Quantitation of Acyl Migration During Lipase-Catalyzed Acidolysis, and of the Regioisomers of Structured Triacylglycerols Formed

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ABSTRACT: Various MLM-type (M, medium-chain fatty acids; L, long-chain fatty acids) structured triacylglycerols were produced in pilot- or small-scale packed-bed reactors by lipase-catalyzed acidolysis. The incorporation and acyl migration of octanoic acid were measured by gas chromatography and Grignard degradation, and ranged from 39.0 to 48.7% and 0.6 to 9.3%, respectively. Quantitation of triacylglycerol molecular species was performed by ammonia negative ion chemical ionization (NICI) mass spectrometry (MS). The proportion of ACN (acyl carbon number) 34 species that contained one C_{18} fatty acid and two $C_{8:0}$, in samples analyzed, varied from 12.5 to 23.2%. The selected regioisomers MLM and MML within the ACN 34 species group were quantified by NICI tandem MS (MS/MS) and were in the range of 97.1 to 98.4% and 1.6 to 2.9%, respectively. There was no correlation between the level of acyl migration during lipase-catalyzed esterification and the level of regioisomers of the selected MLM-type triacylglycerols in the structured lipid samples.

Paper no. J9880 in *JAOCS 78,* 959–964 (September 2001).

KEY WORDS: Acyl migration, lipase-catalyzed acidolysis, regioisomer, quantitation, structured triacylglycerol, tandem mass spectrometry.

Lipase-catalyzed esterification has been used in fat modification to improve absorption properties and nutritional values of lipids (1–4). Acidolysis is one of the most commonly used methods for production of MLM-type (M, medium-chain fatty acid; L, long-chain fatty acid) structured triacylglycerols (STAG) using regiospecific lipase to incorporate the medium-chain fatty acids into the primary positions of triacylglycerols (5,6). The acidolysis proceeds in two steps, i.e., the original triacylglycerols are hydrolyzed into diacylglycerols and eventually monoacylglycerols, followed by the esterification of new fatty acids into the triacylglycerols. Acyl migration occurs between these two steps (7,8). Because acyl migration results in the formation of by-products, the level of acyl migration is normally monitored in order to control product quality.

The most commonly used method to monitor acyl migration is to study the fatty acid profile in the *sn-2* position of the triacylglycerols. Pancreatic lipase-catalyzed degradation of triacylglycerols, as a standard method, has been used to examine fatty acid distribution in lipids containing normal fatty acids (9). As discussed in the standard method, it cannot be applied unreservedly to oils and fats containing substantial amounts of fatty acids with 12 or fewer carbon atoms, or to highly unsaturated fatty acids containing 20 or more carbon atoms, and fatty acids containing oxygenated groups. Milk fat, for example, will not give representative acylglycerols, as short- or medium-chain fatty acids are more readily hydrolyzed by the pancreatic lipase than are long-chain fatty acids (10). Grignard degradation has selectivity for neither fatty acids nor the positions of the acyl groups in the triacylglycerols (11). Therefore, it is the preferred method to study the products of acyl migration in STAG containing medium-chain fatty acids.

Ammonia negative ion tandem mass spectrometry (MS/MS) has been applied to characterize and quantify triacylglycerols and the positional distribution of their acyl groups (12,13). The advantage of MS/MS is the possibility of analyzing individual molecular species within a triacylglycerol mixture (12). Detailed information on molecular species can be obtained by collision-induced dissociation of selected $[M - H]^-$ ions. The daughter ion spectrum provides information on the fatty acid constituents and their distribution between *sn*-2 and *sn*-1/3 positions in triacylglycerols (12,14).

In the present study, several structured lipid samples were produced by lipase-catalyzed acidolysis in packed-bed reactors. The incorporation and acyl migration of octanoic acid were determined by conventional gas chromatography (GC) and Grignard degradation. The triacylglycerol species of the structured lipid samples were quantified by ammonia negative ion chemical ionization (NICI) MS. Selected MLM- and MML-regioisomers were quantified by the NICI MS/MS.

EXPERIMENTAL PROCEDURES

Reagents and solvents. Safflower oil and rapeseed oil were purchased from Róco (Copenhagen, Denmark); sunflower oil, linseed oil, and high-oleic sunflower oil were from FDB (Lyngby, Denmark), Nomeco A/S (Copenhagen, Denmark), and Aarhus Olie A/S (Aarhus, Denmark), respectively. Octanoic acid, linoleic acid, and trioctanoylglycerol were purchased from Sigma Inc. (St. Louis, MO). Novo Nordisk A/S (Bagsvaerd, Denmark) donated the Lipozyme IM, a commercial immobilized lipase from *Rhizomucor miehei*.

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TABLE 1
Interesterification Parameters for the Production of Structured Lipids[a]

Sample	Oil	Reaction	t (min)	Reactor[b]
A	Safflower	Acidolysis	161	Packed-bed ϕ50 mm × 100 cm
B	Rapeseed	Acidolysis	165[c]	Packed-bed ϕ50 mm × 100 cm
C	HO sunflower	Acidolysis	153	Packed-bed ϕ26 mm × 40 cm
D	Linseed	Acidolysis	172	Packed-bed ϕ26 mm × 40 cm
E	Sunflower	Acidolysis	151	Packed-bed ϕ26 mm × 40 cm
F	Rapeseed	Acidolysis	178[c]	Packed-bed ϕ50 mm × 100 cm

[a]Conditions for the production in packed-bed reactors: substrate mol ratio (oil/acyl donor) 1:6, temperature 60°C, water content in substrate 0.08%, enzyme Lipozyme IM (Novo Nordisk A/S, Bagsvaerd, Denmark). HO sunflower oil is high-oleic sunflower oil.
[b]ϕ represents diameter of the packed bed (i.d. × length).
[c]Total residence time from the two steps.

All reagents and solvents were of analytical or chromatographic grade. Acetonitrile, hexane, and isopropanol were from BDH Laboratory Supplies (Poole, England).

Production of structured lipids. Structured lipid samples A to F were produced in packed-bed reactors (Table 1). A jacketed glass column (Pharmacia Biotech, Hørsholm, Denmark) was used to form the bed for the continuous production. Column temperature was maintained by a water bath (Circulator DC30; Haake GmbH, Karlsruhe, Germany). Before being pumped through the enzyme bed, the substrates were preheated to a set temperature in a feed container by circulating heated water from the water bath. Homogeneous distribution of water in the substrate mixture was achieved by stirring at the set temperature. The product container was cooled to 10–15°C by a jacketing system. Both the substrate and product containers were nitrogen protected. With each new production batch at specific set parameters, preheated and conditioned substrates were pumped into the enzyme bed. The first 1.5–2.0 L from the enzyme bed were discarded. For samples B and F, the reaction was conducted in two steps. The mixture produced from the first step was passed through the packed bed again at the same flow rate as the first step.

The major fatty acid composition of the oils used for the production of structured lipids is listed in Table 2.

Purification by short-path distillation. The product mixture of Sample F from the packed-bed reactor was purified with a pilot short-path distillation system (KD-6, UIC GmbH, Alzenau-Hoerstein, Germany). The purifications were conducted in two steps. The first step was set to remove the medium-chain fatty acids and the second step to remove the long-chain fatty acids. Conditions for the first step were as follows: heat exchanger temperature 60°C, evaporator temperature 85°C, condenser temperature 25°C, and vacuum less than 0.001 mbar. Conditions for the second step were as follows: heat exchanger temperature 80°C, evaporator temperature 175°C, condenser temperature 35°C, and vacuum less than 0.001 mbar. The operation was conducted under N_2 and light protection.

Preparation of triacylglycerol standards. The MLM- and MLL-type triacylglycerol standards were prepared by purification of the acidolysis products of safflower oil and octanoic acid. The acidolysis was performed in a small-scale packed-bed reactor containing Lipozyme IM under conditions that have been described previously (5). The LML- and MML-type triacylglycerol standards were prepared by purification of the acidolysis products of trioctanoylglycerol and linoleic acid. The parameters used for their acidolysis were the same as those used for sample D (Table 1).

The required triacylglycerol standards were isolated from the acidolysis products by preparative high-performance liquid chromatography (HPLC). A Waters Delta Prep 3000 HPLC (Millipore Corporation, Milford, MA) was equipped with a Delta-Pak C_{18} column (47 × 300 mm, particle size 15 µm, and pore size 100 Å; Waters Corporation, Milford, MA). Lambda-Max model 481 LC spectrophotometer (Waters Corporation) was used as detector at 210 nm. The column was

TABLE 2
The Major Fatty Acid Composition (mol%) of the Oils Used for the Production of the Structured Lipids[a]

	Sunflower		Safflower		Rapeseed		HO sunflower		Linseed	
Fatty acid	TAG	*sn*-2	TAG	*sn*-2	TAG	*sn*-2	TAG	*sn*-2	TAG	*sn*-2
C16:0	6.6	0.3	7.1	0.3	6.1	0.4	4.5	0.3	5.6	0.7
C18:0	5.1	0.2	2.4	0.1	1.8	0.0	4.7	0.2	3.0	0.3
C18:1n-9	21.9	20.9	11.2	10.9	53.1	44.3	77.0	89.9	19.8	23.1
C18:1n-7	0.7	0.3	0.6	0.1	3.3	0.9	0.9	0.0	0.8	0.3
C18:2n-6	61.0	71.3	75.5	85.6	20.9	34.5	6.4	6.8	15.3	21.1
C18:3n-3	0.6	0.8	0.4	0.5	9.8	16.5	0.0	0.0	53.2	52.4
Others	4.1	6.2	2.8	2.5	5.0	3.4	6.1	2.8	2.3	2.1

[a]TAG, triacylglycerol; *sn*-2, 2-monoacylglycerol. See Table 1 for other abbreviation.

TABLE 3
The Main Fatty Acid Composition (mol%) of Structured Triacylglycerol Standards[a]

	8:0/18:2/8:0		8:0/18:2/18:2		8:0/8:0/18:2		18:2/8:0/18:2	
Fatty acid	TAG	*sn*-2	TAG	*sn*-2	TAG	*sn*-2	TAG	*sn*-2
C8:0	67.2	0.0	35.7	0.0	62.7	95.0	30.8	93.7
C16:0	0.1	0.0	0.2	0.0	0.0	0.0	0.2	0.0
C18:0	0.0	0.0	0.3	0.0	0.0	0.0	0.2	0.0
C18:1n-9	0.1	0.1	0.3	0.8	0.3	0.2	4.3	0.6
C18:2n-6	32.4	99.3	63.3	99.2	36.2	3.5	58.0	4.6
C18:3n-3	0.0	0.0	0.0	0.0	0.2	0.0	3.3	0.0
Others	0.2	0.6	0.2	0.0	0.5	1.3	2.8	1.2

[a]See Table 2 for abbreviations.

maintained at ambient temperature with a flow rate of 60 mL/min (4). A binary solvent system was applied; solvent A was acetonitrile and solvent B was isopropanol/hexane (2:1, vol/vol). The gradient of solvent was changed according to the composition of acidolysis products. Two milliliters of the product solution with a concentration of 0.5 g/mL was injected, and the required STAG were collected.

The composition of the purified triacylglycerol standards was determined by GC after KOH-catalyzed methylation (15). In short, lipids were dissolved in 1 mL hexane and mixed with 60 μL KOH solution (2 M KOH in methanol). After mixing with anhydrous sodium sulfate and centrifuging 15 min at 4000 rpm, the upper phase was transferred to vials for GC analysis. The fatty acids in *sn*-2 position of the STAG were determined by Grignard degradation and GC analysis (Table 3).

GC. The triacylglycerols in the acidolysis products were converted into fatty acid methyl esters with 2 M KOH (in methanol), and these were analyzed with an HP 6890 series gas chromatograph (Hewlett-Packard, Waldbronn, Germany) equipped with a fused-silica capillary column (SP-2380, 60 m × 0.25 mm i.d., film thickness 0.20 μm; Supelco Inc., Bellefonte, PA). Oven temperature was programmed from 70 to 160°C at a rate of 15°C/min, further to 180°C at a rate of 1°C/min, further to 185°C at a rate of 0.5°C/min, and finally to 200°C at a rate of 20°C/min and held for 10 min (4). A flame-ionization detector was used at 280°C, and the injector temperature was 250°C. The injector was used in split mode with a ratio 1:20. Carrier gas was helium with a column flow of 2 mL/min. The fatty acid methyl esters were identified by comparing their retention times with authentic standards (Sigma Chemical Co., St. Louis, MO), and the resulting compositions were calculated using the actual response factors for each fatty acid.

Grignard degradation. About 30 mg of the structured lipid samples or 10 mg of the purified triacylglycerol standards were dissolved in 10 mL diethyl ether. The reaction started after adding 0.3 mL allylmagnesium bromide (1 M in diethyl ether) and lasted for 1 min, and thereafter was stopped by adding acid buffer (0.27 M HCl in 0.4 M boric acid). The organic phase was washed twice with 0.4 M boric acid and dried with anhydrous sodium sulfate and evaporated under nitrogen. The lipid residues were separated on a thin-layer chromatography (TLC) plate precoated with boric acid, and the *sn*-2 monoacylglycerol fraction was scraped off and extracted with diethyl ether. After methylation with 2 M KOH in methanol, the fatty acid methyl esters were analyzed by GC.

The oils used in the acidolysis were also analyzed by GC after Grignard degradation; these results are listed in Table 2.

Mass spectrometry. All the mass spectrometric analyses were performed on a Finnigan MAT TSQ-700 triple quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) using NICI with ammonia (99.998%, Prax Air, Oevel, Belgium). An aliquot of 0.5 μL of structured lipid sample (0.5 mg/mL in hexane) was applied to the rhenium wire of a direct exposure probe (Finnigan MAT). The probe was introduced into the ion source after the solvent had been evaporated, and the heating of the probe wire with the current was started to vaporize the sample. The heating rate of the rhenium wire (40 mA/s), the pressure of ammonia (8500 mtorr), the ion source temperature (200°C), the electron energy (70 eV), and the filament current (400 μA) were selected based on the method optimization reported previously (13). Quadrupole 1 of the instrument was used to select parent ions, whereas quadrupole 3 was used to monitor the fragment ions formed in quadrupole 2. To produce appropriate fragmentation in MS/MS analysis, the pressure of argon (99.998%, AGA, Lidingö, Sweden) was set to 1.4 mtorr and the collision energy was set to 15 eV. Quadrupole 1 was scanned from *m/z* 450 to 800 for the determination of molecular weight distribution, and quadrupole 3 was scanned from *m/z* 100 to 800 for the determination of regioisomeric structures. Each sample was analyzed four times, and an average and SD of results were presented.

The regioisomers of acyl carbon number (ACN) 34 species (MML + MLM) were quantitatively determined by the ratio of $[M - H - 18:2 - 100]^-$ and $[M - H - 8:0 - 100]^-$fragment ion relative intensities in the ammonia NICI MS/MS spectra. By analyzing standard STAG, a linear relation between the proportion of MML in the mixture and the ratio was obtained, enabling the quantification of regioisomers in an unknown mixture. The fragmentation of regioisomers of ACN 44 species (LLM + LML) was less regiospecific, and the proportions could only be estimated when the proportion of MLL in the mixture exceeded 50% (Kurvinen, J.P., H. Mu, H. Kallio, X. Xu, and C.-E. Høy, unpublished results).

RESULTS AND DISCUSSION

Quantification of incorporation and acyl migration during the acidolysis. Incorporation is the most important parameter for evaluating the product quality after acidolysis of conventional long-chain triacylglycerols with medium-chain fatty acids; it is defined as the molar percentage of the medium-chain fatty acids in the STAG. The product mixture contains both triacylglycerols and free fatty acids. Therefore, alkaline-catalyzed methylation procedures are preferred to prepare fatty acid methyl esters from triacylglycerols for analysis, because with these methods free fatty acids cannot be methylated (16). In the present study, we employed an alkaline-catalyzed methylation procedure because most of the structured lipid samples contained free fatty acids (approximately 40 wt%), except for sample F in which free fatty acids were removed by short-path distillation. Incorporation of octanoic acid in the examined acidolysis products was between 39.0 and 48.7% (Table 4).

Another important parameter for evaluating the product quality is the level of acyl migration, which is defined as the molar percentage of the medium-chain fatty acids in the *sn*-2 position of triacylglycerols after acidolysis. Acyl migration is one of the major problems for acidolysis in batch reactors even when highly *sn*-1/3-specific lipases are used. The high ratio between the substrate and enzyme demands long reaction time in order to reach equilibrium, and this consequently results in high degrees of acyl migration (8,17,18). Because acyl migration results in decreased purity of the specific STAG, it should be kept as low as possible during fat modification. Preferred methods for production of structured lipids are based on packed-bed reactors because they reduce acyl migration significantly (5,18). In the present study, all structured lipid samples were synthesized in packed-bed reactors. The levels of acyl migration in the structured lipid samples are listed in Table 4. The structured lipid samples produced in the small packed-bed reactors (C, D, E) had very low acyl migration (0.6 to 2.1%), whereas two of the samples produced in the pilot-scale packed-bed reactor had much higher acyl migration (8–9%). The latter were specially selected for this study to give a range of acyl migrations with similar degrees of incorporation. Both of these samples were prepared by means of a two-step reaction system. This might be the main cause of the higher degrees of acyl migration.

These structured lipid samples represented different levels of acyl migration. The same samples were studied by ammonia NICI MS/MS for quantitation of selected regioisomers of MLM- and MML-type STAG.

Quantitation of triacylglycerol species by ammonia NICI MS. Triacylglycerol composition changes during esterification, and the molecular species can be separated by reversed-phase high-performance liquid chromatography (HPLC) (19). In our previous studies we identified new triacylglycerol species in acidolysis products with atmospheric pressure chemical ionization mass spectrometry (20). In the present study the molecular weight distribution of STAG samples was determined by NICI MS with ammonia as the reagent gas, as reported earlier for natural triacylglycerol mixtures (12, 21–24). The response correction factor of ACN 34 species was calculated to be 0.14 when compared with ACN 44 species, as determined using the standard triacylglycerols listed in Table 3. The relative molar proportions of triacylglycerol ACN/DB molecular species (DB, double bond) are listed in Table 5. Results are reported as the average and standard deviations of four replicate analyses.

A wide range of triacylglycerols was detected in the structured lipid samples after acidolysis. The level of MLL-type STAG was higher than that of MLM-type for all of the structured lipid samples, suggesting that the acidolysis parameters used for the process could be further optimized in order to obtain higher levels of di-incorporated triacylglycerols, i.e., MLM.

Quantitation of regioisomers of the STAG by ammonia NICI MS/MS. NICI MS/MS has been used for the regioisomeric analysis of triacylglycerols based on different fragmentations of fatty acids from *sn*-1/3 and *sn*-2 positions, and it has been proven to be an applicable method for determination of the regioisomeric structure of triacylglycerols in various fats and oils (12,14,21–23). With some modifications, the

TABLE 4
The Main Fatty Acid Composition (mol%) of the Triacylglycerols in the Acidolysis Products[a]

	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F	
Fatty acid	TAG	*sn*-2	TAG	*sn*-2	TAG	*sn*-2	TAG	*sn*-2	TAG	*sn*-2	TAG	*sn*-2
C8:0	48.7	1.8	40.2	8.3	42.1	2.1	44.0	1.5	39.0	0.6	47.6	9.3
C10:0	0.2	0.2	1.8	1.5	—	—	—	—	—	—	0.1	—
C12:0	—	0.2	—	—	—	—	—	—	—	—	—	—
C14:0	0.1	0.7	—	—	—	—	—	—	—	—	0.1	—
C16:0	2.4	0.8	2.8	1.2	1.4	0.4	1.9	0.8	2.6	0.4	1.9	2.0
C18:0	0.7	0.3	1.1	0.6	1.2	0.3	1.3	0.5	1.7	0.2	0.6	0.6
C18:1n-9	6.9	12.6	6.7	8.6	50.5	90.2	9.5	18.5	12.9	19.7	27.0	44.4
C18:1n-7	0.1	0.1	0.3	0.2	—	—	0.3	0.3	0.6	0.2	1.3	0.8
C18:2n-6	40.0	78.4	47.0	61.5	3.8	6.4	9.1	19.1	41.9	76.6	14.2	30.1
C18:3n-3	0.3	0.5	—	—	0.3	0.5	33.7	58.8	0.9	1.7	6.6	12.9
Others	0.6	4.4	—	18.0	0.1	0.1	—	—	—	—	0.7	—

[a]Only triacylglycerols in the acidolysis products were methylated to fatty acid methyl esters. The fatty acid profile was analyzed by gas chromatography. —, fatty acids with concentrations below 0.1% or not detected. See Table 2 for abbreviations.

TABLE 5
Triacylglycerol Composition (mol%) of Structured Lipid Samples Determined by Ammonia Negative Ion Chemical Ionization Mass Spectrometry[a]

ACN/DB	*m/z*	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
24:0	469.4	—	0.1 ± 0.2	—	0.1 ± 0.3	—	—
32:0	581.6	—	—	—	0.2 ± 0.2	—	—
34:3	603.5	0.2 ± 0.2	5.0 ± 0.0	0.2 ± 0.2	19.5 ± 0.7	0.7 ± 0.1	4.4 ± 0.3
34:2	605.5	16.8 ± 2.1	9.7 ± 0.0	1.6 ± 0.1	5.9 ± 0.2	23.2 ± 0.9	8.6 ± 0.5
34:1	607.5	3.2 ± 0.5	14.3 ± 0.4	16.7 ± 1.3	6.2 ± 1.2	5.8 ± 0.1	12.5 ± 1.2
34:0	609.5	0.1 ± 0.1	0.2 ± 0.1	0.6 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
42:3	715.5	—	—	—	4.4 ± 0.6	—	0.5 ± 1.1
42:2	717.6	10.8 ± 3.6	3.0 ± 0.1	—	—	7.1 ± 0.4	2.3 ± 1.5
42:1	719.7	0.2 ± 0.4	4.0 ± 0.3	5.1 ± 0.5	—	0.7 ± 1.4	4.3 ± 0.4
44:6	737.6	—	—	—	21.6 ± 2.6	—	—
44:5	739.6	—	3.3 ± 0.6	—	14.6 ± 3.0	—	3.3 ± 0.5
44:4	741.6	48.6 ± 6.9	13.3 ± 0.7	—	18.0 ± 4.3	30.3 ± 1.8	13.7 ± 1.2
44:3	743.6	17.3 ± 3.3	22.5 ± 2.3	8.9 ± 1.4	5.7 ± 0.9	21.6 ± 1.2	23.0 ± 7.2
44:2	745.7	2.9 ± 3.4	23.3 ± 2.1	60.9 ± 4.3	3.7 ± 1.8	10.5 ± 5.2	26.2 ± 7.5
44:1	747.7	—	1.4 ± 0.6	3.8 ± 2.7	—	—	1.0 ± 0.7
54:3	883.7	—	—	2.2 ± 1.5	—	—	—

[a]ACN, acyl carbon number; DB, double bond; —, not detected. Results are presented as average ± standard deviation (n = 4).

method was found to be applicable also for the analysis of STAG containing medium-chain fatty acids.

Two triacylglycerol ACN/DB species from each sample were selected to analyze their regioisomeric structure (Table 6). It was possible to determine quantitatively the regioisomers of ACN 34 species (MML + MLM), whereas the proportions of regioisomers of ACN 44 species (LLM + LML) could only be approximated. The proportion of ACN 34 species varied from 12.5 to 23.2 mol% (Table 7). The proportions of regioisomers MLM and MML within the ACN 34 species were in the range of 97.1–98.4 mol% and 1.6–2.9 mol%, respectively. From these percentages, the proportions of MLM- and MML-type isomers of total triacylglycerols were calculated; these results are listed in Table 7.

For mono-incorporated STAG (LLM + LML; ACN 44 species), it was not possible to determine quantitatively the proportions of the regioisomers, but they were estimated based on the relation between the ratio of $[M - H - 8:0 - 100]^-$ and $[M - H - 18:2 - 100]^-$ and the proportion of MLL (Kurvinen, J.P., H. Mu, H. Kallio, X. Xu, and C.-E. Høy, unpublished results), that in all studied samples more than 90% of the ACN 44 triacylglycerols were of the LLM type and less than 10% were of the type LML (Table 7).

TABLE 6
The Regioisomers from Different Structured Lipid Samples Quantified by Tandem Mass Spectrometry

Sample	Selected regioisomers	
A	8:0/18:2/8:0, 8:0/8:0/18:2;	8:0/18:2/18:2, 18:2/8:0/18:2
B	8:0/18:1/8:0, 8:0/8:0/18:1;	8:0/18:1/18:1, 18:1/8:0/18:1
C	8:0/18:1/8:0, 8:0/8:0/18:1;	8:0/18:1/18:1, 18:1/8:0/18:1
D	8:0/18:3/8:0, 8:0/8:0/18:3;	8:0/18:3/18:3, 18:3/8:0/18:3
E	8:0/18:2/8:0, 8:0/8:0/18:2;	8:0/18:2/18:2, 18:2/8:0/18:2
F	8:0/18:1/8:0, 8:0/8:0/18:1;	8:0/18:1/18:1, 18:1/8:0/18:1

Correlation between acyl migration and the level of regioisomers of the selected STAG. The primary objective of fat modification by lipase-catalyzed acidolysis was to produce MLM-type STAG by using lipase with 1/3-specificity. The degrees of acyl migration and incorporation of medium-chain fatty acids are usually determined to describe the product quality. In the present study we wanted to investigate if there was any correlation between the level of acyl migration of medium-chain fatty acids and the level of the regioisomers of MLM- and MML-type STAG.

Table 7 summarizes the incorporation and acyl migration of medium-chain fatty acids in the structured lipid samples, and the level of MLM- and MLL-type triacylglycerols and regioisomers of MLM- and MML-type triacylglycerols. We found no correlation between the level of acyl migration and the production of regioisomers. The level of the MML-type regioisomers was quite low for all the structured lipid samples. One of the possible reasons for the low level of these regioisomers could be the wide distribution of medium-chain fatty acids in different triacylglycerol species. The STAG contain several different triacylglycerol species, so the total acyl migration of octanoic acid will hardly be represented by the selected regioisomers.

ACKNOWLEDGMENTS

We thank Jannie F. Agersted and Bert Nielsen for their technical assistance and for the financial support from the Center for Advanced Food Studies (LMC) and the Danish Technological Research Council (STVF).

REFERENCES

1. Haumann, B.F., Structured Lipids Allow Fat Tailoring, *inform 8*:1004–1011 (1997).
2. Ikeda, I., Y. Tomari, M. Sugano, S. Watanabe, and J. Nagata, Lymphatic Absorption of Structured Glycerolipids Containing

TABLE 7
The Level (%) of Incorporation and Acyl Migration of Octanoic Acid, and the Resulting Regioisomers[a]

Sample	Incorporation	Migration	MLM + MML	MLM	MML	LML + MLL[b]
A	48.7	1.8	16.8	16.5	0.34	48.6
B	40.2	8.3	14.3	14.1	0.24	23.3
C	42.1	2.1	16.7	16.2	0.48	60.9
D	44.0	1.5	19.5	19.2	0.45	21.6
E	39.0	0.6	23.2	22.8	0.37	30.3
F	47.6	9.3	12.5	12.2	0.26	26.2

[a]M, medium-chain fatty acid; L, long-chain fatty acid.
[b]>90% of acyl carbon number (ACN) 44 molecular species are approximated to be of MLL-type isomers.

Medium-Chain Fatty Acids and Linoleic Acid, and Their Effect on Cholesterol Absorption in Rats, *Lipids 26*:369–373 (1991).
3. Shimada, Y., A. Sugihara, H. Nakano, T. Yokota, T. Nagao, S. Komemushi, Y. Tominaga, Production of Structured Lipids Containing Essential Fatty Acids by Immobilized *Rhizopus delemar* Lipase, *J. Am. Oil Chem. Soc. 73*:1415–1420 (1996).
4. Mu, H., and C.-E. Høy, Effect of Medium-Chain Fatty Acids on Lymphatic Absorption of Essential Fatty Acids in Specific Structured Lipids, *Lipids 35*:83–89 (2000).
5. Mu, H., X. Xu, and C.-E. Høy, Production of Specific Structured Triacylglycerols by Lipase-Catalyzed Interesterification in a Laboratory Scale Continuous Reactor, *J. Am. Oil Chem. Soc. 75*:1187–1193 (1998).
6. Xu, X., S. Balchen, C.-E. Høy, and J. Adler-Nissen, Production of Specific-Structured Lipids by Enzymatic Interesterification in a Pilot Continuous Enzyme Bed Reactor, *Ibid. 75*:1573–1579 (1998).
7. Mu, H., X. Xu, J. Adler-Nissen, and C.-E. Høy, Production of Structured Lipids by Lipase-Catalyzed Interesterification in a Packed-Bed Reactor: Effect of Reaction Parameters on the Level of Diacylglycerols in the Products, *Fett/Lipid 101*: 158–164 (1999).
8. Xu, X., H. Mu, A.R.H. Skands, C.-E. Høy, and J. Adler-Nissen, Parameters Affecting Diacylglycerol Formation During the Production of Specific-Structured Lipids by Lipase-Catalyzed Interesterification, *J. Am. Oil Chem. Soc. 76*:175–181 (1999).
9. *Official Methods of the American Oil Chemists' Society*, 4th edn., AOCS Press, Champaign 1990, Method Ch 3-91.
10. Entressangles, B., L. Pasero, P. Savary, and P. Desnuelle, Influence de la Nature des Chaines sur la Vitesse de leur Hydrolyse par la Lipase Pancreatique, *Bull. Soc. Chim. Biol. 43*:581–585 (1961).
11. Becker, C.C., A. Rosenquist, and G. Hølmer, Regiospecific Analysis of Triacylglycerols Using Allyl Magnesium Bromide, *Lipids 28*:147–149 (1993).
12. Kallio, H., and G. Currie, Analysis of Natural Fats and Oils by Ammonia Negative Ion Tandem Mass Spectrometry—Triacylglycerols and Positional Distribution of Their Acyl Groups, in *CRC Handbook of Chromatography, Analysis of Lipids,* edited by K.D. Mukherjee and N. Weber, CRC Press, Boca Raton, 1993, pp. 435–458.
13. Laakso, P., and H. Kallio, Optimization of the Mass Spectrometric Analysis of Triacylglycerols Using Negative-Ion Chemical Ionization with Ammonia, *Lipids 31*:33–42 (1996).
14. Kallio, H., and P. Rua, Distribution of the Major Fatty Acids of Human Milk Between *sn*-2 and *sn*-1,3 Positions of Triacylglycerols, *J. Am. Oil Chem. Soc. 71*:985–992 (1994).
15. Christopherson, S.W., and R.L. Glass, Preparation of Milk Fat Methyl Esters by Alcoholysis in an Essentially Nonalcoholic Solution, *J. Dairy Sci. 52*:1289–1290 (1969).
16. Christie, W.W., The Preparation of Derivatives of Fatty Acids, in *Gas Chromatography and Lipids,* edited by W.W. Christie, The Oily Press, Ayr, 1989, pp. 64–84.
17. Xu, X., A.R.H. Skands, C.-E. Høy, H. Mu, S. Balchen, and J. Adler-Nissen, Production of Specific-Structured Lipids by Enzymatic Interesterification: Elucidation of Acyl Migration by Response Surface Design, *J. Am. Oil Chem. Soc. 75*:1179–1186 (1998).
18. Xu, X., Production of Specific-Structured Triacylglycerols by Lipase-Catalyzed Reactions: A Review, *Eur. J. Lipid Sci. Technol. 102*:287–303 (2000).
19. Mu, H., P. Kalo, X. Xu, and C.-E. Høy, Chromatographic Methods in the Monitoring of Lipase-Catalyzed Interesterification, *Ibid. 102*:202–211 (2000).
20. Mu, H., H. Sillen, and C.-E. Høy, Identification of Diacylglycerols and Triacylglycerols in Structured Lipid Samples by Atmospheric Pressure Chemical Ionization Liquid Chromatography/Mass Spectrometry, *J. Am. Oil Chem. Soc. 77*:1049–1059 (2000).
21. Currie, G.J., and H. Kallio, Triacylglycerols of Human Milk: Rapid Analysis by Ammonia Negative Ion Tandem Mass Spectrometry, *Lipids 28*:217–222 (1993).
22. Kallio, H., and G. Currie, Analysis of Low Erucic Acid Turnip Rapeseed Oil *(Brassica campestris)* by Negative Ion Chemical Ionization Tandem Mass Spectrometry. A Method Giving Information on the Fatty Acid Composition in Positions *sn*-2 and *sn*-1/3 of Triacylglycerols, *Ibid. 28*:207–215 (1993).
23. Johansson, A., P. Laakso, and H. Kallio, Molecular Weight Distribution of the Triacylglycerol of Berry Seed Oils Analysed by Negative Ion Chemical Ionization Mass Spectrometry, *Z. Lebensm. Unters. Forsch. 204*:308–315 (1997).
24. Kallio, H., J.-P. Kurvinen, O. Sjövall, and A. Johansson, Tandem Mass Spectrometric Analysis of Regio-Isomerism of Triacylglycerols in Human Milk and Infant Formulae, in *Functional Foods for Disease Prevention,* edited by T. Shibamato, J. Terao, and T. Osawa, American Chemical Society, Washington, DC, 1998, pp. 221–230.

[Received January 29, 2001; accepted July 11, 2001]