Mass Spectrometric Identification of Triacylglycerols of Enzymatically Modified Butterfat Separated on a Polarizable Phenylmethylsilicone Column

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Original butter oil, butter oil interesterified with *Pseudomonas fluorescens* lipase as catalyst, and the saturated, monoene, diene and triene fractions of interesterified butter oil from silver ion chromatography on a *p*-propylbenzene sulfonic acid column were analyzed on a polarizable phenyl(65%)methylsilicone column. Major and also some minor molecular species of triacylglycerols (TG) were identified from the electron-impact (EI) gas chromatography/mass spectrometry analytical data for the original and lipase-modified butter oil and the four fractions. Acyl + 74 and acyl + 128 fragments of EI mass spectral data proved useful, in addition to acyl and M − RCOO fragments, in the identification of molecular species of TG. The TG compositions of the modified butter oils were compared with the composition calculated according to random distribution and with the TG composition of original butter oil.

KEY WORDS: Acyl, acyl + 74, acyl + 128 and (M − RCOO) fragment ions, interesterified butter oil, polarizable phenylmethylsilicone columns, triacylglycerols.

Our recent studies on interesterification reactions of butter oil (BO) and BO/rapeseed oil mixtures, catalyzed by nonspecific lipases, have been carried out with two objectives: To investigate production of modified fats with physical properties suitable for the manufacture of novel fat products and to optimize the reaction conditions so that the competitive hydrolytic side reaction is minimal (1). The focus of our attention has been the changes in the chemical composition during the interesterification reaction, especially those that alter the physical properties and physiological effects. The physical properties depend mainly on the triacylglycerol (TG) composition, especially when the competing hydrolysis is minimal.

We recently determined the TG composition of lipase-modified butter oil by gas–liquid chromatography on polarizable phenyl(50%)methylsilicone columns (1). TG with the same acyl carbon number were separated according to the level of unsaturation, and, in addition, TG isomers in the acyl carbon number range 24–42 were separated into several peaks. Identification was based on the analysis of several calibration standards, natural fats of known TG composition, the saturated and monoene fractions of butterfat separated by argentation thin-layer chromatography (TLC), the saturated fraction of butterfat separated by mercuric adduct method and an interesterified mixture of symmetric TG standards (1,2). The TG isomers separated in the acyl carbon number range 24–42 were identified and quantified as groups with the same acyl carbon number and level of unsaturation.

Since the 1960s, (M − 18), (M − RCOOH), (M − RCOO), (M − $RCOOCH_2$), (RCO), (RCO + 74) and (RCO + 128 + 14n) ions have been used to characterize the molecular species of TG from mass spectral data (3–6). Both electron (EI) and chemical ionization (CI) mass spectra have been used for structure elucidation (7,8).

In gas chromatography/mass spectroscopy (GC/MS) analyses, thermally stable apolar packed (7) and capillary columns (8) were initially used to separate TG into groups with the same number of carbon atoms. Later, Geeraert and Sandra (9) introduced polarizable immobilized phenylmethylsilicone columns for the gas-chromatographic separation; these were superior to apolar columns in thermal stability and resolving power. Myher *et al.* (10) used these columns and fractionation by argentation TLC in the mass spectrometric identification of a large number of molecular species of milk fat TG. Ohshima *et al.* (11) analyzed soybean TG separated on similar polarizable phenylmethylsilicone columns by EI MS (11).

Here we report on the gas-chromatographic quantitative analysis of lipase-modified BO and original butter oil on an immobilized polarizable phenyl(65%)methylsilicone column, and on the identification of major molecular species of TG, by using as an aid argentation chromatography on a cation-exchange solid-phase extraction column loaded with silver ions (12) and EI mass spectral data.

EXPERIMENTAL PROCEDURES

Immobilization of lipase. Celite Hyflo Supercel (Johns-Mansville Co, Ltd., Richmond, Surrey, United Kingdom) (100 g) was slowly added at 4°C to a solution of 10 g *Pseudomonas fluorescens* lipase in 200 mL of 0.3 M TES buffer, pH 7.0. The mixture was dried to constant weight in a vacuum desiccator at 4°C.

Original BO. The fatty acid composition of the original BO used was (mol%): 4:0, 12.8; 6:0, 5.1; 8:0, 2.2; 10:0, 3.8; 12:0, 3.5; 14:0, 11.0; 14:1, 0.9; 15:0, 0.9; 16:0, 24.2; 16:1, 1.3; 17:0, 0.4; 17:1, 0.3; 18:0, 10.0; 18:1, 18.8; 18:2, 1.7; 18:3, 0.5; 20:0, 0.2; other, 2.5.

Lipase-modified BO 1 (LMBO1). BO interesterified at 50°C in the absence of a solvent and with *P. fluorecens* lipase as catalyst was re-examined. The interesterification has been described elsewhere (1).

Lipase-modified BO 2 (LMBO2). BO was bleached and dried under vacuum to a water content of 55–85 ppm. Batches containing 500 g of dried BO and 68.5 g of lipase preparation were stirred at 60°C under vacuum (*ca.* 4 mmHg) for 1 h under argon for 16 h.

Determination of water and free fatty acid (FFA) in reaction mixtures. Water content was measured by coulometric Karl Fischer titration; FFAs were determined by titration with methanolic sodium hydroxide solution.

Fractionation by argentation chromatography. Lipase-modified butterfat was separated into fractions differing in degree of unsaturation on Bond Elut™ (Analytichem International, Harbor City, CA) SCX (*p*-propylbenzene sulfonic acid) solid-phase extraction columns

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loaded with silver ions, as described elsewhere (12).

Determination of FA. The transesterification of fat samples to methyl esters with sodium methoxide in hexane and the determination of their FA compositions are described elsewhere (12).

Determination of TG. TGs were analyzed on a 25-m, 0.25-mm i.d. immobilized phenyl(65%)methylsilicone column, by using high-oven temperature cold-on-column injection as described in detail elsewhere (12).

Temperature programs were: After an isothermal period of 1 min at 200°C, the column temperature was raised 15°C/min to 320°C and, after an isothermal period of 1 min, 7°C/min to 360°C. After an isothermal period of 1 min at 200°C, the column temperature was raised 15°C to 320°C and, after an isothermal period of 1 min, 2°C/min to 360°C.

For calculation of retention indices, symmetric TG 24:0, 30:0 and 36:0 were added to the saturated fraction and modified BOs. To the unsaturated fractions were added all these plus symmetric TG 42:0, 48:0 and 54:0.

For determination of correction factors, a calibration mixture containing trioctanoylglycerol, tridecanoylglycerol, tridodecanoylglycerol, trihexadecanoylglycerol, trioctadecanoylglycerol and tri-*cis*-9-octadecenoylglycerol (Nu-Chek-Prep, Elysian, MN) was analyzed.

Chromatographic data were acquired and integrated with a Microsoft Windows-based SC-Chromatographic Workstation program (Sunicom Ltd., Helsinki, Finland) and calculated with Micman software (Nordion Ltd., Helsinki, Finland).

GC/MS. The mass spectrometric analysis of TG was carried out on a Finnigan MAT INCOS 50 quadruple mass spectrometer (San Jose, CA) connected to a Varian 3400 gas chromatograph (Palo Alto, CA) with a septum-equipped programmable injector (SPI). The same phenyl-(65%)methylsilicone column used in the gas chromatographic study was connected directly to the EI source. The temperatures of the ion source and transfer line were 190 and 350°C, respectively. An on-column injection was made by piercing the septum of the SPI injector with a 40 × 0.7 mm syringe needle and by introducing an approx. 15-cm long, 0.23-mm o.d. silica capillary needle of a 10-μL syringe through that needle into the analytical column. The injection was made at 50°C and the temperature of the injector and column oven was raised by 20°C/min to 320°C, and after an isothermal period of 1 min by 4°C/min to 360°C. The inlet pressure of the helium carrier gas was 20 psi. Recording of full mass spectra (100–610 mass units) at 70 eV every 2 s was started 3 min after injection.

RESULTS

A large number of molecular species of milkfat TG separated on polarizable phenylmethylsilicone columns have been identified from CI mass spectral data, mainly on the basis of (M − RCOOH) fragments (8). Molecular species of vegetable oil TG separated on a similar column have been identified by selected ion monitoring of acyl and (M − RCOO) fragments of EI mass spectral data (9). Because of the large number of molecular species of milkfat TG, only the most abundant TG can be identified from EI solely on these ions; the abundance of most (M − RCOO) fragments is too low for reliable identification and the fragments *m/z* 71 and 99 of lower acyls, butanoyl and hexanoyl, or the alkyl fragments of the same mass, are abundant in the EI mass spectral data of every TG.

High-resolution measurements have shown the acyl + 74 fragments to be derived from (M − RCOO) ions, probably by loss of a substituted ketene (3). Mass spectra of mixed TG have been shown to contain acyl + 74 fragments corresponding to each acyl group. The acyl + 128 + 14n fragments have been suggested to be derived from (M − RCOO) fragments by homolytic cleavage of the alkyl chain (4). Acyl + 74 fragments, produced by positive CI, have been used in the structural characterization of dinitrophenylurethanes of diacylglycerols (13).

Interpretation of mass spectral data. The mass values for acyl, acyl + 74, acyl + 128 ions and the respective (M − RCOO) ions were calculated for saturated, monoene, diene and triene TG with 24–54 acyl carbons for identification of the major and minor molecular species of gas-chromatographic peaks of butterfat TG; also the mass values of the respective ions for odd acyl carbon number TG were calculated. Mass chromatograms of these ions were recorded for natural BO, modified BOs and the four fractions of modified BO from argentation chromatography. The data from these mass chromatograms with a scan number interval of 1–4 were collected to interpretation tables similar to the table for TG with 38 acyl carbons of modified BO 1 (Table 1). In general, positive identification of molecular species was considered when at least two of the three ions indicating each acyl group and the respective (M − RCOO) were observed at the same or close to the same scan number. Detection of acyl + 74 and acyl + 128 fragments was required for confirmation of acyls 4:0 and 6:0, because the acyl fragments *m/z* 71 and 99 or alkyl fragments of the same mass are common in mass spectra of all TG. Detection of both acyl + 74 and acyl + 128 fragments was required, because the acyl + 74 fragments for 16:1 and 18:1 and the acyl + 128 fragments for 12:0 and 14:0 had the same mass, *m/z* 311 and *m/z* 339, respectively. The wide variation in both the proportions of different molecular species and the intensities of their fragment ions made the interpretation of mass spectral data a difficult and time-consuming task. To explore the dependence of the intensity of fragment ions on fragment type and molecular weight, we compared the intensity of fragments per gas-chromatographically determined mol% of the peaks of saturated and monoene fractions of lipase-modified BO, where only one molecular species was identified. For example, for BPO, CyPO and PSO (B = 4:0, P = 16:0, O = 18:1, Cy = 8:0, S = 18:0), the relative intensities/mol% of M-oleoyl were 718, 275 and 48, those of oleoyl 325, 133 and 62, those of (oleoyl + 74) 54, 60 and 26, and that of (oleoyl + 128) 28, 43 and 23, respectively.

TG of different unsaturation eluted in different peaks. However, there was some overlapping of the last saturated and first monoene peaks and the last monoene and first diene peaks. A retention index difference of only 3 or 7 for the last saturated and first monoene peaks for TG 36 and 40 suggests such overlapping.

In using the identified peaks in mass chromatograms to identify the peaks in gas chromatograms, retention indices were calculated with added and intact saturated symmetric TG as reference compounds. The retention indices for mass chromatograms were calculated from scan

TABLE 1

Interpretation of TG 38 of Lipase-Modified Butter Oil[a]

Scan number/ retention indices	RCO	RCO + 74 RCO + 128	M − RCOO	Acyl	TG
478/3785	127	201/255	524	Cy	
	155	229/283	496	Ci	
	183	257/311	467	La	CyMP
	211	285/339	439	M	CiLaP
	239	313/367	411	P	CiMM
481/3807		173/227	552	Co	
	239	313/367	411	P	CoPP
485/3807		145/199	580	B	
	239	313/367	411	P	
	267	341/395	383	S	BPS
488/3859		145/199	578	B	
		173/227	550	Co	
	211	285/339	437	M	
	239	313/367	409	P	BPO
	265	339/393	383	O	CoMO
490/3874		145/199	578	B	
	237	311/365	411	Po	
	267	341/395	381	S	BPoS

[a]Abbreviations: B = 4:0, Co = 6:0, Cy = 8:0, Ci = 10:0, La = 12:0, M = 14:0, P = 16:0, Po = 16:1, S = 18:0, O = 18:1, L = 18:2, Ln = 18:3; RCO = acyl fragment *m/z*; TG, triacylglycerols.

numbers. In making the identifications, we took into consideration peak size and shape, elution order of TG in partly or unresolved peaks of mass chromatograms, and the difference in the resolution of mass chromatograms and gas chromatograms.

Original BO. Figure 1 shows the gas chromatogram of natural BO, which was the starting material for LMBO1; Table 2 shows the identification of molecular species in the separated peaks and the quantitation data. The proportion of TG 24 was so low that it was not detected, and in the TG 26–30 only saturated TG, eluting in one or two peaks, were detected. The TG 32 eluted in one saturated and one monoene peak. In the acyl carbon number range 34–38 saturated TG eluted in 3–4 peaks, and monoene TG were identified in 1–2 peaks, but the proportion of diene TG was so low that they could not be identified. Of the TG 40, saturated TG eluted in 3 and monoene TG in 2 peaks, and diene TG in 2 peaks with overlapping of TG of different unsaturation. Of the TG 42, saturated TG were identified in two peaks and monoene and diene TG in one peak; of the TG 44, saturated TG were identified in one peak and unsaturated TG in two peaks; of the TG 48, saturated TG were identified in one peak and monoene TG in one peak. In the acyl carbon range 50–54, saturated, monoene and diene TG were identified in one peak in each acyl carbon number range.

LMBO1 and its fractions from silver ion chromatography. Interesterification of butterfat with, for example, sodium methoxide or a nonspecific lipase as catalyst changes the distribution of acyl groups among TG so that they are randomly distributed. This leads to an increase in the proportions of TG in the low and high acyl carbon number ranges and a decrease in the proportions of TG in the medium acyl carbon number range. The number of TG peaks existing in sufficient proportions for mass spectrometric identification is thus increased. Fractionation of enzymatically randomized BO by silver ion chromatography further increases the number of peaks large enough to allow mass-spectrometric identification.

The gas-chromatographic analysis of fractions from silver ion chromatography showed several TG peaks with the same retention indices as the TG with different unsaturation. The saturated TG fraction contained 26 small TG peaks with the same retention index as the more unsaturated TG. The monoene TG fraction contained eight small TG peaks with the same retention index as the saturated TG, and eight small TG peaks with the same retention index as the more unsaturated TG. Both the diene and triene TG fractions contained twelve TG of lower unsaturation, and the diene TG fraction contained one triene TG peak.

In general, these small peaks with different unsaturation were not resolved in the mass chromatograms because the low-capacity column had to be overloaded to obtain sufficient amounts of (M − RCOO) ions of minor TG. These minor TG with different unsaturation were eluted in the front or in the tail of major TG components.

Figure 2 shows the gas chromatogram of LMBO1, and Table 3 shows the identification and the proportion of TG 36–42 peaks of LMBO1 and its saturated, monoene, diene and triene fractions. TG with the same acyl carbon number and level of unsaturation were separated into several peaks in the acyl carbon number range 24–42. Major and minor molecular species of these peaks could be identified from mass spectra. However, within acyl carbon number range 24–30, the proportions of the two or three first saturated TG peaks or the differences in their retention times were so small that it was not possible to deduce which of the identified molecular species was the major species in each peak.

Within the acyl carbon number range 24–42, the symmetric saturated TG (added reference compounds 24:0, 30:0 and 36:0) were eluted first, and then TG with acyl chains of slightly different chainlength. Next came TG

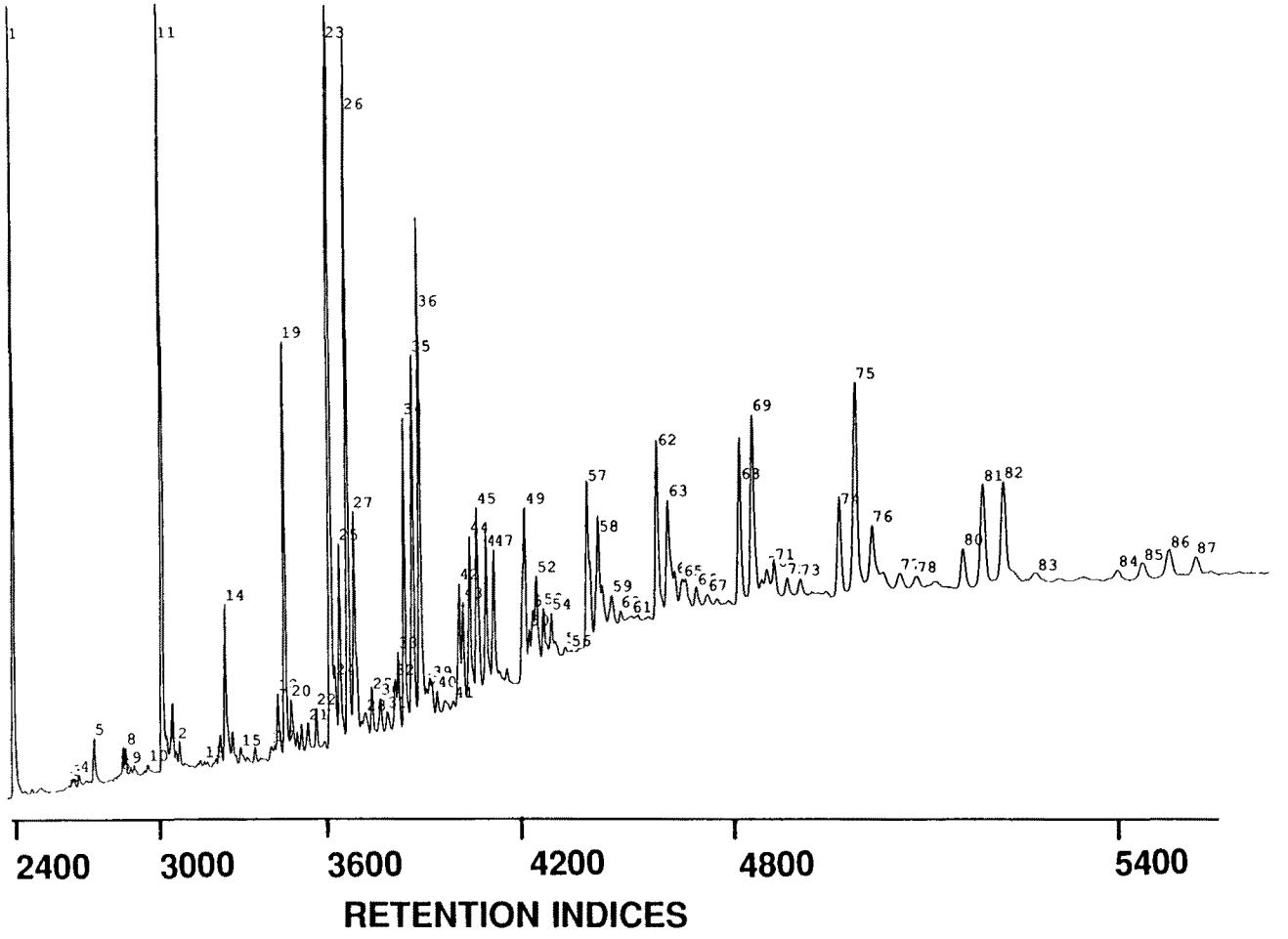

FIG. 1. The gas chromatogram of original butter oil. For conditions see text; temperature program I. For identification see Table 2.

with acyl chains of increasingly different chainlength. For example, for saturated TG 36, the order of elution was LaLaLa, CyLaP and CyCiS, CoMP, BPP, BMS (La = 12:0, Ci = 10:0, Co = 6.0, M = 14:0) (Table 3). The elution of monoene TG seems to follow the same rule, except that the peak composed of CoMO/BPoS/CyPoM elutes after BPO. In this part of the mass chromatogram, acyl chain isomers are only partly separated into the groups of isomers, and the adjacent TG peaks with different unsaturation overlap. However, in the gas chromatogram, some isomer peaks are almost completely separated from each other, and most adjacent peaks of different unsaturation do not overlap significantly.

Above acyl carbon number 42, the acyl chain isomers of saturated and monoene TG did not separate. However, monoene TG 44 and 46 peaks had small shoulders. Diene TG 46 and 48 partially separated into two peaks. The saturated and monoene TG 42, 44 and 46 overlap slightly with each other. The TG with 48 or more acyl carbons and different unsaturation separated from each other nearly to the baseline.

Comparison of the TG composition of original and modified BOs with random TG composition. Interesterification (acyl interchange) with a nonspecific lipase as catalyst leads to a close-to-random distribution of acyl groups among TG. However, in our recent study the products of interesterification reactions catalyzed by *P. fluorescens* lipase had somewhat higher proportions of monoene TG 38 and 48–54 and diene TG 40–54 relative to the calculated random composition (1). To re-examine this deviation, the TG composition of two interesterified fats was compared with the calculated composition and with the composition of natural BO (Figs. 3 and 4). In the acyl carbon range 34–42 (Fig. 3), both modified fats and the calculated composition show a similar proportion of BPP, a similar proportion of CiMP, CiLaP, CyMP, CoPP (column 9) and CiMP, CiLaS, LaMM, CyPP, CyMS, CoPS, BSS (column 13), and a lower proportion of CyCiP, CoMM, CoLaP (column 1), CyLaP, CyCiS, CoMP (column 5), BMS, BMO, BPPo (column 7), CoMS and BPS (column 10), BPO (column 11), CyMO, CiLaO, CoPO, BSO (column 14) and MMM, LaMP, CiPP, CiMS, CyPS, CoSS (column 17). Only the proportions of CiMO, LaLaO, LaMPo, CyPO, CoSO (column 18) were higher in modified fats relative to the calculated composition. In the carbon number range 44–54 (Fig. 4), proportions of TG 46:0, 48:0, 48:1, 50:0, 50:1, 52:0, 52:1, 52:2, 54:0 and 54:2 were clearly higher in both modified fats relative to the calculated composition.

Relative to original BO, the proportions of monoene TG in the acyl carbon number range 46–52 and diene TG 52:2 were clearly higher in the modified fats, the proportions

TABLE 2

Proportions (mol%) of Identified Triacylglycerols (TG) in Original Butter Oil

TG	Peak number	R.i.[a]	Mol%	TG	Peak number	R.i.	Mol%
TG24				BSO/CoPO	45	4060	2.32
CyCyCy	(1)	2400		BSO/BOO	46	4080	1.69
TG26				BSL/BOO	47	4111	1.44
BCyM/CoCoM/BCiLa/CoCyLa	3	2640	0.23	TG42			
BCoP	4	2669	0.18	MMM/LaMP/CiPP/	49	4200	2.64
n.i.[b] (impurity)	5	2727	0.63	CiMS	50	4210	0.54
TG28				CyPO/LaLaO	52	4232	1.49
BCyP/BCiM/CyCiCi/CoCiLa	8	2846	0.20	CoOO	53	4262	0.99
BCyP/BCoS	9	2876	0.21	n.i.	54	4283	0.94
TG30				TG44			
CiCiCi	(11)	3000		MMP/LaPP/CiPS/LaMS	57	4386	2.78
BCiP/CoCiM/CyCiLa/CoCoO	12	3017	0.24	CiPO/LaMO/LaPPo	58	4425	2.78
TG32				CySO	59	4454	0.96
		3220		n.i.	60	4479	0.36
BLaP/BMM/CoCyS/CoLaM	14	3237	2.19	n.i.	61	4519	0.42
BCiO	15	3290	0.28	TG46			
TG34				MPP/LaPS/MMS	62	4587	2.80
CiLaLa	17	3400	0.16	LaPO/MMO/LaPoS	63	4615	1.90
CiLaLa	18	3420	0.63	n.i.	64	4633	0.52
BMP/BLaS/CoMM/CyCiP	19	3447	4.12	n.i.	65	4655	0.80
BLaO	20	3464	0.52	n.i.	66	4692	0.40
n.i.	21	3523	0.29	n.i.	67	4721	0.27
TG36				TG48			
LaLaLa	(23)	3600		PPP/MPS/LaSS	68	4800	2.73
CiLaM/CyMM	24	3613	0.86	MPO/LaSO/PPPo/MSPo	69	4929	3.39
CoMP	25	3630	2.12	n.i.	70	4860	0.35
BMS/BPP/CoLaO/CoMPo	26	3657	6.85	n.i.	71	4875	0.35
BMO/BPPo/	27	3675	3.11	n.i.	72	4903	0.38
n.i.	28	3711	0.44	n.i.	73	4930	0.34
n.i.	29	3732	0.46	TG50			
n.i.	30	3758	0.36	PPS/MSS	74	5005	2.26
n.i.	31	3779	0.24	PPO/MSO/PSPo	75	5030	5.15
TG38				MOO	76	5063	2.33
CiLaP	32	3806	0.48	n.i.	77	5105	0.59
CyMP	33	3816	0.80	n.i.	78	5131	0.58
CoPP/CoMS	34	3834	2.96	TG52			
BPS	35	3861	4.02	PSS	80	5200	1.41
BPO	36	3880	5.45	PSO	81	5228	3.11
n.i.	37	3904	0.25	POO	82	5257	3.57
n.i.	39	3920	0.36	n.i.	83	5297	0.54
n.i.	40	3934	0.29	TG54			
TG40				SSS	84	5400	0.37
CiMP/LaMM	42	4003	1.21	SSO	85	5431	0.76
CyPP/LaLaP	43	4015	1.01	SOO	86	5463	1.82
CoPS/CyMS/CyMO	44	4035	1.73	n.i.	87	5497	1.08

[a]Retention indices; see Table 1 for other abbreviations. [b]Not identified.

of saturated TG with 36, 38 and 42 acyl carbon were clearly lower, and the proportions of saturated TG with 40 and 44–50 acyl carbons were clearly higher. Further, relative to natural BO, the proportion of monoene TG with 42 acyl carbons was higher in modified oils, and that of monoene TG with 36–40 acyl carbons was lower.

DISCUSSION

The resolving power of the immobilized phenyl(65%)-methylsilicone column is superior to that of the phenyl-(50%)methylsilicone column (1). The acyl chain isomers were separated into more peaks in the acyl carbon number range 24–42 than in the earlier study. Also, the overlapping of adjacent peaks of different unsaturation was less than in the separation on the column with lower polarity.

As has been comprehensively demonstrated by Myher *et al.* (10), the prefractionation by argentation TLC into two saturated fractions, two monoene fractions, and diene and triene fractions is invaluable for the identification of major molecular species. The prefractionation in this study into saturated, monoene, diene and triene fractions on a cation exchange column in silver-ion form likewise proved useful for the identification of major molecular species of TG of LMBO. The prefractionation was essential for determination of the elution order of acyl chain isomers. Table 3 shows several examples of acyl chain isomers that were eluted in one peak in the gas

TABLE 3

Proportions (mol%) of Identified Triacylglycerols (TG) with 36–42 Acyl Carbons in Lipase-Modified Butter Oil (LMBO1) and Saturated (S), Monoene (M), Diene (D) and Triene (T) Fractions[a]

TG	Peak number	R.i.	Mol%				
			LMBO1	S	M	D	T
TG24–35							
Saturated			9.30	18.07	1.11		
Monoene			4.56	1.71	10.23		
Diene						1.21	
TG36							
LaLaLa	(35)	3600					
CyLaP/CyCiS	36	3630	0.49	2.22	0.21		
CoMP	37	3640	0.40		0.25		
BPP	38	3657	2.37	5.10	(1.18)	(0.27)	(1.36)
BMS	39	3675	2.22	2.17			
BMO		3678	T		3.36	(0.38)	
BPPo	40	3698	0.66		1.14		
T37:0		3711		0.30			
BML	41	3720–32	0.37	[0.34]	[0.69]	0.56	
T36:2		3741				0.28	
T36:2	42	3758	0.30		[0.28]	1.04	
n.i.		3766					0.77
	43	3779	0.13	0.11			
TG38							
CiMM/CiLaP		3806	T	0.69			
CyMP/CiMM/CiLaP	45	3816	1.53	1.14			
CoPP		3834	⊥	3.04	(0.92)		(0.58)
CoMS/BPS	46	3861	1.92	3.00	(2.08)		
BPO	47	3880–88	2.94	[1.50]	6.60	(1.07)	(0.83)
CoMO/BPoS/CyPoM	48	3904	1.39		2.24		
T39:0		3909		0.33			
n.i.	49	3920–34	0.24	[0.13]		1.77	
T39:1		3989			0.14		
TG40							
n.i.		3995					1.31
CiMP/CiLaS/LaMM	51	4003	0.98	2.07			
CyPP/CyMS	52	4015	0.58	1.51			
CoPS	53	4035	1.35	1.33			
CyMO		4042	T	[0.64]	1.54	(0.30)	(0.51)
CiLaO/CyMO	54	4060–64	1.72	[0.78]	⊥		
CoPO	55	4073–80	1.19	[0.44]	1.16		
BSO		4092	⊥		2.56	(0.65)	
T41:0		4101		0.32		(0.37)	
BOO/CoPoO	56	4111–16	1.38	[0.15]	[1.09]	5.85	
BOO/BSL	57	4136	0.45		[0.22]	2.81	
BOL	58	4152	0.50				4.22
TG42							
MMM/LaMP/CiPP/CiMS/CyPS		4200	1.26	4.27			
CoSS	62	4210	0.27	0.95			
CiMO/LaLaO/LaMPo	63	4232	0.68	[0.35]	1.66		
CyPO	64	4242	1.19	[0.24]	2.27		
CoSO	65	4262	0.50	[0.17]	0.95	(0.31)	
n.i.	66	4273	0.30		0.68	0.56	
CoOO	67	4283	0.39			2.23	
TG43:0	68	4287		0.65			
CoSL	69	4295	0.45			1.06	
CoOL	70	4327	0.15		[0.24]		3.03
TG44–54							
Saturated			17.80	35.31	1.24	1.67	4.19
Monoene			22.65	2.52	44.72	4.50	4.51
Diene			10.81	1.46	0.62	53.32	1.96
Triene			3.50	0.83	0.91	0.40	55.76
TG24–54							
Saturated			41.26	81.82	8.39	2.31	6.87
Monoene TG			37.48	8.35	79.25	8.15	5.85
Diene TG			14.15	1.69	2.90	68.36	1.96
Triene TG			4.15	0.83	1.15	0.40	63.01
Not identified TG			2.91	1.62	2.17	11.43	5.34
Not identified minor peaks			0.05	5.69	6.14	9.35	16.97

[a]R.i. = retention indices; n.i. = not identified; [ ], TG of higher unsaturation; ( ), TG of lower unsaturation. See Table 1 for other abbreviations.

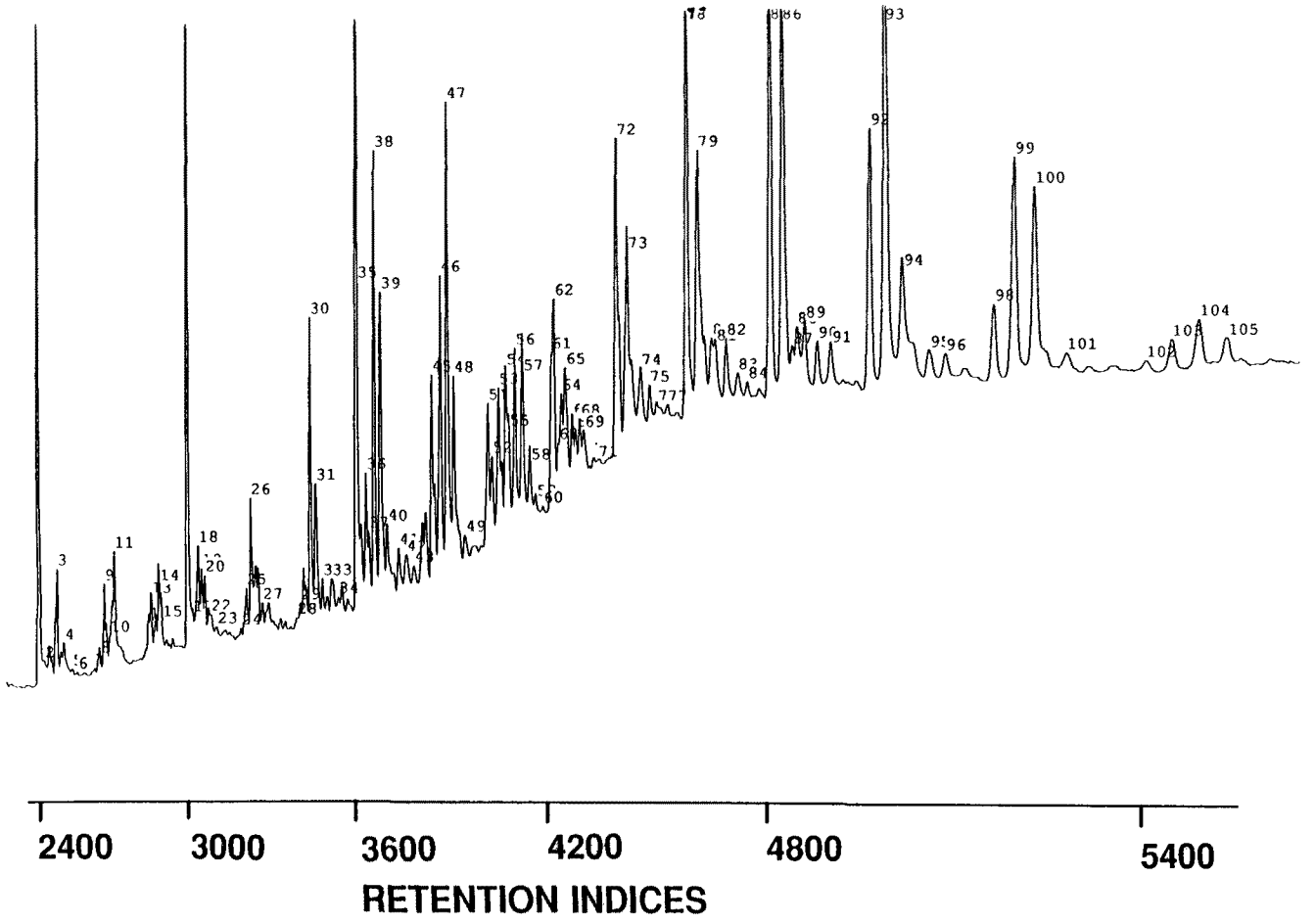

FIG. 2. The gas chromatogram of triacylglycerols of lipase-modified butter oil 1. For conditions see text; temperature program I. For peak identification see Table 3.

chromatogram of LMBO and in several peaks in the gas chromatogram of the appropriate fraction. The calculation of retention indices was useful in the identification of gas chromatogram peaks on the basis of mass chromatogram peaks, and also in the comparison of identification and quantitation data of different fats and fractions (Table 3). However, because of the low capacity of the column, the indices for the same molecular species varied over a few index units in the different samples.

Kuksis *et al.* (14) observed that, on polar packed columns (ethylene glycol succinate), TG with the same acyl carbon number were eluted in the following order: caprates, caprylates, caproates and butyrates. Myher *et al.* (10) presented strong evidence for the elution of the saturated and monoene TG of BO distillate and its argentation TLC fractions in the same order on a polarizable phenyl(50%)methylsilicone column. Our findings show that, on a similar column but with 65% phenyl groups, the elution order of saturated TG in the acyl carbon number range 34–42 is the same.

Except for the earlier elution of BPO than CoMO/BPoS/CyPoM, the elution order of monoene TG 34–42 is also similar. Owing to their different molecular shape, the acyl chain isomers of TG obviously have different vapor pressure and polarity. Because acyl chain isomers evidently are not separated on apolar columns, it is reasonable to conclude that their separation on polar and polarizable columns is due to their differences in polarity. Above acyl carbon number 42, the acyl chain isomers do not separate, probably due to the lack of polarity differences rather than a drastic change in the analytical conditions.

The method used in the interpretation of mass spectral data, including double confirmation for the acyl group (observation of two of the three ions acyl, acyl + 74, acyl + 128) and detection of the respective (M − RCOO) fragment at about the same scan number, should ensure reliable identification. The variation in the abundances of the ions depends not only on peak size and proportion of the respective molecular species in the peak, but also on fragment type and mass range, as was seen for monoene molecular species that contain oleic acid. This variation had to be taken into consideration in the interpretation of mass spectral data.

In our earlier study, relative to the calculated random composition, there were slightly higher proportions of monoene TG 38 and 48–54 and diene TG 40–54 among the products of interesterification reactions catalyzed by *P. fluorescens* lipase (1). The results here show that, in the acyl carbon number range 46–54, the proportions of all saturated TG and diene TG 52:2 were higher in the

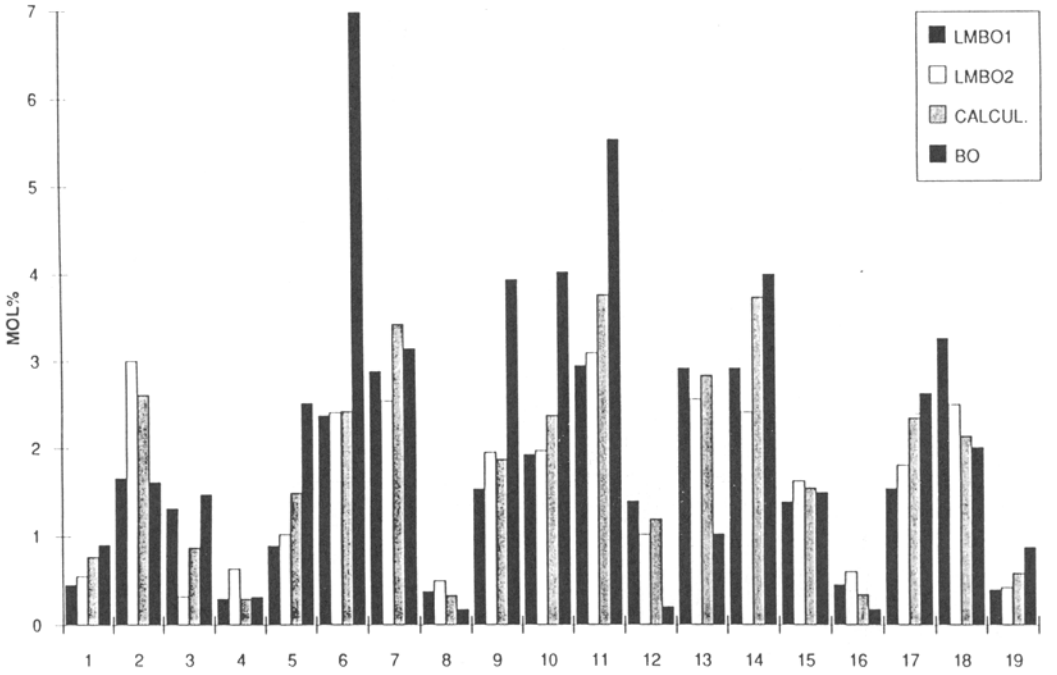

FIG. 3. Proportions (mol%) of TG with 34–42 acyl carbons in lipase-modified butter oils (LMBO1 and LMBO2), in the composition calculated (CALCUL.) according to random distribution, and in natural butter oil (BO). 1. CyCiP/CoMM/CoLaP; 2. BMP/BLaS/CoCiS; 3. CyCyO/CoCiO/BLaO; 4. BMPo; 5. CyLaP/CyCiS/CoMP; 6. BPP; 7. BMS/BMO/BPPo; 8. BML; 9. CiMM/CiLaP/CyMP/CiMM/CiLaP/CoPP; 10. CoMS/BPS; 11. BPO; 12. CoMO/BPoS/CyPoM; 13. CiMP/CiLaS/LaMM/CyPP/CyMS/CoPS/BSS; 14. CyMO/CiLaO/CoPO/BSO; 15. BOO/CoPoO; 16. BOO/BSL; 17. MMM/LaMP/CiPP/CiMS/CyPS/CoSS; 18. CiMO/LaLaO/LaMPo/CyPO/CoSO; 19. CoOO. See Table 1 for abbreviations.

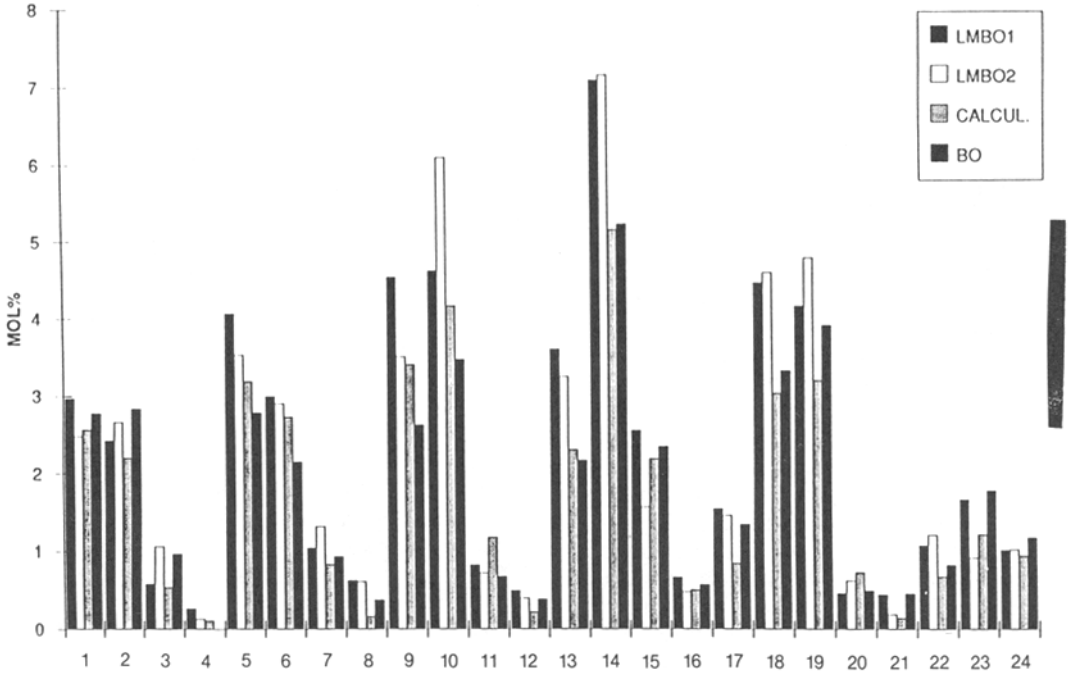

FIG. 4. Proportions (mol%) of triacylglycerols with 44–54 acyl carbons in lipase-modified butter oils (LMBO1 and LMBO2), in the composition calculated (CALCUL.) according to random distribution, and in natural butter oil (BO). 1. MMP/LaPP/LaMS/CiPS; 2. CiPO/LaMO/CySO; 3. CyOO/CiPoO/CiPL; 4. CyOL; 5. MPP/LaPS/MMS/CiSS; 6. LaPO/MMO/MPPo/CiSO; 7. LaPoO/CiOO/MML/CiSL; 8. CiOL; 9. PPP/MPS/LaSS; 10. MPO/LaSO/PPPo/MPoS; 11. LaOO/MPoO/MPL/LaSL; 12. LaOL/MPoLa; 13. PPS/MSS; 14. PPO/MSO/PSPo; 15. MOO; 16. MOL; 17. PSS; 18. PSO; 19. POO; 20. POL; 21. SSS; 22. SSO; 23. SOO/SSL; 24. OOO/SOL. See Tables 1 and 3 for abbreviations.

modified fats relative to the calculated composition (Fig. 4). Moreover, in the acyl carbon number range 34–42, the proportions of most saturated and monoene TG were lower in the modified fats (Fig. 3). The differences on the two columns are due to the lower resolving power of the earlier column coated with phenyl(50%)methylsilicone. Despite the deviations from the random composition, the lipase-catalyzed reaction nevertheless changes the TG composition in the direction of random composition. The changes in the proportions of most TG suggest that, after the random TG composition is reached, the reaction continues further in the same direction; the proportions of most TG exceed or fall below the random proportion. Determination of the FA composition at the *sn*-2 position of LMBO1 by enzymatic deacylation showed the interesterification to change the proportions of major FA in the direction of random distribution (1). The proportions of palmitic, stearic and oleic acids exceeded the random value in the reaction products at 50°C, and more clearly at 60°C (1). The deviation of the TG composition from the random composition found here may be due to positional or fatty acid specificity of the *P. fluorescens* lipase under microaqueous conditions. A complete stereospecific analysis of the LMBOs and determination of FA specificity of the lipase would help to explain the difference between the calculated and determined TG composition.

The present study shows polarizable phenylmethylsilicone column containing 65% phenyl groups to possess higher resolving power than a similar column containing only 50% phenyl groups. Acyl + 74 and acyl + 128 fragments of EI mass-spectral data proved useful, in addition to acyl and M − RCOO fragments, in the identification of molecular species of TG. Interesterification of BO with *P. fluorescens* lipase as catalyst increased the number of molecular species, in the GC/MS analysis existing in sufficient proportion to allow identification by EI MS. The analysis of fractions of different unsaturation from silver ion chromatography on a cation-exchange solid-phase extraction column further increased the number of molecular species of TG that could be identified. Saturated acyl chain isomers were eluted on the phenylmethylsilicone column in order of increasing polarity. A similar trend was observed for acyl chain isomers of monoene TG. Comparison of the TG compositions of LMBOs with original BO, and the TG composition calculated according to random distribution, showed interesterification to change the TG composition in the direction of random distribution.

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