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Enzymatic incorporation of capric acid into a single cell oil rich in docosahexaenoic acid and docosapentaenoic acid and oxidative stability of the resultant structured lipid

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Abstract

Lipase-assisted acidolysis of a single cell oil rich in docosahexaenoic acid (DHA, C22: 6*n*−3) and docosapentaenoic acid (DPA, C22:5*n*−6), commericallly known as the OMEGA-GOLD oil, with capric acid (CA, C10:0) was carried out. Screening of five commercially available lipases was carried out for oil to CA mole ratio of 1:3 at a temperature of 45 °C, a reaction time of 24 h, 4% (w/w of substrates) PS-30 lipase from *Pseudomonas* sp. and 2% (w/w of substrates and enzyme) water content. Stereospecific analysis indicated that CA was present mainly in the sn-1,3 positions of the triacylglycerol (TAG) molecules while DHA and DPA were mainly esterified to the sn-2 position. Enzymatically modified oil generally had higher conjugated diene (CD) and 2-thiobarbituric acid (TBA) values than its unmodified counterpart. However, the oil subjected to the same reaction steps in the absence of any enzyme, exhibited a significantly ($p < 0.05$) lower oxidative stability. Therefore, removal or alteration of endogenous antioxidants during the process may be primarily responsible for the compromised stability of the modified oil.

Keywords: Acidolysis; Lipase; Structured lipids; Medium-chain fatty acids; Capric acid (C10:0); The OMEGA-GOLD oil; Oxidative stability; Conjugated dienes; Thiobarbituric acid reactive substances; Positional distribution

1. Introduction

Structured lipids (SL) are defined as triacylglycerols (TAG) modified to change the fatty acid composition and/or their location in the glycerol backbone via chemical or enzymatic means. Acidolysis is referred to the exchange of acyl group between an acid (fatty acid) and an ester. Acidolysis is an efficient method for incorporating specific fatty acids into triacylglycerols to achieve a desired functionality. SL are also produced via acidolysis in order to enhance or change the physical and/or chemical properties of TAG.

OMEGA-GOLD oil is a commercial oil derived from microalgae *Schizochytrium* sp. via a fermentation process (Zeller, Barclay, & Abril, 2001). It is a rich source of docosahexaenoic acid (DHA, *n*−3) (41%) and docosapentaenoic acid (DPA, *n*−6) (18%). Medium-chain fatty acids (MCFA) are saturated fatty acids with a carbon chain length ranging from 6 to 12 and are prepared mostly from oils of tropical plants, such as those of coconut and palm. Medium-chain triacylglycerols (MCT) exhibit unique structural and physiological features. MCT rapidly clear from the blood (Babayan, 1987). Structured lipids containing MCFA and long-chain fatty acids (LCFA) have modified absorption rates because MCFA are quickly absorbed and oxidized/metabolized for energy while LCFA are oxidized very slowly. These modified lipids are structurally and metabolically different from simple physical mixtures of MCT and long-chain triacylglycerols (LCT).

In order to examine the oxidative stability of the modified and unmodified OMEGA-GOLD oil, a number of stability tests may be employed. These tests

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include chemical and instrumental techniques (Rossell, 1991; Shahidi & Wanasundara, 1998). The tests detect either the primary or secondary products of lipid oxidation. Conjugated diene (CD) assay is a simple and rapid method for evaluation of the primary products of lipid oxidation (hydroperoxide); it does not require chemical reactions and needs only a small amount of sample in the milligramme range. The 2-thiobarbituric acid (TBA) test, meanwhile, is widely used to examine secondary lipid oxidation products.

Jennings and Akoh (1999) successfully incorporated CA (43%) into a fish oil triacylglycerols (containing 40.9% EPA and 33.0% DHA) using immobilized lipase (IM 60) from *Rhizomucor miehei*. Akoh and Moussata (2001) modified fish oil as well as canola oil with caprylic acid using lipozyme IM from *Rhizomucor miehei*. Their results showed that Lipozyme IM incorporated a higher level of caprylic acid (40.1%) into canola oil as than fish oil (29.5%). Shimada et al. (1996) reported that caprylic acid (8:0) was successfully incorporated into tuna oil containing DHA via acidolysis using immobilized lipase from *Rhizomucor delemar*. This specific lipase catalysed the exchange of almost 65% of the fatty acids at the sn-1,3 positions with caprylic acid. Senanayake and Shahidi (2002a, 2002b) used acidolysis to incorporate CA into seal blubber oil. They used immobilized lipase, Lipozyme-IM from *Mucor miehei* and found, incorporation of 25.4% CA into seal blubber oil after 24 h of incubation at 45 °C and a 1% water level. Lipozyme IM catalyzed incorporation of caprylic acid, up to 70%, in the sn-1,3 positions of the modified fish oil (Xu, Balchen, Hoy, & Adier-Nissen, 1998). In another study, Xu (2000) produced SL containing 40% caprylic acid and 35% EPA and DHA with less than 3% caprylic acid at the sn-2 position via acidolysis of menhaden oil with caprylic acid using Lipozyme IM in a solvent-free system.

Iwasaki, Han, Narita, Rosu, and Yamane (1999) reported lipase-assisted acidolysis of a single-cell oil containing docosahexaenoic acid (DHA, *n*−3) (41%) and DPA, *n*−6 (18%) with caprylic acid. Two lipases from *Rhizomucor miehei* and *Pseudomonas* sp. were used as biocatalysts. The end products were modified oils containing caprylic acid in the sn-1,3 positions and DHA and DPA at the sn-2 position of the glycerol backbone. *Pseudomonas* sp. lipase catalysed the exchange of more than 60% of fatty acids in the single-cell oil with caprylic acid, while *Rhizomucor miehei* lipase catalysed incorporation of 23% of caprylic acid into the single-cell oil.

The objectives of this study were to incorporate capric acid, as a rapid source of energy, into the OMEGA-GOLD oil, optimize the reaction conditions for preparation of OMEGA-GOLD-based SL, determine the positional distribution of fatty acids in the enzymatically-modified OMEGA-GOLD oil, and to evaluate the oxidative stability of the resultant SL.

2. Materials and methods

2.1. Materials

Two lipases from *Candida anatrctica* (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 Enzymes (Troy, VA). The OMEGA-GOLD oil was obtained from Monsanto (St. Louis, MO). 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 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, purchased from Fisher Scientific (Nepean, ON).

2.2. Methods

2.2.1. Acidolysis reactions

In general, the OMEGA-GOLD oil (500 mg) was mixed with CA, at different mole ratios of oil to CA, ranging from 1 to 3, in screw-capped test tubes, and then lipase (2–10% by weight of substrates) and water (1–2.5% by weight of substrates and enzyme) were added in 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. All experiments were reported in the Section 2.2 were triplicated.

2.2.2. Separation of acylglycerols following acidolysis

After a given time period, the reaction was stopped by addition of a mixture of acetone and ethanol (20 ml; 1:1, v/v). In order 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 colour of the solution turned pink. The acylgylcerols were then extracted into hexane (25 ml). The two layers (aqueous, hexane) were allowed to separate in a separatory funnel, and the lower aqueous layer was discarded. The hexane layer was passed through a bed of anhydrous sodium sulphate to remove any residual water. The 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.

2.2.3. Preparation of fatty acid methyl esters

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 sulphuric 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 (1ml) 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 twice, each with 1.5 ml of distilled water. The hexane layer (the upper layer) was then separated and the solvent evaporated under a stream of nitrogen. FAMEs were then dissolved in 1.0 ml of carbon disulphide and used for subsequent gas chromatographic analysis.

2.2.4. Analysis of FAMEs by gas chromatography

The FAMEs were analysed using a Hewlett–Packard 5890 Series II gas chromatography (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, 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. Ultra high purity (UHP) helium was used as a carrier gas at a flow rate of 15 ml/min. Data were treated using a Hewlett–Packard 3365 Series II Chem Station Software (Agilent, Palo Alto, CA). 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.

2.2.5. Hydrolysis by pancreatic lipase

Hydrolysis of the modified oil by pancreatic lipase was achieved essentially as described by Christie (1982) with minor modifications. Tris–HCl buffer (5.0 ml; 1 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 addition of 5.0 mg of porcine pancreatic lipase (EC 3.11.3; Sigma). The mixture was subsequently placed in a gyrotory 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 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 removed and washed twice with distilled water and passed through a bed of anhydrous sodium sulphate. 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% (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 hexane/diethyl ether/acetic acid (70:30:1, v/v/v) for 40–50 min and then allowed to dry in 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 earlier.

2.2.6. Oxidative stability tests

The oxidative stabilities of the structured lipid produced from the OMEGA-GOLD oil via acidolysis with CA as well as the original oil, as such, or subjected to process steps in the absence of any enzyme 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 (Thelco, Model, Prcision 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 oxidation stability experiments were also carried out in triplicate.

2.2.7. Conjugated dienes

Conjugated dienes (CD) in the oils were determined according to the IUPAC (1987) method 20505. Oil samples (0.02–0.04 g) were weighted into 50 ml volumetric flasks, dissolved in iso-octane (2,2,4-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, Palo Alto, CA). Conjugated dienes were calculated using the formula $\mathrm{CD} = A/(C \times D)$, where A is the absorbance at 234 nm, C is the concentration of the solution in g/ml, and D is the length of the cell in cm.

2.2.8. Thiobarbituric acid reactive substances determination

The determination of TBARS was carried out as described by the AOCS (1990) method Cd 19–90. Oil samples (0.05–0.10 g) were accurately weighed into 25 ml volumetric flasks and dissolved in a small volume of 1-butanol and made up to the mark with the same solvent. Five millilitres of the mixture (oil and solvent) were transferred into a dry screw-capped test tube, and then 5 ml of freshly prepared TBA reagent (0.5 g TBA in 250 ml 1-butanol) were 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 coloured complex was read at 532 nm. The amount of malonaldehyde (MA) equivalents in μmol per gramme of oil, expressed as TBARS values, was calculated using the formula $C = (0.415 * A_{532})/w$, where the factor 0.415 was obtained from a standard regression line using 1,1,3,3-tetramethoxypropane as a precursor of malonaldehyde. In this formula, C is the concentration as MA equivalents; A represents the absorbance of the coloured complex at 532 nm, and w is the mass of the oil.

2.2.9. Statistical analysis

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

3. Results and discusion

3.1. The fatty acid profile of the OMEGA-GOLD oil

Table 1 shows fatty acid composition of the OMEGA-GOLD oil before and after modification with CA. The OMEGA-GOLD oil served as a concentrated source of DHA (36.8%) and the Ω-6 DPA (14.1%). The oil also contained myristic (8.3%) and palmitic (23.4%) acids. Other fatty acids in OMEGA-GOLD oil were EPA (C20: 5*n*−3, 3.10%) and eicosatrienoic acid (C20: 3*n*−6, 3.21%). The results reported here agree with those of Zeller et al. (2001), who reported that the OMEGA-GOLD oil contained 41% DHA, 18% DPA, 9% myristic acid, and 22% palmitic acid.

Table 1
Fatty acid composition of the OMEGA-GOLD oil triacylglycerols before and after modification with CA (wt%)[a]

Fatty acid	Before modification	After modification[b]
C10:0	0.07 ± 0.01	20.3 ± 0.12
C14:0	8.3 ± 0.03	5.64 ± 0.06
C15:0	0.46 ± 0.01	0.29 ± 0.01
C16:0	23.4 ± 0.23	12.9 ± 0.07
C16:1	1.59 ± 0.03	1.2 ± 0.01
C18:0	0.55 ± 0.01	0.37 ± 0.01
C18:1*n*−9	0.72 ± 0.2	0.45 ± 0.01
C20:3*n*−6	3.21 ± .11	1.46 ± 0.23
C20:4*n*−6	0.29 ± 0.5	0.20 ± 0.03
C20:5*n*−3	3.10 ± .12	3.20 ± 0.03
C22:5*n*−6	14.1 ± .15	13.1 ± 0.01
C22:6*n*−3	36.8 ± 0.39	34.1 ± 0.37
Others	7.33	6.77

[a] Values are mean values of triplicate determinations ± SD.

[b] The reaction mixture contained 500 mg OMEGA-GOLD oil, 315 mg CA, 4% (w/w) enzyme for *Pseudomonas* sp. and 3.0 ml hexane. The reaction mixture was incubated at 45 °C for 24 h in an orbital shaking water bath at 250 rpm.

3.2. Enzymatic incorporation of capric acid into the OMEGA-GOLD oil

Five lipases, from *C. antarctica*, *M. miehei*, *Pseudomonas* sp., *C. rugosa* and *A. niger*, were screened for their ability to incorporate capric acid (CA) into the OMEGA-GOLD oil (Table 2). *Pseudomonas* sp. gave the highest degree of incorporation of CA into the OMEGA-GOLD oil (27.9%). The results reported here agree with the findings of Zhou, Mu, and Adler-Nissen (2000), who reported that lipase from *Pseudomonas fluoresescens* gave the highest incorporation of caprylic acid (C8:0) into fish oil and those of Iwasaki et al. (1999) who reported that *Pseudomonas* sp. lipase catalyzed the exchange of more than 60% of the fatty acids in a single-cell oil with caprylic acid. Because acidolysis of the oil with CA was best achieved with lipase from *Pseudomonas* sp., this enzyme was selected for subsequent experiments.

3.3. Mole ratio effect

The effect of mole ratio of substrates on the incorporation of CA into the OMEGA-GOLD oil is shown in Table 3. As the number of moles of CA increased from 1 to 3 for each mole of triacylglycerol, its incor-

Table 2
Effect of enzyme type on the incorporation (%) of capric acid into the OMEGA-GOLD oil

Enzyme	OMEGA-GOLD
C. antarctia	2.17 ± 0.10[b]
M. miehei	19.1 ± 0.24[d]
Pseudomonas sp.	27.9 ± 0.85[e]
C. rugosa	0.10 ± 0.03[a]
A. niger	4.45 ± 0.18[c]

[*] Values are mean values of triplicate experiments and triplicate determinations ± SD. Values with each column with different superscripts are different ($p < 0.05$) from one another.

Table 3
Effect of mole ratio of substrates on CA incorporation (%) into the OMEGA-GOLD oil[a]

Mole ratio oil /CA[b]	1:1	1:2	1:3
OMEGA-GOLD oil	12.7 ± 0.07[a]	24.5 ± 1.88[b]	27.9 ± 0.85[c]

[a] Values are mean values of triplicate determinations ± SD. Values with each row with different superscripts are different ($p < 0.05$) from one another.

[b] Mole ratios of the original OMEGA-GOLD oil 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% by weight of enzyme and substrates) and 3.0 ml hexane. The reaction mixture was incubated at 45 °C for 24 h in an orbital water bath at 250 rpm.

poration into the OMEGA-GOLD increased steadily. The optimum incorporation of CA into the OMEGA-GOLD oil (27.9%) was 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. Shimada et al. (1996) reported that as the mole ratio of caprylic acid to tuna oil increased from 2 to 8, incorporation of caprylic acid into tuna oil changed only marginally from 41 to 42 mol%. Therefore, the mole ratio of 2:1 for caprylic acid to tuna oil gave the optimum incorporation. On the other hand, the present results agree with those of Soumanou, Bornscheuer, Schmid, and Schmid (1998) who observed that the stoichiometric ratio of 1:3 was optimum for the production of SL via esterification of 2-monoolein and caprylic acid with lipozyme in the presence of hexane.

3.4. Enzyme load effect

The effect of enzyme load (%) on the incorporation of CA into the OMEGA-GOLD oil is shown in Fig. 1. As the enzyme load increased from 2 to 10%, incorporation of CA into the OMEGA-GOLD increased gradually, reaching a maximum (22.4%) at 10%. Above the 10% enzyme load, there was a slight decrease in the incorporation of CA into the oil, possibly due to factors such as deficiency of the available water for hydration of the enzyme. As the amount of lipase in the reaction mixture was raised, the amount of added water remained constant at a 2% (w/w) level. The results presented in this work agree with those of Jennings and Akoh (1999) who reported that the maximum level of CA incorporation (41.4%) into fish oil was achieved with an enzyme load of 10%. Akoh and Huang (1995) also reported that an enzyme load of 10% was adequate for acidolysis of triolein with caprylic acid. Recently, Senanayake and Shahidi (2002a, 2002b) observed similar results when incorporating CA into seal blubber oil; incorporation of CA was increased up to 10% enzyme load. Shimada et al. (1996) found that 2%, 4% and 8% enzyme from *Rhizopus delemar* resulted in the incorporation of 40, 44, and 45 mol% caprylic acid into tuna oil, respectively. Although a better incorporation of CA into the OMEGA-GOLD oil was achieved with a 10% enzyme load, an enzyme load of 4% was selected for subsequent experiments because this amount of enzyme was appropriate when considering a marginal increase in the yield at a higher enzyme level and hence the overall economy of the process.

3.5. Time course

Fig. 2 shows the time course of lipase-assisted acidolysis of OMEGA-GOLD oil with CA using PS-30 from

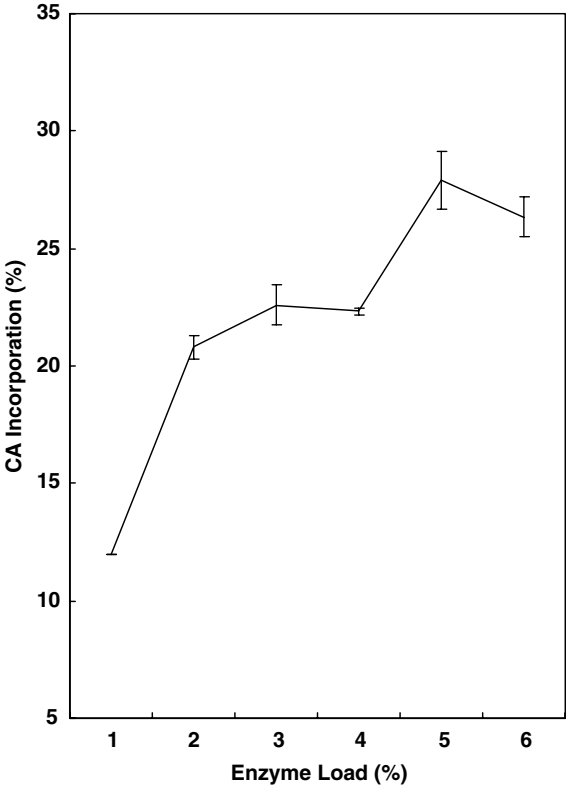

Fig. 1. The effect of enzyme load on the incorporation of capric acid into the OMEGA-GOLD oil.

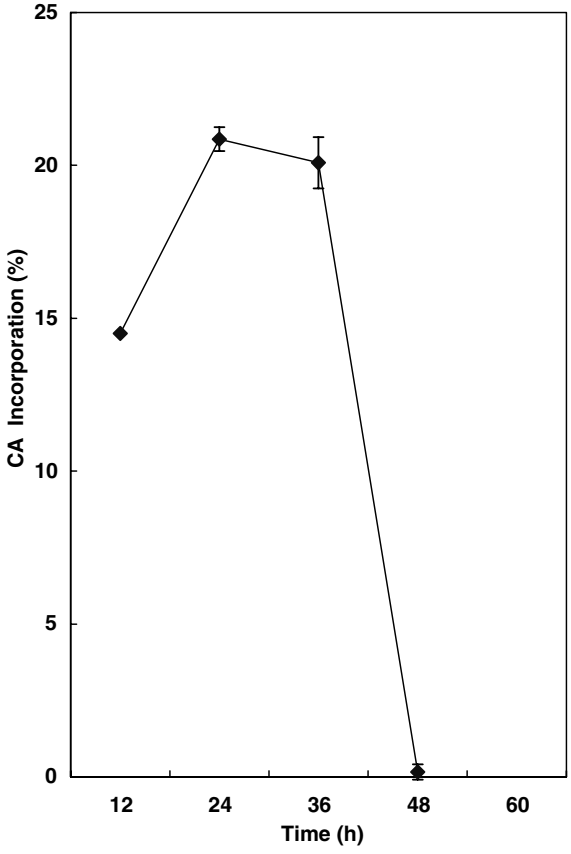

Fig. 2. The effect of time course on the incorporation of capric acid into the OMEGA-GOLD oil.

Pseudomonas sp. As the time progressed from 12 to 24 h, the percent CA incorporation into the OMEGA-GOLD oil increased significantly, reaching a maximum at 24 h, followed by a dramatic decrease in CA incorporation into the OMEGA-GOLD oil (0.16%) at 48 h. This phenomenon may be due to the prolonged incubation time at high temperatures, resulting in denaturation of the lipase and thus loss of its three dimensional structure. Another possible explanation for the decreased incorporation of CA into the OMEGA-GOLD oil may be the occurrence of the reverse reaction. Mu, Xu, and Hoy (1998) made a laboratory scale continuous reactor for the synthesis of SL containing linoleic acid (18:2*n*−6) located on the sn-2 position and capric acid in the sn-1 and sn-3 positions. Their results showed that incorporation of capric acid (48%) into the triacylglycerol containing linoleic acid was obtained after a 7 h reaction. Jennings and Akoh (2000) successfully incorporated capric acid (up to 27%) into rice bran oil in 72 h using immobilized lipase from *Rhizomucor miehei*. The results reported here are in contrast to the findings of Jennings and Akoh (1999) who reported that the highest incorporation (41.2 mol%) into fish oil occurred at 48 h and those of Senanayake and Shahidi (2002a, 2002b) who found that as the reaction time of acidolysis of seal blubber oil with CA increased from 24 to 72 h, the CA incorporation was increased from 25.4% to 29.6%.

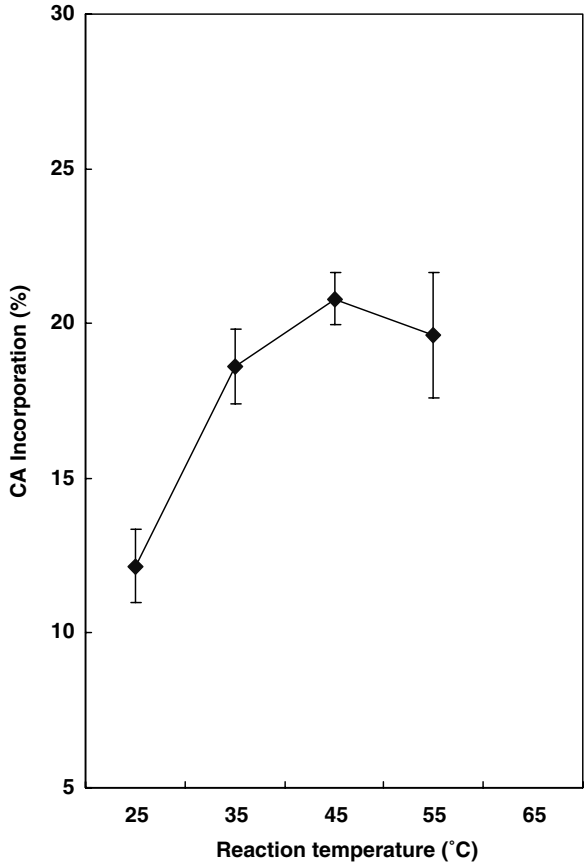

Fig. 3. The effect of reaction temperature on the incorporation of capric acid into the OMEGA-GOLD oil.

3.6. Reaction temperature effect

This reaction parameter was investigated to determine the optimum temperature for PS-30 from *Pseudomonas* sp. lipase for incorporation of CA into the OMEGA-GOLD oil as shown in Fig. 3. The incorporation of CA into the OMEGA-GOLD oil gradually increased as the reaction temperature increased from 25 to 45 °C, reaching an optimum (20.8%) at 45 °C. Low CA incorporation into the oil was noticed at lower temperatures (25–35 °C). When temperature increased above 45 °C, CA incorporation into the OMEGA-GOLD oil declined. In general, a 10 °C increase in temperature results in doubling of the reaction rate and hence a higher incorporation of CA. A similar observation was made by Senanayake and Shahidi (2002a, 2002b) for CA incorporation into seal blubber oil upon increasing temperature from 30 to 50 °C, reaching a maximum (26.9%) at 45 °C. On the other hand, Shimada et al. (1996) observed that incorporation of caprylic acid into tuna oil, rich in DHA and EPA, was almost the same (43–45 mol%) in the temperature range of 30–45 °C.

The results presented here showed 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.

3.7. Effect of added water

Controlling the amount of water is very important in enzymic lipid modification. Presence of a small amount of water on the surface of the enzyme is required for maintaining its three-dimensional structure. However, excess water usually leads to hydrolysis. Fig. 4 shows the effect of water (1–2.5%) on the incorporation of CA into the OMEGA-GOLD oil. CA incorporation (%) into the OMEGA-GOLD oil changed marginally ($p > 0.05$) as the amount of water increased from 1 to 2.5%. The highest incorporation of capric acid into the OMEGA-GOLD oil (20.8%) was observed at a 2% (w/w) water content. Further increase in the content of water, above 2%, led to a decline in CA incorporation into the oil, possibly due to the presence of excess water, which usually leads to hydrolysis.

Senanayake and Shahidi (2002a, 2002b) noticed that the highest CA incorporation (25.4%) into seal blubber oil was obtained at a 1% (w/w) water content. Huang and Akoh (1996) reported that the amount of added water had little impact on incorporation of caprylic acid into triolein when the water content was altered from 0.0% to 0.06%.

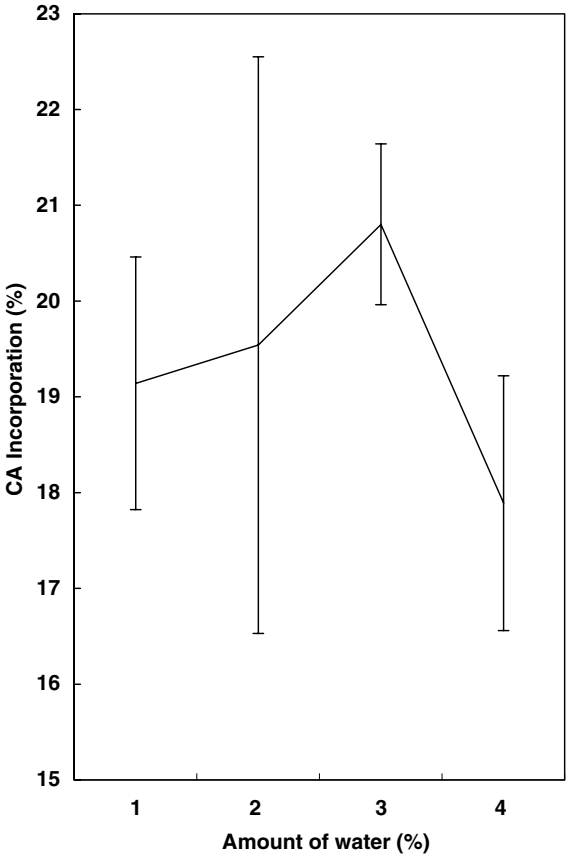

Fig. 4. The effect of added water on the incorporation of capric acid into the OMEGA-GOLD oil.

3.8. Positional distribution of structured lipids

Tables 4 and 5 summarize the results of positional distribution of fatty acids in the unmodified and modified OMEGA-GOLD oil, respectively. DHA and DPA were preferentially located at the sn-2 position in both unmodified and modified oils. Meanwhile, some 87.1% of capric acid was located in position sn-1 and sn-3 of the modified oil (Table 5). Zeller et al. (2001) reported that 23% of DPA + DHA were located at the sn-2 position and 27% present in the sn-1,3 positions of the glycerol backbone of the OMEGA-GOLD oil, using ^{13}C NMR .

Table 4
Positional distribution (sn-2 and sn-1 + sn-3) of fatty acids in the unmodified OMEGA-GOLD oil[a]

Fatty acid	Position	
	sn-2	sn-1 + sn-3
C14:0	5.64 ± 1.19 (22.7)	8.44 ± 0.95 (77.3)
C16:0	22.4 ± 3.16 (31.9)	22.5 ± 0.71 (68.1)
C16:1	1.46 ± 0.28 (30.6)	1.34 ± 0.65 (69.4)
C18:0	1.14 ± 0.39 (69.1)	1.09 ± 0.34 (30.9)
C18:1*n*−9	1.58 ± 0.65 (73.1)	1.57 ± 0.29 (26.9)
C20:4*n*−6	1.84 ± 0.42 (21.1)	2.99 ± 0.39 (78.9)
C20:5*n*−3	1.62 ± 0.86(17.4)	3.31 ± 1.06(82.6)
C22:5*n*−6	15.9 ± 4.34 (37.6)	14.0 ± 3.18 (62.4)
C22:6*n*−3	33.5 ± 6.35 (30.3)	33.4 ± 4.11 (69.7)

[a] 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 acid in triacylglycerols × 3)) × 100; for sn1 + sn-3 = 100−sn-2.

Table 5
Positional distribution (sn-2 and sn-1 + sn-3) of fatty acids in lipase-assisted acidolysis of the OMEGA-GOLD oil with capric acid[a]

Fatty acid	Position	
	sn-2	sn-1 + sn-3
C10:0	7.88 ± 3.16 (12.9)	13.3 ± 1.05 (87.1)
C14:0	4.14 ± 0.42 (24.5)	6.20 ± 0.96 (75.5)
C16:0	14.8 ± 2.97 (38.2)	14.5 ± 0.56 (61.8)
C18:0	0.88 ± 0.03 (79.3)	0.80 ± 0.18 (20.7)
C18:1*n*−9	0.83 ± 0.16 (61.5)	1.00 ± 0.04 (38.5)
C20:4*n*−6	2.95 ± 0.01 (80.6)	3.20 ± 0.15 (19.4)
C20:5*n*−3	1.47 ± 0.03 (15.3)	3.40 ± 0.13 (84.7)
C22:5*n*−6	12.6 ± 1.22 (32.2)	13.0 ± 0.67 (67.8)
C22:6*n*−3	27.9 ± 3.51 (27.3)	33.2 ± 1.12 (72.7)

Fatty acids (C16:1 and C20:5*n*−3) were present at 0–0.5%.

[a] Values in parenthesis 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 acid in triacylglycerols × 3)) × 100; for sn-1 + sn-3 = 100−sn-2.

3.9. Conjugated dienes

The conjugated diene values of the OMEGA-GOLD modified oil and the oil subjected to the same reaction steps in the absence of any lipase as well as the control unmodified oil are shown in Fig. 5. The enzyme-catalyzed structured lipid of the OMEGA-GOLD oil had higher conjugated diene values than the original oil over the entire storage period from 6 to 72 h. As the storage time was extended from 6 to 72 h, the CD values of the modified OMEGA-GOLD and the original oils increased gradually, thus reflecting the formation of more primary products of lipid oxidation. The CD of the modified OMEGA-GOLD oil increased to 55.2 after 72 h of storage under Schaal oven conditions at 60 °C, whereas the corresponding CD value of the unmodified oil was 37.1. This is in contrast to expectation, as incorporation of capric acid (saturated) into the OMEGA-GOLD oil should increase its resistance to oxidation. The present results indicate that enzymatically-modified OMEGA-GOLD oil was less stable than the original oil. This observation might possibly be due to the loss or structural alteration of natural antioxidants present in the oil during the preparation process of SL. This possibility was validated 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 unmodified control oil (Fig. 5). In addition, access of oxygen to the unsaturated sites might be easier in the modified oils containing

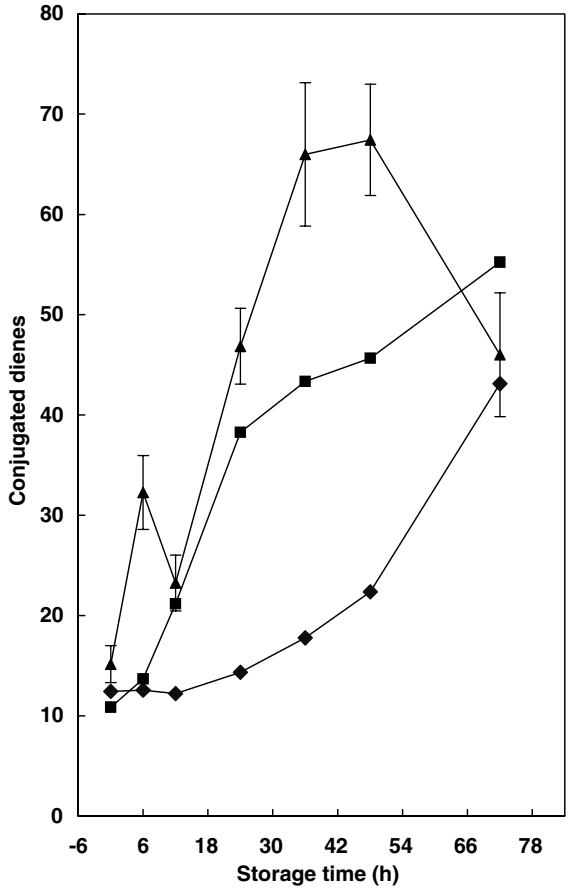

Fig. 5. Conjugated diene values of (■) the modified OMEGA-GOLD oil in the presence of lipase and (●) without lipase as well as (◆) the control unmodified, stored under Schaal oven conditions at 60 °C.

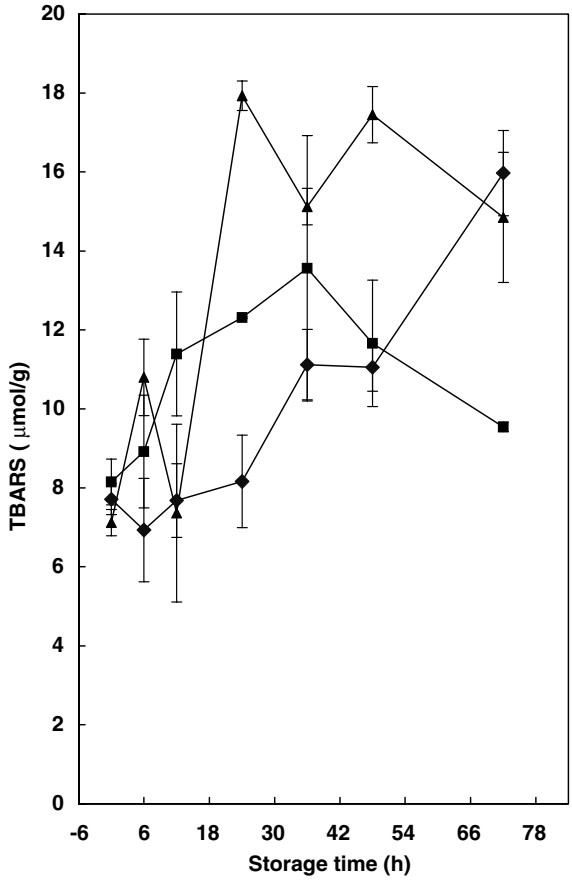

Fig. 6. TBARS values of (■) the modified OMEGA-GOLD oil in the presence of lipase and (●) without lipase as well as (◆) the control unmodified, stored under Schaal oven conditions at 60 °C.

medium-chain CA as compared to long-chain fatty acids in their unmodified counterparts.

3.10. Thiobarbituric acid reactive substances

The TBARS results for the OMEGA-GOLD based-structured lipid and the origin oil are shown in Fig. 6. Results are also shown for subjecting the reactants, in the absence of any enzyme, to the same reaction process. The TBARS values of the unmodified control oil increased progressively as the storage time increased from 6 to 72 h. However, the TBARS values of the modified OMEGA-GOLD oil increased gradually with increasing storage time up to 36 h. After 48 h, TBARS values dramatically decreased. TBARS of the modified oil were considerably higher than those of the original oil over the storage period up to 36 h. The TBARS values of the mixture of reactants subjected to the same reaction conditions in the absence of any enzyme were generally higher than enzymatically modified OMEGA-GOLD oil. Therefore, it appears that removal or structural alteration of antioxidants during the process is responsible for the compromised stability of the modified product. The general increase in TBARS values of the modified OMEGA-GOLD oil during the storage time may be due to the break down of lipid hydroperoxides and production of more secondary oxidation products. These results agree with those of Akoh and Moussata (1998), who reported that SL produced via acidolysis of canola and fish oils with caprylic acid had higher TBA values than their unmodified counterparts.

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