Lipase-Catalyzed Acidolysis of Tristearin with Oleic or Caprylic Acids to Produce Structured Lipids

Vivienne V. Yankah and Casimir C. Akoh*

Department of Food Science and Technology, The University of Georgia, Athens, Georgia 30602-7610

ABSTRACT: Two different structured lipids (SL) were synthesized by transesterifying tristearin with caprylic acid (C8:0) or oleic acid (C18:1). The objective was to synthesize SL containing stearic acid (C18:0) at the *sn*-2 position as possible nutritional and low-calorie fats. The reaction was catalyzed by IM60 lipase from *Rhizomucor miehei* in the presence of *n*-hexane. The effects of reaction parameters affecting the incorporation of caprylic acid into tristearin were compared with those for incorporating oleic acid into tristearin. For all parameters studied, oleic acid incorporation was higher than caprylic acid. The range of conditions favorable for synthesizing high yields of C8:0-containing SL was narrower than for oleic acid. An incubation time of 12–24 h and an enzyme content of 5% (w/w total substrates) favored C8:0 incorporation. The mole percentage of incorporated C18:1 did not increase further at enzyme additions greater than 10%. C18:1 incorporation decreased with the addition of more than 10% water (w/w total substrates) to the tristearin–oleic acid reaction mixture. Increasing the mole ratio of fatty acid (FA) to triacylglycerol increased oleic acid incorporation. The highest C8:0 incorporation was obtained at a 1:6 mole ratio of tristearin to FA. Positional analysis confirmed that C18:0 remained at the *sn*-2 position of the synthesized SL. The melting profiles of tristearin–caprylic acid and tristearin–oleic acid SL displayed peaks between –20 to 30°C and –20 to 40°C, respectively. Their solid fat contents (~25%) at 25°C suggest possible use in spreads or for inclusion with other fats in specialized blends.

Paper no. J9284 in *JAOCS 77,* 495–500 (May 2000).

KEY WORDS: Acidolysis, caprylic acid, lipase, oleic acid, structured lipids, transesterification, tristearin.

The beneficial role of modified fats in nutrition, health, and food applications cannot be overemphasized. Research interest has focused on improving the techniques for their synthesis. The industrial applicability of any fat is limited by the nonrandom distribution of fatty acids (FA), which imparts a given set of physical, chemical, and functional properties. Natural fats lack the variability desired in the food industry. Structured lipids (SL) are triacylglycerols (TAG) that have been restructured or modified by chemical or enzymatic processes so as to change the composition of the FA and/or their positional distribution in glycerol molecules (1). Fats can be modified to meet a nutraceutical need including medical/therapeutic, nutritional, low caloric value, or fortification with a desired FA. Industrial processing technologies use fat modification to control spreadability and improve oxidative/flavor stability and palatability in margarines, shortenings, and cooking fats. Fats are also modified to obtain certain desired performance characteristics (2), or to provide to the food processor a fat similar to a more expensive alternative (3). Some commercially available modified fats include SALATRIM/Benefat (Nabisco Foods Group, Parsippany, NJ/Cultor Food Science, Inc., New York, NY), and caprenin (Procter & Gamble Co., Cincinnati, OH).

Enzymatic interesterification as a tool for fat modification has many advantages. In contrast to hydrogenation and fractionation, interesterification does not always change the FA composition or the unsaturation level of the starting mixture; hence, the inherent stability of the product oil or blend remains predictable. It therefore eliminates any health concerns associated with *trans* FA formed during partial hydrogenation reactions. Compared to chemical interesterification, enzymatic synthesis affords milder processing conditions and the possibility of regiospecificity and FA specificity. It is ideally suited for the production of nutritionally superior fats (4).

Restructuring fat to inhibit absorption or to achieve a lower caloric density is the principle underlying production of low-calorie fats because the metabolism and absorption of the FA depend on their position in the TAG. To effectively reach these goals, the physiological effects of the FA used to assemble SL as well as their positional orientation on the glycerol backbone must be known. Short-chain (SCFA) and medium-chain FA (MCFA) provide fewer calories per unit of weight and can be used to replace long-chain FA on the TAG. With respect to desired positional distribution of the FA as *sn*-1, *sn*-2, or *sn*-3, the enzyme selection is the main determining factor.

Medium-chain triacylglycerols (MCT) have physiological dietary benefits. They are metabolized more like carbohydrates than like normal oils and fats (5) although they have better than twice the caloric density of proteins and carbohydrates. Thermogenesis of MCT may be a factor in their very low tendency to remain in the body as depot fat (6). MCFA are used as a source of quick energy and rapid absorption, especially for immature neonates, hospitalized patients, and individuals with lipid malabsorption disorders. In a rat study,

*To whom correspondence should be addressed at Department of Food Science and Technology, Food Science Bldg., The University of Georgia, Athens, GA 30602-7610. E-mail: cmscakoh@arches.uga.edu

Bourre *et al.* (7) concluded that there is insufficient endogenous synthesizing potential to guarantee the normal FA composition of certain organs (particularly liver) if oleic acid is totally absent in the diet. Oleic acid in the diet can lower low-density lipoprotein as well as total cholesterol. Saturated FA placed in the *sn*-1,3 positions are released by pancreatic lipase during digestion and may form insoluble, poorly absorbed soaps in the presence of calcium. FA placed in the *sn*-2 position form 2-monoacylglycerol (2-MAG), which can be more effectively absorbed. This mechanism was used to explain why saturated FA in milk are well absorbed by infants compared to vegetable oils with the same FA composition, where the saturated FA are mainly situated at the 1,3-positions (8). We transformed tristearin to SL with C8:0 or C18:1 incorporated at the *sn*-1 and *sn*-3 positions. These modified fats will be more nutritionally beneficial to the body and can be useful to the food processor for various applications such as spreads.

In this study, a 1,3-specific lipase from *Rhizomucor miehei* was used in separate acidolysis reactions of tristearin with caprylic acid and oleic acid. The substrates were selected based on their potential functional and nutritional properties. Incorporation of C8:0 or 18:1 at the *sn*-1 and *sn*-3 positions will change the physical properties of tristearin and enhance absorption of stearic acid as 2-MAG. The melting characteristics and solid fat content (SFC) of the produced SL were studied to evaluate their possible industrial applications.

MATERIALS AND METHODS

Materials. Tristearin (~99% pure), caprylic acid (99+% pure), oleic acid (99% pure), and crude lipase from porcine pancreas were purchased from Sigma Chemical Company (St. Louis, MO). Immobilized lipase IM60 (from *R. miehei*, water content 2–3 wt%) was obtained from Novo Nordisk A/S Bioindustrial, Inc. (Bagsvaerd, Denmark). The interesterification activity of IM60 lipase is 5–6 BAUN/g (Batch Acidolysis Units Novo). Thin-layer chromatography (TLC) plates were obtained from Alltech Associates (Deerfield, IL). Sodium sulfate and all organic solvents were purchased from Fisher Scientific (Norcross, GA).

Enzymatic acidolysis. Milligram quantity acidolysis reactions were performed in screw-capped test tubes to synthesize structured lipids. Acidolysis of tristearin with caprylic acid (TCA) was carried out, as well as acidolysis of tristearin with oleic acid (TOA). Typically, 100 mg tristearin and 32.3 mg caprylic acid (1:2 mole ratio) were dissolved in 3 mL hexane, and the test tubes were incubated in a rotary shaking water bath at 200 rpm. The enzyme was used at 10% of the weight of substrates (equivalent to 0.1 BAUN unit of enzyme). The progress of SL synthesis was followed over a period of 72 h at 60°C. Several reaction parameters (mole ratio of substrates, incubation temperature, effects of water and different enzyme equivalents) were studied by sampling reaction mixtures at 48 h. All reactions were carried out in duplicate and are reported as average values.

Extraction and analytical methods. After incubation, reaction mixtures were filtered through an anhydrous sodium sulfate column to remove the enzyme and any residual water. The products were separated by TLC on precoated silica gel G plates; the developing solvent was petroleum ether/diethyl ether/acetic acid (80:20:0.5, vol/vol/vol). Bands were visualized under ultraviolet light after spraying with 0.2% 2,7-dichlorofluorescein in ethanol. Bands corresponding to TAG were scraped and methylated. TCA-SL was methylated with 2 N CH_3ONa (0.15 mL) in 0.5 mL hexane and shaken for 5 min (9). The internal standard was heptadecanoic acid. After adding 0.3 mL hexane and anhydrous $CaCl_2$, the mixture was allowed to stand for 1 h and centrifuged for 2–3 min. The TOA reaction product TAG bands were methylated in 3 mL methanolic HCl (6% HCl in methanol) at 75°C for 2 h with heptadecanoic acid as internal standard. The fatty acid methyl esters (FAME) were extracted with 2 mL hexane and 1 mL of 0.1 M KCl solution, centrifuged at 1,000 rpm for 3 min, and concentrated under nitrogen. The hexane phases were analyzed by gas–liquid chromatography (GLC).

FA composition and identification were obtained with a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA), equipped with a flame-ionization detector and a fused-silica capillary column (DB-225, 30 m × 0.25 mm i.d.; J&W Scientific, Folsom, CA). The column was held at 120°C for 3 min and programmed to 215°C for 10 min at a rate of 10°C/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. Yields of FAME (in mol%) were determined with the aid of an on-line computer.

Pancreatic lipase hydrolysis to determine the FA at the *sn*-2 position of the SL products was modified from Luddy *et al.* (10). SL products were spotted on TLC plates against a standard TAG and developed in petroleum ether/diethyl ether/acetic acid (80:20:0.5, vol/vol/vol). The identified SL TAG bands were scraped and eluted with diethyl ether. At least 2 mg of SL product was hydrolyzed by mixing with 1 mL of 0.4 M Tris-HCl buffer (pH 7.6), containing 0.25 mL 0.05% bile salts, 0.1 mL 2.2% $CaCl_2$, and 8 mg pancreatic lipase. The mixture was incubated in a 37°C water bath for 3 min and then vortexed vigorously. This was repeated two times before centrifugation at 1,000 rpm for 3 min and extraction with 3 mL ethyl ether (two times). The extract was filtered through a sodium sulfate column, and the sample was concentrated to a small volume with nitrogen. The residual 2-MAG were separated by TLC on a silica gel plate, developed with hexane/diethyl ether/acetic acid (50:50:1, vol/vol/vol). Bands corresponding to the *sn*-2 MAG were scraped, methylated, and analyzed by GLC as described above.

Purification of SL. The SL were deacidified by the alkaline extraction procedure outlined by Lee and Akoh (11). Briefly, the prepared mixture of SL (0.5 g) in hexane was added to a mixture of 15 mL hexane, 2 drops phenolphthalein solution, and 8 mL 0.5 N KOH in 20% ethanol in a separatory funnel. The mixture was shaken and the upper hexane layer collected. To the hexane layer was added 30 mL 0.5 N

KOH in 20% ethanol and 60 mL of saturated NaCl solution. The upper hexane layer was separated and passed through a sodium sulfate column. The SL in hexane were concentrated with a rotary evaporator.

The SL were further purified by TLC on silica gel plates. The concentrated SL were spotted against a TAG standard and developed with petroleum ether/diethyl ether/acetic acid (80:20:0.5, vol/vol/vol). The TAG layer was scraped, dissolved in hexane, and passed through a sodium sulfate column and then concentrated by evaporation of solvent. SL was stored at –90°C under N_2.

Melting characteristics. Differential scanning calorimetry (DSC) on a PerkinElmer (Norwalk, CT) Model DSC 7 was used to determine the melting profiles and percentage of SFC of the acidolysis products according to AOCS-recommended DSC procedure Cj 1-94 (12). A sample of 3–5 mg SL was hermetically sealed in an aluminum pan using a sealed empty pan as a reference. Normal standardization of the instrument was performed with indium (mp, 156.6°C) and *n*-decane (mp, –29.7°C) as reference standards. Liquid nitrogen was used as coolant.

RESULTS AND DISCUSSION

Incorporation of oleic acid into tristearin increased with increase in incubation time over the period of 1–72 h. Oleic acid incorporated after 72 h was 55.2 mol%. Caprylic acid incorporation was time dependent and attained a maximum of 37.0% after 12 h incubation, after which no further increase was obtained, suggesting that equilibrium had been reached (Fig. 1).

Reaction temperature of 45°C yielded high oleic acid incorporation (54.4%) into tristearin for TOA acidolysis (Fig. 1B). SL yields did not show much variation for all temperatures studied above 45°C. Higher temperatures, 55 and 60°C, were necessary to yield 31.8 and 27.45 mol% of incorporated C8:0, respectively. TCA-SL yield decreased at temperatures above 60°C (Fig. 1). Temperatures below 55°C also produced low C8:0 incorporation. Generally, a higher reaction temperature reduces the viscosity of the reaction mixture. The optimal temperature for most immobilized lipases falls within 30 to 62°C (13). However, immobilized lipases are generally more thermostable owing to the higher degree of restriction to unfolding and denaturation as compared to the free form of the enzyme. The melting points of tristearin are 55 and 72°C (Merck Index), thus it was suspected that temperatures around the melting point would be most appropriate for high yields of SL. This temperature was not needed for TOA or TCA acidolysis because our reactions were performed in solvent. A similar observation was made for hydrolysis of animal fats (14). The use of organic solvents in enzymatic synthesis aids solubility of hydrophobic substrates and eliminates the use of high temperatures.

The type of enzyme used for catalysis in transesterification process is a major determining factor on the product yield and composition. Enzyme conformation is affected by several factors in the microenvironment including the solvent, solubility of substrates, water, pH and temperature; and any conformational changes may affect the catalytic activity of enzyme. Lipases have been shown to be more thermostable in organic solvents than in water (15). For optimal stereoselectivity, *n*-hexane, as used in this study, has been found to be the best solvent (16). Interesterification is initiated by the hydrolysis of an ester bond and the formation of an acyl-enzyme intermediate, followed by exchange of acyl moieties and formation of new ester bonds (17). The 1,3-specificity of *R. miehei* lipase may result from the inability of the sterically hindered 2-glycerol esters to fit into the active site of the lipase. Reports indicate that this 1,3-stereoselectivity predominantly becomes a 1-position selectivity when water activity (a_w) is almost at zero (16).

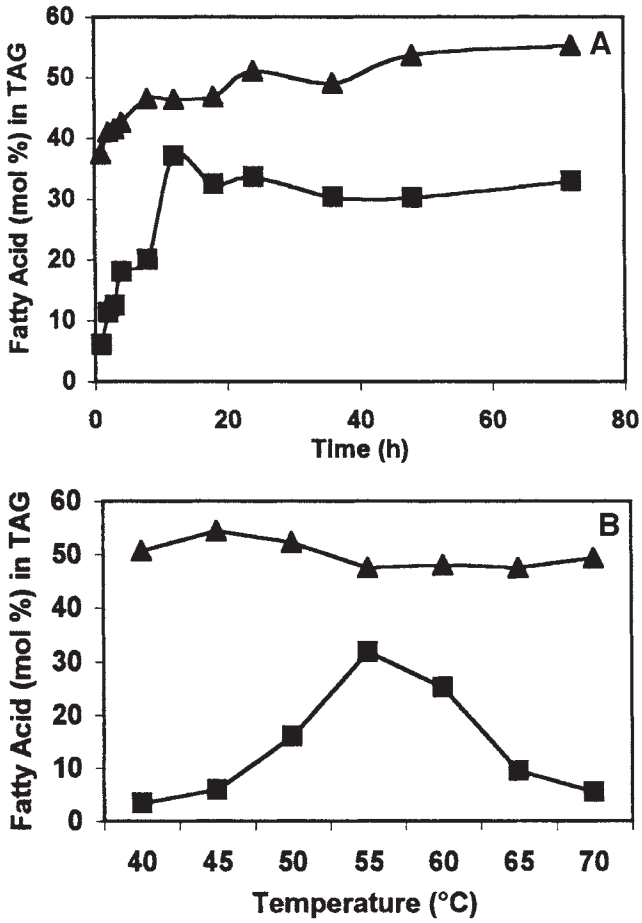

FIG. 1. Effect of time and temperature on the incorporation of caprylic (C8:0, ■) and oleic (C18:1, ▲) acids in stearin. Enzyme was added at 10% the weight of substrates. (A) The time course reaction mixture was incubated at 60°C for 72 h in an orbital shaking water bath at 200 rpm. The ratio of tristearin/caprylic or oleic acid was 1:2. (B) Effect of temperature was conducted by incubating for 48 h. TAG, triacylglycerol.

For TOA acidolysis, incorporated C18:1 remained almost the same at 52.0% for enzyme levels above 10%, and with TCA reactions, the mol% incorporated C8:0 for enzyme levels above 5% remained almost unchanged (data not shown). It appears that IM60 has a preference for longer-chain FA

than C8:0. Incorporation of FA into SL after 48 h incubation peaked at 30 and 48 mol%, respectively, for C8:0 and C18:1 when 5% of enzyme was used.

For the studied mole ratio of substrates from 1:1 to 1:8, the mole percentage of incorporated C18:1 increased with increasing mole ratio of FA (Fig. 2). For C8:0, however, the mole percentage incorporation was almost unchanged around 22% for all substrate ratios except for ratios of 1:5 and 1:6, where 42 and 43% C8:0 were incorporated, respectively.

The conditions for synthesis of both TCA- and TOA-SL were the same; however, substrate inhibition was observed with the synthesis of TCA but not TOA. The difference could be attributed to the differences in acidity and physical properties of the FA in the reaction mixture. The loss of activity by lipase in the presence of high concentrations of free FA (FFA) has been attributed to acidification of the aqueous phase and increased solubility of SCFA (18). High levels of FFA would produce high levels of free or ionized carboxylic acid groups, which would acidify the microaqueous phase surrounding the lipase or cause desorption of water from the interface. In addition, SCFA and MCFA could be partitioning away from the interface into the water shell surrounding the enzyme, owing to their increased solubility in water. Such partitioning limits access by the substrate to the interface. Kuo and Parkin (19) found that there was less inhibition when longer-chain FA such as C13:0 and C17:0 were used during acidolysis compared to C5:0 and C9:0.

The highest yield of SL formed in TOA acidolysis was 55 mol% with added water at 10% by weight of substrates (Fig. 3). Additional water resulted in a slight decrease in SL yield. The mole percentage of C8:0 incorporated into tristearin was maximum, at 30 mol%, with water at 8% by weight of substrates. A substantial decrease in the incorporation of C8:0 was observed on addition of 25% water (Fig. 3). No incorporation was obtained with 48% water added. It is well known

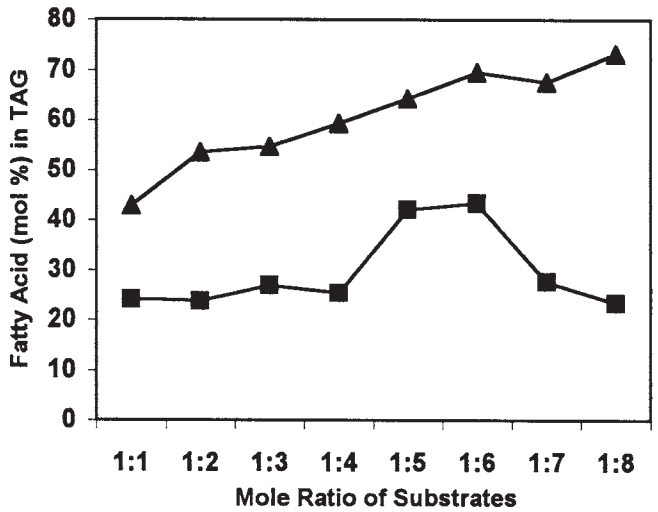

FIG. 2. Effect of mole ratio of tristearin/fatty acid on the incorporation of caprylic (■) and oleic (▲) acids in tristearin. Incubation was for 48 h. For other conditions and abbreviation see Figure 1.

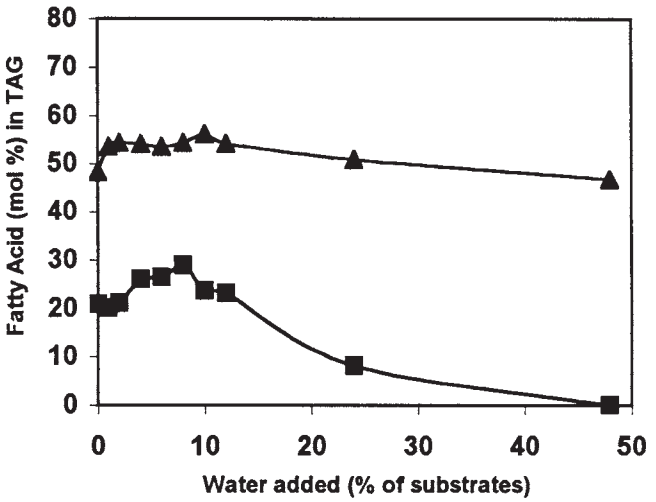

FIG. 3. Effect of water addition on the incorporation of caprylic (■) and oleic (▲) acids in tristearin. The incubation was for 48 h. See Figure 1 for other conditions and abbreviation.

that low a_w shifts the thermodynamic equilibria of hydrolysis reactions toward synthesis in esterifications. With transesterification reactions, water is needed to initiate hydrolysis before synthesis of TAG can occur. However, depending on the enzyme used, increasing the water content of the system above the levels required to maintain the enzyme structure in the activated state will result in deformation of enzyme, as well as the occurrence of acyl migration due to the formation of high levels of MAG instead of ester formation. As hydrolysis dominates, there is a reduction in yields of SL. However, at 10% added water, we did not detect MAG and diacylglycerol in the samples analyzed.

Hydrolysis with pancreatic lipase produced FA profiles with only C18:0 at the *sn*-2 position as determined by GLC. This confirmed the 1,3-specificity of the *R. miehei* enzyme. It also suggests that the expected orientation of the incorporated oleic or caprylic acids at the 1- and 3-positions was achieved without acyl migration of the incorporated FA to the *sn*-2 position.

Melting profiles of the substrates and synthesized SL are shown in Figure 4. In contrast to the sharp melting point of the substrates, the SL have melting characteristics spreading over a wide range, indicating plasticity. Tristearin-caprylic acid structured lipid (TCA-SL) melting was over the range –20 to 30°C, and tristearin-oleic structured lipid (TOA-SL) was over –20 to 40°C. The main peaks observed for TCA-SL were at melting temperatures 0.37, 15 and 28°C, and those for TOA-SL were at 15 and 23°C.

Depending on the crystal conformation of the synthesized SL (α, β′, or β), the SFC varies. The desired polymorphic form for SL intended for use as margarines and spreads is the β′ form. The SFC of the SL compared to those of SALATRIM and cocoa butter are shown in Figure 5. The amount of solid fat at 2 to 10°C determines the spreadability of margarine at refrigerator temperatures, SFC at 25°C influences plasticity at room temperature and the SFC between 33 and 38°C determines the mouthfeel (20). The two SL had comparable SFC

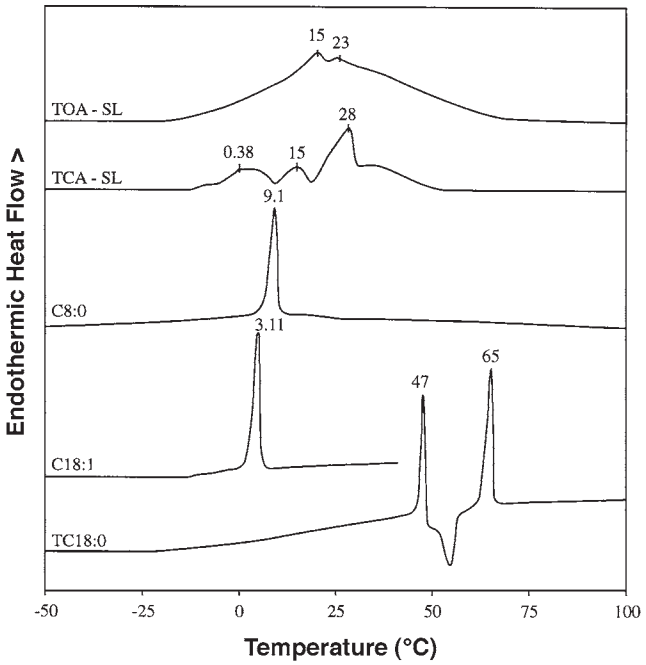

FIG. 4. Differential scanning calorimetry (DSC) heating thermograms of substrates and structured lipids. Samples were rapidly heated at 200°C/min from room temperature to 80°C and held at this temperature for 10 min; cooled to –40°C at 10°C/min and held for 30 min; then heated to 80°C at 5°C/min. TOA-SL, tristearin-oleic acid structured lipid; TCA-SL, tristearin-caprylic acid structured lipid; C8:0, caprylic acid; C18:1, oleic acid; TC18:0, tristearin.

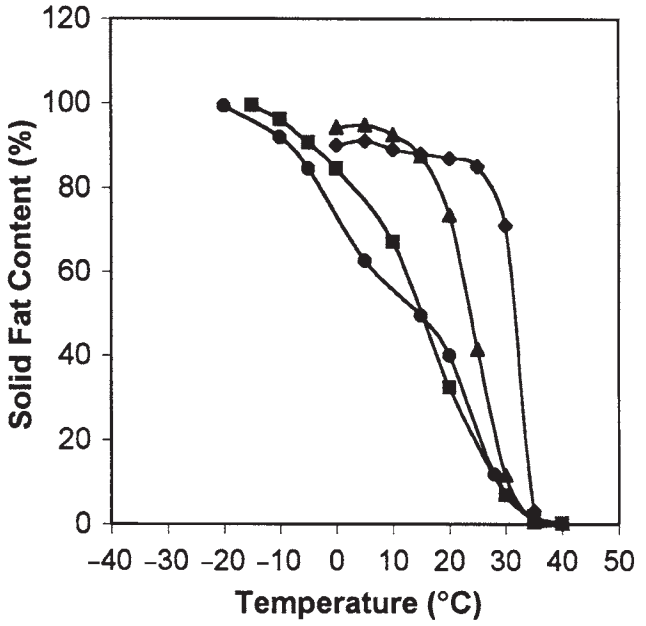

FIG. 5. Solid fat contents of TCA-SL (●) and TOA-SL (■) determined by DSC compared to SALATRIM (◆) and cocoa butter (▲) (commercial products). Data for SALATRIM and cocoa butter were obtained from Ref. 22. For abbreviations see Figure 4.

for temperatures between –20 and –5°C, and also 20 to 40°C. Differences were observed between the temperature ranges of –5 to 20°C. TCA-SL had a lower SFC than TOA-SL. The differences could be due to the levels of incorporated FA as well as the polymorphic crystal structure of the SL. The SFC of the enzymatically synthesized SL at 25°C were between 25 and 28%, suggesting possible use as spreads. The SFC of cocoa butter and SALATRIM at the same temperature are about 40 and 88%, respectively (Fig. 5). We do not have data for the SFC and melting properties of caprenin.

Possible industrial uses of synthesized SL. The structural compositions of the synthesized SL are considered comparable to SALATRIM and caprenin. The chemical interesterification process for production of SALATRIM results in a random distribution of the FA with at least one SCFA and one saturated long-chain FA (preferably stearate) within a TAG. SALATRIM was produced to mimic the physical characteristics of cocoa butter but with a lower caloric value. In feeding experiments with rats and in human clinical studies, it has been demonstrated that SALATRIM provides fewer calories (4.5–6.0 kcal/g) than conventional fats (9 kcal/g) (21). It is possible to control the amount of C8:0 incorporated into tristearin to obtain melting profiles and SFC similar to cocoa butter, although this will not guarantee similar functionality. Macroscopic differences in fat systems have been associated with structural differences at the microstructural level (22), and this has been used to explain in part the differences in functionality between SALATRIM and cocoa butter at 22°C. Caprenin is a randomized TAG comprising caprylic (C8:0), capric (C10:0), and behenic (C22:0) acids. The usable energy value of caprenin is about the same as SALATRIM, and is calculated at 4.3 kcal/g. The TOA-SL is similar to the human milk fat substitute used in the production of Betapol, a product currently in development for application in infant formula (23). Betapol has C16:0 instead of C18:0 in the *sn*-2 position.

ACKNOWLEDGMENT

Research supported by Food Science Research.

REFERENCES

1. Lee, K.-T., and C.C. Akoh, Structured Lipids: Synthesis and Applications, *Food Rev. Int. 14*:17–34 (1998).
2. A.C. HUMKO, *Technical Guide to Products*, Memphis, 1997, pp. 1–49.
3. Lo, Y.C., and A.P. Handel, Physical and Chemical Properties of Randomly Interesterified Blends of Soybean Oil and Tallow for Use as Margarine Oils, *J. Am. Oil Chem. Soc. 60*:815–818 (1983).
4. Rousseau, D., and A.G. Marangoni, Chemical Interesterification of Food Lipids: Theory and Practice, in *Food Lipids: Chemistry, Nutrition and Biotechnology*, edited by C.C. Akoh and D.B. Min, Marcel Dekker, New York, 1998, pp. 251–281.
5. Timmerman, F., Production and Properties of Medium Chain Triglycerides, *Lipid Technol. 6*:61–64 (1994).
6. Babayan, V.K., Medium Chain Triglycerides, in *Dietary Fat Requirements in Health and Development*, edited by J. Beare-Rogers, American Oil Chemists' Society, Champaign, 1988, pp. 73–86.
7. Bourre, J-M.E., O.L. Dumont, M.E. Clement, and G.A. Durand, Endogenous Synthesis Cannot Compensate for Absence of Dietary Oleic Acid in Rats, *J. Nutr. 127*:488–493 (1997).

8. Quinlan, P., and S. Moore, Modification of Triglycerides by Lipases: Process Technology and Its Application to the Production of Nutritionally Improved Fats, *INFORM 4*:580–585 (1993).
9. Christie, W., *Gas Chromatography and Lipids: A Practical Guide. Vol. 1. Lipids, Chemical Analysis I*, 1st edn., Bell and Bain Ltd., Glasgow, 1989, pp. 92.
10. Luddy, F.E., R.A. Barfield, S.F. Herb, P. Magidman, and R.W. Riemenschneider, Pancreatic Lipase Hydrolysis of Triglycerides by a Semimicro Technique, *J. Am. Oil Chem. Soc. 41*:693–696 (1963).
11. Lee, K.-T., and C.C. Akoh, Characterization of Enzymatically Synthesized Structured Lipids Containing Eicosapentaenoic, Docosahexaenoic, and Caprylic Acids, *Ibid. 75*:495–499 (1998).
12. AOCS, *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., American Oil Chemists' Society, Champaign, 1989, Cj 1-94..
13. Malcata, F.X., H.R. Reyes, H.S. Garcia, C.G. Hill, Jr., and C.H. Amundson, Kinetics and Mechanisms of Reactions Catalyzed by Immobilized Lipases, *Enzyme Microb. Technol. 14*:426–446 (1992).
14. Derenobales, M., I. Agudi, J.M. Lascaray, J.C. Mugica, L.C. Landeta, and R. Solozabal, Hydrolysis of Animal Fats by Lipase at Temperatures Below Their Melting Points, *Biotechnol Lett*. *14*:683–688 (1992).
15. Yang, Z., and A.J. Russell, Fundamentals of Non-Aqueous Enzymology, in *Enzymatic Reactions in Organic Media*, edited by A.M.P. Koskinen and A.M. Klibanov, Blackie Academic & Professional, London, 1996, pp. 43–69.
16. Chandler, I.C., P.T. Quinlan, and G.P. McNeill, Lipase-Catalyzed Synthesis of Chiral Triglycerides, *J. Am. Oil Chem. Soc. 75*:1513–1518 (1998).
17. Akoh, C.C., K.-T. Lee, and L.B. Fomuso, Synthesis of Positional Isomers of Structured Lipids with Lipases as Biocatalysts, in *Structural Modified Food Fats: Synthesis, Biochemistry and Use*, edited by A.B. Christophe, AOCS Press, Champaign, 1998, pp. 46–72.
18. Willis, W.M., and A.G. Marangoni, Enzymatic Interesterification, in *Food Lipids: Chemistry, Nutrition and Biotechnology*, edited by C.C. Akoh and D.B. Min, Marcel Dekker, New York, 1998, pp. 665–698.
19. Kuo, S.-J., and K.L. Parkin, Substrate Preference for Lipase-Mediated Acyl-Exchange Reactions with Butteroil Are Concentration-Dependent, *J. Am. Oil Chem. Soc. 70*:393–399 (1993).
20. Brekke, L.O., Soybean Oil Food Products—Their Preparation and Uses, in *Handbook of Soy Oil Processing and Utilization*, edited by D.R. Erickson, E.H. Pryde, L.O. Brekke, T.L. Mounts, and R.A. Falb, American Soybean Association and American Oil Chemists' Society, Champaign, 1980, pp. 389–438.
21. Hayes, J.R., N.H. Wilson, D.H. Pence, and K.D. Williams, Subchronic Toxicity Studies of SALATRIM Structured Triacylglycerols in Rats. I. Triacylglycerols Composed of Stearate and Butyrate, *J. Agric. Food Chem. 42*:528–538 (1994).
22. Narine, S.S., and A.G. Marangoni, The Difference Between Cocoa Butter and SALATRIM Lies in the Difference in the Microstructure of the Fat Crystal Network, *J. Am. Oil Chem. Soc. 76*:7–13 (1999).
23. Van Camp, J., A. Huyghebaert, and P. Goeman, Enzymatic Synthesis of Modified Fats, in *Structural Modified Food Fats: Synthesis, Biochemistry and Use*, edited by A.B. Christophe, AOCS Press, Champaign, 1998, pp. 20–45.

[Received June 14, 1999; accepted December 30, 1999]