Enzymatic Interesterification of Triolein and Tristearin: Chemical Structure and Differential Scanning Calorimetric Analysis of the Products

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ABSTRACT: The structural composition and thermal properties of the products of enzymatic interesterification of triolein and tristearin were investigated. The biocatalyst for the reaction was an immobilized *Candida antarctica* lipase, SP435. Enzyme load of 10% (w/w reactants) produced 72% of desired total products. Oleoyl-distearoyl triglycerides (SSO, OSS) had higher melting points than dioleoyl-stearoyl triglycerides (OOS, SOO) because the sample contained larger amounts of stearic acid than oleic acid residues. SOS and OSO were hardly produced (0.2 to 1.2%), which indicates that SP435 acted as a nonspecific lipase when catalyzing the interesterification of triolein and tristearin. The maximal yield of OSS and SSO (46.9%) was achieved with a 1:2 mole ratio of triolein to tristearin. As the proportion of tristearin was increased, the production of SOO and OOS decreased, the melting profile of the interesterified triglycerides shifted toward higher melting forms, and the solid fat content increased, indicating formation of hard fats.
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KEY WORDS: *Candida antarctica*, fatty acid composition at *sn*-2 position, interesterification, melting properties, solid fat content, triolein, tristearin.

Many new and interesting ideas for employing biotechnology to produce oleochemicals from fats and oils have been studied through hydrolysis, ester synthesis, and interesterification of lipids catalyzed by lipases (1). Among these reactions, interesterification is used in industry to modify crystallization behavior and physical properties of fats (2). Preparation of cocoa butter-like fat from vegetable oil by lipases has received considerable attention because they offer certain advantages over chemical catalysts, such as selective and positional specific fatty acid exchange, and mild reaction conditions (3).

Triglycerides may occur in three main polymorphs, named α, β', and β in order of increasing stability (2). These morphologies directly influence important properties, of the fat such as melting point, softening point, solid content, plasticity, and brittleness (4). One of the interesting features of triglyceride crystallization is the occurrence of a variety of crystal forms (polymorphism) (5). Due to the different packing modes available to the fatty acid residues in triglycerides, complex crystal polymorphism is exhibited (4).

Thus, a fundamental understanding of fat products' physical state and the factors that control the physical state, such as thermal treatment, is of importance for the control of product quality and for the possible extension of the range of products in which they can be used. For example, Garti *et al.* (6) studied the effect of food emulsifiers on the polymorphic behavior of monoacid triglycerides. DeMan and Gupta (7) reported the viscoelastic properties of butter, margarines, and other plastic fat products. The effects of tempering and heating rates on the crystalline content and stability of palm oil in relation to its chemical constitution have been reported (4).

The use of *Candida antarctica* lipase (SP435) in the modification of fats and oils has been studied recently. For example, immobilized SP435 lipase was used as a biocatalyst to alter the fatty acid composition of evening primrose oil by incorporating n-3 polyunsaturated fatty acid (PUFA), such as eicosapentaenoic acid (EPA) (8). Huang and Akoh (9) optimized and scaled up the synthesis of structured lipids by transesterification of caprylic acid ethyl ester and soybean oil and high-oleic sunflower oil with SP435 lipase. Our previous reports showed that SP435 lipase performed best with fatty acid esters in transesterification reactions (10–12).

In this paper, the effects of enzyme load (SP435) and mole ratio of substrates were investigated. Triolein and tristearin are homogeneous or monoacid triglycerides of oleic and stearic acids, respectively. Oleic acid (18:1) is commonly found in vegetable oils, such as canola, peanut, soybean and sunflower oil, while 18:0 is found in saturated fats, such as lard, beef tallow, and cocoa butter. The two triglycerides were enzymatically interesterified with SP435 lipase as the biocatalyst, and their products were analyzed in terms of their fatty acid constitution and physical characteristics, such as melting profiles and solid fat contents. This experiment can, therefore, act as a general and useful model for the interesterification of complex nonhomogeneous triglycerides of fats and oils.

MATERIALS AND METHODS

Immobilized lipase, SP435 (7000 PLU/g) from *Candida antarctica,* was provided by Novo Nordisk Biochem North America Inc. (Franklinton, NC). The activity of SP435 is ex-

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pressed in propyl laurate units (PLU/g). Triolein (99% pure), tristearin (99% pure), and porcine pancreatic lipase (Type II, crude) were purchased from Sigma Chemical Company (St. Louis, MO). Silver nitrate thin-layer chromatography (TLC) plates were purchased from Alltech Associates (Deerfield, IL). All organic solvents were obtained from Fisher Scientific (Norcross, GA).

Enzymatic interesterification. The general synthesis of modified lipids was carried out in screw-capped test tubes in which an equimolar mixture of triolein and tristearin (i.e., 88.54 mg of triolein and 89.15 mg of tristearin) was added to 3 mL *n*-hexane, followed by 10% (w/w of substrates) of immobilized SP435 lipase. The mixture was then incubated in an orbital shaking water bath at 200 rpm for 24 h at 55°C. A control (sample with no enzyme) was also incubated under the same condition. The mole ratio study was conducted by adding a fixed amount of lipase and various ratios of triolein to tristearin under the same conditions. All reactions were conducted in duplicate, and reported values are averages.

Triglycerides separation procedure. The products of the interesterification reaction were passed through a column (~3 cm) of anhydrous sodium sulfate to remove enzymes. Triglyceride species were analyzed by nonaqueous reversed-phase high-performance liquid chromatography (HPLC) in a Hewlett-Packard (Avondale, PA) Model 1090 ternary solvent delivery module, equipped with a Sedex 45 evaporative light-scattering detector (ELSD) (Richard Scientific, Novato, CA). The ELSD was set to 40°C, a nebulizer gas pressure of 2.2 atm, and a gain of 7. A Hewlett-Packard 35900 digital A/D analog interface connected the ELSD electronically to the on-line computer. The injected sample was prepared by mixing 20 µL of the reaction product with 10 µL of internal standard (100 mg/mL of tricaprin) and brought up to 1 mL with chloroform. This sample (20 µL) was automatically injected on a Beckman/Altex (San Ramon, CA) Ultrasphere ODS 5 µm, (4.6 × 250 mm) column. Separations were obtained with acetone (solvent A): acetonitrile (solvent B) as eluent at a flow rate of 1.8 mL/min, and the following gradient profile: initial condition 50:50 A/B, held 8 min at 1.8 mL/min; to 95:5 A/B over 7 min at 2 mL/min; to 50:50 A/B at 1.5 mL/min for 5 min; and returned to original condition. Total run time was 20 min.

Extraction of triglycerides. After the reaction and removal of enzyme, a portion of the reaction mixture was concentrated under nitrogen and dissolved in benzene. This concentrated mixture was analyzed by TLC on a silica gel GHL plate, which contained 20% silver nitrate. The developing solvent was benzene/chloroform (70:30, vol/vol). The bands corresponding to triglyceride products were scraped and extracted twice with 3 mL ether.

Hydrolysis by pancreatic lipase. Determination of the *sn-2* positional distribution of fatty acids in triglycerides species obtained after TLC was conducted by the method of Luddy *et al.* (13). Briefly, 1 mg of triglyceride was mixed with 1 mL of 1 M Tris-HCl buffer (pH 7.6), 0.25 mL of 0.05% bile salts, 0.1 mL of 2.2% $CaCl_2$, and 1 mg pancreatic lipase. The mixture was incubated in a water bath at 37°C for 3 min, vortexed vigorously, centrifuged (1900 rpm, 3 min), extracted with 3 mL diethyl ether (two times), and dried by passing through a column of anhydrous sodium sulfate. TLC analysis was on silica gel G (Fisher Scientific), and the developing solvent system was hexane/diethyl ether/acetic acid (50:50:1, vol/vol/vol). The band corresponding to 2-monoglycerides was scraped and extracted with ether, methylated, and analyzed by gas chromatography (GC).

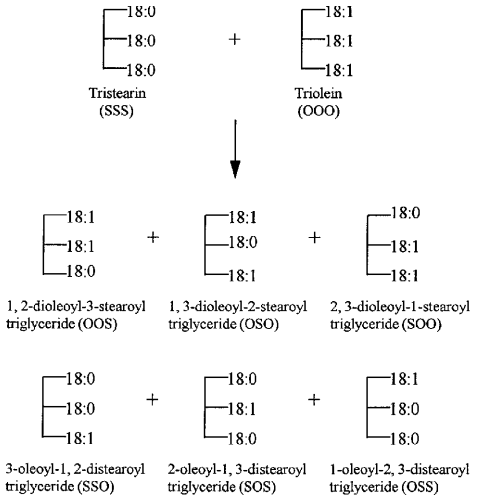
Fatty acid compositional analysis. Fatty acid profiles of triglyceride species and 2-monoglycerides were determined after methylating to fatty acid methyl esters (FAME) with 3 mL of 6% HCl in methanol at 75°C for 2 h. FAME were extracted with hexane (2 mL) and 0.1 M KCl solution (1 mL), centrifuged (1000 rpm, 3 min), and concentrated under nitrogen. FAME were then analyzed with a Hewlett-Packard 5890 Series II gas chromatograph, equipped with a DB-225 fused-silica capillary column (30 × 0.25 mm i.d.) (J&W Scientific, Folsom, CA) and a flame-ionization detector, in a splitless mode. The injector and detector temperatures were 250 and 260°C, respectively. The column temperature was held at 210°C for 10 min, and total helium carrier gas flow was 23 mL/min. The relative content of FAME as mol% was quantitated by an on-line computer with heptadecanoic acid (17:0) as an internal standard.

Melting characteristics. Differential scanning calorimetry (DSC) on a Perkin-Elmer (Norwalk, CT) Model DSC7 was used to determine melting profile and percentage of solid fat content of the interesterified products. A sample of 2–3 mg triglycerides was hermetically sealed in an aluminum pan, with an empty pan serving as a reference. Analysis was performed according to the AOCS recommended DSC procedure Cj 1-94 (15). Briefly, the sample was initially rapidly 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; heated to 80°C at 5°C/min to define the melting profile. Normal standardization of the instrument was performed with *n*-decane (m.p., –30°C) and indium (m.p., 176°C) as reference standards. Liquid nitrogen (–196°C) was used as coolant.

RESULTS AND DISCUSSION

SP435 lipase was used to interesterify triolein and tristearin to produce modified lipids. Six possible products of the interesterification are shown in Scheme 1. A proposed mechanism of interesterification reaction was described by Sreenivasan (15). HPLC analysis separated the interesterified triglycerides into four peaks with the following retention times: triolein (OOO) = 9.32 min, dioleoyl-stearoyl triglycerides (OOS, SOO, and OSO) = 10.15 min, oleoyl-distearoyl triglycerides (SSO, OSS, and SOS) = 10.98 min, and tristearin (SSS) = 12.37 min. Tricaprin, the internal standard, eluted at 3.99 min.

With enzymes costing as much as U.S. $1750/kg (16), the amount of enzyme used in a reaction is crucial for an enzymatic process to be competitive with a chemical process. The



SCHEME 1

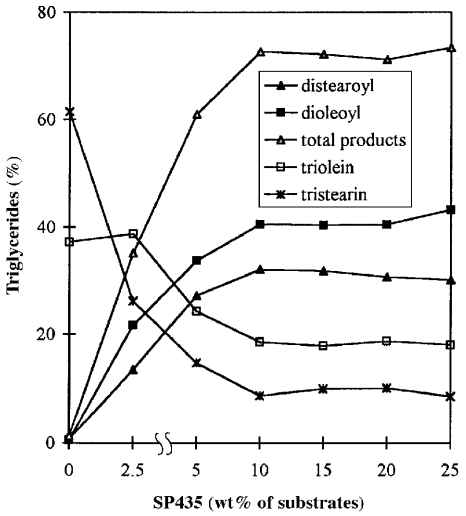


FIG. 1. Effect of enzyme load on interesterification of an equimolar mixture of triolein and tristearin by SP435 lipase. Each mixture was incubated at 55°C for 24 h. Distearoyl: oleoyl-distearoyl triglycerides; dioleoyl: dioleoyl-stearoyl triglycerides; total products: distearoyl + dioleoyl.

effect of enzyme load on the interesterification reaction is shown in Figure 1. As the amount of SP435 was increased from zero to 25% (w/w of reactants), the yields of both distearoyl and dioleoyl triglycerides increased, while triolein and tristearin decreased. This clearly indicates that the reactants were being consumed during the reaction. When 10% of SP435 lipase was used, 72.7% yield of total products was obtained. Only about 1% increase in yield was obtained when 20% of enzyme (73.4%) was used to catalyze the reaction. Therefore, 10% by weight of SP435 lipase was chosen as the optimal enzyme load and used in the later experiments.

TLC analysis was used to separate the interesterified products, besides HPLC. By using TLC plates impregnated with silver nitrate, triglyceride species were separated based on their degree of saturation. Thus, tristearin with three stearic acid (18:0) residues is the most saturated triglyceride and migrated nearest to the solvent front, i.e., it had the highest R_f value. R_f values (±0.05) of the major glyceride bands were as follows: tristearin = 0.496; oleoyl-distearoyl triglycerides = 0.418; dioleoyl-stearoyl triglycerides = 0.284; triolein = 0.154; and monoglycerides, diglycerides, oleic acid (18:1), and stearic acid = 0.04. Oleoyl-distearoyl and dioleoyl-stearoyl triglycerides were further analyzed in terms of their melting profiles and fatty acid compositions.

Figure 2 shows the melting profiles of tristearin, oleoyl-distearoyl triglycerides, dioleoyl-stearoyl triglycerides, and triolein as determined by DSC. All four melting curves seemed to have two melting components. Two polymorphs of tristearin melting at 49.1°C (ΔH = 116.8 J/g) and 66.3°C (ΔH = 171.1 J/g) were found. These were the two large endotherms that corresponded to the α-to-β and the β-to-liquid transitions (17). Lovegreen and Gray (18) observed that the melting range of the β form could be from 66 to 71.5°C when heated at 5°C/min. Garti *et al.* (6) reported that the α form melted at 55.6°C (ΔH = 110.3 J/g), and the β form melted at 71.3°C (ΔH = 194.3 J/g). For triolein, Hagemann and Tallent (20) observed that the β_3' and β_1' forms of triolein melted at −12 and −5°C, respectively. These temperatures are close to our results for triolein, which were −15.2°C (ΔH = 42.4 J/g) and −2.4°C (ΔH = 101.2 J/g), respectively.

The modified lipids, oleoyl-distearoyl and dioleoyl-stearoyl triglycerides, melted between the endotherms of tristearin and triolein. Their peaks were much smaller and broader than the homogenous pure substrates. Busfield and Proschogo (4) reported that the high-melting component of palm stearin was due to triglycerides that consisted of saturated fatty acid residues, such as POP, PPP, POS, and PPS; and the lower melting component was mainly unsaturated fatty acid residues, such as POO, PLP and SOO (where P = palmitic and L = linoleic). The thermogram (Fig. 2) shows that distearoyl triglycerides had higher-melting components than dioleoyl triglycerides. The distearoyl triglycerides sample consisted of SSO, OSS, and SOS with a high amount of 18:0 residues and had a large melting component at 21.2°C (ΔH = 62.5 J/g) and another tiny melting component at 24.8°C (ΔH = 1.5 J/g). Dioleoyl triglycerides contained 18:1 residues as the major fatty acid and melted at lower temperatures, compared to the distearoyl species. An exothermic

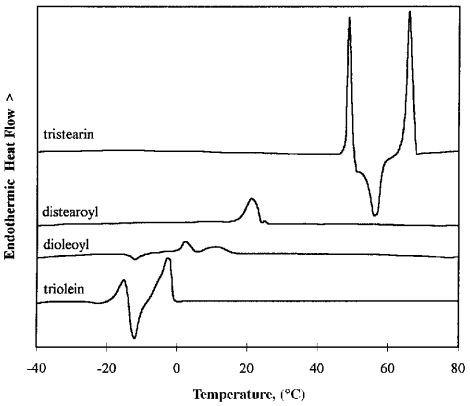


FIG. 2. Differential scanning calorimetry (DSC) heating thermograms of interesterified triolein and tristearin. Pretreatment: heated to 80°C at 200°C/min; cooled to –40°C at 10°C/min; held for 30 min; heating program: heated to 80°C at 5°C/min.

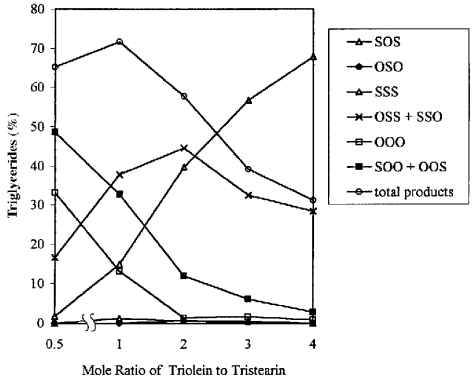


FIG. 3. Percentage yield of products from interesterification of triolein with various mole ratios of triolein to tristearin by SP435 lipase. Each mixture was incubated at 55°C for 24 h. Total product: OSS + SSO + SOS + SOO + OOS + SOS, where OOO = triolein, OOS, SOO, and DSD = dioleoyl-stearoyltriglycerides; SSO, OSS, and SOS = oleoyl-distearoyl triglycerices; SSS = tristearin.

change appeared in the dioleoyl species below the baseline at −12.1°C. According to Lovegreen and Gray (18), this is a measure of ΔH_t (9.6 J/g) for the transition and crystallization to higher-melting polymorphs, which were seen at 2.4°C (ΔH = 30.7 J/g) and 11.4°C (ΔH = 24.0 J/g).

To further analyze the composition of the interesterified products, fatty acid profiles at the *sn*-2 position of distearoyl and dioleoyl triglyceride species were quantitatively determined as shown below:

$$\%SSO + OOS = (\%distearoyl) \times (\%18:0)/100$$
$$\%SOS = (\%distearoyl) \times (\%18:1)/100$$
$$\%OOS + SOO = (\%dioleoyl) \times (\%18:1)/100$$
$$\%OSO = (\%dioleoyl) \times (\%18:0)/100$$

where %distearoyl and %dioleoyl were obtained from HPLC analysis, and %18:0 and %18:1 were obtained from GC analysis. The positional specificity of SP435 (or Novozym® 435) depends on the reactants. In some reactions, SP435 shows *sn*-1,3-positional specificity, whereas in other reactions, the lipase functions as a nonspecific lipase (19). Figure 3 shows the calculated percentage yield of each product that is produced at different mole ratios of triolein to tristearin. The graph indicates that interesterification catalyzed by SP435 lipase hardly produced SOS and OSO (0.2 to 1.2%). This also indicates that SP435 is a nonspecific lipase when hydrolyzing triolein and tristearin during interesterification reaction as assayed here. *sn*-1,3 Hydrolysis and esterification by SP435 would result in higher production of SOS and OSO species than observed. Accordingly, the production of SOO and OOS decreased as the concentration of triolein decreased. In a hypothetical case, triolein (limiting substrate) was supposed to be all consumed to leave an excess of unreacted tristearin in the reaction mixture if the mole ratio of triolein to tristearin continued to increase. Also, the amount of interesterified products should eventually equal the initial amount of triolein. The highest production of OSS and SSO (46.9%) was achieved when a mole ratio of triolein to tristearin of 2 was used. The maximal overall yield of the products (71.8%) was obtained when an equimolar mixture of substrates was used. Wada and Koizumi (21) randomly interesterified an equivalent quantity of triolein and tristearin with sodium methoxide as a catalyst at 80°C. They obtained the following result: 13.7% OOO, 12.6% OSO, 24.4% SOO and OOS, 12.7% SOS, 24.2% OSS and SSO, and 12.8% SSS. Their total product yield was 73.9%, which is comparable to the yield reported here, except for SOS and OSO.

Figure 4 shows the melting profiles of products from the mole ratio study. The peaks at the lower melting polymorphs seemed to be gradually deviating toward higher melting ranges as the proportion of tristearin increased. Here, the peaks were arbitrarily labeled A, B, C, etc., based on the size of the peak. Peak A shifted from 55°C in T0.5 (where T0.5 = mole ratio of triolein to tristearin of 1:0.5) to 71°C and became a major peak, which was sharper and more pronounced in T4. Endotherm B appeared as a minor hump in T0.5 (at 37.5°C) and slowly formed an obvious shoulder at 57°C in T4. Similarly, peak C, well separated from peak B, diminished as it shifted from 25°C in T0.5 to 51°C in T4. Endotherms D and E appeared to be disjoining from peak F in T2 and T3, and as the ratio of tristearin was increased, peak F disappeared in T4. G (−1.5°C) was seen as a big hump in melting curve T0.5 but was absent in T1 to T4. The shifting of the endotherm from a lower to higher melting range could be explained by high 18:0 content in the distearoyl triglyceride moiety in the melt. In addition, the disappearing or di-

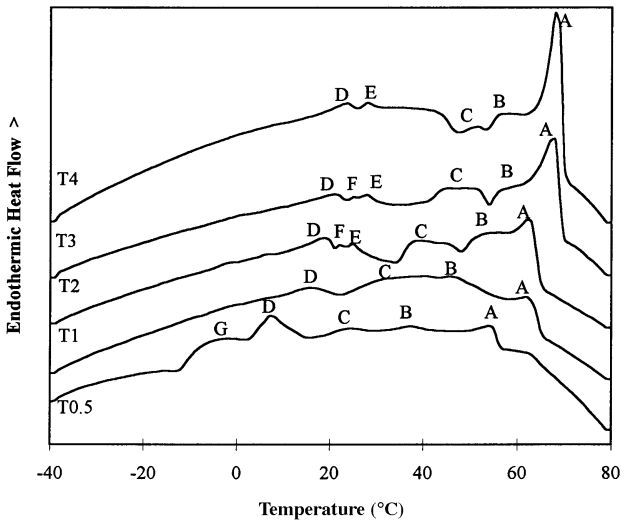


FIG. 4. DSC heating thermograms of interesterified mixtures of triolein with various mole ratios of triolein to tristearin by SP435 lipase. Mole ratios of triolein to tristearin were 1: 0.5 (T0.5), 1 (T1), 2 (T2), 3 (T3), and 4 (T4). Pretreatment: heated to 80°C at 200°C/min; cooled to –40°C at 10°C/min; held for 30 min; heating program: heated to 80°C at 5°C/min. See Figure 2 for abbreviation.

minishing peaks could be due to a decreasing dioleoyl triglyceride moiety, going from T0.5 to T4.

Consequently, the various triglycerides moieties do also contribute to differences in the hardness of fats. For example, Bornaz *et al.* (22) demonstrated that adding high-melting glycerides, such as trimyristin and tripalmitin, to butter oil led to an increase in the solid fat content (SFC) of butter oil. SFC of the interesterified products (Fig. 5) were determined from the area under their heating curves against sigmoidal baselines. These calculations were computed by the software from DSC7 (23). From the graph, interesterifying a 0.5-to-1 mole ratio of triolein to tristearin undoubtedly gave the softest fat because T0.5 contained high dioleoyl species, which is the low-melting triglyceride, made up with unsaturated oleic acid. Similarly, high distearoyl content or high-melting triglycerides resulted in fat mixtures with high SFC or hard fats.

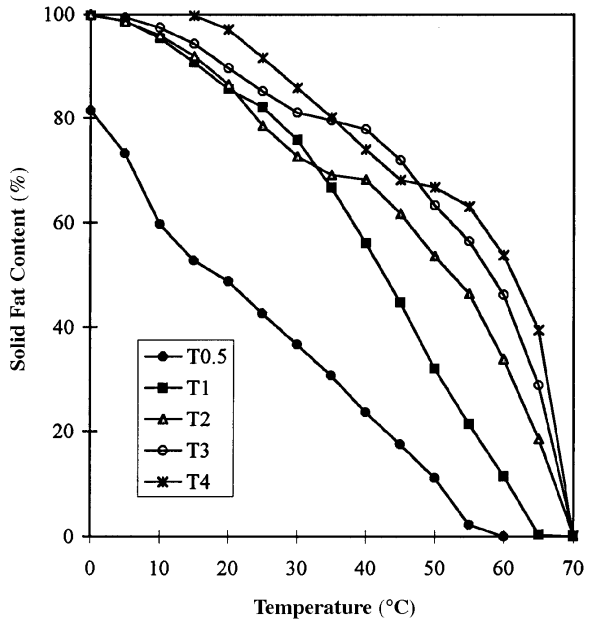


FIG. 5. Solid fat content of interesterified mixtures of triolein with various mole ratios of triolein to tristearin by SP435 lipase. Mole ratios of triolein to tristearin were 1: 0.5 (T0.5), 1 (T1), 2 (T2), 3 (T3), and 4 (T4).

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REFERENCES

1. Cho, S.W., and J.S. Rhee, Immobilization of Lipase for Effective Interesterification of Fats and Oils in Organic Solvent, *Biotechnol. Bioeng. 41*:204–210 (1993).
2. DeMan, J.M., Fats and Oils: Chemistry, Physics and Applications, in *Encyclopedia of Food Science and Technology*, edited by Y.H. Hui, Wiley, New York, 1992, pp. 823–824.
3. Chang, M.K., G. Abraham, and V.T. John, Production of Cocoa Butter-Like Fat from Interesterification of Vegetable Oils, *J. Am. Oil Chem. Soc. 67*:832–834 (1990).
4. Busfield, W.K., and P.N. Proschogo, Thermal Analysis of Palm Stearin by DSC, *Ibid. 67*:171–175 (1990).
5. Birker, P.J.M.W.L., and F.B. Padley, Physical Properties of Fat and Oils, in *Recent Advances in Chemistry and Technology of Fats and Oils*, edited by R.J. Hamilton and A. Bhati, Elsevier Applied Science, New York, 1987, p. 2.
6. Garti, N., J. Schlichter, and S. Sarig, DSC Studies Concerning Polymorphism of Saturated Monoacid Triglycerides in the Presence of Food Emulsifiers, *Fat Sci Technol. 8*:295–299 (1988).
7. DeMan, J.M., and S. Gupta, Viscoelastic Properties of Plastic Fat Products, *J. Am. Oil Chem. Soc. 62*:1672–1675 (1985).
8. Akoh, C.C., B.H. Jennings, and D.A. Lillard, Enzymatic Modification of Evening Primrose Oil: Incorporation of n-3 Polyunsaturated Fatty Acids, *Ibid. 73*:1059–1062 (1996).
9. Huang, K.H., and C.C. Akoh, Optimization and Scale-Up of Enzymatic Synthesis of Structured Lipid Using RSM, *J. Agric. Food Sci. 61*:137–141 (1996).
10. Huang, K.H., and C.C. Akoh, Lipase-Catalyzed Incorporation of n-3 Polyunsaturated Fatty Acids into Vegetable Oils, *J. Am. Oil Chem. Soc. 71*:1277–1280 (1996).
11. Huang, K.H., and C.C. Akoh, Enzymatic Synthesis of Structured Lipids: Transesterification of Triolein and Caprylic Acid Ethyl Ester, *Ibid. 73*:245–250 (1994).
12. Huang, K.H., C.C. Akoh, and M.C. Erickson, Enzymatic Modification of Melon Seed Oil: Incorporation of Eicosapentaenoic Acid. *J. Agric. Food. Sci. 42*:2646–2648 (1994).
13. Luddy, F.E., R.A. Barford, 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).
14. *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.
15. Sreenivasan, B., Interesterification of Fats, *J. Am. Oil Chem. Soc. 55*:796–805 (1978).

16. *Book Pricing*, Novo Nordisk Biochem North America Inc., Franklinton, NC, 1997.
17. Walker, W.W., Aging of Tristearin: Comparison of DSC and Positron Lifetime Results, *J. Am. Oil Chem. Soc*. *64*:754–756 (1987).
18. Lovegreen, N.V., and M.S. Gray, Polymorphism of Saturated Triglycerides: I. 1,3-Distearoyl Triglycerides, *Ibid. 55*:311–316 (1978).
19. *Product Information*, Novo Nordisk Biochem North America Inc., B 665a-GB 200, Franklinton, NC, 1992.
20. Hagemann, J.W., and W.H. Tallent, Differential Scanning Calorimetry of Single Acid Triglycerides: Effect of Chain Length and Unsaturation, *J. Am. Oil Chem. Soc. 49*:119–123 (1972).
21. Wada, S., and C. Koizumi, Influence of the Position of Unsaturated Fatty Acid Esterified Glycerol on the Oxidation Rate of Triglyceride, *Ibid. 60*:1105–1109 (1983).
22. Bornaz, S., J. Fanni, and M. Parmentier, Limit of Solid Fat Content Modification of Butter, *Ibid. 71*:1373–1380 (1994).
23. *7 Series/Unix DSC7 Users Manual*, Software Version 4.0, Perkin Elmer Corporation, Norwalk, CT, 1995.

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