLA) (11, 14). The aim of this study was to (i) produce structured lipids via acidolysis of DHASCO with a medium-chain fatty acid (C10:0, capric acid), (ii) determine the effect of reaction variables such as type of enzyme, enzyme concentration, incubation time, substrate mole ratio, and amount of added water on the extent of incorporation of capric acid into DHASCO, (iii) determine the positional distribution of fatty acids in the modified DHASCO, and (iv) assess the oxidative stability of the resultant SL.

## MATERIALS AND METHODS

**Materials.** Two lipases from *Candida antarctica* (Novozyme-435) and *Mucor miehei* were acquired from Novo Nordisk (Franklinton, NC). Other lipases, namely, *Pseudomonas* sp. (PS-30), *Aspergillus niger* (AP-12), and *Candida rugosa* (AY-30), were obtained from Amano Enzyme (Troy, VA). DHASCO containing 40% DHA was obtained from Martek Bioscience Corp. (Columbia, MD). Standards of fatty acid methyl esters (FAMES; GLC-461) were purchased from Nu-Check (Elysian, MN). Porcine pancreatic lipase (EC 3.11.3), sodium taurocholate, and silica gel thin-layer chromatographic plates (TLC; 20 × 20 cm; 60 Å mean pore diameter, 2–25  $\mu$ m mean particle size, 500  $\mu$ m 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).

**Methods.** *Acidolysis Reaction.* In general, DHASCO (500 mg) was mixed with CA at different mole ratios of oil to CA ranging from 1 to 3, in a screw-capped test tube, and then lipase (2–10 wt % of substrates) and water (1–2.5 wt % of substrates and enzyme) were added in *n*-hexane (3.0 mL). The mixture was incubated for different periods (12–48 h) in an orbital shaker at 250 rpm at 25–55 °C.

*Separation of Acylglycerols after Acidolysis.* After a given time period, the reaction was stopped by the addition of a mixture of acetone and ethanol (20 mL; 1:1, v/v). To neutralize the released and unused free fatty acids, the reaction mixture was titrated with a 0.5 M NaOH solution (using a phenolphthalein indicator) until the color of the solution turned pink. The acylglycerols were then extracted into *n*-hexane (25 mL). The two layers (aqueous, *n*-hexane) were allowed to separate in a separatory funnel, and the lower aqueous layer was discarded. The *n*-hexane layer was passed through a bed of anhydrous sodium sulfate to remove any residual water. The *n*-hexane was evaporated using a rotary evaporator at 45 °C, and the acylglycerol

fraction was recovered and a portion of it transferred to special transmethylation vials.

**Fatty Acid Composition of Products.** *Preparation of FAMES.* Fatty acid profiles of products were determined following their conversion to methyl esters. Transmethylation reagent (2.0 mL; freshly prepared 6.0 mL of concentrated sulfuric acid made up to 100 mL with methanol and 15 mg of hydroquinone as an antioxidant) was added to the sample vial, followed by vortexing. The mixture was incubated at 60 °C for 24 h 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 oxidation; lipids 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 layer (the upper layer) was then separated, and the solvent was evaporated under a stream of nitrogen. FAMES were then dissolved in 1.0 mL of carbon disulfide and used for subsequent gas chromatographic (GC) analysis.

*Analysis of FAMES by Gas Chromatography.* The FAMES were analyzed using a Hewlett-Packard 5890 series II gas chromatograph (Agilent, Palo Alto, CA) equipped with a Supelcowax-10 column (30 m × 0.25 mm, 0.25  $\mu$ m film thickness; Supelco Canada Ltd., Oakville, ON). 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 (flame ionization) and detector (FID) temperatures were both set at 250 °C. UHP helium was used as a carrier gas at a flow rate of 15 mL/min. Data were treated using Hewlett-Packard 3365 series II Chem Station software (Agilent). The FAMES were identified by comparing their retention times with those of an authentic standard mixture (GLC-461; Nu-Check); results were presented as weight percentage.

*Hydrolysis by Pancreatic Lipase.* Hydrolysis of the modified oil by pancreatic lipase was achieved essentially as described by Christie (15) with minor modifications. Tris-HCl buffer (5.0 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) were added to 25 mg of modified oil in a glass test tube. The whole mixture was allowed to stand at 40 °C in a water bath for 1.0 min, followed by the addition of 5.0 mg of porcine pancreatic lipase (EC 3.11.3; Sigma). The mixture was subsequently placed in a gyratory water bath shaker at 250 rpm under a blanket of nitrogen for 1 h at 40 °C. Ethanol (5.0 mL) was added to the mixture to stop the enzymatic reaction, followed by the addition of 5.0 mL of 6.0 M HCl. The hydrolytic products were extracted three times with 50.0 mL of methanol/chloroform (1:1, v/v), and the upper layer was removed and washed twice with distilled water and 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  $\mu$ m mean particle size, 500  $\mu$ m thickness, with 2,7'-dichlorofluorescein, Sigma) were evenly sprayed with 5% (w/v) boric acid and dried at 100 °C for 1 h. The hydrolytic products were separated on a silica gel TLC plate. The plates were developed using *n*-hexane/diethyl ether/acetic acid (70:30:1, v/v/v) for 40–50 min and then allowed to dry in the air. The bands were located by viewing 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, v/v). Fatty acid profile of lipids was obtained by employing the GC method described in the earlier section.

*Oxidative Stability Tests.* The oxidative stabilities of the structured lipid produced from DHASCO via acidolysis with CA as well as the original oil, as such, or subjected to process steps in the absence of enzymes, were evaluated under Schaal oven conditions at 60 °C for a period of 72 h. Oils (0.4–0.5 g) were placed in loosely capped test tubes (10 mm diameter and 4.0 cm height) and stored at 60 °C in a forced-air oven (Thelc model, Precision Scientific Co., Chicago, IL). Samples were removed at 0, 6, 12, 24, 36, 48, and 72 h from the oven, cooled to room temperature, flushed with nitrogen, capped, and stored at –20 °C until analyzed. The experiments were carried out in triplicate.

*Conjugated Dienes (CD).* CD in the oils were determined according to IUPAC (16) method 20505. Oil samples (0.02–0.04 g) were weighted into 50 mL volumetric flasks, dissolved in iso-octane (2,2,4-

**Table 1.** Fatty Acid Composition of DHASCO TAG before and after Modification with CA<sup>a</sup>

fatty acid (wt %)	before modification	after modification <sup>b</sup>
C10:0	0.46 ± 0.01	10.17 ± 0.11
C12:0	3.46 ± 0.05	3.03 ± 0.04
C14:0	12.9 ± 0.08	10.0 ± 0.02
C16:0	10.5 ± 0.05	7.9 ± 0.07
C18:0	0.86 ± 0.02	0.79 ± 0.01
C18:1 <i>n</i> -9	26.6 ± 1.54	26.3 ± 1.19
C18:2 <i>n</i> -6	1.43 ± 0.02	1.21 ± 0.00
C20:5 <i>n</i> -3	0.28 ± 0.01	ND
C22:6 <i>n</i> -3	37.1 ± 0.39	37.1 ± 0.29
others	2.86	

<sup>a</sup> Values are means of triplicate determinations ± standard deviation. ND, not detected. <sup>b</sup> The reaction mixture contained 500 mg of DHASCO, 315 mg of CA, 4% (w/w) enzyme for *Pseudomonas* sp., and 3.0 mL of hexane. The reaction mixture was incubated at 45 °C for 24 h.

trimethylpentane), and made up to the mark with the same solvent; pure iso-octane was used as the blank. The contents were thoroughly mixed, and the absorbance at 234 nm was read using a Hewlett-Packard model 845LA diode array spectrophotometer (Agilent). Conjugated dienes were calculated using the formula  $CD = A/(cd)$ , where  $A$  = absorbance at 234 nm,  $c$  = concentration of the solution in g/mL, and  $d$  = length of the cell in cm.

**Thiobarbituric Acid Reactive Substances (TBARS) Determination.** The determination of TBARS was carried out as described by AOCS (17) method Cd 19-90. Oil samples (0.05–0.10 g) were accurately weighed into 25 mL volumetric flasks, dissolved in a small volume of 1-butanol, and made up to the mark with the same solvent. Five milliliters of the mixture (oil and solvent) was transferred into a dry screw-capped test tube, and then 5 mL of freshly prepared TBA reagent (0.5 g of TBA in 250 mL of 1-butanol) was added. The constituents of the mixture were thoroughly mixed and placed in a water bath at 95 °C for 2 h. Heated samples were cooled in an ice bath, and the absorbance of the resulting colored complex was read at 532 nm. The micromole amount of malonaldehyde (MA) equivalents per gram of oil, expressed as TBARS values, was calculated using the formula  $C = (0.415A_{532})/w$ , where the factor 0.415 is obtained from a standard regression line using 1,1,3,3-tetramethoxypropane as a precursor of malonaldehyde. In this formula,  $C$  is the concentration of MA,  $A$  represents the absorbance of the colored complex at 532 nm, and  $w$  is the mass of the oil.

**Statistical Analysis.** All determinations were performed in triplicate. Data are reported as mean ± standard deviation (SD). Normality was tested using Sigma stat. Analysis of variance and Tukey's standardized test were carried out at a level of  $p < 0.05$  to assess the significance of differences among mean values.

## RESULTS AND DISCUSSION

**Fatty Acid Profile of DHASCO.** The fatty acid profile of DHASCO before and after modification with CA and catalyzed by *Pseudomonas* sp. is shown in **Table 1**.

The original DHASCO contained 37.14% DHA and small amounts of other polyunsaturated fatty acids. Lauric (3.46%), myristic (12.92%), and palmitic (10.52%) acids were the saturated fatty acids in DHASCO. Kyle (18) reported that DHASCO oil prepared from microalgae *Cryptocodinium cohnii* contained 43.53% DHA. The present study indicates that DHASCO contained a relatively high level of oleic acid (26.95%) as compared with 14.67% reported by Kyle (18). The differences between the fatty acid profile presented here and those reported by Kyle (18) might be due to variation in the source and batch of the oil, method of preparation, and fermentation conditions employed.

**Enzymatic Incorporation of Capric Acid into DHASCO.** *Enzyme Screening.* Five lipases from *C. antarctica*, *M. miehei*,

**Table 2.** Effect of Enzyme Type on the Incorporation (Percent) of Capric Acid into DHASCO<sup>a</sup>

enzyme	DHASCO
<i>Candida antarctica</i>	3.32 ± 0.09a
<i>Mucor miehei</i>	21.0 ± 0.56d
<i>Pseudomonas</i> sp.	31.3 ± 0.54e
<i>Candida rugosa</i>	10.9 ± 0.43c
<i>Aspergillus niger</i>	6.6 ± 0.84b

<sup>a</sup> Values are means of triplicate determinations ± standard deviation. Values with different letters are different ( $p < 0.05$ ) from one another.

**Table 3.** Effect of Mole Ratio of Substrates on Capric Acid (CA) Incorporation into DHASCO<sup>a</sup>

oil	mole ratio of DHASCO/CA		
	1:1	1:2	1:3
DHASCO	13.8 ± 0.34a	22.6 ± 2.08b	31.36 ± 0.54c

<sup>a</sup> Values are means of triplicate determinations ± standard deviation. Values with different letters are different ( $p < 0.05$ ). <sup>b</sup> Mole ratios of DHASCO to CA were 1:1, 1:2, and 1:3. The reaction mixture contained 500 mg of oil, 105–315 mg of CA, 10% *Pseudomonas* sp. lipase preparation, distilled water (2 wt % of enzyme and substrates), and 3.0 mL of hexane. The reaction mixture was incubated at 45 °C for 24 h in an orbital water bath at 250 rpm.

*Pseudomonas* sp., *C. rugosa*, and *A. niger* were screened for their ability to incorporate capric acid (CA) into DHASCO. Results indicated that lipase PS-30 from *Pseudomonas* sp. was most effective (**Table 2**). These findings agree with those Zhou et al. (19), who reported that lipase from *Pseudomonas fluorescens* gave the highest incorporation of caprylic acid (C8:0) into fish oil. This is despite the activity of enzymes tested (*C. antarctica*, 554 units; *M. miehei*, 13613 units; *Pseudomonas* sp., 11936 units; *A. niger*, 8142 units; and *C. rugosa*, 38707 units). Because acidolysis of DHASCO with CA was best achieved using lipase from *Pseudomonas* sp., this enzyme was selected for subsequent experiments.

**Mole Ratio Effect.** The effect of mole ratio of substrates on the incorporation of CA into DHASCO is shown in **Table 3**. The mole ratio of DHASCO to capric acid was varied from 1:1 to 1:3. When the mole ratio of substrates increased from 1:1 to 1:3, the incorporation of CA increased accordingly. The optimum incorporation of CA into DHASCO (31.32%) may be obtained at an oil to CA mole ratio of 1:3 because TAG molecules can incorporate a maximum of three fatty acids in their backbone. The results of this study agree with those of Soumannou et al. (20), who observed that the stoichiometric ratio of 1:3 was optimum for the production of SL via esterification of 2-monoolein and caprylic acid in the presence of Lipozyme in hexane. On the other hand, Shimada et al. (21) reported that as the weight ratio of caprylic acid to tuna oil increased from 2 to 8, incorporation of caprylic acid into tuna oil did not change significantly (from 41 to 42 mol %). Therefore, the optimum weight ratio of 2:1 for caprylic acid/tuna oil was selected for optimum incorporation of caprylic acid. Furthermore, Lee and Akoh (2) found that the highest incorporation of caprylic acid into peanut oil (containing 58% oleic acid) was obtained at a substrate (peanut oil/caprylic acid) mole ratio of 1:2.

**Enzyme Load Effect.** The effect of enzyme load (percent) on the incorporation of CA into DHASCO is shown in **Figure 1**. As the enzyme load increased from 2 to 10%, the incorporation of CA into DHASCO increased gradually, reaching a maximum (26.4%) at 10%, but at above 10% enzyme load, a slight



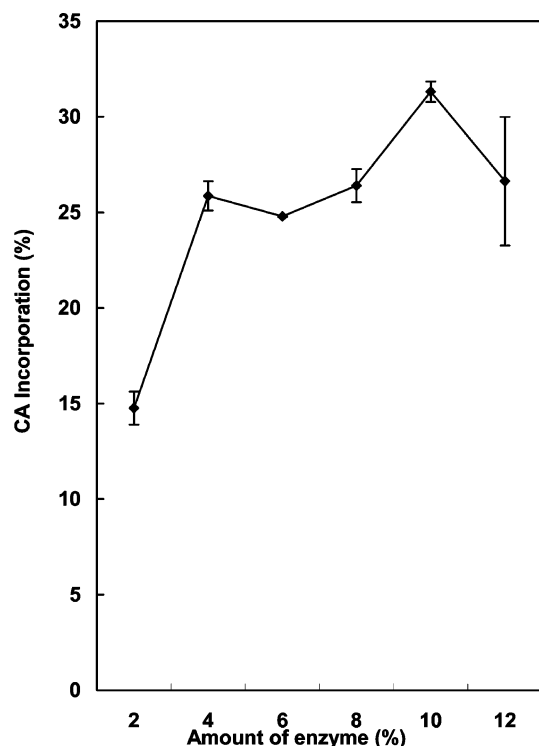


Figure 1. Effect of enzyme load on the incorporation of capric acid into DHASCO.

decrease was noted. This may be due to factors such as deficiency of the available water for hydration of the enzyme because the amount of added water remained constant at a 2% (w/w) level, regardless of the amount of enzyme added. Results of this work agree with those of Jennings and Akoh (10), who reported that a maximum level of CA incorporation (41.4%) into fish oil was achieved with an enzyme load of 10%. Furthermore, results of this study agree with the findings of Akoh and Huang (6), who reported that an enzyme load of 10% was adequate for acidolysis of triolein with caprylic acid. Recently, Senanayake and Shahidi (13) observed similar results when incorporating CA into seal blubber oil; the incorporation of CA was increased up to 10% enzyme load. Although a better incorporation of CA into DHASCO was obtained with a 10% enzyme load, an enzyme amount of 4% was selected for the remaining experiments because this amount of enzyme was appropriate when only a marginal improvement in the yield at a higher enzyme level, and hence the overall economy of the process, was considered.

**Time Course.** The time course of lipase-assisted acidolysis of CA into DHASCO using PS-30 from *Pseudomonas* sp. is shown in Figure 2. As the time progressed from 12 to 24 h, incorporation of CA into DHASCO increased significantly, reaching a maximum at 24 h, followed by a dramatic decrease (0.57%) at 48 h. This phenomenon may be due to denaturation of the lipase or possible hydrolysis of the resultant product. The present results agree with those of Kim et al. (22), who modified perilla oil (containing 60%  $\alpha$ -linolenic acid) with caprylic acid using two lipases. After 24 h of incubation in *n*-hexane, caprylic acid was incorporated at a level of 48.5 mol % with lipase from *Rhizomucor miehei* and at 51.4 mol % with lipase from *Thermomyces lanuginose*. The results reported here are in contrast with the findings of Jennings and Akoh (10), who reported that the highest incorporation (41.2 mol %) into fish oil occurred at 48 h, and those of Senanayake and Shahidi (13), who found that as the reaction time for acidolysis of seal blubber

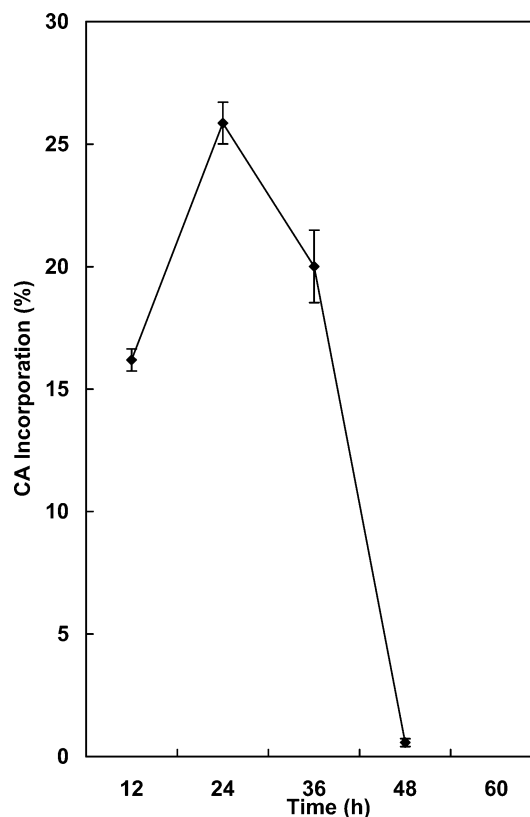
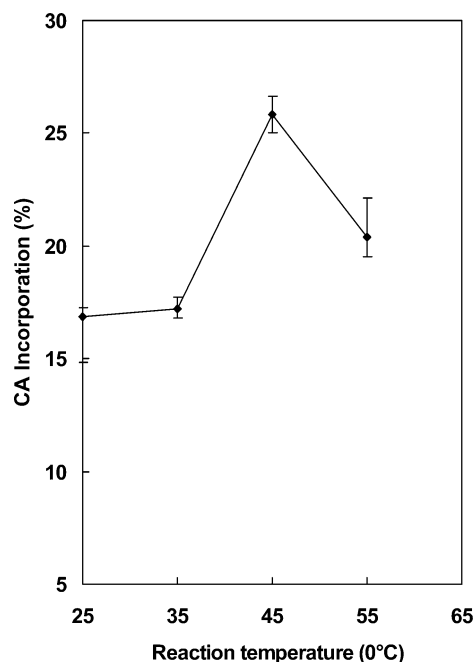


Figure 2. Effect of time course on the incorporation of capric acid into DHASCO.

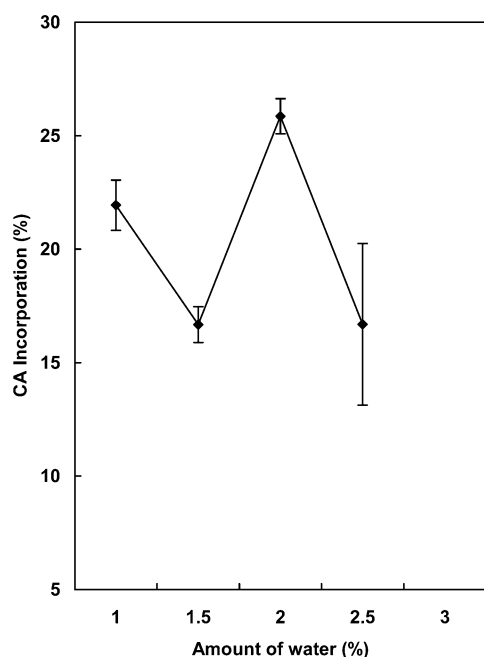
oil with CA increased from 24 to 72 h, the CA incorporation was increased from 25.4 to 29.6%.

**Reaction Temperature Effect.** The effect of temperature on the reaction rate of enzyme-catalyzed reaction, prior to denaturation temperature, is a well-established phenomenon. Thus, to determine the optimum temperature for the incorporation of CA into DHASCO, the temperature was varied from 25 to 55 °C as shown in Figure 3; values gradually increased from 35 to 45 °C. The optimum incorporation of CA into DHASCO (25.86%) was obtained at 45 °C. When the temperature was increased above 45 °C, CA incorporation into DHASCO declined. In general, a 10 °C increase in temperature results in doubling of the reaction rate and hence a higher incorporation of CA at 45 °C. A similar observation was made by Senanayake and Shahidi (13) for CA incorporation into seal blubber oil upon increasing the temperature from 30 to 50 °C, reaching a maximum (26.9%) at 45 °C. On the other hand, Shimada et al. (21) observed that incorporation of caprylic acid into tuna oil, rich in DHA and EPA, was almost the same (43–45 mol %) over the temperature range of 30–45 °C. The results presented here show that *Pseudomonas* sp. lipase is more reactive at 45 °C than at 25–35 °C. Therefore, a reaction temperature of 45 °C was used for the rest of the experiments.

**Effect of Water Content.** The presence of a small amount of water on the surface of the enzyme is required to maintain its three-dimensional structure. However, excess water usually leads to hydrolysis. The effect of water (1–2.5%) on the incorporation of CA into DHASCO is shown in Figure 4. The highest incorporation of capric acid into DHASCO (25.86%) was noted at a 2% (w/w) water level, after which the efficacy of CA incorporation declined, possibly due to dominance of the reverse hydrolysis reaction; this possibility was not explored any further in this study. Senanayake and Shahidi (13) noted that the highest CA incorporation (25.4%) into seal blubber oil was obtained at



**Figure 3.** Effect of reaction temperature on the incorporation of capric acid into DHASCO.



**Figure 4.** Effect of added water on the incorporation of capric acid into DHASCO.

a 1% (w/w) water content. Huang and Akoh (23) reported that the amount of added water had little impact on the incorporation of caprylic acid into triolein when the water content was altered from 0 to 0.06%. On the basis of the above, 2% (w/w) water was used in subsequent experiments.

**Positional Analysis of Structured Lipids.** The positional distribution of fatty acids in modified and unmodified DHASCO was determined (Table 4). Positional distribution analysis of unmodified DHASCO revealed that 29.69% of DHA was located at the sn-2 position of TAG molecules, which is somewhat lower than that reported by Myher et al. (24).

The positional distribution of fatty acids in DHASCO modified with CA showed that DHA was located to a higher

extent in the sn-2 position, as compared to the sn-1 + sn-3 positions. However, CA was mainly located at the sn-1 and sn-3 positions, confirming the preference of pancreatic lipase for medium-chain fatty acids over long-chain fatty acids during hydrolysis. The present results agree with those of Senanayake and Shahidi (13), who reported that CA was mainly located at sn-1 and sn-3 positions (35.9%) of SL produced via acidolysis of seal blubber oil with CA. Approximately 12% of CA was in the sn-2 position, possibly as a result of acyl migration. Furthermore, Shimada et al. (21) reported a similar fatty acid composition for the sn-2 position of tuna oil before and after acidolysis with caprylic acid, thus suggesting that caprylic acid was incorporated in the sn-1 and sn-3 positions of tuna oil.

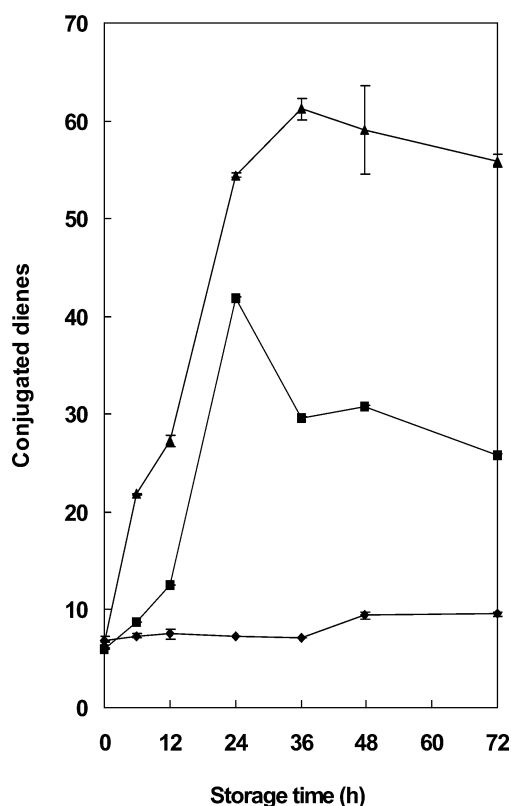
**Conjugated Dienes.** Figure 5 shows the CD values of DHASCO modified in the presence of lipase and subjected to process steps without lipase as well as the control unmodified oil. The CD values of unmodified oil did not change during the storage, from 6 to 72 h, indicating good stability of the unmodified DHASCO. As the storage time was extended to 24 h, the CD values of DHASCO-based SL increased sharply and peaked (41.92) at 24 h. The sharp increase in the CD might be accounted for by the formation of more and more hydroperoxides as primary products of oxidation. After 24 h of storage, the CD values decreased to 27.31, possibly due to the breakdown of unstable hydroperoxides. The oxidative stability of modified DHASCO oil reached a plateau. The present results indicate that modification of DHASCO resulted in its lower stability, possibly due to the loss of natural antioxidants present in the oil during the process of preparation of SL. This possibility was confirmed when starting materials were subjected to the same reaction process in the absence of any enzyme. The oil so treated was indeed considerably less stable than the control unmodified oil (Figure 5). Furthermore, acidolysis of DHASCO with free fatty (CA), with or without lipase, induces a similar degree of oxidation as reflected in CD values. The catalytic effect of the carboxylic groups of free fatty acids might have played a role in this observation (25, 26). In addition, access of oxygen to the unsaturated sites might be easier in the modified oils containing medium-chain CA as compared to long-chain fatty acids in the unmodified counterparts. The modified DHASCO had a lower stability compared to the original oil, as such.

**Thiobarbituric Acid Reactive Substances.** The 2-thiobarbituric acid (TBA) test is widely used for assessing the secondary products of lipid oxidation related to the amount of aldehydes and dialdehydes in the oxidized lipids. TBARS values of modified DHASCO in the presence of lipase and without lipase as well as the control unmodified oils are shown in Figure 6. As the accelerated storage period was extended up to 72 h, TBARS values of enzymatically modified DHASCO increased gradually. TBARS of the modified oil were considerably higher than those of the original oil over the entire storage period. However, there was no difference ( $p > 0.05$ ) between enzymatically modified DHASCO and the mixture of reactants subjected to the same reaction condition in the absence of any enzyme. The presence of free fatty acids (CA) in the reaction mixture, with or without enzyme, induces a similar oxidation degree as indicated by TBARS, possibly due to a catalytic effect of the carboxylic groups of free fatty acids on the formation of free radicals (25, 26). As explained earlier, removal of antioxidants during the process is responsible for the compromised stability of the modified product. Meanwhile, TBARS values of the control unmodified DHASCO, as such, remained nearly constant during the entire storage period (6–72 h), indicating its good stability under Schaal oven conditions at 60 °C. The general

**Table 4.** Positional Distribution (sn-2 and sn-1 + sn-3) of Fatty Acids in Modified and Unmodified DHASCO

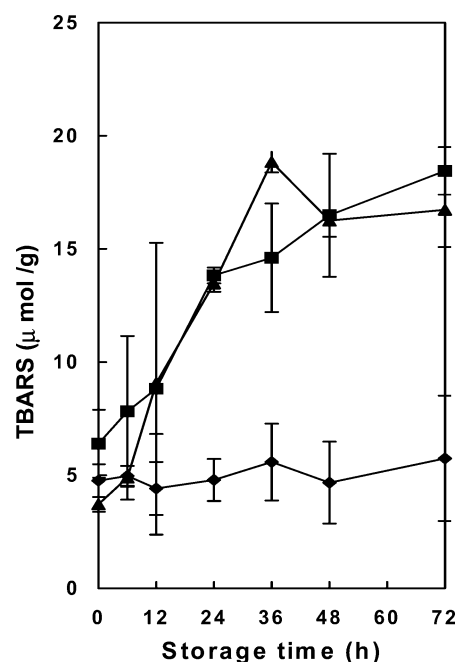
fatty acid	unmodified		modified	
	sn-2	sn-1 + sn-3	sn-2	sn-1 + sn-3
C10:0	1.49 ± 0.13 (41.3)	0.61 ± 0.28 (58.7)	5.61 ± 2.08 (5.97)	16.9 ± 7.11 (94.0)
C12:0	3.81 ± 0.73 (36.70)	3.88 ± 0.52 (63.3)	1.78 ± 0.16 (26.7)	2.72 ± 0.81 (73.3)
C14:0	7.11 ± 1.06 (17.5)	12 ± 2.86 (82.5)	5.69 ± 1.29 (25.3)	7.06 ± 1.28 (74.8)
C16:0	8 ± 1.98 (28.4)	10.8 ± 3.37 (71.6)	8.67 ± 1.62 (45.2)	9.58 ± 1.17 (54.8)
C18:1 <i>n</i> -9	28.8 ± 2.44 (36.1)	24 ± 3.06 (63.9)	33.4 ± 0.59 (41.3)	21.7 ± 1.46 (58.7)
C18:2 <i>n</i> -6	4.51 ± 0.94 (35.2)	1.52 ± 2.80 (64.8)	1.40 ± 0.09 (32.6)	1.23 ± 0.38 (67.4)
C22:6 <i>n</i> -3	25.9 ± 6.00 (23.3)	38.9 ± 3.75 (76.7)	39.9 ± 4.79 (41.4)	35.7 ± 6.91 (58.6)

<sup>a</sup> Values in parentheses indicate percent fatty acid distribution of total triacylglycerols present at the sn-1 + sn-3 and sn-2 positions. These values are % fatty acid at the sn-2 position/% fatty acids in triacylglycerols × 3) × 100; for sn-1 + sn-3 = 100 – sn-2.

**Figure 5.** Conjugated diene values of (■) modified DHASCO in the presence of lipase and (▲) without lipase as well as (◆) the unmodified control stored under Schaal oven conditions at 60 °C.

increase in TBARS values of modified DHASCO during the storage time may be due to the breakdown of lipid hydroperoxides and the production of secondary oxidation products. These results agree with those of Akoh and Moussata (15), who reported that SL produced via acidolysis of canola and fish oils with caprylic acid had higher TBA values than their unmodified counterparts.

**Conclusions.** Lipase-assisted production of structured lipids via acidolysis of DHASCO with CA was better achieved using 4% lipase from *Pseudomonas* sp. and mole ratio of CA to DHASCO of 3:1 at 45 °C over a 24 h incubation period. Examination of positional distribution of fatty acids on the glycerol backbone of modified DHASCO indicated that CA was concentrated in the sn-1,3 positions of the TAG molecules. DHA was present mainly at the sn-2 position of the TAG molecule of modified DHASCO. Modified DHASCO was more prone to oxidation than its unmodified counterpart, as such. Loss of endogenous antioxidants during the processing was found to be responsible for this observation. Therefore, addition of

**Figure 6.** TBARS values of (■) modified DHASCO in the presence of lipase and (▲) without lipase as well as (◆) the unmodified control stored under Schaal oven conditions at 60 °C.

appropriate antioxidants to the resultant modified oils is recommended. Acidolysis of DHASCO with free fatty (CA), with or without lipase, produces a similar degree of oxidation in the resultant product/mixture as reflected in CD and TBARS values.

#### ABBREVIATIONS USED

CD, conjugated dienes; DHA, docosahexaenoic acid; DHASCO, docosahexaenoic acid single cell oil; MCFA, medium-chain fatty acids; MCT, medium-chain triacylglycerol; PUFA, polyunsaturated fatty acids; TBA, 2-thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TLC, thin-layer chromatography; TAG, triacylglycerols; CA, capric acid.

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