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ARTICLE

Fatty Acid Selectivity of Lipases during Acidolysis Reaction between Triolein and Saturated Fatty Acids Varying from Caproic to Behenic Acids

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The chain length selectivity of three immobilized lipases, namely, Lipozyme TL IM from *Thermomyces lanoginosus*, Lipozyme RM IM from *Rhizomucor miehei*, and Novozym 435 from *Candida antarctica*, was determined in acidolysis performed in hexane using the homologous series of even carbon number, saturated fatty acids (SFAs) of 6−22 carbons. Triolein with individual SFAs or a mixture of equimolar quantities of SFAs was used as the substrate. The effects of operating variables including the mole ratio of fatty acid to triolein, temperature, enzyme dosage, and time on incorporation were also investigated. Incorporation abilities of the enzymes tested were found to be significantly different for most of FAs at the experimental conditions evaluated. Lipases acted weakly on SFAs of which the carbon chain length was shorter than eight carbon atoms and higher than 18 carbon atoms. Lipases showed a bell-shaped distribution in incorporation vs chain length plot with a maximum around C12−C16. Among the experimental parameters tested, the effect of the substrate mole ratio was greater than those of the others, and the highest incorporation was observed for C12 (36.98%), C14 (37.63%), and C16 (38.66%) at a 4:1 substrate mole ratio with Lipozyme TL IM. Lipases caused significantly different levels of acyl migration from sn-1,3 to sn-2 positions.

KEYWORDS: Lipase; selectivity; acidolysis; saturated fatty acid; triolein; *Thermomyces lanoginosus*; *Rhizomucor miehei*; *Candida antarctica*

INTRODUCTION

Lipases [triacylglycerol (TAG) acylhydrolases, E.C. 3.1.1.3] are used in several applications including detergent, food, flavor, pharmaceutical, leather, textile, cosmetic, and paper industries. Modification of fats and oils via lipase-catalyzed reactions for the production of fats free from trans fatty acids (TFAs) or structured lipids (SLs) has attracted great attention in the last two decades. TAGs with polyunsaturated fatty acids (PUFAs) and short or medium chain fatty acids (SCFAs and MCFAs, respectively) at sn-1,3 positions and functional fatty acids (FAs) at sn-1,3 positions or at the sn-2 position are known as SLs (*1*). SLs can be synthesized to provide specific metabolic effects, such as nutritive or therapeutic purposes, or to improve physical and/or chemical characteristics of lipids (*2*,*3*). One of the common routes reported in the literature to the synthesis of SLs, which have been successfully used for restructuring lipids, is based on a simple lipase-catalyzed acidolysis between TAG and FAs, leading to exchange of acyl groups.

Lipases have various degrees of selectivity toward FAs involved in fat and oil modification. Some FAs are incorporated at higher levels than others, which might be influenced by the composition of substrates, nature of solvents, and source of lipase (*4*). The ability of lipases to discriminate among FAs could be utilized to facilitate new applications. A number of studies have focused on determining FA selectivity of various lipases or reactivity of FAs in different lipase applications. Shimada et al. (*5*) investigated the FA specifity of *Rhizopus delemar* lipase in hydrolysis and acidolysis with randomly interesterified oil as the substrate. The specifity of the lipases from *R. delemar* and *Rhizopus miehei* has been evaluated for hydrolysis (*6*). Significant selectivities of *Geotrichum candidum* (*7*, *8*) and *Penicillium* sp. (*9*) lipases have also been reported. Useful information related to the specifity of the lipase in partial hydrolysis has been reviewed (*10*). FA selectivity of lipases is related to their ability to distinguish between particular FAs or acyl moieties. Knowledge about the substrate selectivity of lipases is therefore essential for their utilization (*11*).

To the authors' knowledge, no report has been published on the selectivity of lipases in acidolysis with the homologous series of even carbon number, saturated fatty acids (SFAs) of 6−22 carbons. We aimed to incorporate different SFAs into triolein using different immobilized lipases, namely, Lipozyme TL IM from *Thermomyces lanoginosus*, Lipozyme RM IM from *Rhizomucor miehei*, and Novozym 435 from *Candida antarctica* lipase B. The incorporation abilities of the enzymes for each FA were determined. Triolein with individual SFAs or a mixture of equimolar quantities of SFAs was used as the substrate. The effect of operating variables such as mole ratio of FA to triolein, temperature, enzyme dosage, and time on the incorporation was also investigated.

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MATERIALS AND METHODS

Materials. Lipases were provided by Novo Nordisk A/S (Bagsvaerd, Denmark). Lipozyme TL IM is *Thermomyces lanuginosa* lipase immobilized on granulated silica particles. Lipozyme RM IM is *R. miehei* lipase immobilized on anion exchange resin particles. Novozym 435 is *C. antarctica* lipase type B, immobilized on macroporous acrylic resin beads. Caproic (C6), caprylic (C8), capric (C10), lauric (C12), myristic (C14), palmitic (C16), stearic (C18), arachidic (C20), and behenic (C22) acids and triolein (tri C 18:1) were purchased Sigma-Aldrich Chemie GmbH (Steinheim, Germany). A fatty acid methyl ester (FAME) mixture (37 component FAME mix) and mono-, di-, and triglyceride mixtures were obtained from Supelco (Bellefonte, PA). Sodium sulfate (anhydrous) was supplied by J. T. Baker (Deventer, Holland). All other chemicals and reagents for the analysis were analytic or chromatography grades.

Acidolysis Reaction. Binary mixtures of triolein (100 mg) with each of the FAs (C6, C8, C10, C12, C14, C16, C18, C20, and C22) were used in acidolysis. Reactions were carried out in tightly closed, screw-capped glass vials (20 mL) containing FA−triolein mixture dissolved in 3 mL of hexane. The vials were incubated in a shaking water bath at 200 rpm. The effects of substrate mole ratios (SFA:triolein) ranged from 1:1 to 4:1, temperatures ranged from 40 to 60 °C, reaction times ranged from 3 to 24 h, and enzyme dosages ranged from 5 to 20% (by total weight of substrates) on the incorporations that were studied. To determine the effects of different parameters on the incorporation of FAs into triolein, the default conditions were chosen as follows: substrate mole ratio, 1:1; temperature, 50 °C; reaction time, 6 h; enzyme dosage, 10%; and no extra water addition.

In another set of experiments, equimolar quantities of FAs (a total of 0.11 mmol) were combined in hexane. A volumetric amount of SFAs solution was mixed with triolein and used as the substrate. The experimental conditions were the same as those mentioned above. At the end of the reaction, the suspensions were filtered through syringe membrane filter (0.45 μm) to remove the enzyme particles, and filtrates (hexane solutions) were used for subsequent analysis.

Analysis of Product. One hundred microliters of the hexane solution was applied to thin-layer chromatography (TLC) plates (20 cm × 20 cm) coated with silica gel 60 F_{254} (Merck) in a thin uniform line by means of an applicator (Linomat 5, Camag, Muttenz, Switzerland). The developing solvent was hexane/diethyl ether/acetic acid (80/20/1, v/v/v). The bands were visualized under UV light after spraying with 0.2% 2,7-dichlorofluorescein in ethanol. The TAG band was scraped off into a screw-capped vial and methylated with 3 mL of 6% HCl in methanol at 75 °C for 2 h (*12*). At the end of the incubation, vials were cooled on ice bags, and 2 mL of hexane was added before centrifugation. The upper phase containing FAMEs was transferred to a vial containing anhydrous sodium sulfate by Pasteur pipet and used for FA composition analysis.

FA Composition Analysis. The FAMEs were analyzed by gas−liquid chromatography. The gas chromatograph (GC) was an Agilent 7890A with a fused capillary column (DB-23, 60 m × 0.25 mm i.d, 0.25 μm film thickness; J&W Scientific, Folsom, CA), an autoinjector (Agilent 7683B), and a flame ionization detector (FID) and was operated in split mode with the split ratio of 1:30. The injector and detector temperatures were maintained at 250 °C. The column temperature was held at 140 °C for 5 min and ramped to 240 °C for 10 min at the rate of 4 °C/min. The carrier gas was helium, and the total flow rate was 30 mL/min. The FAMEs were identified with those of standard mixtures (37 FAMEs mixtures, Sigma-Aldrich Inc., St. Louis, MO), and the results are presented as average molar percentage of two determinations.

sn-2 FA Analysis. The FA composition of TAG separated by TLC was determined by the pancreatic lipase (porcine pancreatic lipase, crude type II) digestion method as described AOCS Official Method Ch. 3−91 (*13*). This method entails several steps: selective hydrolysis of the 1,3-position of FAs in the TAG with pancreatic lipase; separation of the obtained monoacylglycerols (MAG) by TLC, using silica gel 60 plates and a developing solvent mixture of hexane, diethyl ether, and formic acid in the proportions 70/30/1 (v/v/v); identification of the MAG band (R_f about 0.035) under UV light; and, finally, analysis of the MAGs by GC following conversion of the MAGs to methyl esters as described above. The sn-2 FA analysis was performed in duplicate, and average values were reported for single samples.

Statistics. SPSS version 9.0 (SPSS Inc., Chicago, IL) was used to perform statistical calculations. Significant differences in the means of incorporated FAs (mol %) between three lipases catalyzed acidolysis were determined by using a least significant difference test and analysis of variance procedure ($P < 0.05$).

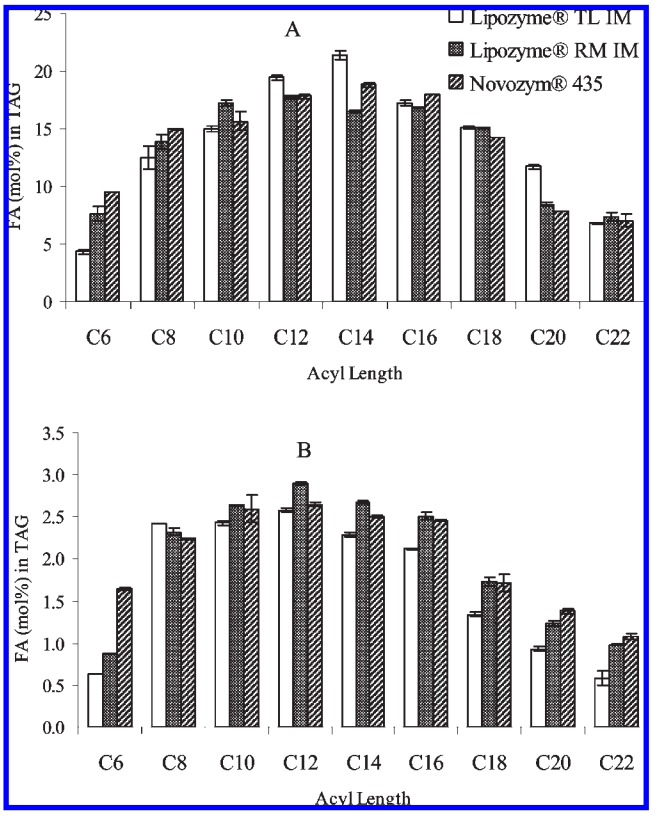

Figure 1. Effect of lipases on incorporation (mol %) of SFAs into triolein. Panels **A** and **B** show individual and a mixture of SFAs, respectively. The reaction conditions are as follows: substrate mole ratio, 1:1; temperature, 50 °C; reaction time, 6 h; and enzyme dosage, 10%.

RESULTS AND DISCUSSION

Three lipases, namely, Lipozyme TL IM, Lipozyme RM IM, and Novozym 435, were screened for their ability to incorporate SFAs into triolein at the following conditions (default conditions): substrate mole ratio, 1:1; temperature, 50 °C; reaction time, 6 h; and enzyme dosage, 10%. The degree of incorporation (mol percentage) is shown in **Figure 1A**,**B** for individual and a mixture of SFAs (competitive substrate medium), respectively. Lipases showed a bell-shaped distribution in incorporation vs chain length plot in acidolysis reactions with a maximum around C12−C16. In general, there are significant differences between lipases for incorporation of SFAs into triolein ($P < 0.05$). Lipases acted weakly on SFAs of which the carbon chain length was shorter than eight carbon atoms and higher than 18 carbon atoms in uses of both individual and competitive substrate mediums. This result is in agreement with those obtained by Pleiss et al. (*14*) who reported similar distribution for *R. miehei* lipase. In another study, it has been shown that C14−C18 FAs formed TAGs more rapidly than C10−C12 FAs in a *R. miehei*-catalyzed synthesis reaction. Especially, tetradecanoic acid (C14) esterified much more rapidly than the others (*15*).

Lipases showed different incorporation abilities for SFAs in acidolysis when they were used individually or as a mixture of them as acyl donor. Lipozyme TL IM from *T. lanoginosus* had a much higher activity than Lipozyme RM IM and Novozym 435 in the enzymatic acidolysis of triolein with C12 and C14 FAs (**Figure 1A**,**B**) when they were used individually as acyl donors.

However, incorporation of the C10−C18 FAs was higher in Lipozyme RM IM-catalyzed acidolysis than those of other enzymes in competitive substrate medium. These results clearly demonstrated that the three lipases show quite different selectivity for SFAs at default conditions, and the competitive medium has a great effect on the incorporation abilities of the enzymes.

It was reported that esterification of SCFAs is slower than that of LCFAs, and higher rates have been obtained mainly for MCFAs (*16*). This result was also confirmed in our study. A similar trend has been observed for the higher incorporation rate for C16 into triolein than that of C18 (*17*). In contrast to our findings, it has been reported that the Novozym 435 exhibited selectivity for SCFAs, while Lipozyme TL IM and Lipozyme RM IM transesterified LCFAs faster than SCFA in ethanolysis reactions (*18*).

Novozym 435 from *C. antarctica* lipase B had a higher incorporation ability for C6 and C8 FAs than those of two other lipases examined when the SFAs were used individually as acyl donors. A similar finding was reported that *C. antarctica* lipase B has a small hydrophobic area and thereby shows higher activity for SCFAs and MCFAs (*14*). Like this assessment, Lipozyme TL IM might have a long active site, which is consistent with the structure of C12 and C14 FAs. However, when the SFAs were combined as acyl donors, in addition to C6, Novozym 435 has strong incorporation ability for C20 and C22 as well as C6 FAs. SFA selectivity of the lipases was also different in the competitive medium composed of SFAs as a mixture.

Acidolysis of triolein and a SFA can be written as given following equations for the initial reactions:

$$\mathrm{SFA} + \mathrm{OOO} \leftrightarrow \mathrm{SFAOO} + \mathrm{O} \quad (1)$$

ΔG can be calculated at a particular instant in time (*19*). Therefore, at any instance in time:

$$\Delta G\ \mathrm{SFA} \leftrightarrow G\ \mathrm{SFAOO} + G\ \mathrm{O} - G\ \mathrm{OOO} - G\ \mathrm{SFA} \quad (2)$$

where ΔG is a free energy change for the acidolysis of SFA and triolein. G SFAOO, G OOO, G O, and G SFA are free energies of SFAOO, OOO, O, and SFA, respectively. The magnitude of the free energy determines the direction in which the reaction proceeds. The lower ΔG for a reaction, the higher will be the completion of that reaction (*19*). Because G for medium chain SFAOO is less than the short chain and greater than C18, the reaction between MCFAs and triolein is more favorable than the others. Substrates with short acyl chain lengths cannot release enough energy, which may be used for conformational changes of the lipase to form an efficient structure, so that substrates can be properly oriented to the active site of the lipase (*20*). From this point of view, in this study, the incorporation ability of the lipases for C10−C18 FAs was found to be higher than SCFAs.

FAs with shorter less chain lengths have stronger effects on lowering pH values than LCFAs at the same concentrations. The three-dimensional structure of the enzyme might have been influenced by different pH values, and hence, catalytic activities decreased. A previous report showed an inhibitory effect of SCFAs on the reactivity of some lipases. It has been attributed that acidic substrate inhibition decreased the amount of incorporated SCFAs (*21*). An alternative explanation for the decrease in the incorporation of SCFAs into triolein could be related to the water activity. The solubility of water in the FAs decreases as the chain length of the FAs increases (*22*). The higher solubility of water causes a strong preference for the hydrolysis of triolein; thus, esterification of the SCFAs present in reaction medium might be hindered. Therefore, incorporation of the SCFAs might be reduced. In this context, a strong preference for hydrolysis of MCFAs has been reported, whereas there is no hydrolysis for SCFAs and little activity toward FAs with 16 or more carbon atoms (*21*).

The physical state of FAs could also affect the incorporation ability or reactivity. Physicochemical properties of FAs are quite dependent on their acyl chain lengths. Especially their melting points may cause a molecular rigidity at relatively lower temperatures. Saturated MCFAs and LCFAs are solid at ambient temperature, and their melting points increase with the increasing carbon number. In acidolysis reactions, the solid state of the FA could cause obstacles to the access of SFAs to the active site of the lipase, even if it is solved in an organic solvent during acidolysis. Hence, weak solubility led FAs to incorporate into triolein at lower rates.

Substrate Mole Ratio (SFA to Triolein). The mole ratio of SFAs to triolein was varied from 1:1 to 4:1. Three-dimensional plots for the interaction of acyl chain length with mole ratio on the amount of SFAs incorporated into TAG molecule are shown in **Figure 2** for individual and combined substrate forms. At individual substrate conditions (**Figure 2**, upper row), incorporation abilities of the three lipases were significantly different ($P < 0.05$) for C6 and C10−C16, C8−C18, and C8, C12, and C16−C22 FAs at substrate mole ratios of 2:1, 3:1, and 4:1, respectively. As the substrate mole ratio increased, SFAs incorporation increased especially for MCFAs for both individual and competitive substrate mediums. When the substrate mole ratio reached 3:1, the reaction reached a steady state for LCFAs (≥C18) and the amount of incorporation decreased for individual FA conditions (**Figure 2**, upper row). On the other hand, incorporation of SFAs increased with the substrate mole ratio increases for the competitive substrate medium.

Among the SFAs, the highest incorporation ratios were 38.66% for C16, 30.10% for C14, and 27.19% for C14 at a mole ratio of 4:1 with Lipozyme TL IM-, Lipozyme RM IM-, and Novozym 435-catalyzed acidolysis, respectively, at individual substrate conditions (**Figure 2**, upper row). The incorporation ability of the Lipozyme TL IM was higher than those of other two enzymes for C8−C18 FAs with the exception of C10 at a mole ratio of 4:1. It has been reported that an increase in the substrate mole ratio causes a decrease in the incorporation degree for SCFAs (*2*, *23*). In addition to SCFAs, the amount of C10 and C12 FAs incorporated was also decreased at the mole ratio of higher than 3:1 with the Lipozyme RM IM- and Novozym 435-catalyzed acidolysis due to the possible inhibition of lipase activity caused by excessive substrate concentration. On the contrary, incorporation of C6 was gradually increased with the increasing mole ratio for the three enzymes.

Incorporation of the SFAs was increased when the substrate mole ratio increased for the three enzymes at competitive substrate medium (**Figure 2**, bottom row). In most cases of various substrate mole ratios, the incorporation ability of Lipozyme RM IM was higher than those of the two other enzymes. The sum of the incorporated SFAs increased from 15.34 to 31.23% when the substrate mole ratio increased from 1:1 to 4:1. In a similar manner, previous studies have shown that a high substrate mole ratio would certainly move the reaction equilibrium to the product formation and improve the acyl incorporation (*24*). The incorporation abilities of three lipases were found to be significantly different ($P < 0.05$) for C6 and C12−C16, C6, C10, C16, and C18 and C6, C10−C14, C18, and C20 FAs at substrate mole ratios of 2:1, 3:1, and 4:1, respectively.

Reaction Temperature. The reaction temperature can affect parameters such as enzyme stability, affinity of enzyme for substrate, and preponderance of competing reactions. The thermostability of enzymes is a factor considered in their industrial

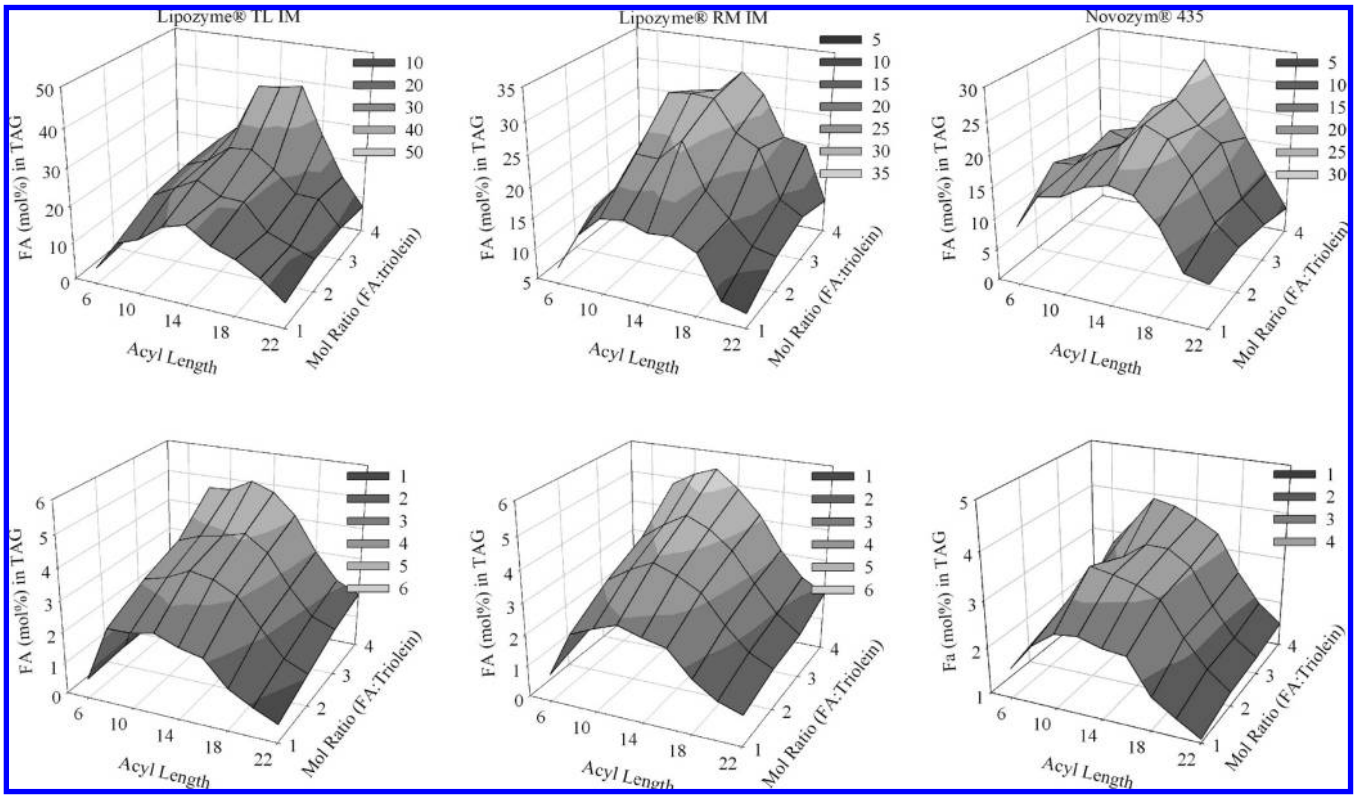

Figure 2. Interaction of acyl chain length with substrate mole ratio on incorporation (mol %) of SFAs into triolein. Upper and bottom rows show individual and mixtures of SFA substrate forms, respectively. The reaction conditions are as follows: substrate temperature, 50 °C; reaction time, 6 h; and enzyme dosage, 10%.

use, mostly because of the potential for minimizing thermal degradation (*25*). To determine the effect of temperature on acidolysis-catalyzed Lipozyme TL IM, Lipozyme RM IM, and Novozym 435 enzymes, temperatures of 40, 50, and 60 °C were tested. It was reported that higher temperature favors higher yields for endothermic reactions due to the shift of thermodynamic equilibrium. An elevated temperature can also make the operation easy, since a higher temperature increases the solubility of the substrate and decreases the viscosity of the solutions (*26*). Three-dimensional plots for the interaction of acyl chain length with temperatures on the amount of SFAs incorporated into TAG molecule are shown in **Figure 3**. In our study, MCFAs were more incorporated into the triolein molecule rather than those of SCFAs and LCFAs at all temperatures tested for three lipases. A bell-shaped distribution was obtained for acyl chain lengths in temperature-dependent acidolysis reactions when the SFAs were used as acyl donors either individually or in combined forms.

When the SFAs were used as acyl donors individually, the incorporation ability of the three lipases was significantly different ($P < 0.05$) for SFAs with the exception of C10, C12, and C22 at 40 °C. When the temperature rose to 60 °C, relatively smaller differences were observed between the lipases for SFAs. The higher acidolysis temperature eliminated the differences between the lipases for incorporation of C8, C10, and C20. Among the SFAs with different acyl chain lengths, the highest incorporation for Lipozyme TL IM was 18.12, 21.36, and 19.42% for C14, for Lipozyme RM IM was 18.10, 17.75, and 20.47% for C12, and for Novozym 435 was 20.25, 18.82, and 23.40% for C14 at 40, 50, and 60 °C, respectively. As a result of probable better mass transfer rates for C20 and C22 FAs at higher temperatures, the amount of incorporation for these FAs was increased smoothly with the increasing temperatures. It was reported that higher temperature reduces the viscosity of the lipid and certainly increases the substrate and product transfer on the surface or inside the enzyme particles (*27*).

These results showed that the lipases tested were more active at higher temperatures as compared to lower temperatures. Our findings are in accordance with the previous reports for SCFAs (*28*, *29*). In contrast to our results for LCFAs, Kojima et al. (*17*) have reported that the acidolysis reaction rates increased with the decreasing temperature for long chain PUFAs. In our study, the highest incorporation was obtained at 50 °C when the mixture of SFAs was used as the acyl donor. MCFAs were more incorporated into the triolein molecule rather than those of SCFAs and LCFAs in both substrate forms at varied temperatures. The thermostability of Novozym 435 was higher than those of two enzymes when the temperature rose to 60 °C, whereas Lipozyme RM IM was more active at 40 °C. At combined substrate conditions, the incorporation abilities of the three lipases were significantly different ($P < 0.05$) for C6, C8, C14, C18, and C20 FAs and for C6, C10, and C14−C20 at temperatures of 40 and 60 °C, respectively.

Reaction Time. To show the differences in incorporation abilities of the enzymes with the reaction times ranging from 3 to 24 h, acidolysis-catalyzed Lipozyme TL IM, Lipozyme RM IM, and Novozym 435 were performed. Three-dimensional plots for the interaction of acyl chain length with reaction times on the amount of SFAs incorporated into TAG molecule are shown in **Figure 4**. The amount of SFAs incorporated in TAG molecule was increased with the increasing acidolysis time. Once again, as shown in **Figure 4**, the lipases have a high preference for C10−C16 FAs as in the conditions previously mentioned for both substrate forms. At individual substrate conditions, incorporation abilities of the enzymes were significantly different ($P < 0.05$) for C6, C10, C12, C16, and C18 at 3 h of acidolysis. The highest incorporation was 16.49% for C14 with the Lipozyme RM IM at 3 h of

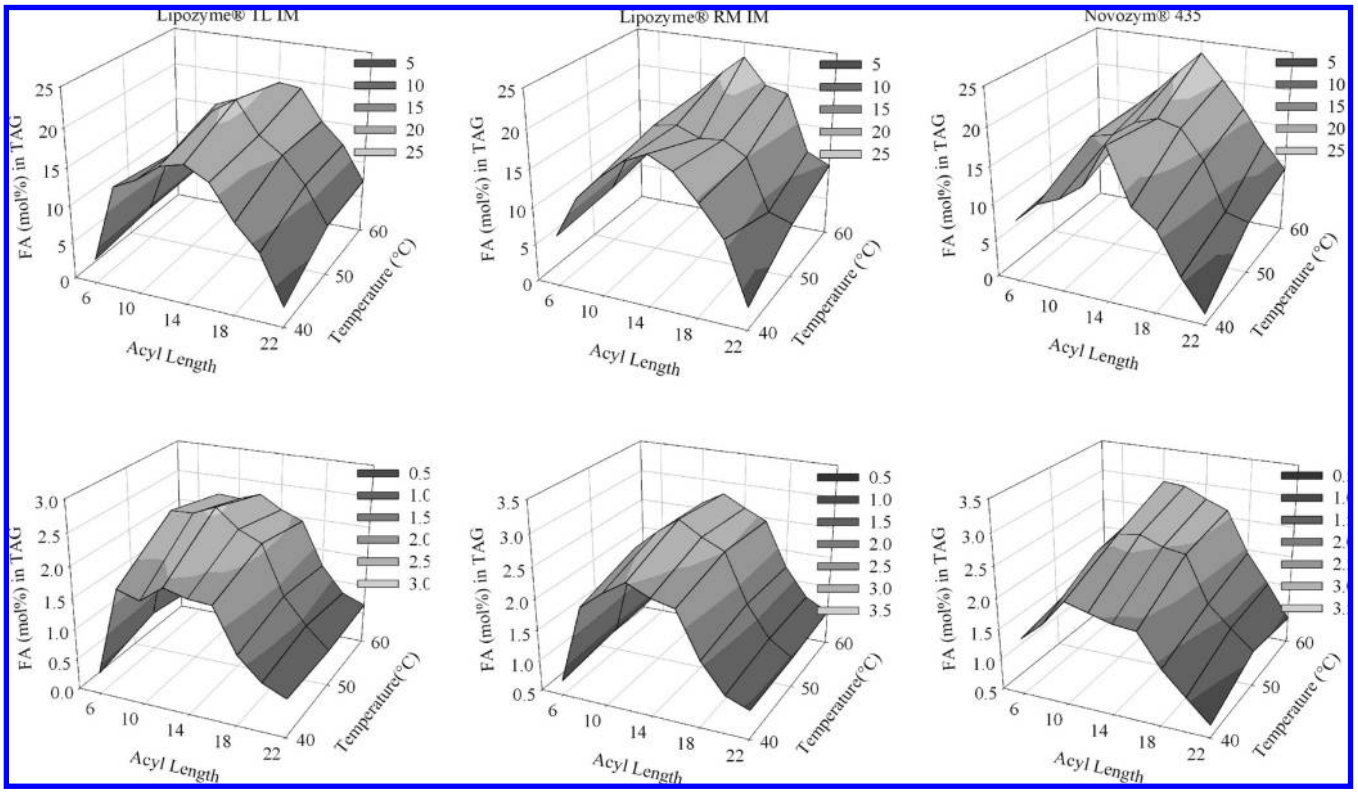

Figure 3. Interaction of acyl chain length with reaction temperature on incorporation (mol %) of SFAs into triolein. Upper and bottom rows show individual and mixtures of SFA substrate forms, respectively. The reaction conditions are as follows: substrate mole ratio, 1:1; reaction time, 6 h; and enzyme dosage, 10%.

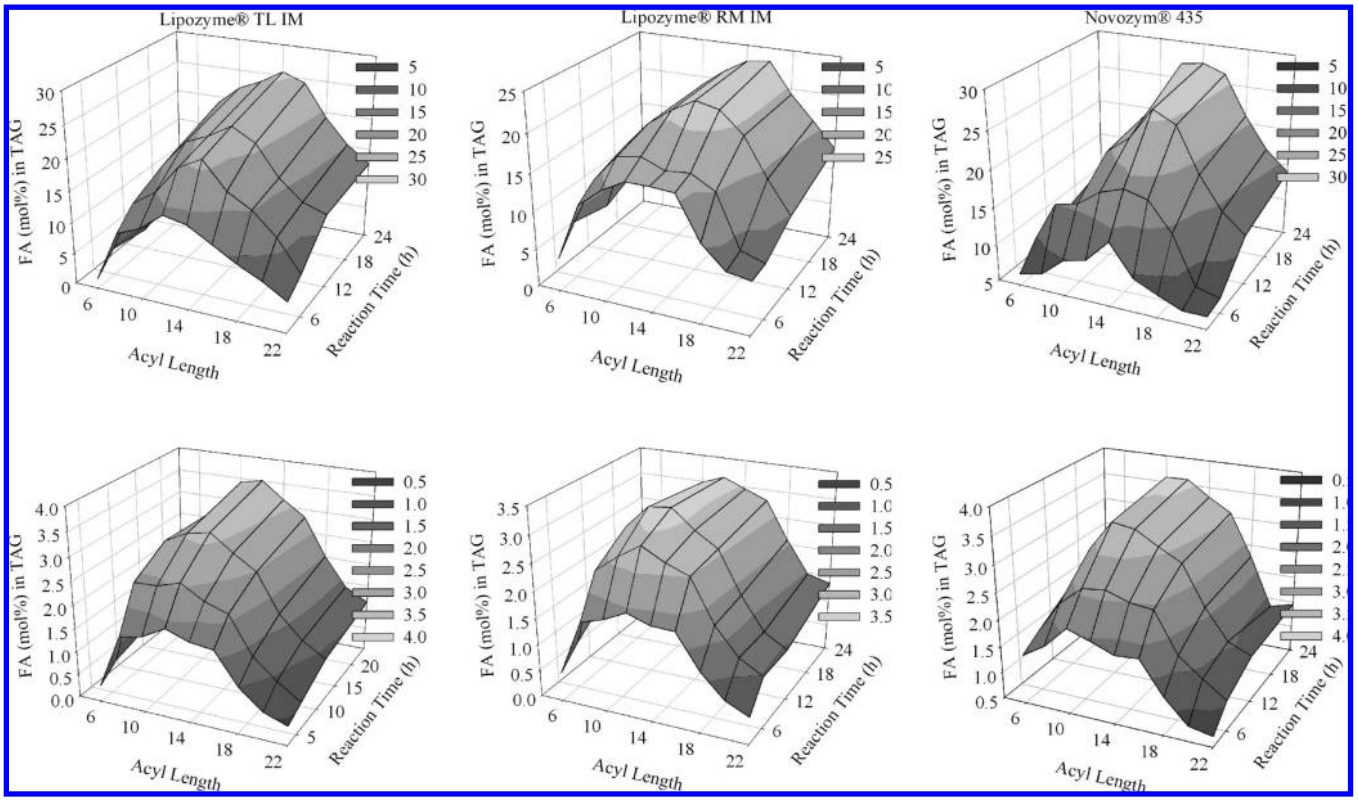

Figure 4. Interaction of acyl chain length with reaction time on incorporation (mol %) of SFAs into triolein. Upper and bottom rows show individual and mixtures of SFA substrate forms, respectively. The reaction conditions are as follows: substrate mole ratio, 1:1; temperature, 50 °C; and enzyme dosage, 10%.

acidolysis. As compared to Lipozyme TL IM and Novozym 435, Lipozyme RM IM has great incorporation ability at this time course. The lowest incorporation for SFAs at 12 and 24 h of acidolysis was obtained with Lipozyme TL IM and Lipozyme RM IM. The highest incorporation was 26.08 and 27.40% for C14 with Novozym 435 at 12 and 24 h of acidolysis, respectively.

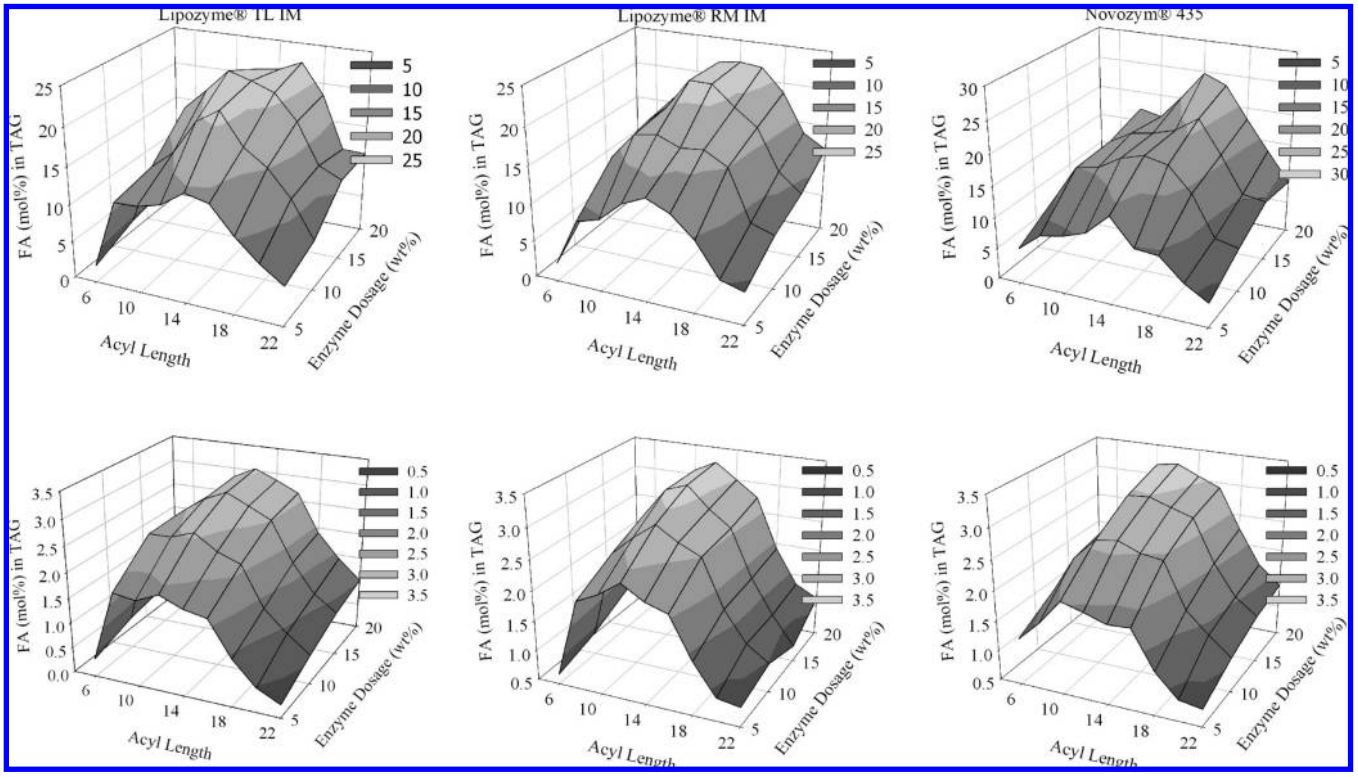

Figure 5. Interaction of acyl chain length with enzyme dosage on incorporation (mol %) of SFAs into triolein. Upper and bottom rows show individual and mixtures of SFA substrate forms, respectively. The reaction conditions are as follows: substrate temperature, 50 °C; and reaction time, 6 h.

At competitive substrate conditions, in contrast to individual substrate forms, the incorporation ability of Novozym 435 was higher than those of two enzymes for most of SFAs at all time−course applications. The amount of total SFAs incorporated increased from 13.50 to 23.65% when the reaction time increased from 3 to 24 h. The incorporation abilities of three lipases were significantly different ($P < 0.05$) for C6 and C18 FAs and for C6 and C16 FAs at reaction times of 3 and 12 h, respectively. Lipozyme RM IM, which was significantly different from the others ($P < 0.05$), has the lowest incorporation ability at 24 h of acidolysis.

Enzyme Dosage. To show the differences in incorporation abilities of the enzymes, different enzyme dosages based on the weight of total reactants ranging from 5 to 20% were tested. Three-dimensional plots for the interaction of acyl chain length with enzyme dosages on the amount of SFAs incorporated into TAG molecule are shown in **Figure 5**. In general, for the acidolysis reactions, the amount of SFAs incorporated is directly proportional to the dosage of enzyme. It was observed that the amount of SFAs incorporated was gradually increased with the enzyme dosages increased. These results are consistent with the data obtained by Paez et al. (*22*) and Turan et al. (*2*). Similar observations have also been reported by Akoh and Huang (*30*) who studied the transesterification of triolein and caprylic acid and by Fomuso and Akoh (*23*) who studied the incorporation of caproic and butyric acids into triolein. Lipases showed a bell-shaped distribution for acyl chain lengths with a maximum around C12−C16 in acidolysis reactions. At individual substrate conditions, the incorporation abilities of three lipases were significantly different ($P < 0.05$) for C16 and C20, C12, C18, and C20, and C16 FAs at the enzyme dosage of 5, 15, and 20%, respectively. Novozym 435 has the highest incorporation ability for C6, C8, C14, and C16 than those of others. Among the SFAs, the highest incorporation was 22.44% for C16, 22.07% for C14, and 24.68% for C14 at the enzyme dosage of 20% with Lipozyme TL IM-, Lipozyme RM IM-, and Novozym 435-catalyzed acidolysis, respectively. It should be noted that when the enzyme dosage increased to 15%, the reaction seems to have reached the equilibrium; hence, the amount of incorporated C8−C14 and C20, C12, and C6, C10, and C22 decreased at 20% enzyme dosage for Lipozyme TL IM-, Lipozyme RM IM-, and Novozym 435-catalyzed acidolysis, respectively. Similar observations have also been reported that the incorporation of the C10 reached its maximum at 15% enzyme dosage at the acidolysis between C10 FA and lard (*26*).

At competitive substrate conditions, the incorporation ability of Lipozyme TL IM was lower than those of two enzymes for SFAs. The total amount of SFAs incorporated with Lipozyme RM IM- (14.51%) and Novozym 435- (13.42%) catalyzed acidolysis was not significant ($P > 0.05$), while Lipozyme RM IM was more active than Novozym 435 for incorporation of C12 to C18 FAs. The amount of total incorporated SFAs elevated at other enzyme dosages with the Novozym 435 and the highest incorporation were obtained for C6, C16, C18, and C22 and C6 and C16−C20 FAs at 15 and 20% enzyme dosages, respectively.

FAs at sn-2 Position. Despite the specifity of lipase to the sn-1,3 position, incorporation of FAs into acylglycerols at sn-2 position occurs due to acyl migration. To compare the amount of acyl migration of SFAs caused by commercial lipases tested, the product of acidolysis reaction performed at competitive substrate conditions was used. The mole percentages of FAs at sn-2 positions of TAGs are given in **Figure 6**. As can be seen, acidolysis reaction performed at default conditions given above with three lipases caused significantly different ($P < 0.05$) acyl migration from sn-1,3 to sn-2 positions. FA chain lengths that varied from C12 to C18 and C10 to C22 were found at sn-2 positions of TAG obtained from Lipozyme TL IM/RM IM- and Novozym 435-catalyzed acidolysis, respectively. No migration was observed for FAs chain length smaller than 10 carbons. Besides, the amount of C16 migrated was greater than those of other FAs with three

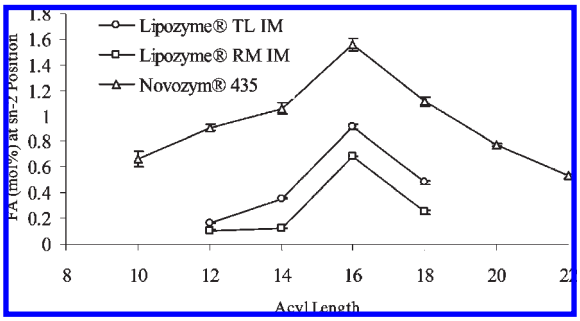

Figure 6. Effect of lipases on acyl migration of SFAs at competitive substrate medium in acidolysis between SFAs and triolein.

enzymes catalyzed acidolysis. Among the enzymes, the lowest acyl migration occurred with Lipozyme RM IM, immobilized on anion exchange resin particles.

In conclusion, nine SFAs were used as acyl donors in three immobilized lipases catalyzed acidolysis reactions to determine the possible preferences for the FAs, which have different acyl chain lengths, between the enzymes tested. As compared to the other FAs, lipases preferably incorporated the C12−C16 FAs. Selectivity of the lipases notably depends on the chain length of FA, which provides different physicochemical properties. It was estimated that the physical or conformational changes on the active site of the lipase strongly affected such parameters during acyl−enzyme complex forming. The incorporation abilities of the enzymes tested were found to be significantly different for the majority of FAs at the experimental conditions evaluated. Among all of the experimental conditions tested, the effect of substrate mole ratio was greater than those of others, and the highest incorporation was observed for C12 (36.98%), C14 (37.63%), and C16 (38.66%) at a 4:1 substrate mole ratio in Lipozyme TL IM-catalyzed acidolysis reaction. Our findings related to acyl chain length selectivity of different lipases may provide helpful information for future lipase applications and will be conducted in academia and industry.

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