JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Enrichment of Anhydrous Milk Fat in Polyunsaturated Fatty Acid Residues from Linseed and Rapeseed Oils through Enzymatic Interesterification

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Lipozyme TL IM was used in a solvent-free batch and microaqueous system for enzymatic interesterification of anhydrous milkfat (AMF) with linseed oil (LO) in binary blends and with rapeseed oil (RO) in one ternary blend. The aim was to obtain and characterize physicochemically fats enriched with unsaturated C_{18} fatty acids (oleic, linoleic, and, especially, linolenic acids) from natural vegetable oils. Binary blends of AMF/LO 100/0, 90/10, 80/20, 70/30, and 60/40 (w/w) were interesterified. The change in triacylglycerol (TAG) profiles showed that quasi-equilibrium conditions were reached after 4–6 h of reaction. Free fatty acid contents <1%. The decrease in solid fat content and in dropping point temperature obtained with increasing content of LO and interesterification resulted in good plastic properties for the products originating from the blends 70/30 and 60/40. This was confirmed by textural measurements. Melting profiles determined by differential scanning calorimetry showed complete disappearance of low-melting TAGs from LO and the formation of intermediary species with a lower melting temperature. Oxidative stability of the interesterified products was diminished with increasing LO content, resulting in low oxidation induction times. A ternary blend composed of AMF/RO/LO 70/20/10 gave satisfactory rheological and oxidative properties, fulfilling the requirements for a marketable spread and, moreover, offering increased potential health benefits due to the enriched content in polyunsatured fatty acid residues.

KEYWORDS: Anhydrous milk fat; interesterification; linseed oil; lipase; rapeseed oil; spread; structured lipids

INTRODUCTION

The production of structured lipids can be achieved through lipase-catalyzed interesterification of oils and fats, which leads to the rearrangement of acyl moieties. The use of a *sn*-1,3-specific lipase confines the exchange of fatty acid residues to the *sn*-1 and *sn*-3 positions of triacylglycerols (TAGs) and generates products with characteristics that cannot be obtained through chemical processing or blending. Such solvent-free reactions require mild conditions and yield no unhealthy trans fatty acids; this justifies the stepped-up interest for enzymatic interesterification for the production of margarines and other food fats (*1*−*4*). The use of the 1,3-specific lipase from *Thermomyces lanuginosa* stands out for interesterifications (*3*−*6*), and a commercial immobilized form (Lipozyme TL IM) can be reused many times efficiently in microaqueous media (*6*−*8*).

Worldwide, milkfat is the third main lipid source for human nutrition, with a flavor and mouthfeel superior to those of any other edible fat. It is a very complex mixture of thousands of different TAGs with a high percentage of saturated fatty acids, which are known to be hypercholesterolemic (*9*). Butter consumption may thus be an aggravating factor in cardiovascular diseases. On the other hand, it was established that polyunsaturated fatty acids (PUFA) and specifically linoleic (18:2n-6) and

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10.1021/jf0722203 CCC: $40.75
Published on Web 02/14/2008

linolenic (18:3n-3) acids are effective in the prevention and treatment of coronary heart diseases (*10*). Moreover, they are the only fatty acids considered to be "essential" for humans. Linolenic acid has received special attention, given its numerous health benefits and its crucial role in the development of the brain and retinal tissues of infants (*11, 12*). The industry has thus developed fats enriched with unsaturated fatty acids as an answer to growing consumer demand. Indeed, tests involving human consumption of dairy products enriched in PUFA showed a resulting reduction of blood cholesterol (*13, 14*).

Interesterification of lipids by lipases is a convenient way to obtain modified fats, and various examples of applications leading to the generation of products enriched with unsaturated fatty acids have been described (*7, 8, 15*−*19*). Examples of lipase-catalyzed acidolysis of canola oil or milkfat and pure long-chain PUFA have been also reported (*20*−*24*). Linseed oil (LO) (also named flaxseed oil) presents very high contents of unsaturated C_{18} fatty acid residues [α-linolenic ($C_{18:3}$), linoleic ($C_{18:2}$), and oleic ($C_{18:1}$) acids], but only scarce examples of its use in structured lipids have been reported. For example, LO was esterified with methyl oleate by *Mucor miehei*lipase in presence of hexane with the aim of reducing its content in $C_{18:2}$ and $C_{18:3}$ (*25*). It was also interesterified with palm olein and stearin (ratios of 10/90) to generate softer fats (*26*).

The high content of unsaturated C_{18} on the *sn*-2 position of TAGs of LO (*12*) makes them efficiently utilized by animal metabolism (*1, 27*). Filtered cold-pressed LO is mostly used in India and eastern Europe as edible oil (*12*). However, its use in food is seriously limited in some countries due to its instability resulting from the high content of unsaturated fatty acids, the oxidation of which leads to the formation of carcinogenic compounds (*28*). However, it is permitted by the U.S. FDA, for instance (*29*), as a supplement and as an additive in salad oils and in spreads.

The objective of the present work was to carry out a study leading to the production of new fats by enzymatic interesterification of anhydrous milkfat (AMF) and LO. Lipozyme TL IM was used in solvent-free batch reactions. TAG profiles, interesterification degree (ID), and free fatty acid (FFA) content were followed throughout the reactions. Determination of dropping points (DP) and solid fat contents (SFC) enabled rheological characterization of the products. Differential scanning calorimetry (DSC) was used to monitor the melting profile of the new products. End products' oxidative stability and textural properties were also characterized. Finally, a ternary blend containing RO was proposed as a way to obtain a fat with increased oxidative stability. These results should contribute to obtain a butter-based spread offering additional nutritional benefits due to its high content in unsaturated C_{18} and diminished saturated fatty acid ratios.

MATERIALS AND METHODS

Materials. Lipozyme TL IM, a silica granulated *T. lanuginosa* lipase, was kindly supplied by Novozymes A/S (Bagsværd, Denmark). Anhydrous cow's milk fat (AMF) was purchased from Corman (Goé, Belgium) and stored at −20 °C until used. RO was purchased from Vandemoortele (Izegem, Belgium), and refined LO was obtained from Vandeputte Co. (Mouscron, Belgium). Both were kept at 4 °C in the dark until used. All other reagents and solvents used were of analytical grade (Fisher Bioblock Scientific, Tournai, Belgium).

Methods. *The water content in fats* was determined by volumetric Karl Fischer titration, carried out with a Karl Fischer titrator DL31 (Mettler Toledo, Zurich, Switzerland) using "Solvent oils & fats" (Merck, Darmstadt, Germany) and the one-component reagent for volumetric Karl Fischer titration Hydranal composite 5 (5 mg of H_2O/mL) (Riedel-de Haën, Seelze, Germany).

Enzymatic Reactions. Six hundred grams of oil blend with 4% (w/w) Lipozyme TL IM was used for the solvent-free interesterification reaction in a 1 L batch reactor (Pierre E. SPRL, Vilvoorde, Belgium) equipped with an impeller-type stirrer. N_2 was blown into the medium during the reaction. As fresh Lipozyme TL IM contained 4–5% water (w/w) (determined by gravimetry), this content was reduced prior to the interesterification of the blends to avoid byproduct (FFA and diacylglycerols) formation caused by hydrolysis of the fat. Hence, 3 volumes of RO (600 g) were successively interesterified for 30 min at 70 °C. Water from the enzyme and the medium was thus consumed in hydrolytic side reactions (*30*). The lipase was then quickly rinsed with the blend that was going to be interesterified to remove the RO. Interesterifications were then conducted and samples were withdrawn every 15 min during 1 h and then every hour. They were filtered on a nylon membrane (Sefar Nitex, Switzerland) to remove enzyme particles.

Analytical Procedures. Fatty acid methyl esters were prepared by hydrolysis of TAGs (20 mg) and methylation with BF_3−methanol (0.5 mL) in pentane (0.2 mL) for 90 min at 70 °C. At the end of the reaction the medium was cooled and 4.2 mL of pentane was added; 1 volume of this solution was diluted 10 times before analysis. Three independent replicates were performed. The samples were analyzed by gas chromatography (GC) on a 30 m CP-Wax 52 CB column (0.25 mm internal diameter, 0.25 μm phase thickness) with split injection mode. Temperature programming was from 40 to 150 °C (30 °C/min) and then to 250 °C (5 °C/min) and kept for 10 min at 250 °C. Helium was the carrier gas.

TAG profiles were analyzed by GC equipped with a capillary column (CP-TAP CB for triglycerides; 25 m, 0.25 mm internal diameter, 0.1 μm phase thickness), and hydrogen was the carrier gas. The samples were dissolved in hexane, and 1 μL was injected manually, with on-column mode. The temperature was programmed at a rate of 3 °C/min from 280 to 360 °C and then maintained for 30 min at 360 °C.

The chromatograms were integrated manually with the aid of HP Chemstation software (Agilent Technologies, Diegem, Belgium) by carbon number (CN), that is, the sum of the carbons of the fatty acids from one TAG.

A soybean oil standard for triglycerides, "Testmix CP0024" (Varian Instruments, Middelburg, The Netherlands), was used to identify the groups of TAGs by CN. For the identification of the whole spectrum of TAGs from AMF, we reported the certification of the TAG contents of AMF from the Community Bureau of Reference Materials (*31*).

The degree of interesterification was defined as

$$\mathrm{ID} = \left(\frac{\mathrm{areaCN50}}{\mathrm{areaCN44}}\right)_{\mathrm{IE}} - \left(\frac{\mathrm{areaCN50}}{\mathrm{areaCN44}}\right)_{\mathrm{NE}}$$

where CN50 and CN44 are the peaks with CN equal to 50 and 44, corresponding, respectively, to a highly variable and the most stable group of peaks during the reaction, for interesterified (IE) and noninteresterified (NIE) samples.

Free fatty acid contents were determined according to the AOCS official method (*32*). An average molecular weight of 256.4 (palmitic acid) was used for calculations.

Dropping points (DPs) were determined with Mettler dropping point apparatus model FP90 (Mettler Toledo, Zurich, Switzerland). When the sample reaches the dropping temperature, it flows and traverses through an IR beam, defining the DP. Liquefied samples were first placed into chilled holders and held at −20 °C for 1 h before heating was initiated (1 °C/min), starting 12–15 °C below the expected dropping point.

Solid Fat Content (SFC) was measured at different temperatures by nuclear magnetic resonance using a pulsed NMR spectrometer (Minispec-mq20, Bruker, Karlsruhe, Germany). Melting curves were obtained according to the standard IUPAC method 2.150 (*33*).

Differential scanning calorimetry (DSC) analyses were carried out with a Q1000 DSC (TA Instruments, New Castle, DE). Calibration was done with indium, eicosane, and dodecane standards. An empty aluminum pan was used as reference. Melted samples were weighed (1.5–3 mg) into solid fat index aluminum pans (TA Instruments) and then heated to 80 °C and held for 10 min to melt all of the crystals.

The samples were then cooled to −80 °C at 10 °C/min and maintained for 30 min. Following this, the melting profiles were obtained by heating the samples to 80 °C at 15 °C/min (*34*).

Oxidative Stability. Oxidation induction times (OIT) were measured with the Rancimat 679 apparatus (Metrohm AG, Herison, Switzerland) on 2.5 g of fat sample heated to 100 °C under a purified air flow rate of 15 L/h. The volatile degradation products are trapped in distilled water, and its conductivity is followed. The induction time is defined as the time necessary to reach the inflection point of the conductivity curve.

Texture Measurements. The samples were first heated for 10 min at 80 °C to destroy crystal memory and then kept in liquid form at 50 °C. They were then crystallized at −20 °C for 30 min. Before measurements, samples were tempered at 5 ± 0.5 °C for 48 h in a temperature-controlled room (5 ± 0.5 °C) using an SMS TA.XT2i/5 texturometer (Stable Micro Systems, Surrey, U.K.). A constant penetration speed was adopted, and a cone probe (P/45C) was used in the penetration tests. The probe penetrated the product at a constant speed of 0.5 mm/s to a distance of 5 mm. The maximum penetration force and the final penetration force were recorded (*34, 35*). At least five penetration tests were run on each sample.

RESULTS AND DISCUSSION

Composition of the Fats. Fatty acid compositions of AMF and LO are given in **Table 1**. The AMF used in this study contained 30.2% of palmitic ($C_{16:0}$) and 11% of stearic ($C_{18:0}$) acids. The total unsaturated C_{18} summed 90.3% of LO fatty acids. A series of blends of AMF and LO, containing from 0 to 40% (w/w) LO, were chosen for interesterification. **Table 2** shows the calculated unsaturation content of the different blends, based on the determined fatty acid contents. The first column reports the molar quantity of unsaturations from C_{18} for 100 g of the blend; it ranged from 0.25 for AMF alone to 0.92 for the blend with 40% LO, which represented an unsaturations enrichment factor of 3.7. When linolenic acid alone was considered, the enrichment factor was equal to 36.6; that is, the blend with 40% LO contained 36.6 times more $C_{18:3}$ than AMF. In column 4 are indicated the weight percents of the sum of unsaturated C_{18} in the blend equal to 26.7% in AMF and doubling to 52.2% in the blend with the ratio 60/40.

GC analyses enabled a good separation of the fats' TAGs as shown in **Figure 1**. TAG profiles of the fats were established on the basis of the percent area of CN peaks (indicated in **Figure 1**). The very high diversity of TAGs from AMF spread from CN of 20 to 54 (**Figure 1A**). It is noteworthy that diacylglycerols (DAGs) were also detected, and they may appear mixed with CN from 20 to 36–38. This was demonstrated by analyzing commercial DAGs and also the strips corresponding to 1,2- and 1,3-DAGs obtained by thin-layer chromatography of the different IE fats (not shown). However, the content of DAGs here was very low. LO has a much more reduced range of TAGs, and they were detected here from CN of 50 to 54 (**Figure 1B**). The major peaks corresponding to TAG species of LO are composed of tri-$C_{18:3}$, $C_{18:3}C_{18:3}C_{18:1}$, and $C_{18:3}C_{18:3}C_{18:2}$ by decreasing order (*12*).

AMF and LO were characterized by water contents of 0.035 and 0.018%, respectively, according to Karl Fischer measurements (**Table 1**). Such contents were in accordance with microaqueous conditions enabling a good esterification activity of this lipase (*6, 8*).

TAG Profile Changes during Interesterification. TAG profiles were followed throughout the reactions by plotting the percents of the areas of CN peaks groups. This was done until quasi-equilibrium conditions were reached. The redistribution of the acyl moieties on TAGs was thus followed. **Figure 2A** shows the percents of TAGs by CN in AMF alone and in the tested blends. TAGs of AMF divide in two main groups, one from CN32 to CN42 and another from CN44 to CN54. By increasing the content of LO in the blend, a clear increase in CN54 occurs, reaching >20% of the total TAGs for the blend with 40% LO. **Figure 2B** shows TAGs distribution after 6 h of interesterification. The enzymatic reaction led in all cases to a redistribution of TAG species, which appears clearly in **Figure 3**. This figure shows the changes of the percents of TAGs reported for the NIE blend AMF/LO 80/20. CN30, CN32, and CN44 showed no or low changes; a decrease of CN34–42 and CN54 occurred as well as an increase of CN46–52. These variations indicate a decrease of TAGs with medium-chain acyl moieties and of tri-C_{18} species, leading to an increase of intermediate species. This was the general trend for all of the tested blends (**Figure 2**).

An increase in CN20–28 can also be observed (**Figures 2** and **3**), corresponding notably to DAG formation during the reaction (not shown).

Interesterification Degree (ID). Dashed lines in **Figure 3** indicate peaks with a high variation and peaks with the highest stability during the reaction, that is, CN50 and CN44, respectively. The ratio of these values for a given reaction time, minus

Table 1. Fatty Acid Composition and Water and FFA Contents of the Fats Used in This Study (Percent Weight)

	AMF	linseed oil	rapeseed oil
fatty acid composition			
4:0	4.52 ± 0.09		
6:0	2.24 ± 0.13		
8:0	1.31 ± 0.09		
10:0	3.08 ± 0.08		
10:1	0.34 ± 0.02		
12:0	3.88 ± 0.04		
13:0	0.08 ± 0.01		
14:0	12.05 ± 0.04		
14:1	1.15 ± 0.02		
15:0	1.18 ± 0.13		
16:0	30.17 ± 0.13	5.08 ± 0.00	4.87 ± 0.02
16:1	1.80 ± 0.02		0.21 ± 0.01
17:0	0.53 ± 0.02		
18:0	10.97 ± 0.05	3.71 ± 0.13	1.60 ± 0.05
18:1	22.65 ± 0.06	19.03 ± 0.36	61.84 ± 0.33
18:2	3.44 ± 0.06	16.32 ± 0.10	20.38 ± 0.23
18:3	0.61 ± 0.03	55.01 ± 0.63	8.94 ± 0.22
20:0		0.15 ± 0.01	0.47 ± 0.03
20:1		0.15 ± 0.01	1.09 ± 0.03
22:0		0.11 ± 0.01	0.23 ± 0.02
22:1		0.18 ± 0.00	0.19 ± 0.00
22:2		0.25 ± 0.03	
24:0			0.07 ± 0.00
24:1			0.10 ± 0.03
water content (% w/w)	0.035 ± 0.006	0.018 ± 0.005	0.045 ± 0.006
FFA (% w/w)	0.27 ± 0.01	0.11 ± 0.01	0.04 ± 0.01

Table 2. Calculations Made from the Determined Fatty Acid Composition of AMF and LO: Unsaturation Number (UN = Moles of unsaturations of C_{18} per 100 g, Considering the Molar Mass of All Fatty Acid Residues of the Blend), Unsaturation Enrichment Factor ($UEF = UN_{blend}/UN_{AMF}$), $C_{18:3}$ Enrichment Factor ($C_{18:3}\ EF = C_{18:3}UN_{blend}/C_{18:3}UN_{AMF}$), and Percent of Total Unsaturated C_{18} in the Blend (TUC_{18})

AMF/LO (w/w)	UN[a] (mol/100 g)	UEF	$C_{18:3}$ EF	TUC_{18} (% w)
100/0	0.25	1	1	26.7
90/10	0.41	1.6	9.9	33.1
80/20	0.57	2.3	18.8	39.4
70/30	0.74	3.0	27.7	45.8
60/40	0.92	3.7	36.6	52.2

[a] Moles of $C_{18:1}$ + 2 × moles of $C_{18:2}$ + 3 × moles of $C_{18:3}$ for 100 g of fat.

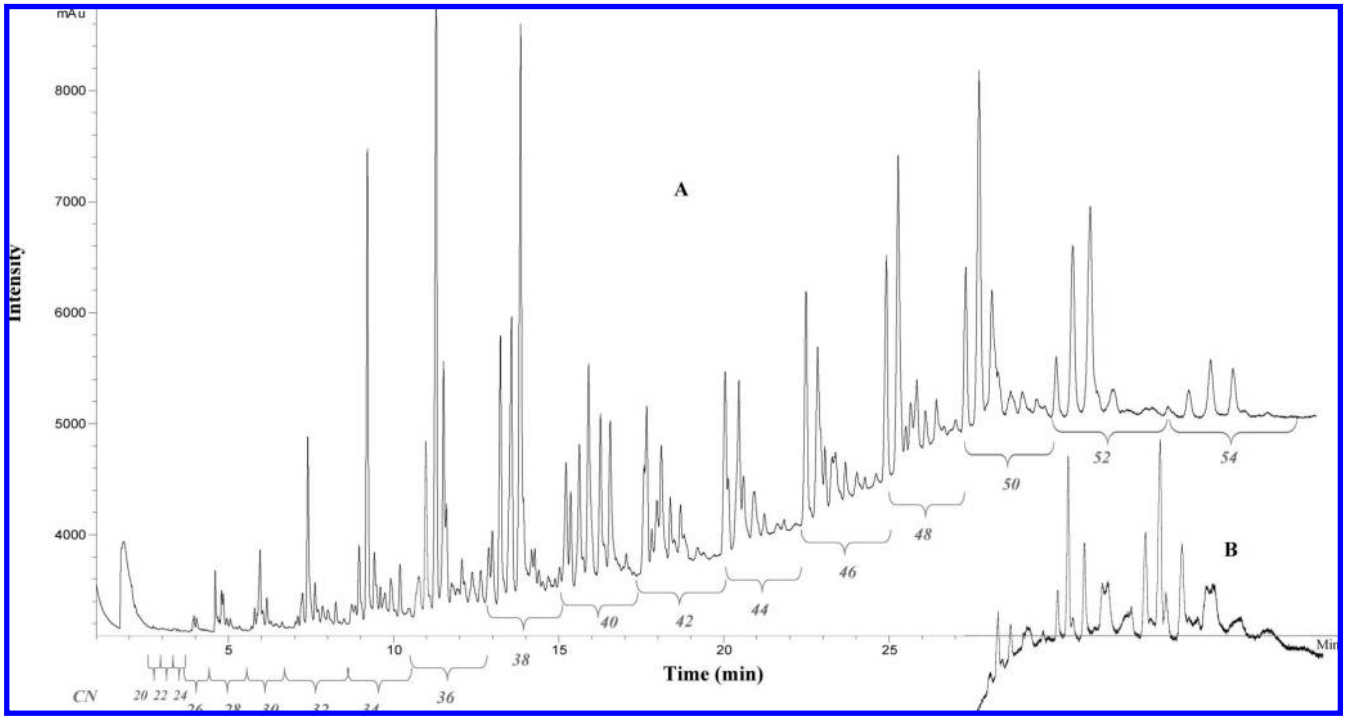

Figure 1. Gas chromatograms of AMF (**A**) and LO (**B**). The braces indicate the groups of peaks with the same carbon number (CN), which is indicated below.

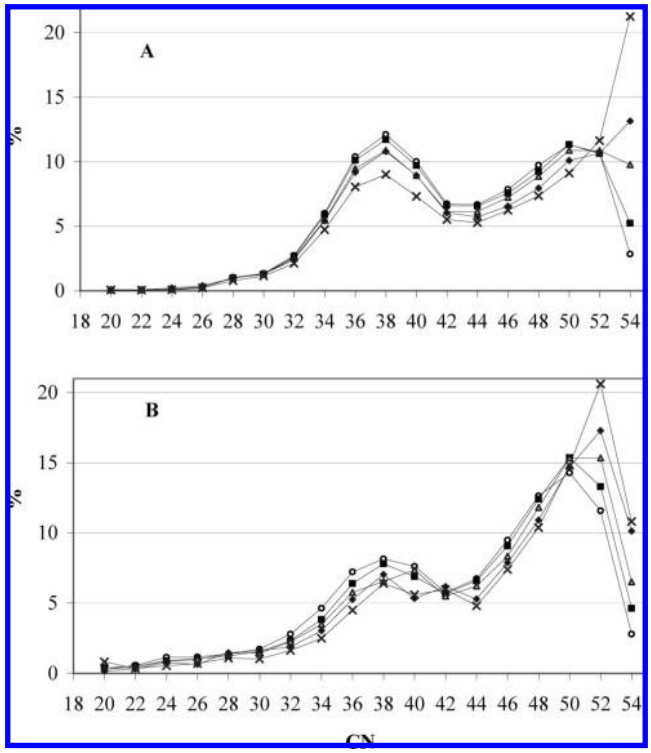

Figure 2. Distribution of TAGs (%) by CN in the non-interesterified blends (**A**) and at quasi-equilibrium conditions after 6 h of interesterification (**B**): blends AMF/LO of 100/0 (○), 90/10 (■), 80/20 (△), 70/30 (◆), and 60/40 (×).

the same ratio for the NIE blend, defines the ID. The ID was determined for each blend throughout the reaction and was used to monitor the reaction (**Figure 4**). The interesterification occurred quickly during the first hour of reaction and reached a quasi-equilibrium state after 4–6 h with no further significant change until 24 h. The relationship between ID values at 6 h against LO in the blend is illustrated in **Figure 4** (inset).

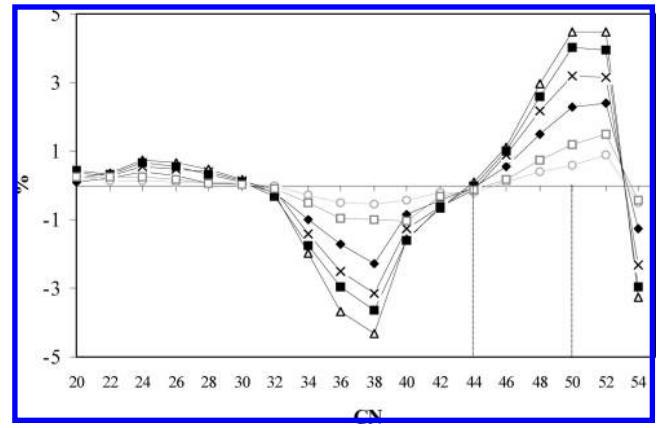

Figure 3. Variations of TAGs (%) with respect to the NIE blend, along the interesterification reaction for the blend AMF/LO 80/20 (w/w), after 15 min (○), 30 min (■), 1 h (◆), 2 h (×), 3 h (■), and 6 h (△).

Lipozyme TL IM is a very specific *sn*-1,3 lipase, meaning that within a few hours the fatty acid residues on *sn*-1 and *sn*-3 positions of TAGs are totally randomized. Very few substitutions occur at the *sn*-2 position of TAGs with this enzyme (*19*); however, nonenzymatic migration on that position was reported (*1, 6*), which may explain the slight detectable variations of ID going on with time after the quasi-equilibrium state was reached.

Considering the high content of tri-$C_{18:3}$ in LO (and of $C_{18:3}C_{18:3}C_{18}$) (*12*) and the observed changes of TAG species (**Figures 2** and **3**), good ratios of TAGs with $C_{18:3}$ residues on the *sn*-2 position are expected in the IE product. This is an asset because essential fatty acids are more efficiently utilized from the *sn*-2 position in TAGs; once hydrolyzed by pancreatic lipase, they are efficiently absorbed by mucosal cells (*1*).

Free Fatty Acids (FFA). The water content of the reaction medium influences the release of FFA from TAGs during interesterification, and hydrolysis reactions occur in the extent of the available water (*3*). As can be seen in **Figure 5**, the

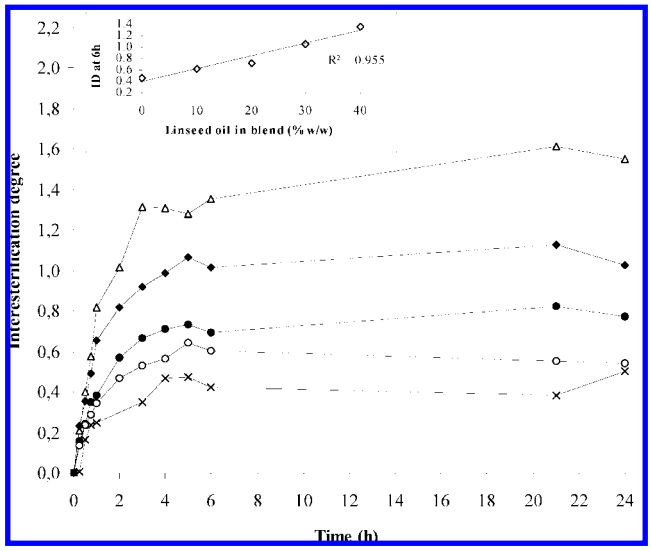

Figure 4. Interesterification degree as a function of time for the blends AMF/LO of 100/0 (×), 90/10 (○), 80/20 (●), 70/30 (◆), and 60/40 (△). (Inset) ID as a function of LO ratio in the blend.

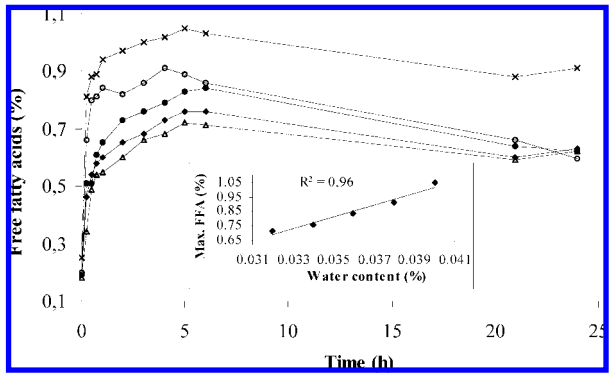

Figure 5. Free fatty acids (%) as a function of reaction time for the blends AMF/LO of 100/0 (×), 90/10 (○), 80/20 (●), 70/30 (◆), and 60/40 (△). (Inset) Maximum FFA content determined (%) as a function of water content calculated for the blend (%).

profiles of FFA contents in the samples resembled the evolution of ID (**Figure 4**), and they reached a maximum after 4–6 h of reaction. The more AMF in the blends, the higher were the concentrations of FFA; this may be linked to the water content of AMF, which was twice that of LO (**Table 1**), and indeed a linear relationship was found between the maximum FFA contents and the water content of the blend (calculated on the basis of the fat's water content) (**Figure 5**, inset). In the 21 and 24 h samples, a slight FFA decrease was observed. A possible explanation may be the high volatility of short-chain FFA, the content of which is high in AMF (**Table 1**); butyric acid ($C_{4:0}$), for instance, is almost exclusively located at the *sn*-3 position of TAGs (*9*) and thus primarily released by the lipase. The FFA content of IE products has to be minimized to avoid off-flavors if a marketable fat is considered. FFA contents obtained here were <1%, which is lower than typical values from other published works (*3, 8, 36*).

Dropping Points. The blends and the reaction products are semisolid fats with a rheological behavior that is governed by interactions between fat crystals in an aggregated three-dimensional solid–liquid fat matrix (*37*). As the sample is heated solid fats melt, progressively weakening the crystal network until the oil is no more retained, flowing out at DP. DP of AMF alone was at 33.8 °C. Blending AMF with 40% LO led to a decrease of DP by 2.5 °C. The interesterification of the blends 100/0 and 90/10 resulted in a slight increase (0.5–1 °C) of the DP temperatures, which may be explained by a slightly denser crystal network. For the three other blends a decrease of DP was observed, all the more important as LO content was high; DP occurred at 27 °C (decrease of 4 °C) in the case of the blend with 40% LO and at 30 °C (minus 1.2 °C) for the blend with 30% LO (**Figure 6A**). These variations are clearly visible in **Figure 6B**, which shows the relationship between the measured DP and the initial content of LO in the blend. Rousseau et al. (*17*) reported similar increases of DP for chemically IE blends of butterfat and canola oil, whereas they observed decreases in DP only for the blends with an oil content of at least 50%.

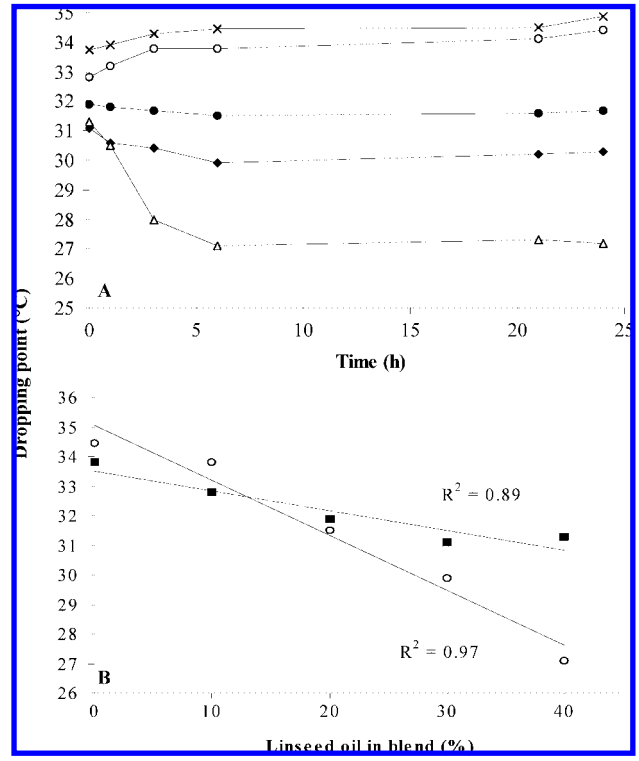

Figure 6. Dropping points (°C) as a function of reaction time for the blends AMF/LO of 100/0 (×), 90/10 (○), 80/20 (●), 70/30 (◆), and 60/40 (△) (**A**) and as a function of LO in the blend for NIE blends (■) and after 6 h of interesterification (○) (**B**).

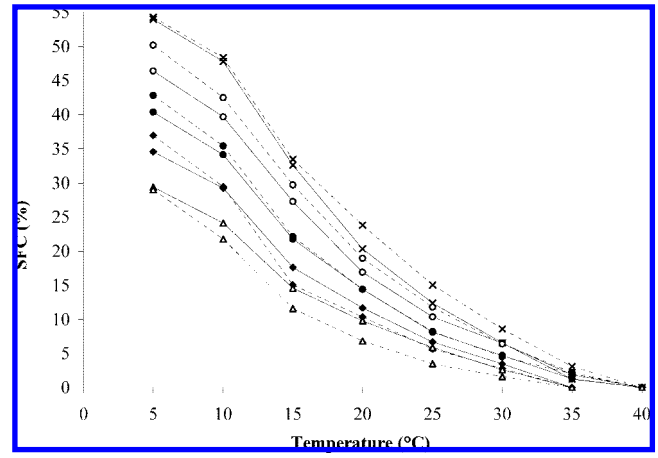

Figure 7. Solid fat content (%) at temperatures between 5 and 40 °C for NIE blends (solid lines) and after 6 h of interesterification (dashed lines): blends AMF/LO of 100/0 (×), 90/10 (○), 80/20 (●), 70/30 (■), and 60:40 (△).

The crystals of semihard and hard fat of AMF are composed of TAGs with CN42–54 and the oil phase of TAGs with CN26–40 (*9*). LO brings mainly liquid TAGs with CN54. As described here before, interesterification of the blends led to a decrease of TAGs with CN30–40 and CN54 and to an increase of TAGs with CN46–52 and CN20–26 (**Figure 2**). The increase of DP in the blends 100/0 and 90/10 may be explained by the low initial content and scarce variations of the content of TAGs with CN54 (compared to the higher initial contents observed on TAG profiles of the other blends), making the interesterification balance positive for harder fats. This shows in the present case the major influence of the initial content of TAGs with CN54 on the physical structure of the IE products.

Solid Fat Content. SFC profiles can give indications on physical (meltability, spreadability, plasticity, etc.) and organoleptic properties of edible fats, that is, major concerns for the consumers. The variations of SFC with temperature and the sharpness of melting range determine the range within which a fat can be considered plastic. SFC values given between 4 and 10 °C determine the spreadability of the product at refrigeration temperatures, and <32% SFC at 10 °C is necessary for good spreadability. Between 33 and 38 °C the values of SFC influence "mouthfeel" or waxy sensations (*37*).

The simple blending of AMF with LO reduced the SFC from 55% (0% LO) to around 30% (40% LO) at 5 °C. The blends displayed a nonlinear melting behavior between 5 and 40 °C. The sharpest drop occurred between 10 and 15 °C. Interesterification resulted in an increase of SFC above 5 °C for the blends 100/0 and 90/10. This observation is correlated with the increased DP (**Figure 6A**,**B**). An increase of SFC was also detected at 5 and 10 °C for the blend 80/20 and at 5 °C for the blend 70/30. SFC was lowered for the blend 60/40 above 10 °C and above 15 °C for the blend 70/30 (**Figure 7**). It is noteworthy that DP occurs below 5% SFC (**Figures 6A** and **7**), which shows the small amount of solid fat necessary to maintain a cohesive network of crystals and oil.

Considering that the temperature range within which fats are plastic corresponds to SFC values in the range of 15–35% (*35*), only IE products derived from the blends 70/30 and 60/40 could be considered as such at 10 °C. Moreover, both of these products were totally liquid (SFC = 0%) at mouth temperature (37 °C), a required property for an edible spread.

Differential Scanning Calorimetry. Melting thermograms obtained by DSC analyses reveal transition temperatures and heats of fusion, providing complementary results to DP and SFC data (*19, 35*). The thermal profiles of the tested blends before (solid lines) and after 6 h of interesterification (dashed lines) are presented in **Figure 8**. AMF alone was characterized by three major endotherms (a_1, b_1, and c_1) spreading from around −20 to 35 °C; blending with LO resulted in their attenuation. The other consequences of blending with LO were a shift of the maximum peak temperature (peaks c_{1-5}) to lower temperatures and the occurrence of a fourth endotherm between −40 and −30 °C (d). The interesterification of AMF alone had only slight impacts on the DSC profile, namely, a deepening of endotherm b_1 (**Figure 8**, curves 100/0). This means a slight increase in total high melting species, which is in total accordance with DP (**Figure 6A**) and SFC (**Figure 7**) data.

LO alone was characterized by two endotherms between −45 and −20 °C (not shown), corresponding to the low melting constituents, namely, TAGs with unsaturated C_{18}. When blended with AMF, these peaks from LO (**Figure 8**, peaks d_{3-5}) were detectable by DSC only for a LO content of >20%, remaining small in all cases. The interesterification resulted in the total disappearance of those peaks, that is, of the low-melting TAGs from LO. Thus, following by DSC the peak c (when LO content is sufficient to enable its detection) may be a way to monitor the reaction because the total disappearance of the TAG species from LO can be detected.

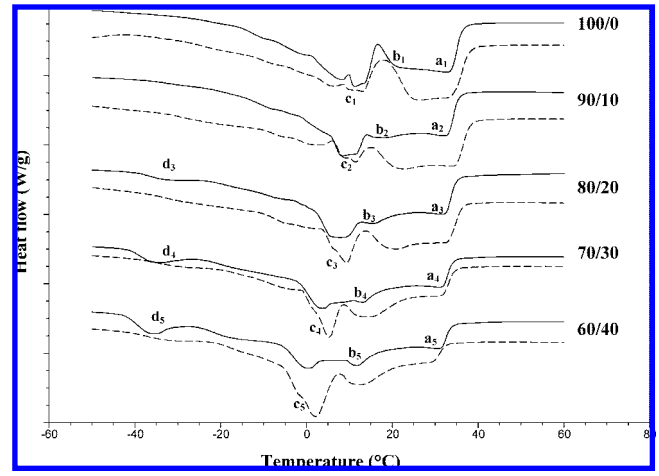

Figure 8. DSC melting curves for NIE blends (solid lines) and after 6 h of interesterification (dashed lines) for different AMF/LO blends (ratios indicated on the curves).

The randomization of high- and low-melting TAGs occurring during interesterification, forms new intermediate TAG species. The consequences on DSC profiles were a deepening of peaks c, the more pronounced as LO content was high, and the formation of shoulder peaks b (**Figure 8**). The new TAGs of IE products were shifted to lower melting temperatures when compared to the TAG species in NIE products.

Texturometry. The rheological properties of the end reaction products were evaluated by textural measurements of penetration force, and typical resulting curves are shown in **Figure 9**. The numerous irregularities of the curve corresponding to 100% AMF (**Figure 9A**) indicate that the sample is very brittle, that is, AMF is not easily spreadable (*35*). After interesterification, the sample was softened, but kept a brittle character (**Figure 9B**). On the contrary, regular curves like those obtained for the blend 70/30, for instance, indicate a very smooth product like a typical plastic shortening (*34*). For the blends 90/10 and 80/20, interesterification also yielded products with a decreased brittleness as can be evaluated by the more regular curves obtained; however, they were not totally regular and smooth.

The hardness of the samples can be estimated by the slope of the curve and by the maximum and final penetration forces. The enzymatic interesterification resulted in lower hardness products except for AMF, which was not significantly different before and after interesterification (**Figure 9**, inset). On average, the IE products were 30% softer than the NIE blends. The hardness of the blends decreased linearly with the tested LO contents.

Oxidative Stability. Oxidation of unsaturated fatty acid residues leads to rancidity, off-flavors, and musty odors. The products presented here are characterized by increased contents of unsaturated fatty acid residues; therefore, it may be problematic.

Table 3 shows the values of oxidation induction times (OIT), which give an indication of the oxidative stability of the fat. The times to reach the oxidation of the IE products were not significantly different from those of the initial blends. This can be explained in part by efficient N_2 flowing in the medium during the reaction. OIT diminished clearly with the LO content of the blend. LO alone oxidized in 2.7 h, due notably to its

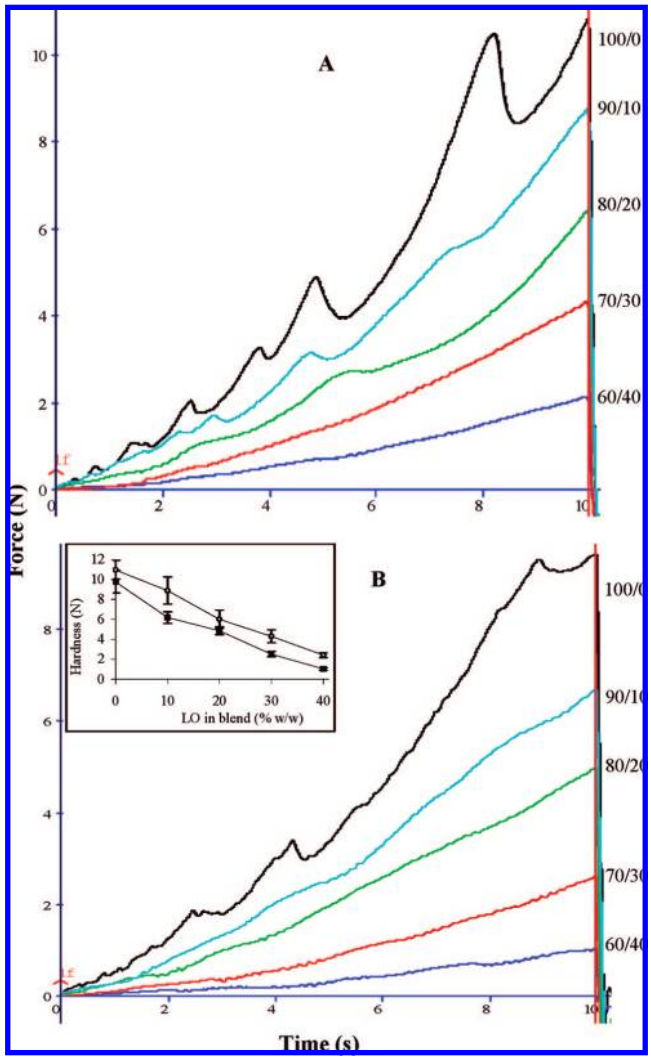

Figure 9. Typical curves obtained by penetration test on NIE blends (**A**) and on blends after 24 h of interesterification (**B**). The composition of the different AMF/LO blends is indicated on the right of the curves. (Inset) Hardness (maximum penetration force in N) as a function of LO in the blend for NIE blends (○) and after 6 h of interesterification (■).

Table 3. Oxidation Induction Times Determined by the Rancimat Method

	oxidation induction time (h)	
blend	NIE	24 h
100/0	28.6 ± 0.7	27.6 ± 0.6
90/10	17.6 ± 0.2	18.0 ± 0.1
80/20	9.7 ± 0.2	9.8 ± 0.5
70/30	9.4 ± 0.3	9.2 ± 0.0
60/40	7.0 ± 0.2	6.2 ± 0.2
linseed oil	2.7 ± 0.1	
rapeseed oil	17.7 ± 0.1	

high content in tri-$C_{18:3}$, which is highly unstable (*38*) and, thus, constitutes an instability factor within the oil.

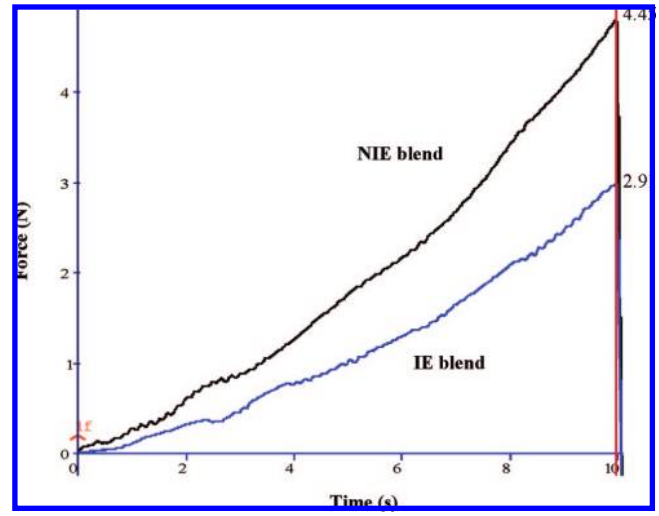

Figure 10. Penetration test curves for the NIE and IE ternary blend (AMF/RO/LO 70/20/10 w/w/w).

Both products that were demonstrated to have interesting plastic characteristics, that is, blends 70/30 and 60/40, presented low OIT values, 9.2 and 6.2 h, respectively, which may be incompatible with a marketable product.

Ternary Blend with Rapeseed Oil. RO is also a good source of unsaturated C_{18}, although with less $C_{18:3}$ than LO (**Table 1**), making it also oxidatively more stable than LO (**Table 3**). Therefore, as an alternative to the binary blends of AMF/LO, a ternary blend containing also RO was tested. **Table 4** gathers the physicochemical data determined for the interesterification of a blend composed of AMF/RO/LO with the ratio 70/20/10. This blend contained 12.6 times more $C_{18:3}$ than AMF alone, and its content in unsaturated C_{18} represented 45.8% of total fatty acid residues. The value of ID at quasi-equilibrium conditions was close to that obtained with the blend AMF/LO 60/40 (**Figure 4**). FFA content in IE samples was very low (0.7%). DP was detected at 29.5 °C for the 6 h IE product, which is very close to the value obtained for the 6 h IE AMF/LO 70/30 sample (**Figure 6A**,**B**). SFC at 10 °C was equal to 29.8% before and after 6 h of interesterification, which is in the plastic range. The effects of the reaction on SFC are visible at 15 and 30 °C (**Table 4**). SFC at 35 °C was equal to 0 in every instance (not shown).

Concerning the textural properties, interesterification gave a product with a 35% lower hardness. The regularity and smoothness of the curve indicate that the product presents the spreadable character of a good shortening (**Figure 10**).

OIT was equal to 21.8 h, which was much higher than the times obtained for the binary blends, still lower than OIT for AMF alone (**Table 3**). The resistance to oxidation was thus improved with respect to the binary blends.

A ternary blend of AMF, RO, and LO led to a structured fat enriched in unsatured C_{18} with rheological and textural properties of a plastic spreadable fat and with an improved oxidative

Table 4. Data Determined for the Ternary Blend MGLA/RO/LO 70/20/10: UEF, $C_{18:3}$ EF, and TUC_{18} (See **Table 2**), ID, FFA, DP, SFC, and Oxidation Induction Times (OIT)

							% SFC			
	UEF	$C_{18:3}$ EF	TUC_{18} (% w)	ID	FFA (%)	DP (°C)	10 °C	15 °C	30 °C	OIT (h)
NIE	2.4	12.6	45.8	0.0	0.2	31.6 ± 0.8	29.8	16.5	3.3	21.8 ± 0.3
IE 6 h				1.3	0.7	29.5 ± 0.1	29.8	15.6	2.5	
IE 24 h				1.2	0.6	30.4 ± 0.4	30.3	16.0	3.0	21.8 ± 0.3

stability. These data seem to be conclusive for a commercial fat with increased potential health benefits.

The next step would be to consider a sensory evaluation and possibly a deodorization of the IE products (*39, 40*).

ABBREVIATIONS USED

AMF, anhydrous milk fat; C_{18}, fatty acid with 18 carbons; DP, dropping point; DSC, differential scanning calorimetry; CN, carbon number; FFA, free fatty acids; GC, gas chromatography; ID, interesterification degree; IE, interesterified; LO, linseed oil; NIE, non-interesterified; OIT, oxidation induction time; PUFA, polyunsaturated fatty acids; RO, rapeseed oil; SFC, solid fat content; TAG, triacylglycerol; DAG, diacylglycerol.

ACKNOWLEDGMENT

We are very grateful to Novozymes for the donated samples of enzymes and also for help and advice, especially from David Cowan.

LITERATURE CITED

(1) Xu, X. Production of specific structured triacylglycerols by lipase catalyzed reaction: a review. *Eur. J. Lipid Sci. Technol.* **2000**, *102*, 287–303.

(2) Pal, P. K.; Bhattacharyya, D. K.; Ghosh, S. Modifications of butter stearin by blending and interesterification for better utilization in edible fat products. *JAOCS* **2001**, *78*, 31–36.

(3) Rønne, T. H.; Pedersen, L. S.; Xu, X. Triglyceride selectivity of immobilized *Thermomyces lanuginosa* lipase in interesterification. *JAOCS* **2005**, *82*, 737–743.

(4) Houmøller, L. P.; Kristensen, D.; Rosager, H. Determination of SFC, FFA, and equivalent reaction time for enzymatically interesterified oils using NIRS. *Talanta* **2007**, *71*, 868–873.

(5) Yang, T.; Fruekilde, M.-B.; Xu, X. Applications of immobilized *Thermomyces lanuginosa* lipase in interesterification. *JAOCS* **2003**, *80*, 881–887.

(6) Zhang, H.; Xu, X.; Nilsson, J.; Mu, H.; Adler-Nissen, J.; Høy, C.-E. Production of margarine fats by enzymatic interesterification with silica-granulated *Thermomyces lanuginosa* lipase in a large scale study. *JAOCS* **2001**, *78*, 57–64.

(7) Criado, M.; Hernández-Martín, E.; Otero, C. Optimized interesterification of virgin olive oil with a fully hydrogenated fat in a batch reactor: effect of mass transfer limitations. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 474–485.

(8) Osório, N. M.; da Fonseca, M. M. R.; Ferreira-Dias, S. Operational stability of *Thermomyces lanuginosa* lipase during interesterification of fat in continuous packed-bed reactors. *Eur. J. Lipid Sci. Technol.* **2006**, *108*, 545–553.

(9) Balcão, V. M.; Malcata, X. M. Lipase catalysed modification of milkfat. *Biotechnol. Adv.* **1998**, *16*, 309–341.

(10) Wijendran, V.; Hayes, K. C. Dietary n-6 and n-3 fatty acid balance and cardiovascular health. *Annu. Rev. Nutr.* **2004**, *24*, 597–615.

(11) MacLean, C. H.; Mojica, W. A.; Morton, S. C.; Pencharz, J.; Hasenfeld Garland, R.; Tu, W.; Newberry, S. J.; Jungvig, L. K.; Grossman, J.; Khanna, P.; Rhodes, S.; Shekelle, P. Effects of omega-3 fatty acids on lipids and glycemic control in type II diabetes and the metabolic syndrome and on inflammatory bowel disease, rheumatoid arthritis, renal disease, systemic lupus erythematosus, and osteoporosis; AHRQ Publication 04-E012-2; Agency for Healthcare Research and Quality: Rockville, MD, 2004; Vol. 89, 351 pp.

(12) Krist, S.; Stuebiger, G.; Bail, S.; Unterweger, H. Analysis of volatile compounds and triacylglycerol composition of fatty seed oil gained from flax and false flax. *Eur. J. Lipid Sci. Technol.* **2006**, *108*, 48–60.

(13) Heguy, J. M.; Juchem, S. O.; DePeters, E. J.; Rosenberg, M.; Santos, J. E. P.; Taylor, S. J. Whey protein gel composites of soybean and linseed oils as a dietary method to modify the unsaturated fatty acid composition of milk lipids. *Anim. Feed Sci. Technol.* **2006**, *131*, 370–388.

(14) Noakes, M.; Nestel, P. J.; Clifton, P. M. Modifying the fatty acid profile of dairy products through feedlot technology lowers plasma cholesterol of humans consuming the products. *Am. J. Clin. Nutr.* **1996**, *63*, 42–46.

(15) Rodrigues, J. N.; Gioielli, L. A. Chemical interesterification of milkfat and milkfat-corn oil blends. *Food Res. Int.* **2003**, *36*, 149–159.

(16) Rønne, T. H.; Yang, T.; Mu, H.; Jacobsen, C.; Xu, X. Enzymatic interesterification of butterfat with rapeseed oil in a continuous packed bed reactor. *J. Agric. Food Chem.* **2005**, *53*, 5617–5624.

(17) Marangoni, A. G.; Rousseau, D. Chemical and enzymatic modification of butterfat and butterfat−canola oil blends. *Food Res. Int.* **1998**, *31*, 595–599.

(18) Akoh, C. C.; Moussata, C. O. Characterization and oxidative stability of enzimatically produced fish and canola oil-based structured lipids. *JAOCS* **2001**, *78*, 25–30.

(19) Otero, C.; López-Hernandez, A.; García, H. S.; Hernández-Martín, E.; Hill, C. G. Continuous enzymatic transesterification of sesame oil and a fully hydrogenated fat: effects of reaction conditions on product characteristics. *Biotechnol. Bioeng.* **2006**, *94*, 877–887.

(20) Hamam, F.; Shahidi, F. Synthesis of structured lipids containing medium-chain and omega-3 fatty acids. *J. Agric. Food Chem.* **2006**, *54*, 4390–4396.

(21) Sehanputri, P. S.; Hill, C. G. Lipase-mediated acidolysis of butteroil with free conjugated linoleic acid in a packed bed reactor. *Biotechnol. Bioeng.* **2003**, *83*, 608–617.

(22) Kim, J. S.; Maeng, I. K.; Kim, C. K.; Kwon, Y. J.; Lee, B. O.; Kim, Y. J. Lipase catalysed acidolysis of butterfat with α-linolenic acid from perilla oil. *Food Sci. Biotechnol.* **2002**, *11*, 66–70.

(23) Garcia, H. S.; Keough, K. J.; Arcos, J. A.; Hill, C. G. Interesterification (acidolysis) of butterfat with conjugated linoleic acid in a batch reactor. *J. Dairy Sci.* **2000**, *83*, 371–377.

(24) Balcão, V. M.; Kemppinen, A.; Malcata, F. X.; Kalo, P. J. Lipase-catalyzed acidolysis of butterfat with oleic acid: characterization of process and product. *Enzyme Microb. Technol.* **1998**, *23*, 118–128.

(25) Sridhar, R.; Lakshminarayana, G.; Kaimal, T. N. B. Modification of selected edible vegetable oils to high oleic oils by lipase-catalyzed ester interchange. *J. Agric. Food Chem.* **1991**, *39*, 2069–2071.

(26) Long, K.; Zubir, I.; Hussin, A. B.; Idris, N.; Ghazali, H. M.; Lai, O. M. Effect of enzymatic transesterification with flaxseed oil on the high-melting glycerides of palm stearin and palm olein. *JAOCS* **2003**, *80*, 133–137.

(27) Jandacek, R. J.; Whiteside, J. A.; Holcombe, B. N.; Volpenhein, R. A.; Taulbee, J. D. The rapid hydrolysis and efficient absorption of triglycerides with octanoic acid in the 1 and 3 positions and long-chain fatty acid in the 2 position. *Am. J. Clin. Nutr.* **1987**, *45*, 940–945.

(28) French Ministry of Health. Avis du 14 octobre 1997 du Conseil supérieur d'hygiène publique de France (section de l'alimentation et de la nutrition) relatif à l'emploi d'huile de lin en alimentation humaine. Bulletin Officiel 98/14, 1997; http://www.sante.gouv.fr/adm/dagpb/bo/1998/98-14/a0140936.htm.

(29) Glade, M. J. The dietary supplement health and education act of 1994—focus on labeling issues. *Nutrition* **1997**, *13*, 999–1001.

(30) Zhang, H.; Pedersen, L. S.; Kristensen, D.; Adler-Nissen, J.; Holm, H. C. Modification of margarine fats by enzymatic interesterification: evaluation of a solid-fat-content-based exponential model with two groups of oil blends. *JAOCS* **2004**, *81*, 653–658.

(31) Precht, D.; Molkentin, J. The certification of the triglyceride contents of an anhydrous butter fat reference material with additional value for free cholesterol. European Comission, Community Bureau of Reference Materials; CRM 519; 1997; http://www.irmm.jrc.be/html/reference_materials_catalogue/catalogue/attachements/BCR-519_report.pdf.

(32) *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th ed.; AOCS Press: Champaign, IL, 1993; Method Ca 5a-40.

(33) International Union of Pure and Applied Chemistry. Solid content determination in fats by NMR. In *Standard Methods for the Analysis of Oil Fats and Derivatives*, 7th revised and enlarged ed.; Paquot, H., Hautenne, A., Eds.; Blackwell Scientific Publication: Oxford, U.K., 1987; IUPAC Norm version 2.150.

(34) Danthine, S.; Deroanne, C. Influence of SFC, microstructure and polymorphism on texture (hardness) of binary blends of fats involved in the preparation of industrial shortenings. *Food Res. Int.* **2004**, *37*, 941–948.

(35) Rousseau, D.; Marangoni, A. G. Tailoring the textural attributes of butter fat/canola oil blends via *Rhizopus arrhizus* lipase-catalyzed interesterification. 2. Modifications of physical properties. *J. Agric. Food Chem.* **1998**, *46*, 2375–2381.

(36) Zhang, H.; Xu, X.; Nilsson, J.; Mu, H.; Adler-Nissen, J.; Høy, C.-E. Lipozyme IM-catalyzed interesterification for the production of margarine fats in a 1 kg scale stirred tank reactor. *Eur. J. Lipid Sci. Technol.* **2000**, *102*, 411–418.

(37) Rao, R.; Sankar, K. U.; Sambaiah, K.; Lokesh, B. R. Differential scanning calorimetric studies on structured lipids from coconut oil triglycerides containing stearic acid. *Eur. Food Res. Technol.* **2001**, *212*, 334–343.

(38) Russin, T. A.; van de Voort, F. R.; Sedman, J. Novel method for rapid monitoring of lipid oxidation by FTIR spectroscopy using disposable IR. *JAOCS* **2003**, *80*, 635–641.

(39) Rousseau, D.; Marangoni, A. G. The effects of interesterification on physical and sensory attributes of butterfat-canola oil spreads. *Food Res. Int.* **1999**, *31*, 381–388.

(40) Rønne, T. H.; Yang, T; Mu, H.; Jacobsen, C.; Xu, X. Deodorization of lipase-interesterified butterfat and rapeseed oil blends in a pilot deodorizer. *Eur. J. Lipid Sci. Technol.* **2006**, *108*, 182–192.

Received for review July 25, 2007. Revised manuscript received December 21, 2007. Accepted December 26, 2007.

JF0722203