JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Acidolysis of Tristearin with Selected Long-Chain Fatty Acids

FAYEZ HAMAM[†] AND FEREIDOON SHAHIDI*[,†,§]

Departments of Biology and Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3X9, Canada

Five lipases, namely, *Candida antarctica* (Novozyme-435), *Mucor miehei* (Lipozyme-IM), *Pseudomonas* sp. (PS-30), *Aspergillus niger* (AP-12), and *Candida rugosa* (AY-30), were screened for their effect on catalyzing the acidolysis of tristearin with selected long-chain fatty acids. Among the lipases tested *C. antarctica* lipase catalyzed the highest incorporation of oleic acid (OA, 58.2%), γ-linolenic acid (GLA, 55.9%), eicosapentaenoic acid (EPA, 81.6%), and docosahexaenoic acid (DHA, 47.7%) into tristearin. In comparison with other lipases examined, *C. rugosa* lipase catalyzed the highest incorporation of linoleic acid (LA, 75.8%), α- linolenic acid (ALA, 74.8%), and conjugated linoleic acid (CLA, 53.5%) into tristearin. Thus, these two lipases might be considered promising biocatalysts for acidolysis of tristearin with selected long-chain fatty acids. EPA was better incorporated into tristearin than DHA using the fifth enzmyes. LA incorporation was better than CLA. ALA was more reactive than GLA during acidolysis, except for the reaction catalyzed by *Pseudomonas* sp., possibly due to structural differences (location and geometry of double bonds) between the two fatty acids. In another set of experiments, a combination of equimolar quantities of unsaturated C18 fatty acids (OA + LA + CLA + GLA + ALA) was used for acidolysis of tristearin to C18 fatty acids at ratios of 1:1, 1:2, and 1:3. All lipases tested catalyzed incorporation of OA and LA into tristearin except for *M. miehei*, which incorportaed only OA. *C. rugosa* lipase better catalyzed incorporation of OA and LA into tristearin than other lipases tested, whereas the lowest incorporation was obtained using *Pseudomonas* sp. As the mole ratio of substrates increased from 1 to 3, incorporation of OA and LA increased except for the reaction catalyzed by *A. niger* and *C. rugosa*. All lipases tested failed to allow GLA or CLA to participate in the acidolysis reaction, and ALA was only slightly incoporated into tristearin when *M. miehei* was used.

KEYWORDS: Acidolysis; interesterification; lipase; tristearin; structured lipids; oleic acid; α-linolenic acid; γ-linolenic acid; docosahexaenoic acid; eicosapentaenoic acid; conjugated linoleic acid; linoleic acid

Acidolysis is one type of interesterification, defined as the exchange of acyl groups between an ester (triacylglycerol, TAG) or phospholipids and an acid (fatty acid). Yang et al. (*1*) compared acyl incorporation of linoleic acid (LA) and conjugated linoleic acid (CLA) into tristearin (SSS) in a solvent-free system at 60 °C using 5% Lipozyme RM IM from *Rhizomucor miehei*. LA was more easily incorporated into tristearin than CLA. At 5 h of reaction time, linoleic acid was more easily incorporated (up to 50 mol %) into tristearin than conjugated linoleic acid (only 28 mol %). Formation of trilinolein (LA-LALA) was 5 times higher than that of triconjugated linolein (CLACLACLA), suggesting that linoleic acid was more reactive than conjugated linoleic acid, probably due to the rigidity of the latter (*1*). Yankah and Akoh (*2*) reported the highest incorporation (55.2%) of oleic acid (OA) into tristearin after 72 h at 45 °C using 10% immobilized IM-60 from *Mucor miehei* in the presence of hexane.

Senanayake and Shahidi (*3*) reported that lipase Novozyme-435 from *Candida antarctica* exhibited the highest degree of docosahexaenoic acid (DHA) incorporation (25.8−28.7%, after 24 h) into borage and evening primrose oils. On the other hand, the highest degree of incorporation of (28.7−30.7%, after 24 h) of eicosapentaenoic acid (EPA) in both oils was obtained with lipase PS-30 from *Pseudomonas* sp. In addition, Akoh and Yee (*4*) produced structured lipids (SL) upon enzyme-catalyzed transesterification of tristearin and tricaprylin. The specific enzyme from *Rhizomucor miehei* produced SL with a total carbon number of 41 (C_{41}, 44.2%) and 49 (C_{49}, 40.5%). In another study, Huang et al. (*5*) produced a concentrate containing 52.0% γ-linolenic acid (GLA) in acylglycerols derived from borage oil via acidolysis using the enzyme from *Candida rugosa*. Akoh and Huang (*6*) screened 12 lipases for their capacity to produce SL via acidolysis of triolein and caprylic acid. Monocaprylolein was the major constituent at 57.4 mol

* Corresponding author [telephone (709) 737-8552; fax (709) 737-4000; e-mail fshahidi@mun.ca].
[†] Department of Biology.
[§] Department of Biochemistry.

10.1021/jf062553k CCC: $37.00
Published on Web 02/09/2007

%, and IM-60 lipase from *R. miehei* served as the best biocatalyst tested.

Little attention has been paid to incorporating different fatty acids (OA; LA; CLA; GLA; ALA, α-linolenic acid; EPA; and DHA) into tristearin using different lipases, namely, Novozyme-435 from *C. antarctica*, AY-30 from *C. rugosa*, PS-30 from *Pseudomonas* sp., AP-12 from *Aspergillus niger*, and Lipozyme-IM from *Mucor miehei*, to catalyze the acidolysis of tristearin with selected long-chain unsaturated fatty acids. Therefore, this study aimed to examine the effect of chain length, number of double bonds, and the location and geometry of double bonds on the incorporation of these fatty acids (OA, LA, CLA, GLA, ALA, EPA, and DHA) into tristearin. This paper discusses reasons behind different degrees of incorporation of selected long-chain fatty acids into tristearin on a molecular basis in order to fill an important gap in the existing scientific literature regarding the differences in the reactivity of different fatty acids. The work is expected to expand the existing knowledge, both basic and applied, in the area of lipid biotechnology. The information obtained in this work would allow scientists and manufacturers to design and/or predict reaction results for incorporating different fatty acids of interest into triacylglycerols.

MATERIALS AND METHODS

Materials. Two lipases from *C. antarctica* (Novozyme-435) and *M. miehei* (Lipozyme-IM) were acquired from Novo Nordisk (Franklinton, NC). Other lipases, namely, *Pseudomonas* sp. (PS-30), *Aspergillus niger* (AP-12), and *C. rugosa* (AY-30), were obtained from Amano Enzyme (Troy, VA). All lipases used in this work were in the powder form, and all solvents used were of analytical grade and purchased from Fisher Scientific (Nepean, ON, Canada). Four fatty acids, namely, OA, LA, GLA, and ALA, were purchased from Sigma Chemical Co. (St. Louis, MO). EPA (>99% pure) was from Fuso Pharmaceutical Industries Ltd. (Osaka, Japan) and kindly provided by Dr. K. Miyashita. Algal oil containing DHA (40.0%) was provided from Martek Biosciences Corp. (Columbia, MD). Tristearin (tri C18:0) was purchased from Sigma Chemical Co.

Preparation of Free Fatty from Algal Oil and Concentration of DHA by Urea Complexation. Preparation of free fatty acids from algal oil was conducted according to the method described by Wanasundara and Shahidi (*7*). Urea complexation was followed as described by Wanasundara and Shahidi (*7*).

Preparation of CLA by Alkali Isomerization. CLA was prepared according to the method described by Chin et al. (*8*). Ethylene glycol (100 g) was placed in a 500 mL round-bottom flask, which was heated in an oil bath at 180 °C for 10 min and then removed from the oil bath as the temperature decreased to 160 °C; 26 g of potassium hydroxide was subsequently added to its content. The flask was placed again in the oil bath, and the temperature was increased to 180 °C and held there for 10 min. LA (50 g) was added to the mixture in the flask after it was removed from the oil bath, and then it was stirred for few seconds. The flask was returned to the oil bath and the temperature maintained at 180 °C for 2 h under a stream of nitrogen to prevent autoxidation; the flask was then cooled to room temperature using tap water. Methanol (200 mL) was subsequently added to the mixture, then transferred to a 1 L separatory funnel, and acidified (pH <2) with 250 mL of 6 M HCl. Water (200 mL) was added to the mixture for dilution. CLA was extracted from the mixture with 200 mL of hexane. A washing step was conducted using 30% methanol in water (3 × 200 mL) and then double-distilled water (3 × 200 mL). The extract was passed through a bed of anhydrous sodium sulfate to remove any residual water; hexane was then removed at 45 °C using a rotary evaporator.

Acidolysis Reaction. Tristearin (100 mg) was mixed with different fatty acids (OA, GLA, LA, CLA, and ALA) at a mole ratio of acid to tristearin of 3:1 in a screw-capped test tube, and then lipase (10% by weight of substrates) and water (2% by weight of substrates and enzyme) were subsequently added in hexane (3.0 mL). The mixture was incubated at 45 ± 2 °C for 24 h in a shaking water bath at 250 rpm. In another set of experiments, a combination of equimolar quantities of unsaturated C18 fatty acids (OA + LA + CLA + GLA + ALA) at tristearin to C18 fatty acids ratios of 1:1, 1:2, and 1:3 was used to investigate the effect of substrate mole ratio on incorporation of these fatty acids into tristearin. One mole of tristearin was mixed with 0.2 mol of each of the five C18 fatty acids at a tristearin to C18 fatty acids ratio of 1:1. For a mole ratio of tristearin to C18 fatty acids of 1:2, 1 mol of tristearin was mixed with 0.4 mol of each of the five C18 fatty acids, and for a mole ratio of 1:3, this was 0.6 for each fatty acid to 1 mol of tristearin. The experimental conditions were the same as those mentioned earlier.

In another set of experiments, a mixture of equimolar amounts of n-3 fatty acids (ALA + EPA + DHA) at tristearin to n-3 fatty acid ratios of 1:1, 1:2, and 1:3 was mixed in a screw-capped test tube, and then lipase (10%) and water (2%) were added in hexane (3.0 mL). The mixture was incubated at 45 ± 2 °C for 24 h in a shaking water bath at 250 rpm.

Fatty Acid Composition of Products. *Preparation of Fatty Acid Methyl Esters (FAMEs).* Fatty acid profiles of products were determined following their conversion to the corresponding 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 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 layer was 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 separated and evaporated under a stream of nitrogen. FAMEs were then dissolved in 1.0 mL of carbon disulfide and used for subsequent gas chromatographic 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 length, 0.25 mm diameter, 0.25 μm film thickness; Supelco Canada Ltd., Oakville, ON, Canada). The oven temperature was first set at 220 °C for 10.25 min and then raised to 240 °C at 30 °C/min and held there for 15 min. The injector (flame ionization) and detector (FID) temperatures were both set at 250 °C. Ultrahigh-purity (UHP) helium was used as a carrier gas at a flow rate of 15 mL/min. The data were treated using Hewlett-Packard 3365 series II Chem Station software (Agilent). The FAMEs were identified by comparing their retention times with those of authentic standard mixtures GLC-461 from Nu-Check (Elysian, MN), and the results are presented as weight percentage.

Statistical Analysis. All experiments were performed in triplicate. Data reported are mean ± standard deviation (SD). Analysis of variance and Tukey's test were carried out at a level of $p < 0.05$ to assess the significance of differences among mean values.

RESULTS AND DISCUSSION

Table 1 shows the degree of incorporation of C18 fatty acids (OA, GLA, LA, CLA, and ALA) into tristearin. Novozyme-435 from *C. antarctica* catalyzed significant incorporation ($p < 0.05$) of LA into tristearin (46.7%) compared to CLA (31.8%). The acidolysis of tristearin (SSS) and LA and CLA can be summarized by the following equations, respectively; the orders shown do not necessarily relate to the location of fatty acids in the triacylglycerol molecules:

$$\mathrm{LA} + \mathrm{SSS} \rightleftharpoons \mathrm{LASS} + \mathrm{S} \quad (1)$$

$$\mathrm{CLA} + \mathrm{SSS} \rightleftharpoons \mathrm{CLASS} + \mathrm{S} \quad (2)$$

According to thermodynamic laws, the free energy change ΔG can be calculated at a particular instant in time (*9*). The more extended shape of CLA, due to the conjugation of

Table 1. Effect of Different Lipases on Percent Incorporation of C18 Fatty Acids into Tristearin[a]

enzyme source	OA	LA	CLA	ALA	GLA
Candida antarctica	58.2 ± 1.24 c	46.7 ± 0.52 b	31.8 ± 1.44 a	65.2 ± 3.20 c	55.9 ± 2.34 c
Mucor miehei	50.0 ± 3.79 d	47.7 ± 1.94 c	30.3 ± 1.52 a	43.4 ± 2.10 b	39.6 ± 1.15 b
Pseudomonas sp.	35.3 ± 2.07 a	46.2 ± 2.35 c	31.8 ± 1.02 a	41.4 ± 2.71 b	44.8 ± 2.46 b
Candida rugosa	47.2 ± 3.24 b	75.8 ± 2.82 d	53.5 ± 1.89 c	74.8 ± 0.95 d	38.3 ± 1.34 a
Aspergillus niger	51.4 ± 0.74 b	66.9 ± 0.90 c	38.32 ± 0.82 a	53.7 ± 1.93 b	38.6 ± 1.48 a

[a] Results are mean values of triplicate determinations ± standard deviation. Values within each row with different letters are different ($p < 0.05$) from one another. a–e: the order of C18 fatty acid incorporation into tristearin, from lowest to highest, was as follows: a < b < c < d < e. OA, oleic acid; LA, linoleic acid; CLA, conjugated linoleic acid; ALA, α-linolenic acid; GLA, γ-linolenic acid; ND, not detected. The reaction mixture contained tristearin (100 mg), fatty acid to tristearin at a mole ratio of 3:1, enzyme amount (10%, by weight of substrates), water (2%, by weight of enzyme and substrates), and 3.0 mL of hexane. The mixture was kept at 45 ± 1 °C for 24 h in a shaking water bath at 250 rpm.

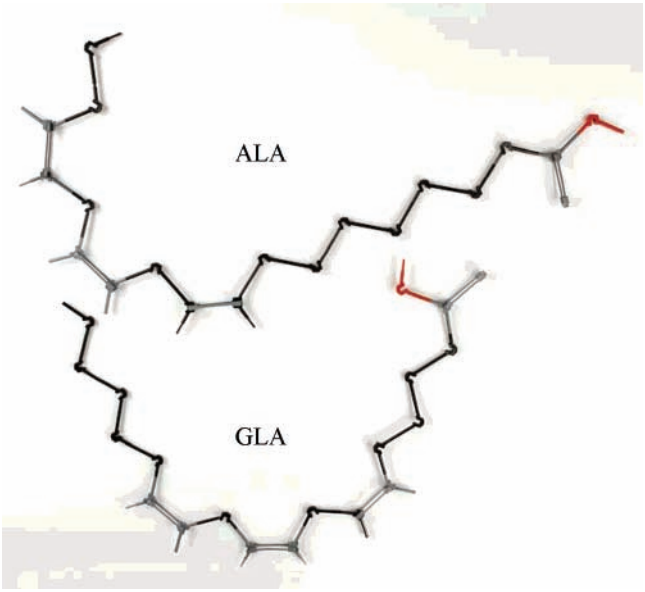

Figure 1. Representations of α-linolenic (ALA) and γ-linolenic (GLA) acids using chemical models.

Table 2. Effect of Different Lipases on Percent Incorporation of n-3 Fatty Acids into Tristearin[a]

enzyme source	EPA	DHA
Candida antarctica	58.2 ± 2.05 b	47.7 ± 2.45 a
Mucor miehei	47.7 ± 1.86 b	33.1 ± 2.42 a
Pseudomonas sp.	68.8 ± 1.84 b	30.8 ± 1.84 a
Candida rugosa	66.8 ± 1.59 b	47.7 ± 1.54 a
Aspergillus niger	69.9 ± 3.56 b	38.8 ± 1.61 a

[a] Results are mean values of triplicate determinations ± standard deviation. Values within each row with different letters are different ($p < 0.05$) from one another. a, b: the order of n-3 fatty acid incorporation into tristearin, from lowest to highest, was as follows: a < b. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. The reaction mixture contained tristearin (100 mg), fatty acid to tristearin at a mole ratio of 3:1, enzyme amount (10%, by weight of substrates), water (2%, by weight of enzyme and substrates), and 3.0 mL of hexane. The mixture was kept at 45 ± 1 °C for 24 h in a shaking water bath at 250 rpm.

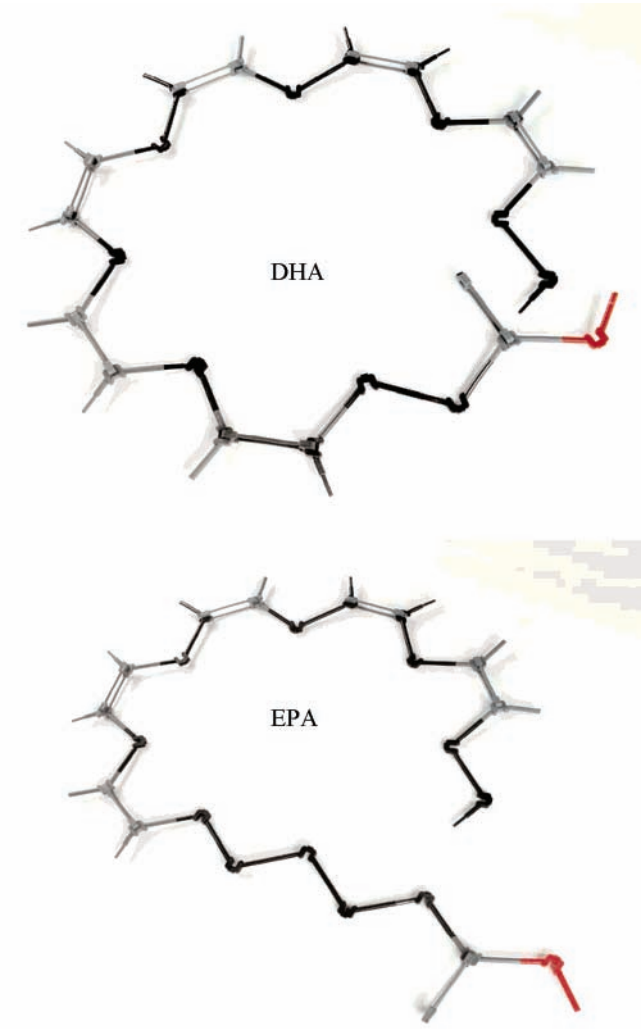

Figure 2. Representations of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids using chemical models.

the double bonds, results in less stability of CLASS compared to LASS (*1*). Therefore, the free energy of LASS (G LASS; eq 1) is less than that of CLASS (G CLASS; eq 2). When the free energies on both sides of the reaction are equal, equilibrium is reached (*9*). The lesser the ΔG for a reaction, the more complete a reaction will be (*1*, *2*). Because G LASS is less than G CLASS, the reaction between LA and tristearin is more favored than that between CLA and tristearin. The results from this study are consistent with those of Yang et al. (*1*), who reported that LA incorporation into tristearin was up to 50 mol %, whereas CLA incorporation was only 28 mol % in the same acidolysis reaction catalyzed by Lipozyme RM IM from *R. miehei*. Furthermore, Yang et al. (*1*) suggested that LA was more reactive than CLA, probably due to the rigid structure of the latter arising from conjugation of its double bonds. The rigidity and hindrance of CLA could produce obstacles for its access to the active site of the lipase and hence lead to its low percent incorporation into tristearin.

Table 3. Effect of Mole Ratio of Substrates on Percent Incorporation of C18 Fatty Acids into Tristearin[a]

mole ratio of tristearin to C18 FA	enzyme source	OA	LA	CLA	ALA	GLA
1:1	*Candida antarctica*	10.9 ± 0.74 b	8.29 ± 1.58 a	ND	ND	ND
	Mucor miehei	5.87 ± 0.38 b	ND	ND	0.25 ± 0.01 a	ND
	Pseudomonas sp.	5.74 ± 0.75 a	4.86 ± 0.17 a	ND	ND	ND
	Candida rugosa	16.7 ± 0.72 b	14.4 ± 0.81 a	ND	ND	ND
	Aspergillus niger	14.1 ± 0.46 a	13.0 ± 0.96 a	ND	ND	ND
1:2	*Candida antarctica*	11.0 ± 0.97 a	10.9 ± 0.73 a	ND	ND	ND
	Mucor miehei	7.74 ± 0.51 a	ND	ND	0.33 ± 0.14 a	ND
	Pseudomonas sp.	9.80 ± 1.01 b	7.05 ± 0.49 a	ND	ND	ND
	Candida rugosa	18.1 ± 0.49 b	14.5 ± 0.98 a	ND	ND	ND
	Aspergillus niger	12.8 ± 0.74 b	10.0 ± 0.46 a	ND	ND	ND
1:3	*Candida antarctica*	14.4 ± 0.38 b	11.5 ± 0.73 a	ND	ND	ND
	Mucor miehei	8.22 ± 0.12 b	ND	ND	0.24 ± 0.01 a	ND
	Pseudomonas sp.	8.09 ± 0.55 b	6.85 ± 0.01 a	ND	ND	ND
	Candida rugosa	17.9 ± 0.86 b	14.9 ± 0.19 a	ND	ND	ND
	Aspergillus niger	10.2 ± 0.26 a	9.66 ± 0.31 a	ND	ND	ND

[a] Results are mean values of triplicate determinations ± standard deviation. Values within each row with different letters are different ($p < 0.05$) from one another. a–e: the order of C18 fatty acid incorporation into tristearin, from lowest to highest, was as follows: a < b < c < d < e. FA, fatty acids; OA, oleic acid; LA, linoleic acid; CLA, conjugated linoleic acid; ALA, α-linolenic acid; GLA, γ-linolenic acid; ND, not detected.

Table 4. Effect of Mole Ratio of Substrates on Percent Incorporation of n-3 Fatty Acids into Tristearin[a]

mole ratio of tristearin to n-3 FA	enzyme source	ALA	EPA	DHA
1:1	*Candida antarctica*	17.5 ± 0.74 c	15.9 ± 0.27 b	6.62 ± 0.21 a
	Mucor miehei	10.3 ± 0.67 b	13.1 ± 0.06 c	4.70 ± 0.30 a
	Pseudomonas sp.	11.5 ± 0.47 b	25.3 ± 1.30 c	4.69 ± 0.33 a
	Candida rugosa	15.3 ± 1.09 b	29.2 ± 0.84 c	7.59 ± 0.89 a
	Aspergillus niger	15.9 ± 0.42 b	41.2 ± 0.92 c	7.11 ± 0.43 a
1:2	*Candida antarctica*	19.8 ± 0.50 b	25.7 ± 0.27 c	10.9 ± 0.58 a
	Mucor miehei	13.7 ± 0.25 b	17.6 ± 0.83 c	5.72 ± 0.58 a
	Pseudomonas sp.	11.5 ± 1.08 b	28.7 ± 0.38 c	6.65 ± 0.17 a
	Candida rugosa	20.4 ± 1.19 b	58.1 ± 0.55 c	7.80 ± 0.39 a
	Aspergillus niger	15.8 ± 2.50 b	50.0 ± 0.85 c	8.23 ± 0.64 a
1:3	*Candida antarctica*	28.3 ± 0.84 b	33.0 ± 2.04 c	14.4 ± 0.47 a
	Mucor miehei	20.1 ± 1.18 b	19.2 ± 0.02 b	5.89 ± 0.04 a
	Pseudomonas sp.	13.7 ± 0.92 b	33.4 ± 0.46 c	7.26 ± 0.71 a
	Candida rugosa	12.5 ± 0.32 b	60.6 ± 0.57 c	6.37 ± 0.57 a
	Aspergillus niger	11.7 ± 0.69 b	59.9 ± 2.00 c	5.22 ± 0.58 a

[a] Results are mean values of triplicate determinations ± standard deviation. Values within each row with different letters are different ($p < 0.05$) from one another. a–c: the order of n-3 fatty acid (ALA + EPA + DHA) incorporation into tristearin, from lowest to highest, was as follows: a < b < c. FA, fatty acids; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

ALA incorporation (65.2%) into tristearin was significantly ($p < 0.05$) higher than that of GLA (55.9%). Whereas ALA and GLA shared the same chain length and number of double bonds, the first double bond in ALA is located between C3 and C4 from the methyl end of the molecule, whereas that in GLA is located between C6 and C7 (**Figure 1**). From the carboxylic acid end group, the first double bond in ALA is located at C9, whereas in GLA it is positioned at C6. Therefore, ALA might have a less bent structure because its three double bonds are positioned on one side of the molecule, whereas GLA might have a more bent shape because the three double bonds are located in the middle of it (**Figure 1**). This was confirmed when chemical models were constructed for ALA and GLA, as shown in **Figure 1**, demonstrating that GLA has a more bent shape than ALA. Therefore, structural differences between ALA and GLA related to the location of double bonds as well as specificity of the enzymes used might lead to variation in their reactivity.

The highest incorporation (50.0%) of C18 fatty acids examined, catalyzed by Lipozyme-IM from *M. miehei*, was for OA. This lends further support to the findings of Yankah and Akoh (*2*), who reported the highest incorporation (55.2%) of OA into tristearin after 72 h at 45 °C using immobilized IM-60 from *M. miehei*. Moreover, Soumanou et al. (*10*) reported that lipase from *M. miehei* afforded the highest yield of structured lipids (73% at 40 °C) using peanut oil and tricaprylin, whereas the lowest incorporation was reported for CLA. The percent incorporation (43.4) of ALA was higher than that of GLA (39.6).

The results presented in this study showed that lipase PS-30 from *Pseudomonas* sp. catalyzed significant ($p < 0.05$) incorporation (46.2%) for LA compared to that of CLA (31.8%). Incorporation of GLA into tristeasrin was higher than that of ALA: 44.8 versus 41.4. This result contrasts those from the reactions catalyzed by lipases from *C. antarctica* and *M. miehei*. Incorporation (35.3%) of OA into tristearin corresponded with the findings of Moussata and Akoh (*11*), who reported 53.0% incorporation of OA into melon seed oil at 55 °C after 24 h using lipase from *Pseudomonas* sp.

The incorporation of C18 fatty acids into tristearin, using lipase AY-30 from *C. rugosa* as a biocatalyst, is also shown in

Table 1. Although the highest incorporation (74.8%) was observed for ALA followed by LA (75.8%), there was no significant ($p > 0.05$) difference between these two molecules, whereas the lowest incorporation (38.3%) was for GLA. These results are contrary to those of Huang et al. (*5*), who reported that IM-60 lipase from *C. rugosa* catalyzed acidolysis reaction between the GLA-rich free fatty acids and the unhydrolyzed acylglycerols, increasing the GLA content from 52.1 to 75% at 50 °C after 4 h (*12*). Incorporation of ALA (74.8%) was almost double that of GLA (38.3%). Meanwhile, OA was incorporated at 47.2% into tristearin, whereas a higher percentage of conversion (>90) of OA was obtained with esterification of dodecyl alcohol with OA using *C. rugosa* at 40 °C after 4 h (*13*).

Table 2 shows the results for incorporation of EPA and DHA into tristearin. The reactions were catalyzed by lipases from *C. antarctica*, *M. miehei*, *Pseudomonas* sp., *C. rugosa*, and *A. niger*. EPA incorporation (58.2%) was significantly ($p < 0.05$) higher than that of DHA (47.7%) using *C. antarctica* as a biocatalyst. EPA has a shorter chain length and one fewer double bond than DHA; therefore, EPA has a less bent shape compared to DHA (**Figure 2**). These differences (the chain length and the number of double bonds) lead to decreased incorporation of DHA into tristearin compared to EPA. These results lend further support to the findings of Senanayake and Shahidi (*3*), who reported that maximum incorporation of EPA (28.7−30.7%) in borage and evening primrose oils was obtained with lipase from *Pseudomonas* sp., and those of Akoh et al. (*14*), who found that EPA was more easily incorporated into trilinolein than DHA. In general, the extent of incorporation of EPA, using the five selected lipases, was higher than that of DHA. These results suggest that EPA was more reactive than DHA.

A combination of equimolar quantities of unsaturated C18 fatty acids (OA + LA + CLA + GLA + ALA), at tristearin to C18 FA ratios of 1:1, 1:2, and 1:3, was used to investigate the effect of substrate mole ratio on the incorporation of these fatty acids into tristearin; results are shown in **Table 3**. As the mole ratio of tristearin to C18 fatty acids increased from 1:1 to 1:3, incorporation of OA and LA increased except for the reactions catalyzed by *A. niger* and *C. rugosa*. All lipases tested failed to allow GLA or CLA to participate in the acidolysis reaction, and ALA was only slightly incorporated into tristearin when *M. miehei* was used. Incorporation of OA and LA into tristearin was also assisted by all lipases tested except for *M. miehei*, which incorporated only OA. *C. rugosa* lipase better catalyzed incorporation of OA and LA into tristearin than other lipases examined, whereas lowest incorporation was observed for *Pseudomonas* sp.

The effect of mole ratio of substrates on the incorporation of a combination of equimolar amounts of n-3 fatty acids into tristearin is shown in **Table 4**. The mole ratio of tristeasrin to a mixture of n-3 fatty acids (ALA, EPA, and DHA) was varied from 1:1 to 1:3. When the mole ratio of substrates increased from 1:1 to 1:3, incorporation of n-3 fatty acids increased accordingly. In general, the highest incorporation of n-3 fatty acids into tristearin was obtained at a mole ratio of tristearin to n-3 fatty acids of 1:3 because triacylglycerol molecules can incorporate a maximum of three fatty acids in their backbone. EPA was significantly ($p < 0.05$) more reactive than ALA and DHA during acidolysis using the five selected enzymes employed in this study. In general, the order of incorporation of a mixture of n-3 fatty acids into tristearin was as follows: EPA > ALA > DHA. Theortically, the order of degree incorporation of these acids should be in the order of ALA > EPA > DHA when chain length and number of double bonds are taken into consideration. Although EPA has a longer chain length (20 in EPA compared to 18 in ALA) and more double bonds (five in EPA and three in ALA) than ALA, its percent incorporation into tristearin was significantly ($p < 0.05$) higher than that of ALA. Reasons behind the higher reactivity of EPA compared to ALA remain unclear, but might be influenced by the specificity of the enzymes in the acidolysis reaction.

In general, incorporation of long-chain fatty acids into triacylglcerols (e.g., tristearin) may be affected by many factors, including chain length, number of double bonds, the location and geometry of the double bonds, as well as reaction conditions and reactivity and specificity of lipases used. LA was more reactive than CLA due to the rigidity of the latter. EPA was more reactive than DHA, due to the structural differences between the two (number of double bonds, chain length). Novozyme-435 enzyme from *C. antarctica* catalyzed highest incorporation percentage of OA (58.2), GLA (55.9), EPA (81.6), and DHA (47.7) into tristearin. AY-30 from *C. rugosa* catalyzed highest percent incorporation of LA (75.8), ALA (74.8), and CLA (53.5) into tristearin. Thus, these two lipases might be considered as promising biocatalysts for acidolysis of tristearin and selected long-chain fatty acids.

The high percent incorporation of fatty acids into tristearin using lipase from *C. antarctica* or *C. rugosa* might be due to the experimental conditions employed in this study, which were suitable for these two enzymes. Thus, further studies are needed to verify the optimum reaction conditions for each enzyme examined.

LITERATURE CITED

(1) Yang, T.; Xu, X.; Li, L. Comparison of linoleic and conjugated linoleic acids in enzymatic acidolysis of tristearin. *J. Food Lipids* **2001**, *8*, 149−161.

(2) Yankah, V. V.; Akoh, C. C. Lipase-catalyzed acidolysis of tristearin with oleic or caprylic acids to produce structured lipids. *J. Am. Oil Chem. Soc.* **2000**, *77*, 495−500.

(3) Senanayake, S. P. J. N.; Shahidi, F. Enzyme-assisted acidolysis of borage (*Borage officinalis* L.) and evening primrose (*Oenothera biennis* L.) oils: incorporation of omega-3 polyunsaturated fatty acids. *J. Agric. Food Chem.* **1999**, *47*, 3105−3112.

(4) Akoh, C. C.; Yee, L. N. Enzymatic synthesis of position-specific low-calorie structured lipids. *J. Am. Oil Chem. Soc.* **1997**, *74*, 1409−1413.

(5) Huang, F. C.; Ju, Y. H.; Huang, C. W. Enrichment of γ-linolenic acid from borage oil via lipase-catalyzed reactions. *J. Am. Oil Chem. Soc.* **1997**, *74*, 977−981.

(6) Akoh, C. C.; Huang, K. H. Enzymatic synthesis of structured lipids: transesterification of triolein and caprylic acid. *J. Food Lipids* **1995**, *2*, 219−230.

(7) Wanasundara, U. N.; Shahidi, F. Concentration of omega-3 polyunsaturated fatty acids of seal blubber oil by urea complexation: optimization of reaction conditions. *Food Chem.* **1999**, *65*, 41−49.

(8) Chin, S. F.; Liu, J. W.; Storkson, M.; Ha, Y. L.; Pariza, M. W. Dietary sources of conjugated dienoic isomers of linleic acid, a newly recognized class of anticarcinogens. *J. Food Compos. Anal.* **1992**, *5*, 185−197.

(9) Campbell, P. N.; Smith, A. D. The structure and function of enzymes. In *Biochemistry Illustrated*; Campbell, P. N., Smith, A. D., Eds.; Churchill Livingstone: Edinburgh, U.K., 1994; pp 55−78.

(10) Soumanou, M. M.; Bornscheuer, U. T.; Menge, U.; Schmid, R. D. Synthesis of structured triglycerides from peanut oil with immobilized lipase. *J. Am. Oil Chem. Soc.* **1997**, *74*, 427−433.

(11) Moussata, C. O.; Akoh, C. C. Lipase-catalyzed incorporation of oleic acid into melon seed oil. *J. Am. Oil Chem. Soc.* **1997**, *74*, 177−179.

(12) Huang, F. C.; Ju, Y. H.; Chiang, J. C. γ-Linolenic acid rich triacylglycerol derived from borage oil via lipase-catalyzed reactions. *J. Am. Oil Chem. Soc.* **1999**, *7*6, 833−837.

(13) Rao, N. M.; Shanmugam, V. M. Lipase-mediated synthesis of dodecyl oleate and oleyl oleate in aqueous foams. *J. Am. Oil Chem. Soc.* **2000**, *77*, 605−608.

(14) Akoh, C. C.; Jennings, B. H.; Lillard, D. A. Enzymatic modification of trilinolein: incorporation of n-3 polyunsaturated fatty acids. *J. Am. Oil Chem. Soc.* **1995**, *72*, 1317−1321.

Received for review September 5, 2006. Revised manuscript received December 20, 2006. Accepted January 4, 2007.

JF062553K