Enzymatic Acidolysis of Tristearin with Lauric and Oleic Acids to Produce Coating Lipids[1]

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ABSTRACT: Triacylglycerols with potential for coating application were prepared by acidolysis of tristearin with lauric and oleic acids using Lipozyme IM60 lipase in *n*-hexane. The effects of reaction parameters such as time, temperature, substrate mole ratio, water content, enzyme load, and enzyme reuse were studied. Five-gram scale synthesis was carried out to obtain the melting profile of products by differential scanning calorimetry (DSC). An acceptable melting profile was obtained for the product obtained with a 1:4:1 (tristearin/lauric acid/oleic acid) mole ratio of reactants. The DSC melting peak for this product was 31.4°C. Synthesis of 1200 g of this product was carried out at a 1:4:1 substrate ratio in a stirred tank batch reactor under optimal conditions. The reaction product, purified by short-path distillation, was coated onto crackers and studied for its moisture inhibition ability, under water vapor-saturated atmosphere, in a desiccator over different time intervals. The effectiveness of the synthesized lipid as a coating material was compared against uncoated crackers as a control and with cocoa butter-coated crackers. The synthesized lipid was better in preventing moisture absorption than cocoa butter.

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KEY WORDS: Acidolysis, coating lipid, cocoa butter, Lipozyme IM60, moisture inhibition, transesterification, tristearin.

Lipase-catalyzed modification is used to alter the fatty acid composition and physicochemical, nutritional, and functional properties of fats and oils to meet particular applications. Lipases can catalyze a variety of reactions including hydrolysis, esterification, and transesterification with a wide range of substrates (1). The ability of lipases to catalyze reactions under low water conditions with various reactivities, specificities, and selectivities toward fatty acids, and their positional distribution in triacylglycerol (TAG) make them remarkable biocatalysts for fats and oils modification (2–4). With the current understanding of lipase-catalyzed lipid modification, it is possible to synthesize or tailor-make specific TAG compositions. The final product end use and cost of the overall process are the two determining factors in adopting this technology.

Many value-added lipids have been synthesized using lipases. For example, synthesis of cocoa butter substitutes (5), human milk fat substitutes (6), flavors and fragrances (7,8), position-specific low-calorie lipids (9), and modified lipids that incorporate eicosapentaenoic and docosahexaenoic acids (10) have been reported. Meanwhile, the functionality of lipids in food products varies from one product to another. For instance, high- and sharp-melting fats are preferred in confectioneries, whereas liquid and odorless oils are favored in salad dressings. This variation in functionality from one product to another demands a very specific type of lipid for a particular application. One such important application is as a coating material for foods.

Coatings are applied on food products for variety of reasons. The edible coating is prepared basically from polysaccharides, proteins, and lipids (11). The first two components are effective in preventing or minimizing the transport of gases at relatively low humidity. Unlike these hydrophilic components, lipids are very effective as moisture barriers. Lipids can control mass transfer by preventing the movement of moisture, permeable gases, and aromas between the food and its external environment. Lipids are preferred as coating materials mainly to inhibit the loss of moisture and to improve the surface appearance of the finished product. Many types of lipids have been used as a coating material, including waxes, fats, and oils. Other than waxes, acetylated glyceride monoesters of long-chain fatty acids are used. For many applications, waxes are used as coating materials to protect against moisture loss (12) especially for edible coatings. Other than moisture prevention, lipid coatings are used for other ingredients such as nuts, or for protecting encapsulated materials from moisture absorption. Food products like fruits and vegetables, confectioneries, nuts, refrigerated and frozen meat, baked products, and moisture-sensitive ingredients are coated with lipids (13). Waxes form flexible films at room temperature and retain their flexibility at low temperature and are fairly effective moisture barriers. TAG from various sources and with various compositions could be used for this purpose.

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The terms "fats" and "oils" are used interchangeably to describe lipids but they are not equivalent terms. The major physical property difference between a fat and an oil is melting point. Fats are solid at ambient temperature whereas oils are liquid. Hydrogenation of many unsaturated oils such as cottonseed, peanut, soybean, or other vegetable oils makes them solid or wax-like substances with suitable physical characteristics for many applications. This increase in melting point also improves their viscoelastic and mechanical properties and imparts greater chemical stability and increased moisture resistance (14,15). Hydrogenation increases the melting point and at the same time increases the *trans*-fatty acid content of final products.

Many higher-value-added products have been obtained by enzymatic modification of lipids such as cocoa butter equivalent (CBE) (16), compound coating lipids (17), margarine-type fats (18), and plastic fats (19). After considering these possibilities, an attempt was made enzymatically to produce a new kind of lipid that may be suitable for coating applications, specifically as a moisture barrier. The synthesis and effectiveness of such a lipid is reported in this paper.

MATERIALS AND METHODS

Tristearin (1,2,3,-trioctadecanoylglycerol), lauric and oleic acids of 99% purity were obtained from Sigma Chemical Co. (St. Louis, MO). Gallium, 99.9999% purity was purchased from Aldrich Chemical Co. (Milwaukee, WI). Immobilized *sn*-1,3 specific lipase IM60, 7.1 BAUN/g (Batch Acidolysis Units Novo) from *Rhizomucor miehei* was obtained from Novo Nordisk Biochem North America, Inc. (Franklinton, NC). Cocoa butter was from Loders Croklaan (Aawormerveer, Holland). *n*-Hexane and all other chemicals were purchased from Fisher Scientific (Norcross, GA).

Enzymatic acidolysis. Acidolysis reactions were carried out in screw-capped test tubes containing of 89.15 mg of tristearin (0.1 mM), 80.12 mg of lauric acid (0.4 mM), 113.00 mg of oleic acid (0.4 mM), 39.51 mg (0.28 BAUN, 14% w/w of reactants) of lipase, and 3 mL of hexane. The reaction mixture was incubated in a gyratory shaking water bath at 60°C for 24 h at 200 rpm unless otherwise stated. All reactions were conducted in duplicate.

Extraction and analysis. After a predetermined incubation time the reaction was stopped by filtering the reaction mixture through an anhydrous sodium sulfate column (~3 × 0.6 cm) to remove the enzyme and any residual water. A 50-μL aliquot of the mixture was analyzed by thin-layer chromatography (TLC) on precoated silica gel G plates (Fisher Scientific), developed with petroleum ether/diethyl ether/acetic acid (90:10:1, vol/vol/vol). The bands were visualized under ultraviolet light after spraying with 0.2% 2,7-dichlorofluorescein in ethanol. Bands corresponding to TAG were scraped off and methylated with 3 mL methanolic HCl at 75°C for 2 h. The fatty acid methyl esters (FAME) were isolated by the addition of 2 mL hexane and 1 mL of 0.1 M KCl, and centrifuged at 1000 rpm for 10 min. The top hexane layer was separated into 5-mL-capacity vials and concentrated under nitrogen. The FAME residue was redissolved in 50 μL of hexane.

Fatty acid composition and identification were determined using a Hewlett-Packard 5890 Series II gas chromatograph, equipped with a flame-ionization detector (FID) and a fused-silica capillary column (DB-225, 30 m × 0.25 mm i.d.; J&W Scientific, Folsom, CA) using pure standard FAME. The column was held at 120°C for 3 min and programmed to 215°C at a rate of 10°C/min and held at 215°C for 3 min. Helium was the carrier gas, and the total flow rate was 23 mL/min. Injector and detector temperatures were set at 250 and 260°C, respectively. One microliter of sample was injected for analysis. Heptadecanoic acid was used as internal standard by adding 30 μL (10 mg/mL) to each sample before methylation. The mol% of FAME was determined with the aid of an on-line computer.

Large-scale synthesis. Gram-scale (4–6 g) synthesis was carried out by using 1:4:1 to 1:4:4 substrate mole ratio, enzyme (14%, w/w), and water (4%, v/w) and by incubating at 60°C for 24 h. After the reaction, the free fatty acids and modified TAG were extracted and purified twice using an alkaline alcoholic extraction method previously described (10). The purified products were analyzed for fatty acid and melting profiles. A desirable melting profile was obtained at a substrate mole ratio of 1:4:1, which had a melting point of 31.4°C.

Kilogram scale (1200 g) of glyceride synthesis was performed in a stirred tank batch reactor at 1:4:1 substrate mole ratio, enzyme (14%, w/w), 1.4 L hexane and water (4%, vol/wt) at 60°C, and 200 rpm stirring for 24 h. The reaction was terminated after 24 h reaction by filtration of the catalyst. The hot reaction mixture was filtered through a 500-mL column (32 × 6.5 cm) plugged with glass wool and sodium sulfate to approximately the 150-mL mark. The setup was preheated to 75°C to facilitate filtration and keep the reaction mixture liquid. Filtration was done by suction using a vacuum assembly to remove enzyme particles and any trace amounts of water. The filtered reaction product was purified by short-path distillation. The melted reaction mixture (60°C) was transferred to the reservoir and introduced dropwise into the wiped-film KDL-4 short-path distillation apparatus (UIC Inc., Joliet, IL) to separate residual solvent at 75°C under atmospheric pressure. Solvent-free products were distilled at 195°C at 800 mTorr at a feed rate of 80–85 mL/h. The reservoir was heated with external hot water at 70°C to keep the feed material in a liquid state. The internal cooling trap condenser temperature was maintained at 15°C, which condensed the evaporated free fatty acids. The nondistilled portion of the feed was collected and redistilled under the same conditions to remove any free fatty acid. The purified TAG were analyzed for fatty acid composition and melting point and used in a moisture absorption study as described below.

Differential scanning calorimetry (DSC). The melting profile of transesterified and purified product was determined by DSC on a PerkinElmer (Norwalk, CT) Model DSC7. A sample of 7–9 mg TAG was hermetically sealed in a 30-μL capacity aluminum pan, with an empty pan as reference. DSC procedure Cj 1-94 (20), as described in American Oil

Chemists' Society recommended methods, was followed. Samples were initially heated (200°C/min) from room temperature to 80°C and held at this temperature for 10 min to destroy crystal memory; cooled to −40°C at 10°C/min and held for 30 min; and heated to 80°C at 20°C/min to determine the melting profile. Normal standardization of the instrument was performed with gallium (mp, 29.78°C) as reference standard. Dry ice was used as coolant.

Moisture absorption study. Commercially available crackers, approximately 5 cm in diameter and 3 mm in thickness, weighing between 3.0–3.5 g, were selected for this study. The crackers were dried at 75°C for 1 h to remove any absorbed moisture. Upon removal from oven, the crackers immediately were dipped into the premelted (60°C) synthesized lipid or cocoa butter for 20 s. The oil-coated crackers were removed from the dip and coatings allowed to solidify at room temperature for 15 min. The crackers were re-dipped for 20 s and cooled for 15 min at room temperature to solidify. Completely coated crackers and uncoated but predried crackers (control) were carefully transferred with waxed paper (to prevent handling damage and moisture uptake) into a desiccator at room temperature. The bottom of the desiccator was filled with 100 mL distilled water and allowed to presaturate for 24 h at room temperature. A mesh support kept the crackers above the liquid water in the air space within the desiccator to avoid any contact with liquid water. The desiccator was sealed firmly. The lid was later opened very briefly to atmosphere to transfer the crackers for weighing. All the crackers were weighed before and after coating to ±1 mg. Moisture absorption of individual crackers was determined by weighing at 8-h time intervals for 72 h.

RESULTS AND DISCUSSION

The lipase IM60 acidolysis (transesterification) of tristearin with lauric and oleic acids was studied in terms of substrate mole ratio, water content, enzyme load, temperature, reaction time, and enzyme reuse. Lauric and oleic acids were selected because of their melting points and to help modulate the melting point of tristearin to obtain a TAG with melting profile of 30–37°C suitable for coating applications.

The time course of the reaction was studied, and products were analyzed at selected times. Figure 1 shows the incorporation level of lauric and oleic acids. In the initial 8 h of reaction, low incorporations of lauric (14.9 mol%) and oleic (31.7 mol%) acids were obtained. However, as the reaction time increased, more incorporation of lauric (29.8 mol%) and oleic (42.9 mol%) acids were obtained at 24–32 h. Residual tristearin content was approximately 3%. Oleic acid was incorporated at a higher mole percentage than lauric acid at all times. The reaction seemed to have reached equilibrium after 32 h; beyond this time there was not much increase in the incorporation of these acids.

The effect of the mole ratio of tristearin to lauric and oleic acids was studied from 1:1:1 to 1:5:5 and is shown in Figure 2. In general, as the mole ratio of oleic acid increased, the incorporation increased from 22.2 to 39.7 mol%. The maximal incorporation was obtained at 1:4:4. Incorporation of oleic acid remained higher than lauric acid in spite of the equal mole ratio of these acids at all mole ratios studied. This may reflect a higher reactivity and/or selectivity of oleic acid over lauric acid by Lipozyme IM60 lipase. At a 1:1:1 mole ratio of reactants, 12.3 mol% lauric acid was incorporated into the product. Increasing the ratio to 1:5:5 increased the level of lauric acid incorporation to only 17.3 mol%. Incorporation of high mole percentages of oleic acid into the product resulted in a lower melting point. To improve the melting point, further experiments were conducted, keeping the tristearin to lauric acid ratio constant at 1:4 and varying the oleic acid ratio from 1 to 5 (data not shown). Reduction of the oleic acid ratio to 1:4:1 resulted in a product high in lauric acid and low in oleic acid.

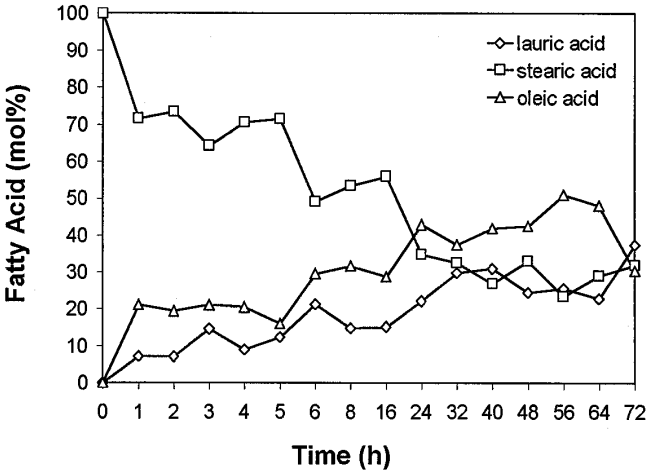

FIG. 1. Time course of acidolysis of tristearin with lauric and oleic acids. Substrate mole ratio was maintained at 1:4:4. Each mixture was incubated at 60°C with 14% Lipozyme (reactant-based weight percentage, w/w) and 4% water (reactant-based weight percentage, vol/wt).

Water content played an important role in the enzymatic

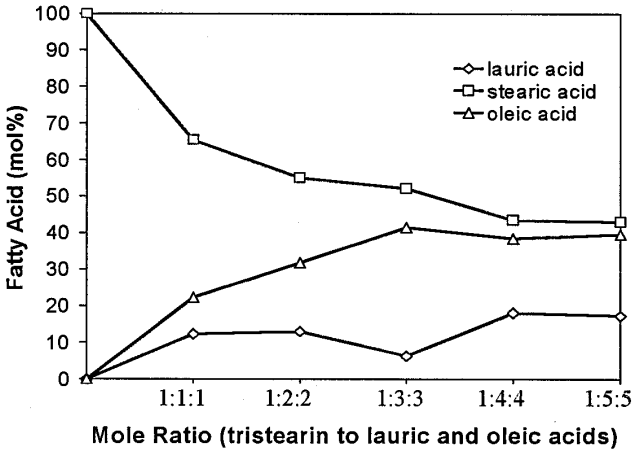

FIG. 2. Effect of substrate mole ratio of tristearin, lauric and oleic acids. Each mixture was incubated in the presence of 4% water (reactant-based weight percentage, vol/wt) at 55°C for 24 h with 14% Lipozyme (reactant-based weight percentage, w/w).

synthesis of coating lipid. Water influenced the incorporation level of lauric and oleic acids differently. As water content increases the amount of water around the enzyme or enzyme particle increases. This in turn influences the polarity, flexibility, and accessibility of the active site toward the substrate. The substrate accessibility toward the active site can be influenced by chain-length variation, saturation, and solubility (21). Figure 3 shows the effect of water on the incorporation of lauric and oleic acids. Equal incorporation of both acids (21 mol%) was obtained without addition of water. Addition of water from 2 to 50% (vol/wt) based on reactant weight influenced the fatty acid profile of the final product. Oleic acid incorporation remained basically unchanged with increase in water content from 0 to 15% (w/w). Highest incorporation of oleic acid (27.7 %) was observed at 4% water content. Lauric acid incorporation was optimal at 2% water content. High water content inhibited the synthesis due to its influence on reaction equilibrium. Such effects are known for Lipozyme IM60 catalyzed reactions (22). Higher mole percentage incorporation of oleic acid than of lauric acid may be due to the high specificity of the enzyme toward unsaturated fatty acids rather than saturated ones (23,24). Water content also plays an important role in substrate selectivity (25). By considering the combined lauric and oleic acid incorporation level, the optimal level of 4% water content was selected for further study.

The effect of enzyme load was investigated based on two variables: water content based on reactant weight (Fig. 4A) and water content based on enzyme weight (Fig. 4B). In both cases, water content was maintained at 4% (vol/wt) level. Incorporation of oleic acid was higher than of lauric acid at all levels of enzyme load. In the first case, when the water content was maintained based on reactant weight (theoretically less water), an increase in enzyme load did not enhance or inhibit the incorporation of either acid to any noticeable level. Almost equal incorporation of these acids was observed for all enzyme loads. However, slightly less incorporation at a lower enzyme load reflects a slower reaction rate. The overall water content within the system remained the same in spite of an increasing enzyme load. The water activity at low enzyme load is expected to be higher than high enzyme load. Introduction of high enzyme load may lead to the absorption of some amount of water. This in turn may decrease the water activity as the enzyme load increases. However, overall water content within the system remained at a lower level and hence the same synthetic activity was achieved. Nevertheless, in the second system where the water content was maintained on the basis of enzyme weight (theoretically more water), the incorporation profiles of these acids varied dramatically and sometimes were erratic. Lauric acid incorporation was inhibited at 32% enzyme load (Fig. 4B). This is because the overall water content in the system increased with increases in enzyme load since the water content was based on enzyme load. That means, the water content was high at high enzyme load for the same amount of reactants. This increase in water content affected the equilibrium unfavorably toward hydrolysis. This

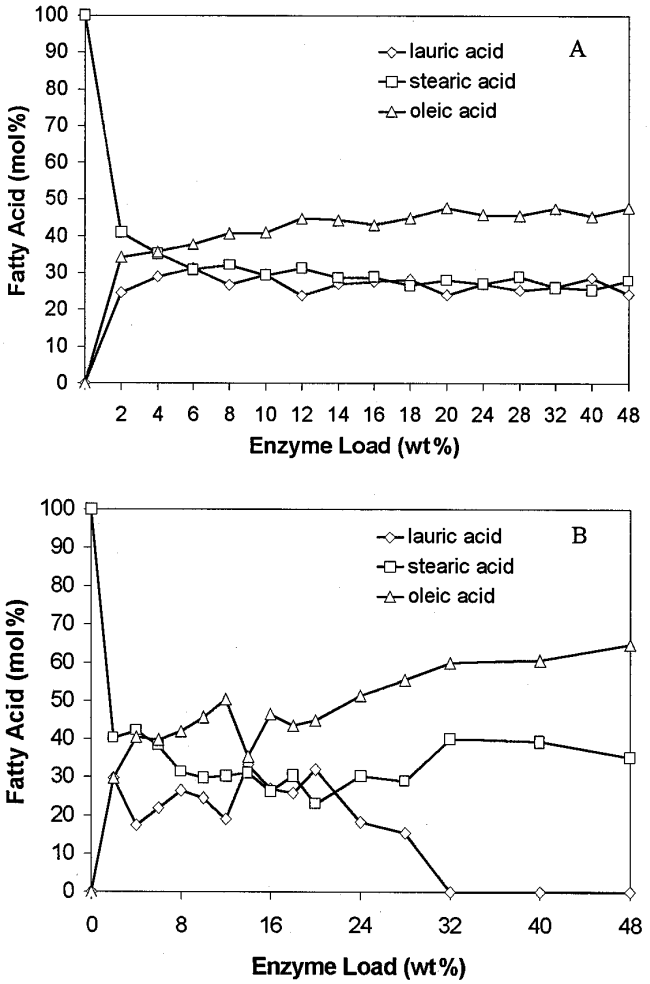

FIG. 4. Effect of enzyme load on acidolysis of tristearin with lauric and oleic acids. Each mixture was incubated at 55°C for 24 h with substrate mole ratio of 1:4:4. (A) Water content was maintained at 4% (vol/wt) level based on reactant weight; (B) water content was maintained at 4% (vol/wt) level based on enzyme weight.

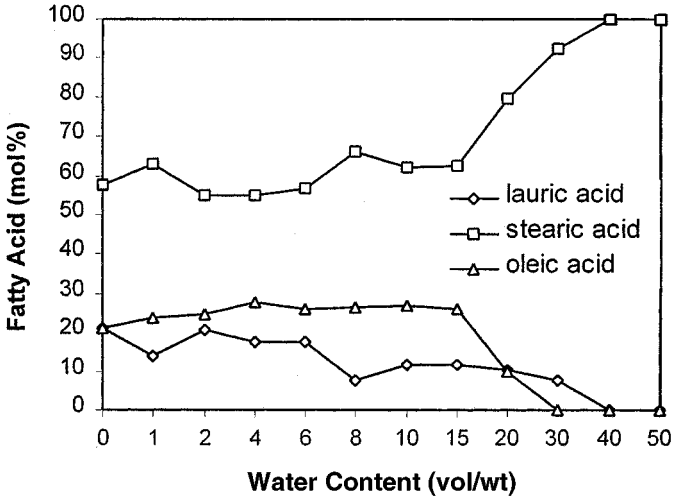

FIG. 3. Effect of water content (vol/wt) on acidolysis of tristearin with lauric and oleic acids at substrate mole ratio of 1:1:1. Each mixture was incubated at 55°C for 24 h with 10% Lipozyme (reactant-based weight percentage, w/w).

observation prompted us to adjust water content based on reactant weight rather than enzyme weight.

Temperature can affect the enzyme activity in two ways: (ii) an increase in temperature can lead to an increase in the reaction rate, and (ii) denaturation can occur by heat-induced structural modification. The synthesis of structured coating lipids by Lipozyme IM60 was influenced by temperature as shown in Figure 5. Lower incorporation was observed below 55°C. Increases in temperature increased activity without inactivation of enzyme up to 55°C. At this temperature the incorporation of lauric and oleic acids reached 22.9 and 46.3 mol%, respectively. Further increase in temperature to 65°C did not enhance fatty acid incorporation to any appreciable level. Temperature plays a very important role in solubilization of reactants apart from its influence on reaction equilibrium and enzyme activity. The overall incorporation of oleic acid remained higher than lauric acid at all temperatures. This effect again shows the selectivity of Lipozyme IM60 toward oleic acid. Reactions at a temperature above 65°C were not studied since the reactants were solubilized with *n*-hexane (boiling point 69°C).

Figure 6 shows the reusability and stability of Lipozyme IM60 under our assay conditions. After each use, the enzyme was isolated from reaction products by filtration, washed with distilled water (three times), and dried under vacuum for 24 h at room temperature before the next use. The filtrate was analyzed for TAG as described in the Materials and Methods section. The enzyme retained high activity even after six uses. The stability may be due to immobilization and low water content. Water content plays an important role in enzyme stability, as water is responsible for the internal structural flexibility and for heat-induced inactivation (26). The good stability of Lipozyme demonstrates its potential for repeated use to synthesize this particular lipid.

Figure 7 shows the melting profiles of products synthesized at 1:4:1 to 1:4:4 mole ratios of tristearin to lauric and oleic acids. The peaks from the lower-melting polymorphs seem to shift gradually toward higher melting ranges as the proportion of oleic acid decreases (top to bottom). The peaks were arbitrarily labeled A, B, and C from high-melting temperature toward low temperature. The major peak in all samples was B. The melting point of peak B shifted from 20.4 to 31.4°C (i.e., from 1:4:4 to 1:4:1 mole ratio). Melting ranges of peak B at substrate mole ratios 1:4:4 to 1:4:1 were 10.8–22.5°C, 18.9–37.9°C, 21.0–39.4°C, and 23.2–40.9°C, respectively. The higher mole percentage of oleic acid at a 1:4:4 ratio resulted in TAG species with lower melting points. Increased incorporation of lauric acid due to a decrease in oleic acid ratio resulted in a TAG species with a higher and sharper melting point of 31.4°C (1:4:1 ratio). Smaller melting

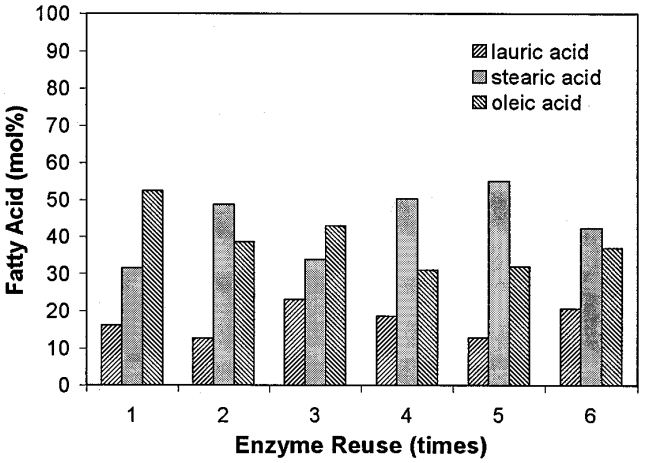

FIG. 6. Reusability of Lipozyme IM60. Substrate mole ratio was maintained at 1:4:4. Each mixture was incubated at 60°C for 24 h with 14% Lipozyme (reactant-based weight percentage, w/w) and 4% water (reactant-based volume percentage, vol/wt).

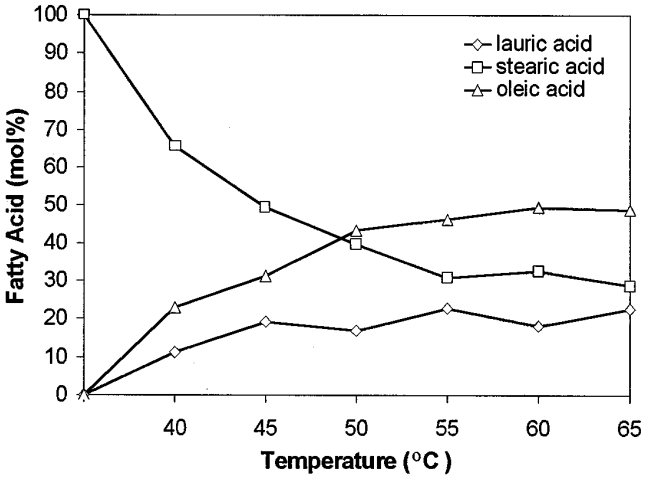

FIG. 5. Effect of temperature on transesterification of tristearin with lauric and oleic acids. Substrate mole ratio was maintained at 1:4:4. Each mixture was incubated for 24 h with 14% Lipozyme (reactant-based weight percentage, w/w) and 4% water (reactant-based weight percent, vol/wt).

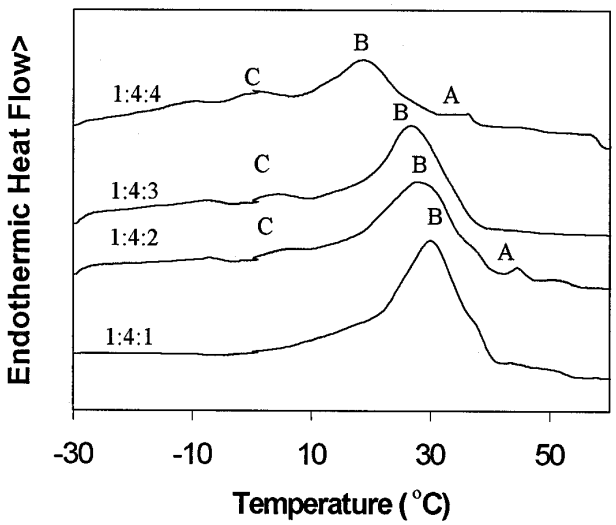

FIG. 7. Differential scanning calorimetry (DSC) of synthesized lipids at substrate mole ratios of 1:4:1 (1200 g synthetic reaction) to 1:4:4 of tristearin to lauric and oleic acids. See the Materials and Methods section for DSC conditions.

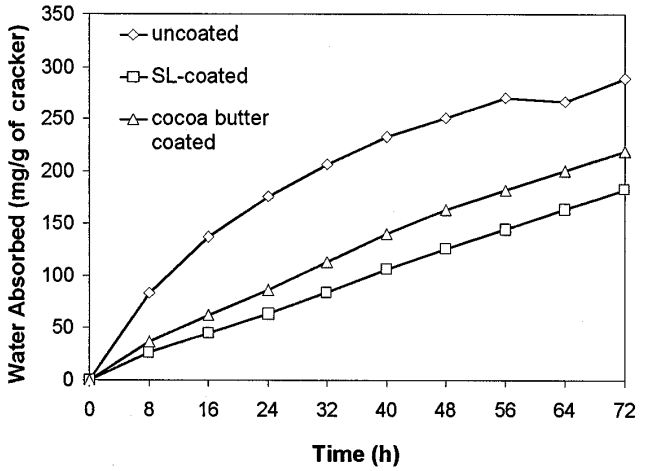

FIG. 8. Inhibition of moisture at 25°C by lipid coating: synthesized lipid (SL)-coated crackers and cocoa butter-coated crackers compared to control (uncoated). Lipid was synthesized at substrate mole ratio 1:4:1 of tristearin to lauric and oleic acids (1200 g synthetic reaction).

point peaks A and C slowly disappeared as the ratio of oleic acid was decreased from 4 to 1. The minor peaks indicate the presence of some minor TAG species, however, only one dominant peak B was present in all endotherms. The shift of the endotherm from a lower to a higher melting range could be explained by a reduction in oleic acid content and increased lauric and stearic acids content.

Prebaked and oven-dried crackers were used to compare the effect on moisture absorption of lipid coating with enzymatically synthesized lipid (using 1:4:1 mole ratio of reactants) and cocoa butter against uncoated control. The lipid-coated crackers showed an altered pattern of moisture absorption under controlled conditions. Crackers were exposed to moisture in a closed and water vapor-saturated environment. Both the cocoa butter and synthesized lipid showed a better inhibition of moisture absorption than the uncoated control (Fig. 8). However, the moisture inhibition ability of synthesized lipid seemed better than cocoa butter. Improved ability of synthesized structured lipid in preventing moisture absorption may be due to the rigidity or compactness of the solidified structured fat. Further study may explain the actual mechanism involved in this effect. However, this new kind of lipid may be a potential ingredient for new food applications such as food coating or as carrier for other ingredients.

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REFERENCES

1. Gunstone, F.D., Enzymes as Biocatalysts in the Modification of Natural Lipids, *J. Sci. Food Agric. 79*:1535–1549 (1999).
2. Christensen, M.S., A. Mullertz, and C.-E. Høy, Absorption of Triglycerides with Defined or Random Structure by Rats with Biliary and Panceatic Diversion, *Lipids 30*:521–525 (1995).
3. Christensen, M.S., C.-E. Høy, C.C. Becker, and T.G. Redgrave, Intestinal Absorption and Lymphatic Transport of Eicosapentaenoic (EPA), Docosahexaenoic (DHA), and Decanoic Acids: Dependence on Intramolecular Triacylglycerol Structure, *Am. J. Clin. Nutr. 61*:56–61 (1995).
4. Lee, K.T., and C.C. Akoh, Effect of Selected Substrate Forms on the Synthesis of Structured Lipids by Two Immobilized Lipases, *J. Am. Oil Chem. Soc. 74*:579–584 (1997).
5. Goh, S.H., S.K. Yeong, and C.W. Wang, Transesterification of Cocoa Butter by Fungal Lipases—Effect of Solvent on 1,3-Specificity, *Ibid. 70*:567–570 (1993).
6. Mukherjee, K.D., and I. Kiewitt, Structured Triacylglycerols Resembling Human Milk Fat by Transesterification Catalyzed by Papaya (*Carica papaya*) Latex, *Biotechnol. Lett. 20*:613–616 (1998).
7. Chulalaksananukul, W., J.-S. Condoret, and D. Combes, Kinetics of Geranyl Acetate Synthesis by Lipase-Catalyzed Transesterification in Normal-Hexane, *Enzyme Microb. Technol. 14*:293–298 (1992).
8. Yee, L.N., C.C. Akoh, and R.S. Phillips, Lipase PS-Catalyzed Transesterification of Citronellyl Butyrate and Geranyl Caproate: Effect of Reaction Parameters, *J. Am. Oil Chem. Soc. 74*:255–260 (1997).
9. Akoh, C.C., and L.N. Yee, Enzymatic Synthesis of Position-Specific Low-Calorie Structured Lipids, *Ibid. 74*:1409–1413 (1997).
10. Lee, K.T., and C.C. Akoh, Characterization of Enzymatically Synthesized Structured Lipids Containing Eicosapentaenoic, Docosahexaenoic, and Caprylic Acids, *Ibid. 75*:495–499 (1998).
11. Gennadios, A., M.A. Hanna, and L.B. Kurth, Application of Edible Coatings on Meats, Poultry and Seafoods: A Review, *Food Sci. Technol.-Leb. 30*:337–350 (1997).
12. Guilbert, S., Technology and Application of Edible Protective Films, in *Food Packaging and Preservation*: *Theory and Practice*, edited by M. Mathlouthi, Elsevier Applied Science, London, 1986, pp. 371–394.
13. Shellhammer, T.H., and J.M., Krochta, Edible Coatings and Film Barriers, in *Lipid Technologies and Applications*, edited by F.D. Gunstone and F.B. Padley, Marcel Dekker, New York, 1997, pp. 453–479.
14. Nesaretnam, K., N. Robertson, Y. Basiron, and C.S. Macphie, Application of Hydrogenated Palm Kernel Oil and Palm Stearin in Whipping Cream, *J. Sci. Food Agric. 61*:401–407 (1993).
15. Warner, K., E.N. Frankel, J.M. Snyder, and W.L. Porter, Storage Stability of Soybean Oil-Based Salad Dressings—Effects of Antioxidants and Hydrogenation, *J. Food Sci. 51*:703–708 (1986).
16. Mojovic, L., S.S. Marinkovic, G. Kukic, and G.V. Novakovic, *Rhizopus arrhizus* Lipase-Catalyzed Interesterification of the Midfraction of Palm Oil to a Cocoa Butter Equivalent Fat, *Enzyme Microb. Technol. 15*:438–443 (1993).
17. Williams, S.D., K.L. Ransom-Painter, and R.W. Hartel, Mixtures of Palm Kernel Oil with Cocoa Butter and Milk Fat in Compound Coatings, *J. Am. Oil Chem. Soc. 74*:357–366 (1997).
18. Seriburi, V., and C.C. Akoh, Enzymatic Transesterification of Triolein and Stearic Acid and Solid Fat Content of Their Products, *Ibid. 75*:511–516 (1998).
19. Lai, O.M., H.M. Ghazali, and C.L. Chong, Effect of Enzymatic Transesterification on the Melting Points of Palm Stearin-Sunflower Oil Mixtures, *Ibid. 75*:881–886 (1998).
20. *American Oil Chemists' Society, Official Methods and Recommended Practices of the American Oil Chemists' Society,* 3rd edn., edited by D. Firestone, American Oil Chemists' Society, Champaign, 1988, Cj 1-94.
21. Flores, M.V., J.J.W. Sewalt, A.E.M. Janssen, and A. van der Padt, The Nature of Fatty Acid Modifies the Equilibrium Position in the Esterification Catalyzed by Lipase, *Biotechnol. Bioeng. 67*:364–371 (2000).

22. Graille, J., Providing Biocatalysts Through Customizing Lipases by Different Processes, *OCGL—Oleagineux Corps Gras Lipides 6*:365–372 (1999).
23. Borgdorf, R., and S. Warwel, Substrate Selectivity of Various Lipases in the Esterification of *cis*- and *trans*-9-Octadecenoic Acid, *Appl. Microbiol. Biotechnol. 51*:480–485 (1999).
24. Schmid, U., U.T. Bornscheuer, M.M. Soumanou, G.P. McNeill, and R.D. Schmid, Highly Selective Synthesis of 1,3-Oleoyl-2-palmitoylglycerol by Lipase Catalysis, *Biotechnol. Bioeng. 64*: 678–684 (1999).
25. Broos, J., A.J.W.G. Visser, J.F.J. Engbersen, W. Verboom, A. vanHoek, and D.N. Reinhoudt, Flexibility of Enzymes Suspended in Organic Solvents Probed by Time-Resolved Fluorescence Anisotropy. Evidence That Enzyme Activity and Enantioselectivity Are Directly Related to Enzyme Flexibility, *J. Am. Chem. Soc. 117*:12657–12663 (1995).
26. Tang, K.E.S., and K.A. Dill, Native Protein Fluctuations: The Conformational-Motion Temperature and the Inverse Correla-

tion of Protein Flexibility with Protein Stability, *J. Biomol. Struct. Dyn. 16*:397–411 (1998).

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