Production of Margarine Fats by Enzymatic Interesterification with Silica-Granulated *Thermomyces lanuginosa* Lipase in a Large-Scale Study[1]

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ABSTRACT: Interesterification of a blend of palm stearin and coconut oil (75:25, w/w), catalyzed by an immobilized *Thermomyces lanuginosa* lipase by silica granulation, Lipozyme TL IM, was studied for production of margarine fats in a 1- or 300-kg pilot-scale batch-stirred tank reactor. Parameters and reusability were investigated. The comparison was carried out between enzymatic and chemical interesterified products. Experimentally, Lipozyme TL IM had similar activity to Lipozyme IM for the interesterification of the blend. Within the range of 55–80°C, temperature had little influence on the degree of interesterification for 6-h reaction, but it had slight impact on the content of free fatty acids (FFA). Drying of Lipozyme TL IM from water content 6 to 3% did not affect its activity, whereas it greatly reduced FFA and diacylglycerol contents in the products. Lipozyme TL IM was stable in the 1-kg scale reactor at least for 11 batches and the 300-kg pilot-scale reactor at least for nine batches. Due to regiospecificity of the lipase (*sn*-1,3 specific), enzymatically interesterified products had different fatty acid distribution at *sn*-2 position from the chemically randomized products, implying the potential nutritional benefits of the new technology.

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KEY WORDS: Batch reactor, coconut oil, interesterification, Lipozyme IM, Lipozyme TL IM, margarine fat, palm stearin.

Interest in applications of enzyme technology for the production of plastic fats for food uses is increasing in both academia and industry. Currently the application of enzyme technology is limited since the price of current commercial lipases is too high for industry to produce low-price plastic fats.

A new immobilized *Thermomyces (Humicola) lanuginosa* lipase, named Lipozyme TL IM, was recently developed with the immobilization method of silica granulation (1). Lipozyme TL IM has *sn*-1,3 specificity and hydrophilic character. It is less expensive than the commonly applied commercial lipase Lipozyme IM (*Rhizomucor miehei* lipase) and offers an opportunity for industry to reduce the process cost and make the production of low-price plastic fats economically competitive to the conventional chemical randomization.

Previously *T. lanuginosa* lipase was investigated mostly in solvent systems in free-state or immobilized forms (2–14). From kinetic studies, it was found that immobilized *T. lanuginosa* lipase had different characteristics depending on the solvent systems and the hydrophobicity of carriers (2,7). Transesterification and alcoholysis reactions catalyzed by the immobilized lipases of *R. miehei* and *T. lanuginosa* on Silica Gel 60 showed less regiospecificity in hexane media, whereas they showed strong 1,3-specificity in a more polar system using diethyl ether as the medium (2). The lipolytic activity of immobilized *T. lanuginosa* lipase depended largely on the substrate used and hydrophobicity of its carrier. A highly hydrophobic carrier enhanced lipase activity for alcoholysis reactions, whereas the reverse effect was observed for acylglycerol synthesis (7). Comparison of hydrolysis and esterification behavior of *T. lanuginosa* and *R. miehei* lipases was also carried out in AOT-stabilized water-in-oil microemulsions (2,3). For the immobilized *T. lanuginosa* lipase on resin, the optimal temperature was 10°C higher than that of the free lipase. Furthermore variation of pH had little effect on the activity, and the stability was well-maintained for repeated batch hydrolysis of triacylglycerols (TAG) (15).

Lipase immobilization usually increases the thermostability of lipases and may improve the mechanical strength, hydrophobic/hydrophilic character, regeneration, and remaining functionality of the immobilized lipase (16). The objective of this study was to use this new immobilized lipase—Lipozyme TL IM—for the production of margarine fats in solvent-free systems and to compare the activities of Lipozyme TL IM and Lipozyme IM (*R. miehei* lipase) toward ester-ester exchange between TAG. The study was focused on large-scale applications in order to supply a toolbox for industrial applications. It is expected that the cost efficiency of the Lipozyme TL IM-catalyzed process will be able to compete with chemical randomization for the production of low-price margarine fats.

In this study, a blend of palm stearin and coconut oil was used. The reaction was carried out in a 1- or 300-kg batch-stirred tank reactor. Lipozyme TL IM was used as the biocatalyst. The parameters, such as enzyme dosage, water content, temperature, and reaction time, were investigated. The

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reusability of Lipozyme TL IM was studied in both 1- and 300-kg pilot-scale batch reactors. The products from enzymatic interesterification and chemical randomization were compared with respect to the overall fatty acid composition and the distribution at *sn*-2 position. The interesterified products were monitored by the analysis of TAG profiles with high-performance liquid chromatography (HPLC). The hydrolysis was monitored by the analysis of the content of diacylglycerols (DAG) and free fatty acid (FFA) in the products. The physical properties of products were monitored by their solid fat content (SFC) profiles.

MATERIALS AND METHODS

Materials. The blend of refined, bleached, and deodorized palm stearin and coconut oil (75:25) and the chemically randomized product from the same oil blend were supplied by Karlshamns AB (Karlshamn, Sweden). The compositional characteristics of the oils and blend were given in a previous paper (17). Lipozyme TL IM, a silica-granulated *T. lanuginosa* lipase, and Lipozyme IM, which both are *sn*-1,3 specific lipases, were supplied by Novo Nordisk A/S (Bagsvaerd, Denmark). Lipases were stored at 0°C, and the blend was stored at −20°C before use. The water contents of Lipozyme TL IM and the oil blend were 6.0 and 0.06%, respectively. The monoacid TAG standards (18:0, 24:0, 30:0, 36:0, 42:0, 48:0, 52:0, 54:3, 54:6, and 54:9, where the first number represents the total carbon number of the acyl-groups and the second number represents the total number of double bonds) for HPLC analysis were from Sigma Inc. (St. Louis, MO) with the purity more than 97%. All other chemicals and reagents for the analysis were of analytical or chromatographic grades.

Interesterification (ester-ester exchange) in 1-kg scale batch reactor. The same reactor was used as described previously (17). The temperature was controlled in the range of ±0.2°C during the reaction. The stirring was by an impeller stirrer that imitates industrial batch reactors. The reactor was connected to a nitrogen supply and a vacuum pump for protection against oxidation during the process. The blend of palm stearin and coconut oil, from 800 to 900 g, was used for the lipase-catalyzed interesterification in a solvent-free system. The enzyme dosage was studied in the range of 4–20 wt%. Temperature was used in the range of 50–80°C. Default conditions were chosen as: temperature 60°C, stirring 700 rpm, no extra water addition, enzyme dosage 10 wt%, and reaction time 6 h.

The blend was melted in an oven at 60°C before use and transferred to the reactor. When the blend reached the set temperature, the lipase was added into the system to start the reaction and distributed evenly by the impeller stirrer. Samples were withdrawn during the reaction from the sampling valve and filtered by syringe membrane filters, or the final product was directly filtrated through an *in situ* filter under 0.25 MPa pressure.

For the reusability test, the reaction conditions were: reaction time 3 h, 10 wt% lipase dosage, and temperature 60°C. The oil was filtered by the *in situ* filter under 0.25 MPa pressure after the reaction and collected by a flask. New preheated blend was fed in to start the next batch. To adjust the water content of the system, water was added before adding the oil blend.

Interesterification (ester-ester exchange) in 300-kg pilot batch reactor. A conventional batch reactor for chemical randomization was used for the enzymatic reaction. The blend of 300 kg applied for the reusability study was first dried by heating to 90°C for 30 min under vacuum and cooled down to 70°C afterward. The lipase was added to start the reaction. The reaction was carried out at 70°C, 8 wt% enzyme dosage, 300 rpm stirring, 6 h reaction time, and 4×10^{-3} MPa vacuum. After the 6-h reaction, the lipase was sedimented for 1 h without stirring and the product was decanted. New preheated blend was pumped into the reactor and the second batch was started. The reaction conditions were the same as the first batch except that no drying of oil blend was conducted. Samples were taken from the reaction mixture after decanting from the reactor.

Triacylglycerol profiles were analyzed by a reversed-phase HPLC. A JASCO HPLC (JASCO Corporation, Tokyo, Japan) was equipped with two PU-980 pumps, an HG-980-30 solvent-mixing module, an AS-950 auto-sampler, and a SEDEX 55 evaporative light-scattering detector (ELSD; SEDERE, Alfortville, France) that operated at 40°C. The separation was performed on a YMC C30 column (l = 10 cm, i.d. = 4.6 mm, particle size = 3 μm; YMC, Kyoto, Japan) with a binary solvent system of acetonitrile (solvent A) and isopropanol/hexane (solvent B, 2:1, vol/vol). A linear gradient of solvent B from 20 to 46% over 45 min was applied at a flow rate of 1 mL/min, followed by 100% solvent B for 6 min and then reversed to the initial solvent A for equilibration of the system. The product samples were dissolved in chloroform (20 mg/mL) and 8 μL aliquots were injected for the HPLC analysis with double determinations. Tridecanoic acid (39:0) solution (10 mg/mL) was used as the internal standard. The TAG were expressed in terms of the relative percentages of the total TAG after normalization. Peak identification was performed by using standard TAG with known equivalent carbon number (ECN). The calculation of ECN value for a triacylglycerol is: ECN = CN − 2 × (DB), where CN is the total carbon numbers of the fatty acids in the TAG and DB is the total number of double bonds. After comparing the TAG profiles of the blend and the chemically randomized product of the blend, we found that the TAG of Peak 11 in Figure 1 can be used as the "marker TAG" to monitor the degree of interesterification, whereas it was under the detection limit in the blend. Therefore, the degree of interesterification can be expressed as:

$$\text{degree of interesterification} = \frac{P_e - P_o}{P_c - P_o} = \frac{P_e}{P_c} \qquad [1]$$

where P_e is the area percentage of Peak 11 of the enzymatically produced products, P_o is the area percentage of Peak 11 of the blend, and P_c is the area percentage of Peak 11 of the

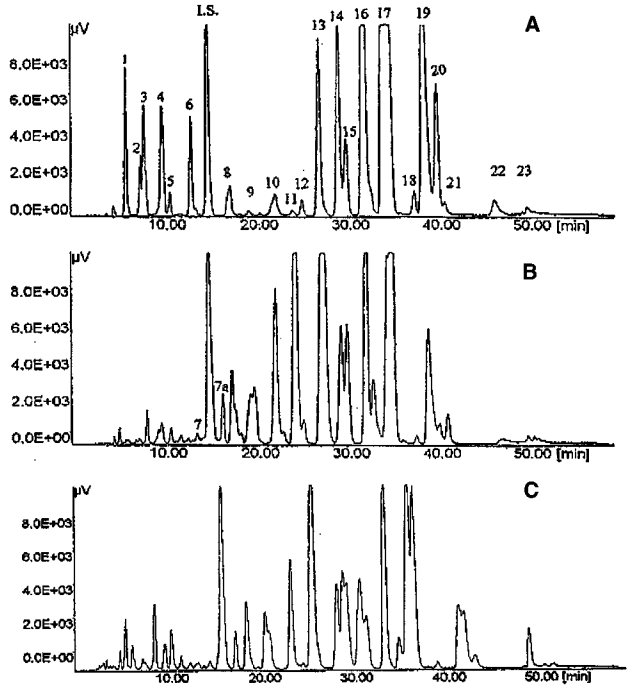

FIG. 1. High-performance liquid chromatographic chromatograms of the blend (feedstock, 75:25, palm stearin and coconut oil) (A), products from chemical randomization (B), and lipase-catalyzed interesterification (C). I.S. is the internal standard. Peaks 1–3 (ECN34); Peaks 4–6 (ECN36); Peak 7 (ECN38); Peak 7a (ECN40); Peaks 8,9 (ECN42); Peaks 10–12 (ECN44); Peaks 13–15 (ECN46); Peaks 16–18 (ECN48); Peaks 19–23 (ECN50). ECN, equivalent carbon number.

chemically randomized product. In order to compare different lipase-catalyzed products and to make the comparison clear, the relative degree of interesterification was calculated as follows:

$$\text{Relative degree of interesterification} = \frac{\text{Degree of interesterification at specific conditions}}{\text{Degree of interesterification at default condition}} \times 100 \quad [2]$$

DAG content in the interesterified products was analyzed on an HP narrow-bore silica column (l = 10 cm, i.d. = 2.1 mm, particle size = 5 µm; Hewlett-Packard, Waldbronn, Germany) with the same HPLC instrument as for the analysis of TAG profiles. A binary solvent system of heptane and heptane/tetrahydrofurane/acetic acid (80:20:1, vol/vol/vol) was used (19). The product samples were dissolved in chloroform (5 mg/mL), and 20 µL aliquots were injected for the HPLC analysis with double determinations. Calibration curves were established to quantify the amount of DAG in the samples; 1,3- and 1,2-dipalmitin were used as external standards. The calculation of DAG content was based on the entire samples containing FFA. The concentration of DAG was expressed as the wt% of the product samples.

Grignard degradation. A previous method was used with slight modification (20). Thin-layer chromatography (TLC) plates for Grignard analysis were coated with 0.4 M boric acid, air-dried overnight, and stored in a desiccator until used. The samples were partially degraded with allyl magnesium bromide (AMB). A triple determination of each sample was performed. About 30 mg of the sample was dissolved in diethyl ether (10 mL) in a 50-mL round-bottom flask. Under vigorous stirring, 0.3 mL AMB was added, and the reaction was stopped with 8 mL acid buffer (0.27 M HCl in 0.4 M boric acid) after precisely 1 min. The mixture was transferred to glass tubes, and the water phase was removed with a Pasteur pipette. The diethyl ether extract was washed twice with boric acid and dried with anhydrous sodium sulfate. After transferring to another small glass tube, the diethyl ether was evaporated under nitrogen and the residue redissolved in 150 µL diethyl ether. The 2-monoacylglycerol (MAG) fractions were separated by TLC on boric acid-impregnated silica gel plates with 100 mL of chloroform/acetone (90:10). The plates were developed 2 × 45 min, with a 10-min period of drying in between. The 2-MAG bands were removed and extracted three times with 1 mL of diethyl ether. Standard samples were used to identify the bands. The following bands were observed: 1(3)-MAG (containing capric acid); 1(3)-MAG (containing long-chain fatty acids); 2-MAG; 1,3-DAG, 1,2(2,3)-DAG; FFA; tertiary alcohol, and TAG. All 2-MAG bands were scraped off and extracted three times with diethyl ether.

Methylation of oils and 2-MAG. Oils and extracted 2-MAG were methylated by the potassium hydroxide method (21) with a little modification. The diethyl ether in the extract was first evaporated with nitrogen. The 2-MAG was redissolved in 0.3 mL of heptane and methylated with 30 µL of 2 M KOH in methanol solution. After drying and centrifuging, the supernatant was transferred to gas chromatographic vials.

Gas chromatography of fatty acid methyl esters. The methyl esters were analyzed on a Hewlett-Packard gas chromatograph HP 6890 equipped with a flame-ionization detector as described before (22).

SFC were measured by pulsed nuclear magnetic resonance using a Bruker PC/20 analyzer (Karlsruhe, Germany) according to the standard IUPAC method 2. 323 .

FFA contents (%) were determined by the American Oil Chemists' Society official method (21). An average molecular weight of 249.57 of the blend was used for calculation.

Water contents were analyzed by the Karl Fischer titration with a 720 KFS Titrino (Herisau, Switzerland). Triplicate measurements were made.

RESULTS AND DISCUSSION

Characteristics of Lipozyme TL IM. The density of Lipozyme TL IM is 0.54 g/mL. The activity of free *T. lanuginosa* lipase is 294-311 KLU/g, and Lipozyme TL IM is 140 IUN/g measured by the internal standard method of Novo Nordisk. The particle size is in a range of 0.3–1 mm. It was not changed after 11 batches of reaction in the 1-kg batch reactor in the reusability test. Particle size changes were also not observed in the 300 kg trial after nine batches. Lipozyme TL IM had similar activity to Lipozyme IM (Fig. 2) under the same reaction conditions (10% lipase dosage, 60°C, 700 rpm stirring) in the ester-ester exchange reaction between palm stearin and coconut oil.

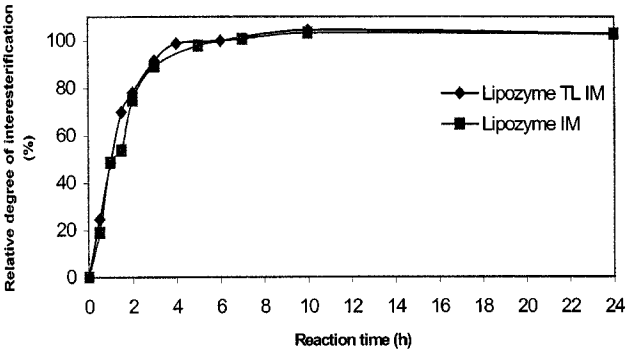

FIG. 2. Comparison of Lipozyme TL IM and Lipozyme IM in the lipase-catalyzed interesterification between palm stearin and coconut oil. Reaction conditions: reaction time 6 h, temperature 60°C, stirring 700 rpm, lipase dosage 10%, and no additional water added.

Parameter study. Lipase-catalyzed interesterification involves water participation during the reactions and is accompanied by the formation of new TAG products as well as DAG and FFA by-products in the system. The following reactions describe the process of lipase-catalyzed interesterification between TAG based on the formation of acyl enzyme complexes (24):

$$TAG_1 + E \rightleftharpoons TAG_1 \cdot E \rightleftharpoons DAG_1 + FA_1 \cdot E \quad [3]$$

$$TAG_2 + E \rightleftharpoons TAG_2 \cdot E \rightleftharpoons DAG_2 + FA_2 \cdot E \quad [4]$$

$$DAG_1 + FA_2 \cdot E \rightleftharpoons TAG_3 \cdot E \rightleftharpoons TAG_3 + E \quad [5]$$

$$DAG_2 + FA_1 \cdot E \rightleftharpoons TAG_4 \cdot E \rightleftharpoons TAG_4 + E \quad [6]$$

$$TAG_3 \cdot E \rightleftharpoons \cdots\cdots \quad [7]$$

$$TAG_4 \cdot E \rightleftharpoons \cdots\cdots \quad [8]$$

$$FA_1 \cdot E + H_2O \rightleftharpoons FA_1 + E \quad [9]$$

$$FA_2 \cdot E + H_2O \rightleftharpoons FA_2 + E \quad [10]$$

The reaction will continue until reaching equilibrium in the system; this process will involve the production of new intermediates and the formation of new TAG products (Eqs. 3–8). If the water content increases in the system, the FFA content will increase (Eqs. 9–10), whereas $FA_1 \cdot E$ or $FA_2 \cdot E$ decreases. As a consequence, the content of DAG will also increase (Eqs. 3,4). The yield of interesterified new TAG products will, therefore, decrease. This theoretical background explains the following results of parameter study.

The enzyme dosage study shows that the degree of interesterification was positively influenced by enzyme dosage (Fig. 3A). However, at enzyme dosages above 6% in 6 h, the reaction degree reached equilibrium. On the other hand, the contents of FFA and DAG were increased linearly with increase of enzyme dosage, since more water was brought into the system with higher enzyme dosage. For the *sn*-1,3 specific lipase-catalyzed interesterification, FFA and *sn*-1,2 (2,3)-DAG were formed as by-products by hydrolysis. The *sn*-1,2 (2,3)-DAG are chemically unstable, and acyl migration occurs to produce 1,3-DAG. That is why the content of 1,3-DAG was higher than the content of 1,2 (2,3)-DAG in the products (Fig. 3A). Lipozyme TL IM contained more water (6%) than Lipozyme IM (3.65%). Consequently it had a larger slope for FFA and DAG formation as the function of enzyme dosage (0.35 and 0.44, respectively) than Lipozyme IM (0.28 and 0.27, respectively) with linear regression from the curves. For Lipozyme TL IM–catalyzed interesterification, at least 6% lipase was needed to reach the equilibrium degree of interesterification in 6 h reaction at 60°C.

The optimal reaction temperature for free *T. langinosa* lipase is around 35–40°C in a solvent system (10). After silica-granulation, Lipozyme TL IM was relatively stable in the solvent-free system in the range from 55 to 80°C (Fig. 3B). The content of FFA was slightly increased with the increase of temperature. That might result from the increased mass transfer of water at higher temperature, which slightly increased the hydrolysis in the system.

In the time-course experiments, the reactions nearly reached equilibrium within 3 h at 60°C, 10% lipase dosage, and 700 rpm stirring conditions both for the normal immobilized lipase (6% water) and the dried immobilized lipase (3% water) (Fig. 3C). The reduction of water content of the lipase from 6 to 3% did not influence the lipase activity. However, the formation of DAG in the system was tremendously decreased. This suggests that the water content of Lipozyme TL IM can be further reduced to minimize the by-product formation without affecting its activity. This also indicates that water in the system, whether brought in by the lipase or by the feedstock, could increase the formation of by-products, such as FFA and DAG. The hydrolysis of lipids can be minimized by the reduction of water content in the system without loss of lipase activity. On the other hand, the yield can be improved by minimizing the water content. Therefore, the selection of optimal water content in the system is very important to achieve a high yield and to improve the product quality.

Reusability study. Based on parameter studies, the following experimental conditions for the reusability study were selected in the 1-kg scale reactor: enzyme dosage 10 wt%, temperature 60°C, stirring 700 rpm, and reaction time 3 h. Eleven batch cycles were conducted. The relative degree of interesterification was stable during the 11 batches (Fig. 4A). The contents of FFA and DAG were effectively decreased after the second or the third batch and gradually stabilized afterward under the same reaction conditions. The reduction of FFA and DAG relates to the decrease of water content in the system. The same phenomenon was also observed in the 300-kg scale studies with nine cycles (Fig. 4B) under conditions of 4×10^{-3} MPa vacuum, 6 h reaction time, 8 wt% enzyme dosage, and temperature 70°C.

At the 1-kg scale reusability test, 4 wt% water, based on lipase, was added into the system at batches 10 and 11. The content of FFA and DAG increased immediately. However, the degree of interesterification was not affected (Fig. 4A), indicating that Lipozyme TL IM can sustain sufficient water to maintain its activity during repeated uses, at least for 11 batches.

Compositional comparison of interesterified products. Lipozyme IM is a commercial lipase and has been widely studied in the last decade. Products from chemical random-

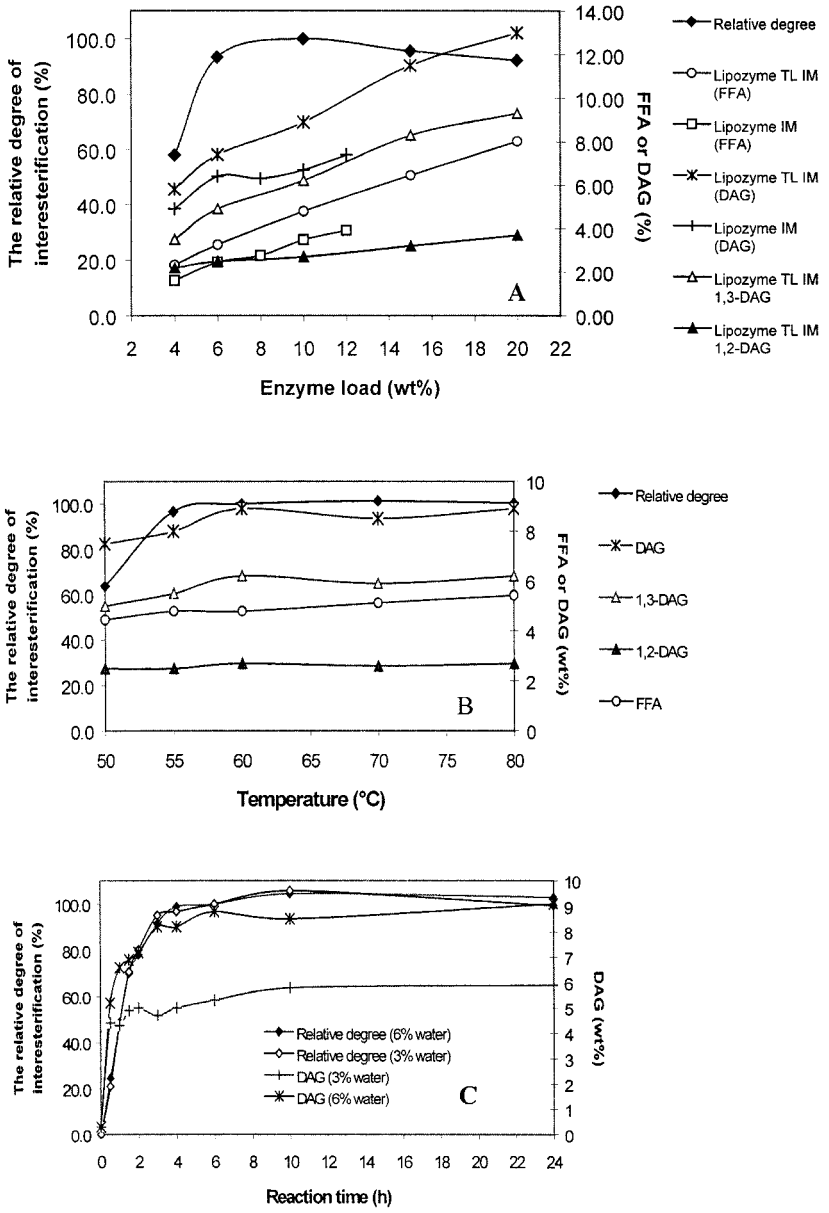

FIG. 3. Parameter effects on the degree of interesterification. (A) Effect of enzyme dosage on the relative degree of interesterification and the contents of free fatty acids (FFA) and diacylglycerols (DAG). Reaction conditions: temperature 60°C, stirring 700 rpm, time 6 h, and no extra water addition. (B) Effects of temperature on the relative degree of interesterification and the contents of FFA and DAG. Reaction conditions: enzyme dosage 10%, stirring 700 rpm, reaction time 6 h, and without extra water addition. (C) Effects of water content in Lipozyme TL IM and reaction time on the relative degree of interesterification and the contents of DAG. Reaction conditions: temperature 60°C, enzyme dosage (wt%) 10%, stirring 700 rpm, and with original (water content 6%) and dried (water content 3%) lipases.

ization and Lipozyme TL IM- and Lipozyme IM-catalyzed interesterification were compared (Table 1). Products from chemical or enzymatic interesterification had the similar changes in TAG species such as ECN34, 36, 48, and 50 (decreased) and ECN 38, 40, 42, 44, and 46 (increased) compared to the blend. For Lipozyme TL IM, the content of TAG species, ECN34, 36, 48, and 50, decreased by 5.5, 5.1, 4.4, 13.4%, respectively, after enzymatic interesterification, whereas these species decreased by 6.7, 6.0, 7.1, and 12.9%, respectively, after chemical randomization. Those of ECN38, 40, 42, 44, and 46 increased by 0.2, 1.4, 12.3, 11.1, 4.3%, respectively, after enzymatic reaction, in comparison with the increase of those species after chemical interesterification, 0.2, 1.5, 6.5, 17.0, 9.2%, respectively.

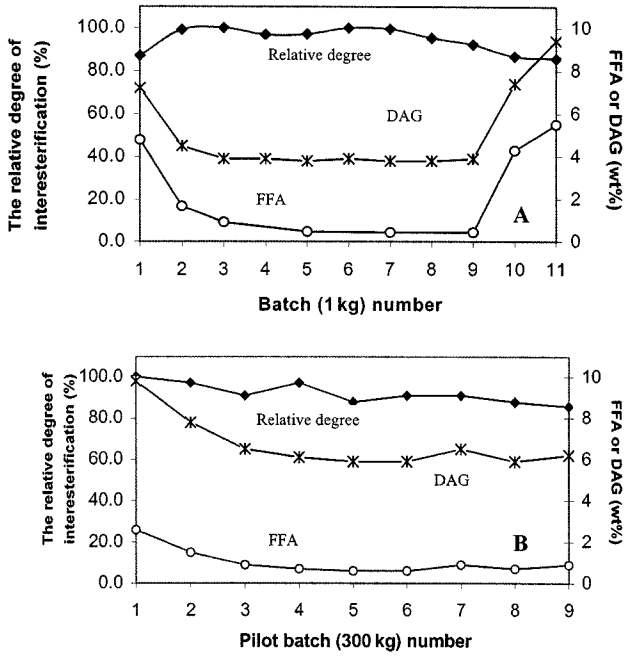

FIG. 4. Reusability studies. (A) Reusability test of Lipozyme TL IM in the 1-kg scale batch reactor. Reaction conditions: temperature 60°C, enzyme dosage (wt%) 10%, stirring 700 rpm, reaction time 3 h, and 4% extra water added after the ninth batch. (B) Reusability test of Lipozyme TL IM in the 300-kg scale batch reactor. Reaction conditions: temperature 70°C, enzyme dosage (wt%) 8%, stirring 300 rpm, reaction time 6 h under 4×10^{-3} MPa vacuum. For abbreviations, see Figure 3.

Lipozyme TL IM and Lipozyme IM both are *sn*-1,3 specific lipases. Similar products were obtained, but some differences are also apparent (Table 1), which might reflect the different fatty acid selectivity or different reaction degree of the lipases or reactions.

SFC profiles of products. In order to check the physical properties of products from the enzymatic interesterification, SFC were analyzed at different temperatures. Products from enzymatic interesterification were compared with that from the chemical randomization (Table 2). SFC were effectively decreased after chemical or enzymatic interesterification within the temperature range of 35–40°C. According to the reusability test, SFC of the products increased with the increased batch numbers from the first batch to the nineth batch in the 1- or 300-kg pilot-scale batch reactors. This was probably related to the decrease of FFA or DAG contents (Fig. 3). However, SFC decreased after adding 4% water to the system in the last two batches in the 1-kg scale reactor, where FFA and DAG contents were increased. Therefore, it is certain that FFA or DAG contents in the products had great influence on the SFC profiles.

Fatty acid distributions of products from both enzymatic and chemical interesterification. Lipozyme TL IM is a *sn*-1,3 specific lipase. Therefore the fatty acids originally located at the *sn*-2 position should largely remain in this position even though some degree of acyl migration into *sn*-1,3 position might occur. However, products from chemical interesterification should be randomized. In order to confirm the difference between the chemical randomization and lipase-catalyzed interesterification, the fatty acid distribution at the *sn*-2 position was analyzed. The enzymatically-interesterified product contains more oleic acid and lauric acid at the *sn*-2 position (Table 3). This agrees with the fact that palm stearin contains more oleic acid at the *sn*-2 position, and high content of lauric acid is located at the *sn*-2 position of the coconut oil. This indicates that enzymatically produced margarine fats are different from chemically randomized products. The fatty acids, which are located at the *sn*-2 position of starting materials, can be maintained at the position after reaction. This might bring special properties of nutritional perspectives for some special cases.

ACKNOWLEDGMENTS

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TABLE 1
Comparison of Different Products from Chemical Randomization, Lipozyme TL IM- and Lipozyme IM-Catalyzed Interesterification

Equivalent carbon number (ECN)	Blend (75:25, palm stearin/coconut oil)		Interesterified products: Chemical randomization		Interesterified products: Lipozyme TL IM-catalyzed interesterification[a]		Interesterified products: Lipozyme IM-catalyzed interesterification[a]	
ECN34	7.4		0.7		1.9		1.3	
ECN36	7.2	14.6	1.2	1.9	2.2	4.1	1.3	2.6
ECN38	0.2		0.4		0.4	1.3		
ECN40	—		1.5		1.4		1.6	
ECN42	1.7	22.4	8.2	56.8	14.0	51.7	8.5	55.1
ECN44	2.2		19.2		13.3		18.9	
ECN46	18.3		27.5		22.6		24.8	
ECN48	39.3		32.2		34.9		33.5	
ECN50	21.8	61.1	8.9	41.1	8.5	43.4	8.1	41.6
Unknown	1.9		0.2		0.8		0.7	

[a]Reaction conditions: temperature 60°C, enzyme load (wt%) 10%, stirring 700 rpm, reaction time 6 h for both Lipozyme TL IM- and Lipozyme IM-catalyzed interesterification. Both enzymes were supplied by Novo Nordisk/Bagsvaerd, Denmark.

TABLE 2
Solid Fat Contents of the Initial Oil Blend, Chemically Randomized Products and Enzymatically Interesterified Products in Different Batches

		Solid fat content (%)				
		10°C	20ºC	30ºC	35ºC	40ºC
Oil blend		64.4	37.4	18.5	12.3	6.9
Chemical randomization		67.9	44.0	18.5	9.3	1.5
Lipozyme TL IM	1st batch	60.3	34.4	14.5	7.3	<0.5
(1 kg scale)[a]	9th batch	65.3	40.0	17.6	9.7	2.9
	10th batch[c]	59.2	32.7	13.8	7.1	<0.5
Lipozyme TL IM	1st batch	65.1	40.7	18.4	10.1	2.3
(300 kg scale)[b]	9th batch	66.2	42.3	18.8	10.7	2.7

[a]Conditions: temperature 60°C, stirring 700 rpm, reaction time 3 h, lipase load (wt%) 10%, and no additional water. See Table 1 for the enzyme supplier.
[b]Conditions: temperature 70°C, stirring 275 rpm, reaction time 6 h, lipase load (wt%) 8%, 4×10^{-3} MPa vacuum and no additional water.
[c]4% extra water was added. Other conditions were the same as the other batches.

TABLE 3
The *sn*-2 Position Fatty Acid Distribution of Oil Blend, Chemical Interesterification, and Lipozyme TL IM-Catalyzed Interesterification

	Blend (mol%)		Chemical randomization (mol%)		Enzymatic interesterification (mol%)	
Fatty acids	TAG	*sn*-2	TAG	*sn*-2	TAG	*sn*-2
C6:0	0.3	—	0.3	—	0.2	—
C8:0	3.2	0.2	3.4	2.8	3.1	0.9
C10:0	2.0	0.8	2.1	2.3	2.0	1.4
C12:0	14.1	21.7	14.9	16.4	14.8	20.6
C14:0	5.9	3.3	6.0	5.7	6.0	4.6
C16:0	40.6	21.6	39.8	39.9	40.7	28.9
C18:0	3.8	1.5	3.7	3.4	4.0	2.3
C18:1n-9	23.1	40.1	22.8	19.1	22.8	32.7
C18:1n-7	0.5	0.4	0.5	0.2	0.9	0.0
C18:2n-6	5.1	9.7	5.0	4.3	5.1	7.9
C20:0	0.3	0.0	0.2	0.1	0.0	0.0
Others	1.1	0.6	1.3	5.8	0.4	0.7

[a]See Table 1 for the enzyme supplier. TAG, triacylglycerol.

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