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ORIGINAL PAPER

C18 Unsaturated Fatty Acid Selectivity of Lipases During the Acidolysis Reaction Between Tripalmitin and Oleic, Linoleic, and Linolenic Acids

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Abstract The C18 unsaturated fatty acid (UFA) selectivity of three immobilized lipases, namely, Lipozyme TL IM from *Thermomyces lanuginosa*, Lipozyme RM IM from *Rhizomucor miehei*, and Novozym 435 from *Candida antarctica*, was determined in acidolysis conducted in hexane. Tripalmitin with a mixture of equimolar quantities of C18 UFAs was used as the substrate. Significantly different incorporation rates were observed for C18 UFAs used ($p < 0.05$). The highest incorporation was obtained for all three C18 UFAs with Novozym 435 followed by Lipozyme RM IM and Lipozyme TL IM catalyzed acidolysis under default conditions (substrate mole ratio 1:1; temperature 50 °C; reaction time 6 h; enzyme dosage 10%). Incorporation of the equimolar quantities of C18 UFAs was in the order C18:3 > C18:2 > C18:1 which also reflects C18 UFAs preferences of the lipases. The effects of operating variables on incorporation or UFA selectivity of lipases were also investigated. Among the experimental parameters including the mole ratio of fatty acid to triolein, temperature, enzyme dosage, and time on incorporation, the effect of the substrate mole ratio on UFA selectivity was greater than those of the others.

Keywords Lipase · Selectivity · Acidolysis · Unsaturated fatty acid · Tripalmitin · *Thermomyces lanuginosa* · *Rhizomucor miehei* · *Candida antarctica*

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Introduction

Lipases [triacylglycerol (TAG) acylhydrolases, E.C. 3.1.1.3] are very versatile enzymes that catalyze a large number of reactions. Lipases can be used as biocatalyst for hydrolysis, esterification, acidolysis, interesterification and modification of fats and oils [1]. The acidolysis activity of the lipases has been widely used for transesterification between TAG and fatty acid (FA) to produce structured lipids (SLs) [2].

Lipases have various degrees of selectivity towards FAs involved in fat and oil modification. Lipase specificity may be due to the structural features of the substrate, such as FA chain length, unsaturation, stereochemistry, physicochemical factors at the interface, and differences in the binding site of the enzyme. The reactivity of FAs may vary depending on the composition of substrates, water activity, nature of solvents and source of lipase [3]. There are numerous reports in the literature evaluating the selectivity of commercially available lipases in hydrolysis, esterification and transesterification reactions. The FA selectivity of commercially available lipases evaluated in acidolysis with substrate combinations of different acyl donors and the same TAG [4] or the same FA (acyl donor) and different TAGs [5]. Hamam and Shahidi [6, 7] examined the effect of chain length, number of double bonds, the location and geometry of double bonds, the reaction conditions, and the reactivity of five lipases on the incorporation of long-chain fatty acids (LCFAs) into TAGs, such as tristearin, trilinolein and trilinolenin. Shimada et al. [8] determined FA specificity of *Rhizopus delemar* lipase in acidolysis using randomly interesterified oil. FA selectivity of lipase from *Geotrichum candidum* was determined in esterification reaction using LCFAs and 1-butanol by Sonnet et al. [9].

Here we report an evaluation of the C18 unsaturated fatty acid (UFA) selectivity of the lipases in acidolysis between tripalmitin and UFA including oleic, linoleic and linolenic acids. In addition to selectivity of the lipases, the information given here will be useful for studies on the synthesis of human milk fat substitute (HMFS) by lipase catalyzed acidolysis. In general, human milk fat contains 20–25% of palmitic acid, and about 70% of that esterified to the *sn*-2 position of the glycerol backbone, and the *sn*-1 and *sn*-3 positions are mostly occupied by UFAs such as oleic acid [15]. Thus, the fats and oils containing abundantly the TAG structure of tripalmitin [10–12] and/or tripalmitin alone [13, 14] have been used to synthesize the HMFS by acidolysis with UFAs using lipase, and clarification of C18 UFA selectivity of lipase is required.

The purpose of the present work was to investigate C18 UFA selectivities of the commercially lipases, namely, Lipozyme® TL IM from *Thermomyces lanuginosa* (immobilized on granulated silica particles), Lipozyme® RM IM from *Rhizomucor miehei* (immobilized on anion exchange resin particles), and Novozym® 435 from *Candida antarctica* lipase B (immobilized on macroporous acrylic resin beads) in acidolysis between tripalmitin, and oleic, linoleic and linolenic acids. In order to emphasize the incorporation attitude of the lipases, different reaction conditions, effect of operating variables such as mole ratio of C18 UFA to tripalmitin, temperature, enzyme dosage and reaction time on incorporation was also investigated.

Materials and Methods

Materials

Lipases were provided by Novo Nordisk A/S (Bagsvaerd, Denmark). Oleic (C18:1, *cis*-9-octadecenoic acid, catalog number: O1008, 99%), linoleic (C18:2, *cis*,*cis*-9,12-octadecadienoic acid, catalog number: L1376, 99%), linolenic (C18:3, *cis,cis,cis*-9,12,15-octadecatrienoic acid; α-linolenic acid, catalog number: L2376, 99%) acids and tripalmitin (catalog number: T5888, 99%) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). 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

Experimental design was similar to those reported in a previous study [4]. Equimolar quantities of C18 UFAs (C18:1 + C18:2 + C18:3, a total of 0.12 mmol) were combined in hexane. The mixture of tripalmitin with a volumetric amount of C18 UFAs solution was used in acidolysis. Reactions were carried out in tightly closed, screw-capped glass vials (20 mL) containing C18 UFAs solution-tripalmitin mixture. The total volume of the reaction mixture was 3 mL. The vials were incubated in a shaking water bath at 200 rpm. The effects of substrate mole ratios (C18 UFAs: tripalmitin) ranging from 1:1 to 4:1, temperatures ranging from 40 to 60 °C, reaction times ranging from 3 to 24 h, and enzyme dosages ranging from 5 to 20% (by total weight of substrates) on the incorporations were studied. To determine the effects of different parameters on the incorporation of FAs into triolein, the default conditions were chosen as the following: substrate mole ratio 1:1, temperature 50 °C, reaction time 6 h, enzyme dosage 10%, and no extra water addition.

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 [16]. 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 auto injector (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 per 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.

Statistical Analysis

SPSS version 9.0 (SPSS Inc., Chicago, IL, USA) was used to perform statistical calculations. Significant differences in the means of incorporated C18 UFAs (mol%) between UFAs and lipases catalyzed acidolysis were determined by using a LSD test and ANOVA procedure ($p < 0.05$).

Results and Discussion

Selectivity of Lipases under Default Conditions

The amount of C18-UFAs incorporated into tripalmitin with the lipase catalyzed acidolysis at default conditions (substrate mole ratio of 1:1, temperature of 50 °C, reaction time of 6 h, and enzyme dosage of 10%) are shown in Fig. 1. In general, significant differences were observed for the level of C18 UFAs incorporated in TAG tripalmitin ($p < 0.05$) with the enzymes tested. The highest to the lowest incorporation for all three C18 UFAs was obtained with Novozym 435, Lipozyme RM IM and Lipozyme TL IM catalyzed acidolysis, respectively. Incorporation of the equimolar quantities of C18 UFAs was in the order of C18:3 > C18:2 > C18:1 ($p < 0.05$). This result showed that as the unsaturation degree of C18 FA increased, the degree of incorporation also increased. This finding is not

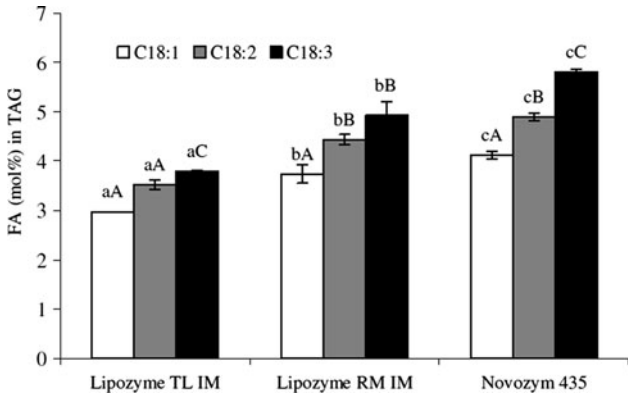

Fig. 1 Effect of lipases on incorporation (mol%) of C18 UFAs into tripalmitin. (The reaction conditions are as follows: substrate mole ratio of 1:1, temperature of 50 °C, reaction time of 6 h and enzyme dosage of 10%). Different *lower case* letters indicate statistical differences in incorporation between the enzymes for the same UFA while different *capital letters* indicate statistically significant differences between the amounts of UFAs incorporated for the same enzyme

consistent with the mechanism proposed by Hamam and Shahidi [6, 7]. In the mentioned studies, the researchers screened lipases for their catalytic effect on the acidolysis of selected LCFA with tristearin [6], trilinolein and trilinolenin [7]. A reverse relationship between the unsaturation degree and incorporation rates was found in their studies. They claimed that structural differences related to the number of double bonds and their location in the UFA molecule might affect the reactivity of FA. Especially, it was emphasized that three double bonds form a hooked shape which makes their incorporation more difficult than the FAs including fewer double bonds. The results of the present study were found to be different with their findings most probably because of the use of tripalmitin instead of trilinolein/trilinolenin as TAG in the substrate mixture.

It has been shown that the degree of unsaturation of FA is responsible for the lower rate of TAG synthesis [17]. In contrast to this finding, reactivity of FA was affected positively with the increasing unsaturation degree in the current study. Our results are in agreement with those obtained by Rønne et al. [18] who reported a similar trend for Lipozyme TL IM. In this study, Lipozyme TL IM was shown to be more selective toward C18 FAs with a higher degrees of unsaturation in interesterification reactions.

As mentioned in the previous reports [4, 5], incorporation of FA into target TAG in acidolysis is governed by many factors. Some regions of the molecular structure responsible for the catalytic action of lipase are exposed to a serious of changes during formation of the acyl–enzyme complex as explained in the report by Pleiss et al. [19]. The geometry of the binding sites of the lipases has a strong effect on the selectivity. For this reason, shape or geometry of the substrate has to be fit with this region of the enzyme. The substrate selectivity of the lipases in acidolysis is dependent on the geometry of FAs to be incorporated into the glycerol moiety, as well as on the geometry of acyl groups of the glycerol backbone. The substrates including UFA and tripalmitin have different geometrical structures which affect the amount of acyl–enzyme complex formed. As confirmed in previous reports, the properties of the substrate have a strong impact on FA selectivity of the lipases. Different incorporation rates were observed with the substrate combinations of different acyl donors and the same TAG [4] or the same FA (acyl donor) and different TAGs [5].

The other factors that affected the activity of lipases have been mentioned in our previous report [4]. The factors include free energy changes between the substrates and products, variation in pH values that may alter three-dimensional structure of the enzyme responsible for the catalytic activity, the effect of the chain length of FAs on solubility of the water which determines the direction of the

reaction (hydrolysis or esterification), and finally the physical state of the substrate may affect reactivity of the FAs.

Substrate Mole Ratio (UFAs to tripalmitin)

A combination of equimolar quantities of C18 UFAs, at C18 UFA to tripalmitin ratios of 1:1, 2:1, 3:1, and 4:1, was used to investigate the effect of substrate mole ratio on the incorporation of these FAs into tripalmitin as presented in Fig. 2. For different enzymes investigated, significant differences were observed ($p < 0.05$) for incorporation of C18 UFAs into tripalmitin at different substrate mole ratios with the exception of a substrate mole ratio of 2:1. The amounts

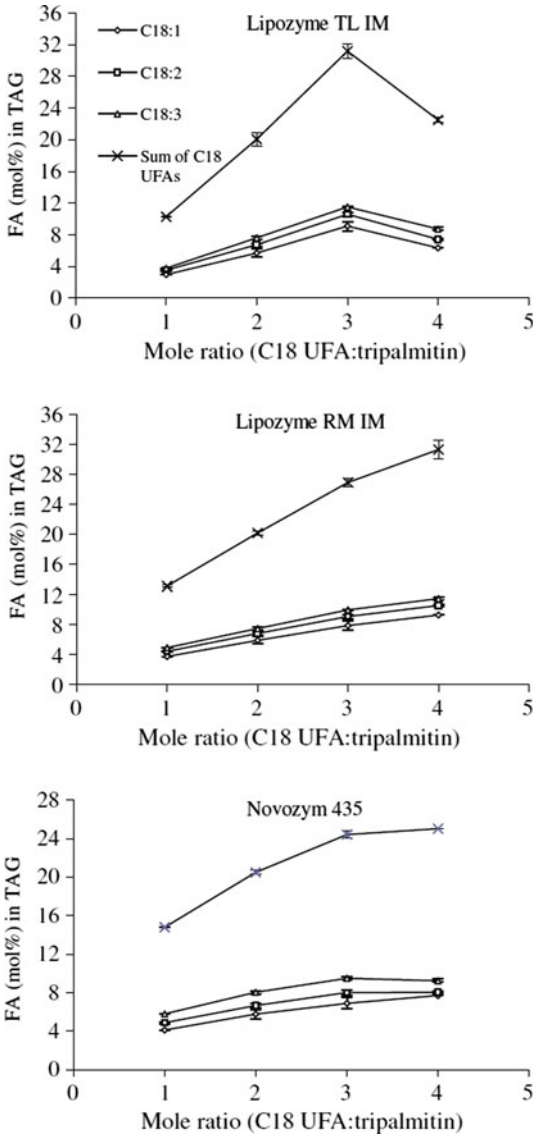

Fig. 2 Interaction of C18 UFAs with substrate mole ratio on incorporation (mol%). The reaction conditions are as follows: reaction temperature 50 °C, reaction time 6 h; and enzyme dosage 10%

of C18:1, C18:2 and C18:3 incorporated were also significantly different ($p < 0.05$) for each mol ratio. As the mole ratio increased from 1:1 to 4:1, incorporation of UFA increased smoothly for the reaction catalyzed by Lipozyme RM IM. The reaction started to deviate from a linear relationship between incorporation and the ratio of UFA to TAG at substrate mole ratio of 3:1 for UFAs with the Lipozyme TL IM and Novozym 435 catalyzed acidolysis. It was reported that an increase in substrate mole ratio lead to a decrease in the incorporation due to possible inhibition of lipase activity caused by higher substrate concentration [20]. The decrease in the incorporation of C18 UFAs with Lipozyme TL IM at a mole ratio of 4:1 may be caused by the same reason.

Similar to the incorporation order of the C18 UFAs observed at default conditions, the order of C18:3 > C18:2 > C18:1 was observed for all substrate mole ratios tested. When the substrate mole ratio increased from 1:1 to 3:1, the sum of the incorporated C18 UFAs increased from 10.29 to 31.20% and from 14.81 to 24.45% with Lipozyme TL IM and Novozym 435 catalyzed acidolysis, respectively. The sum of incorporated C18 UFAs increased from 13.10 to 31.32% when the substrate mole ratio increased from 1:1 to 4:1, respectively, with Lipozyme RM IM. This was the highest value for the sum of incorporated C18 UFAs among the experimental parameters tested.

Reaction Temperature

To clarify the effect of temperature on the C18 UFAs selectivity of the lipases, reaction temperatures of 40, 50, and 60 °C were applied in the acidolysis reactions. The reaction temperature may affect parameters such as enzyme stability, affinity of enzyme for substrate, and preponderance of competing reactions. It was reported that higher temperatures favor higher yields for endothermic reactions due to the shift of thermodynamic equilibrium. An elevated temperature can also make the operation easy, since it increases the solubility of the substrate and decreases the viscosity of the solutions [21]. The plot for the incorporation of C18 UFAs versus reaction temperature is given in Fig. 3. Lipozyme TL IM and RM IM incorporated C18:2 into tripalmitin in the same level ($p > 0.05$) at the reactions performed at 40 °C. Among these enzymes, the highest amount of C18 UFAs incorporation (8.78%) in total was obtained with Novozym 435 at this temperature ($p < 0.05$). Differences in the sum of incorporated C18 UFAs were also significant ($p < 0.05$) for each enzyme at 50 °C. Lipozyme RM IM and Novozyme 435 had the same incorporation level when the temperature rose to 60 °C. The optimum temperature has been found as 60 °C in a study conducted to determine the effect of temperature on the incorporation of C18:1 into tripalmitin [22]. When the

temperature increased from 40 to 60 °C, the sum of the incorporated C18 UFAs increased from 7.41 to 12.30% and from 7.16 to 14.80%, with Lipozyme TL IM and RM IM catalyzed acidolysis, respectively in the present study.

Interestingly, it has been reported that the thermostability of Novozym 435 was higher than those of other two enzymes when the temperature rose to 60 °C [4]. In contrast to this finding, the incorporation ability of Novozym 435 decreased at a higher temperature (60 °C) in the current study. The main difference between previous and current studies was the use of different substrates. It was concluded that relationship between reaction temperature and substrate properties could help to determine the limits of the reaction temperature for best activity of lipases in acidolysis. In addition, due to the lack of antioxidant protection in our experimental conditions, oxidative reactions might have also decreased the actual level of incorporated UFAs.

Reaction Time

Effect of various reaction times ranging from 3 to 24 h on the amount of C18 UFAs incorporated into tripalmitin was investigated. The interaction between unsaturation degrees of C18 FA with reaction times are shown in Fig. 4. The amount of C18 UFAs incorporated in tripalmitin molecule was increased with the increasing of acidolysis time. Lipases showed a preference for incorporation of UFAs in the order of C18:3 > C18:2 > C18:1.

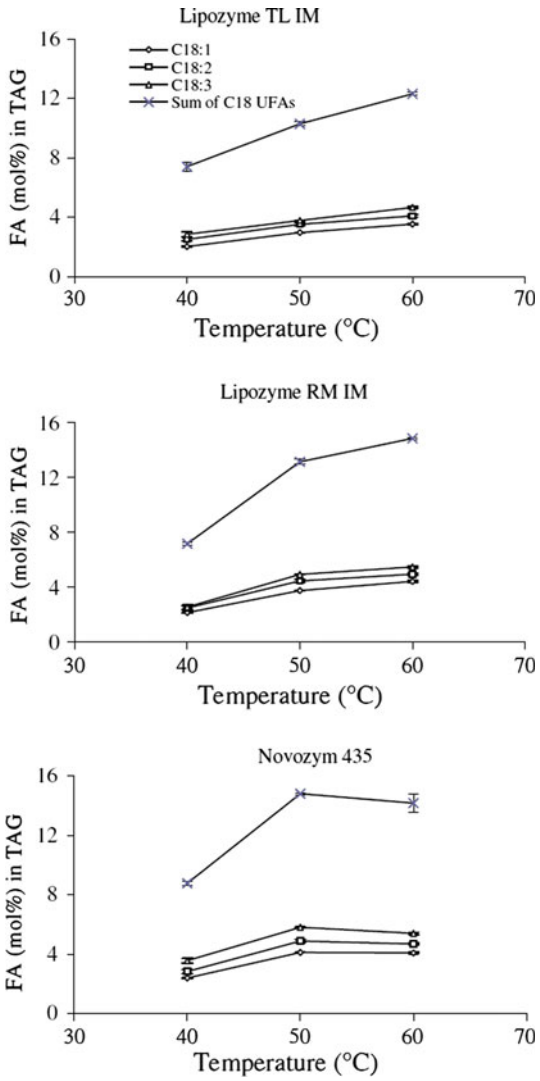

Fig. 3 Interaction of C18 UFAs with reaction temperature on incorporation (mol%). The reaction conditions are as follows: substrate mole ratio 1:1; reaction time 6 h; and enzyme dosage 10%

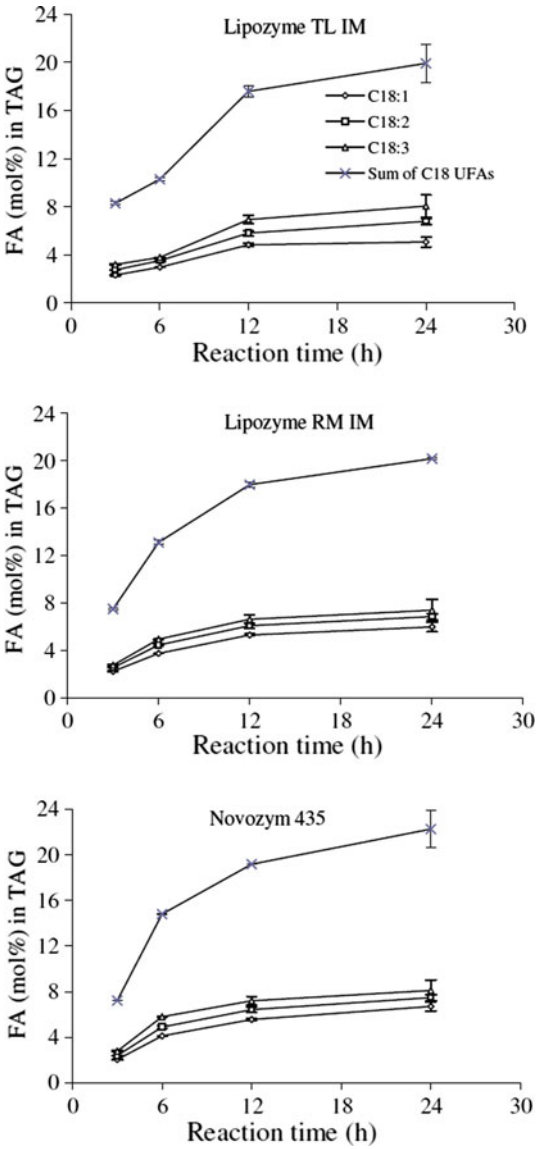

Fig. 4 Interaction of C18 UFAs with reaction time on incorporation (mol%). The reaction conditions are as follows: substrate mole ratio 1:1, reaction temperature 50 °C, and enzyme dosage 10%

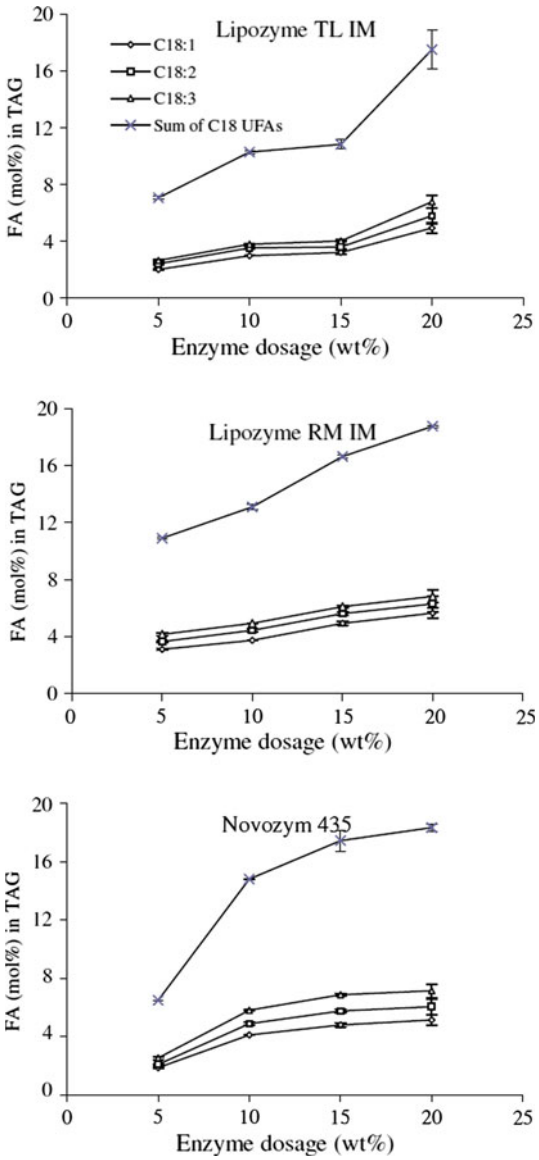

Fig. 5 Interaction of C18 UFAs with enzyme dosage on incorporation (mol%). The reaction conditions are as follows: substrate mole ratio 1:1, reaction temperature 50 °C, and reaction time 6 h

Incorporation abilities of the Lipozyme TL IM at 3 h were slightly different ($p < 0.05$) compared to other lipase at 3 h: the highest value (8.28% for sum of C18 UFA) was achieved in the reaction with the Lipozyme TL IM. In contrast to this result, the lowest incorporation for C18 UFAs at 6, 12 and 24 h of acidolysis was obtained with Lipozyme TL IM ($p < 0.05$). The highest incorporation at 12 and 24 h of acidolysis was achieved in the reaction with Novozym 435 (19.17 and 22.27% for sum of C18 UFAs at 12 and 24 h, respectively).

Enzyme Dosage

To determine the effect of enzyme dosage on the C18 UFAs selectivity of the lipases, different enzyme dosages based on the weight of total reactants ranging from 5 to 20%, with 5 increments, were tested. The interaction between unsaturation degrees of C18 FA with enzyme dosages are shown in Fig. 5. In general, for the acidolysis reactions, the amount of C18 UFAs incorporated is directly proportional to the dosage of enzyme. It was observed that the amount of C18 UFAs incorporated was gradually increased as the enzyme dosages increased. These results are consistent with the data obtained by Paez et al. [23] and Turan et al. [24]. Compared to the others, the highest difference for sum of C18 UFAs incorporated was obtained with the changing of enzyme dosage from 15 to 20% (10.83 ± 0.33 and 17.50 ± 1.38%, respectively) with Lipozyme TL IM catalyzed acidolysis. There were no differences ($p > 0.05$) between the different enzymes for the incorporation level of each C18 UFAs at the enzyme dosage of 20%. Incorporation ability of Lipozyme TL IM was lower than those of other two enzymes at all enzyme dosages. This might be due to the reaction conditions employed which were more suitable for Lipozyme RM IM and Novozym 435. A lot of factors such as the structure of the immobilization matrix which may affect the three-dimensional structure of the active site, the amount of the lipase in immobilized form, lower activity of the enzyme and the factors explained before may be responsible for this result.

Conclusions

C18 UFAs were used as acyl donors in three immobilized lipases catalyzed acidolysis reactions to determine the possible preferences for the C18 UFAs or investigate the effect of unsaturation degree of FA on incorporation into tripalmitin. Incorporation of the C18 UFAs was in the order of C18:3 > C18:2 > C18:1 which reflects the selectivity of the lipases notably depends on the unsaturation degree of FA. Among all of the experimental conditions tested, the effect of substrate mole ratio was greater than those of others. This study gives useful information related to the interactions between the lipases and the substrates regarding to the C18 UFA selectivity of the lipases in acidolysis.

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References

1. Gandhi NN (1997) Application of lipase. JAOCS 74:621–634
2. Xu X (2000) Production of specific-structured triacylglycerols by lipase-catalyzed reactions: a review. Eur J Lipid Sci Technol 102:287–303

3. Yang Y, Xu X, Li L (2001) Comparison of linoleic and conjugated linoleic acids in enzymatic acidolysis of tristearin. J Food Lipids 8:149–161
4. Karabulut I, Durmaz G, Hayaloglu AA (2009) Fatty acid selectivity of lipases during acidolysis reaction between triolein and saturated fatty acids varying from caproic to behenic acids. J Agric Food Chem 57:7584–7590
5. Karabulut I, Durmaz G, Hayaloglu AA (2009) Fatty acid selectivity of lipases during acidolysis reaction between oleic acid and monoacid triacylglycerols. J Agric Food Chem 57:10466–10470
6. Hamam F, Shahidi F (2007) Acidolysis of tristearin with selected long-chain fatty acids. J Agric Food Chem 55:1955–1960
7. Hamam F, Shahidi F (2008) Incorporation of selected long-chain fatty acids into trilinolein and trilinolenin. Food Chem 106:33–39
8. Shimada Y, Sugihara A, Nakano H, Nagao T, Suenaga M, Nakai S, Tominaga Y (1997) Fatty acid specificity of *Rhizopus delamar* lipase in acidolysis. J Ferment Bioeng 4:321–327
9. Sonnet PE, Foglia TA, Baillargeon NW (1993) Fatty acid selectivity: the selectivity of lipases of *Geotrichum candidum*. JAOCS 70:1043–1045
10. Christensen TC, Hølmer G (1993) Lipase catalyzed acyl-exchange reactions of butter oil. Synthesis of a human milk fat substitute for infant formulas. Milchwissenschaft 48:543–547
11. Yang T, Xu X, He C, Li L (2003) Lipase-catalyzed modification of lard to produce human milk fat substitutes. Food Chem 80:473–481
12. Yang TK, Fruekilde MB, Xu XB (2003) Applications of immobilized *Thermomyces lanuginosa* lipase in interesterification. JAOCS 80:881–887
13. Schmid U, Bornscheuer UT, Soumanou MM, McNeill GP, Schmid RD (1999) Highly selective synthesis of 1,3-oleoyl-2-palmitoylglycerol by lipase catalysis. Biotechnol Bioeng 64:678–684
14. Sahin N, Akoh CC, Karaali A (2005) Lipase-catalyzed acidolysis of tripalmitin with hazelnut oil fatty acids and stearic acid to produce human milk fat substitutes. J Agric Food Chem 53:5779–5783
15. Martin JC, Bougnoux P, Antoine JM, Lanson M, Couet C (1993) Triacylglycerol structure of human colostrums and mature milk. Lipids 28:637–643
16. Jennings BH, Akoh CC (1999) Enzymatic modification of triacylglycerol of high eicosapentaenoic and docosahexaenoic acids content to produce structured lipids. JAOCS 76:1133–1137
17. Selmi B, Gontier E, Ergan F, Thomas D (1998) Effects of fatty acid chain length and unsaturation number on triglyceride synthesis catalyzed by immobilized lipase in solvent-free medium. Enzyme Microb Technol 23:182–186
18. Rønne TH, Pederson LS, Xu X (2005) Triglyceride selectivity of immobilized *Thermomyces lanuginosa* lipase in interesterification. JAOCS 82:737–743
19. Pleiss J, Fischer M, Schmid RD (1998) Anatomy of lipase binding sites: the scissile fatty acid binding site. Chem Phys Lipids 93:67–80
20. Sellappan S, Akoh CC (2001) Synthesis of structured lipids by transesterification of triolein catalyzed by Lipozyme IM 60. J Agric Food Chem 49:2071–2076
21. Zhao H, Lu Z, Bie X, Lu F, Liu Z (2007) Lipase catalyzed acidolysis of lard with capric acid in organic solvent. J Food Eng 78:41–46
22. Guncheva M, Zhiryakova D, Radchenkova N (2008) Acidolysis of tripalmitin with oleic acid catalyzed by a newly isolated thermostable lipase. JAOCS 85:129–132
23. Paez BC, Medina AR, Rubio FC, Cerdan LE, Grima EM (2003) Kinetics of lipase-catalyzed interesterification of triolein and caprylic acid to produce structured lipids. J Chem Technol Biotechnol 78:461–470
24. Turan S, Karabulut I, Vural H (2006) Effects of reaction parameters on the incorporation of caprylic acid into soybean oil for production of structured lipids. J Food Lipids 13:306–317