Silver Ion Adsorption Thin Layer Chromatography and Capillary Gas Chromatography in the Study of the Composition of Milk Fat Triglycerides

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Fatty acid and triglyceride (TG) composition of milk fat and of the six fractions obtained by $AgNO_3$-TLC were determined using capillary GC. The saturated (SSS) and monounsaturated (SSM) TGs accounted with two fractions each that were separated into butyrates and all the others; the remaining fractions were identified as di- and triunsaturated TGs, respectively. The total TGs and the SSM were distributed in similar proportions with respect to chain length. The molar percentages of SSS in short- and medium-chain TGs (60.2 and 45.0%, respectively) were higher than those of polyunsaturated TGs (11.5 and 21.6%, respectively). Most of the peaks found in the chromatogram of milk fat TGs were located predominantly in one of the $AgNO_3$-TLC fractions. Thirty-seven peaks were >1%; from the most important in quantitative terms, six (three SSS and three SSM) contain butyric acid and four contain three long-chain fatty acids. Eighty-one molecular species of TGs were identified.

Keywords: *Milk fat; triglycerides; molecular classes; molecular species; silver ion adsorption TLC; capillary GC*

INTRODUCTION

The composition and structure of triglycerides (TGs) determine an important part of the physical and nutritional properties of milk fat. A better understanding of those aspects could be useful to (1) predict melting and crystallization points, which influence the texture and flavor of derivative products; (2) determine the origin of the fat as a means of detecting possible adulteration of dairy products; (3) predict nutritional value on the basis of the differences in digestion of the various triglycerides; and (4) improve the knowledge of TG biosynthesis systems in the mammary gland.

Because of the wide variety of fatty acids contained in milk fat, the characterization of its TGs is a complex and difficult task. Before quantitative analysis, TGs must be grouped on the basis of some of their common characteristics (molecular weight, degree of unsaturation, etc.).

The TLC technique on silica gel with silver nitrate ($AgNO_3$-TLC) separates milk fat TGs according to the degree of unsaturation because of weak interactions between the π electrons of the double bonds and the silver ions. Other factors, such as geometric configuration and chain length (Parodi, 1980; Myher et al., 1988), are also involved in TG separation on silica gel. This separation, which is carried out prior to gas chromatography and/or mass spectrometry, has been used by Myher et al. (1988) to identify and quantify over 100 TGs (the most significant from a quantitative standpoint) from a butter distillate. Reversed phase HPLC prior to final analysis of milk fat TGs has been used by Barron et al. (1990), Gresti et al. (1993), Spanos et al. (1995), and Ruiz-Sala et al. (1996) on unmodified milk fat samples.

The purpose of this study was to identify and quantify the TGs in milk fat using a combination of $AgNO_3$-TLC and capillary GC.

MATERIALS AND METHODS

Samples and Standards. Samples were obtained from butter by melting and filtering the fat at 50 °C. Different dilutions of the fat were prepared in hexane for chromatographic analysis. To identify the TG, a mixture of synthetic TGs (trilinolein, triolein, tristearin, tripalmitin, trimyristin, trilaurin, tricaprin, and tricaprylin) was first analyzed to determine both the best chromatographic conditions and the retention times of these components. The other molecular species were identified following previous studies carried out in similar chromatographic conditions (Myher et al., 1988; Hinshaw and Seferovic, 1986; Kalo and Kemppinen, 1993). A reference butter oil, with known composition, which had served as test fat in EC collaborative trials (Precht, 1991), was used to determine the response factors (RF) for quantitative studies of the TG composition of both milk fat and TLC bands as described in a previous paper (Lozada et al., 1995). The RF determined by comparing the percentage to each TG of the reference fat with experimental values was applied to the corresponding peaks for each TG with the same carbon number.

For quantitative determination of fatty acid composition a BCR reference fat (RM 164) to calculate the RF was used.

To quantify the relative percentages of TLC bands, a solution of the TG trinanoin (C27, Sigma Chemical Co., St. Louis, MO) was added as internal standard to both milk fat and each TLC band scraped off from the $AgNO_3$-TLC plate before GC analysis.

The relative proportion of milk fat TGs was also quantified by adding a solution of trinanoin as internal standard to the scraped bands obtained from TLC without $AgNO_3$.

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Separation of TGs by $AgNO_3$-TLC. TLC glass plates (20 × 20 cm) with silica gel (0.25 mm) (Merck, Darmstadt, Germany) were incubated with 20% aqueous solution of $AgNO_3$ (Panreac) overnight. The plates were then activated at 100 °C for 30 min, and 0.5 mL of the fat solution (12 mg/mL in chloroform, containing 0.5−0.8% ethanol) was applied to the plate. Triolein and tristearin were also applied to the border of the plate as qualitative standards. The plate was developed twice in a saturated chamber, in chloroform with 15 cm migration. After drying, the plates were sprayed with a 0.15% ethanol solution of 2′,7′-dichlorofluorescein and the bands were visualized under UV light. Bands were scraped off, and 20 μL of 3.15 mg/mL solution of C27 was added to each band as internal standard. The TGs were extracted four times with 20 mL of diethyl ether protected with butylhydroxytoluene (BHT, Panreac) and filtered, and the solvent was evaporated under reduced pressure. The residue was resuspended in 140 μL of hexane, and the solution was used for analysis of TGs and fatty acids by GC. Fractionation was twice repeated, and the corresponding fractions were pooled to obtain sufficient material for GC analysis.

GC Analysis of TGs. For analysis of total TGs in butter, 20 mg of fat was dissolved in 0.5 mL of hexane. For analysis of both, butter fat and fractions obtained by $AgNO_3$-TLC, 0.2 μL was injected into the gas chromatograph.

The triglyceride analyses were performed on a Perkin-Elmer gas chromatograph (Beaconsfield, U.K.) model Autosystem, Gion 4072042, equipped with an automatic injector (split/splitless) and programmed temperature. A capillary column of 30 m length, Rtx-65 TG (35% dimethyl, 65% diphenyl polysiloxane) (d_f = 0.10 μm), supplied by Restek (Bellefonte, PA), was used. Experimental chromatography conditions were as follows: the initial temperature (220 °C) was raised to 320 °C at a rate of 15 °C/min, then to 355 °C at a rate of 7 °C/min, and then held at this temperature for 20 min. The injector and detector temperatures were 355 and 370 °C, respectively. The pressure at the top of the column was 25 psig, the split ratio was 1:4, and helium was the carrier gas.

GC Analysis of Fatty Acids. For preparation of methyl esters of milk fat, 0.1 g of fat was dissolved in 1 mL of hexane and 0.5 mL of 2 N potassium hydroxide in methanol added as described by Christopherson and Glass (1969). For analysis of the fractions obtained by $AgNO_3$-TLC the final hexane solution was methylated according to the same procedure. In both cases, 0.2 μL was injected into a gas chromatograph. A Perkin-Elmer model 8420 gas chromatograph was used, equipped with programmed temperature vaporizer inlet, flow splitter, and hydrogen flame ionization detector. Helium was the carrier gas, the split ratio was 1:20, and the pressure at the top of the column was 25 psig. The column was a WCOT silica capillary (50 m × 0.22 mm i.d.) containing a Silar 5CP (50% phenyl, 50% cyanopropyl) stationary phase (d_f = 0.22 μm) (Chrompack, Middelburg, The Netherlands). Experimental chromatography conditions were as follows: the initial temperature, 60 °C, was maintained for 3 min then raised to 190 °C at a rate of 15 °C/min. The final temperature was maintained for 30 min. The injector and detector temperatures were 300 and 250 °C, respectively.

The content of cis/trans isomers was determined using a column BPX-70 (60 m × 0.22 mm i.d.) containing 70% cyanopropyl siloxane, stationary phase (d_f = 0.25 μm) (SGE, Waddinxveen, The Netherlands). The initial temperature, 70 °C, was maintained for 3 min, then raised to 185 °C at a rate of 11 °C/min, and maintained for 30 min. The split ratio was 1:20, and nitrogen was the carrier gas. The injector and detector temperatures were 250 °C.

RESULTS AND DISCUSSION

TG Separation by $AgNO_3$-TLC: Fatty Acid Composition of the Different Fractions. Using $AgNO_3$-TLC, TGs from the milk fat were separated into six bands (Figure 1). There were other bands adjacent to bands A, C, and F, but they were too narrow and faint to be individualized. A great part of fatty acids of the bands A and B (Table 1) were saturated (96 and 94%, respectively), and hence the TGs contained in both bands were identified as trisaturates (SSS). Band B contained 93% of the butyric acid detected in SSS bands, whereas band A contained larger amounts of C6, C8, C10, and C12 fatty acids (74, 61, 65, and 65% of total SSS bands, respectively). The SSS are located in a single band in most of the references consulted, but Myher et al. (1988) reported that they can be separated into two bands differentiated by the chain length of the short fatty acid. The nonindividualized narrow band located at the bottom of band A could possibly include the SSSs containing the remaining 7% of butyric acid not located in band B.

The TGs in bands C and D contained nearly one-third of the monounsaturated fatty acids (average = 27%) and hence were identified as monounsaturates (SSM). The lower percentage of monounsaturates in band C with regard to the theoretical value (33%) could have been due to overlapping with the saturated bands, which also

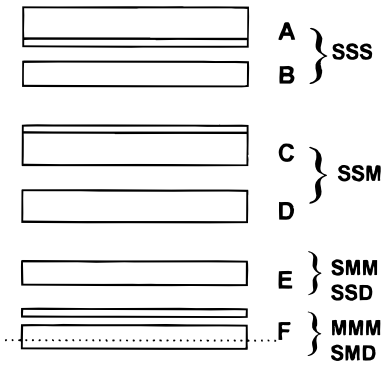

Figure 1. Fractionation of TGs of milk fat by $AgNO_3$-TLC.

Table 1. Fatty Acid Composition (Molar Percent)[a] of TGs of Milk Fat and of Fractions Obtained by $AgNO_3$-TLC

		$AgNO_3$−TLC fraction					
fatty acid	total	A	B	C	D	E	F
C4	10.64	1.10	17.89	3.58	11.44	4.95	6.19
C6	4.77	6.89	2.89	2.53	4.55	2.15	2.19
C8	2.70	4.40	3.22	3.06	5.36	1.96	1.90
C10	4.65	7.07	4.54	4.16	4.64	2.23	2.69
C10:1	0.42	0.26		0.27	0.93	0.48	0.76
C12	4.13	7.85	4.89	3.58	4.43	2.79	2.77
C12:1	0.13	0.15	0.36	0.26	0.79	0.05	
C14	11.17	14.14	12.63	10.12	7.40	5.88	5.23
C14:1+iC15	0.92	0.49	0.41	0.62	1.21	0.51	1.40
C15	1.62	2.97	2.52	2.25	2.77	1.43	1.72
C15:1	0.10			0.28	0.69	0.16	0.84
C16	22.57	30.53	29.04	23.24	16.04	15.53	11.43
C16:1	1.29			2.38	3.47	3.64	5.22
C17	1.98	3.06	2.64	2.62	2.32	2.09	1.13
C17:1	0.38			0.55	0.56	0.81	0.44
C18	8.86	16.88	12.91	12.67	5.53	8.37	5.51
C18:1	17.94	1.44	3.43	22.78	20.35	35.15	30.56
C18:2	1.72			1.44	3.60	7.54	9.48
C19	0.58	0.09	0.11	0.10			3.10
C18:3	0.52			0.01			3.75
$C18:2^{c,t-conj}$	0.75			1.25	0.85	1.31	0.82
C20	0.03	0.49	0.39	0.34		0.23	
C20:1	0.07			0.25	0.07		
others	1.99	2.19	2.14	1.68	3.08	2.71	3.22
$C18:1^{t}$ % of total C18:1	12.9		62.3		8.5	3.9	9.3
total wt %[b]		25.1	21.9	17.1	15.8	10.0	10.2

[a] Mean values of three replicates. [b] Total wt % of $AgNO_3$−TLC fractions.

contained some monounsaturated acids (band A, 1.8%; band B, 3.8%). On the other hand, band D (containing 65% saturated fatty acids) overlapped with the most unsaturated bands (E and F). The presence of C18: $2^{c,t-conj}$ in the SSM bands (1.25 and 0.85% in bands C and D, respectively; Table 1) has also been reported by Parodi (1980), who likewise found a higher concentration of this acid in the *trans*-SSM band (SSM^t) than in the *cis*-SSM band (SSM^c).

Comparison of bands C and D again showed a higher molar percentage of butyric acid in the latter (11.4%) than in the former (3.6%). This could mean that, in the present case, bands C and D separated in the same way as bands A and B. Laakso and Kallio (1993) also found differences (if smaller) in the butyric acid content of the SSM bands. Although it does not give data on the fatty acid composition of the bands, the paper by Myher et al. (1988) reports that the length of the short-chain fatty acid affected the SSMs separation, although this influence was less effective than in the case of the SSSs. However, this effect has not been found in other experiments, such as that of Parodi (1980), where the low, medium, and high molecular weight fractions were separated previously. According to the above-cited authors, the main cause of separation of the SSMs into two bands is the geometric configuration (cis or trans) of the unsaturated fatty acid. The retention time of SSM^ts is closer to that of SSSs and that of SSM^cs closer to that of SMMs, since trans-monounsaturated fatty acids form weaker π complexes than cis-monounsaturated do. According to that, the proportion of the band where the SSM^ts are located with respect to total SSMs should be close to the ratio of $C18:1^t$ to total C18:1 normally found in milk fat TGs (according to different authors, the trans isomer content is in the range of 2−11%). Our own data show that the ratios of $C18:1^t$ to total C18:1 in the milk fat and in the bands B and D were 13, 62, and 8.5%, respectively; in bands A and C measurable amounts of $C18:1^t$ were not found (Table 1). This may indicate that the great part of SSM^t is in band B, the rest of the SSM being distributed in similar proportions in bands C and D, which suggests that in the given experimental conditions the length of the chain was a basic factor in the separation of the SSMs.

The TGs in bands E and F were identified, respectively, as di- (SMM, SSD) and triunsaturates (SMD and MMM). Band E contained 40.3% monounsaturated fatty acids, predominantly C18:1 (87% of the total). It is not surprising that they should not have reached a higher percentage (close to the theoretical value of 66%) since it was to be expected that band E would contain some SSM^cs. On the other hand, some C18:2 (7.5%) and $C18:2^{c,t-conj}$ (1.3%) were also found in band E. Similar results on fatty acid composition of diunsaturated bands were obtained by other authors (Shehata et al., 1972; Taylor and Hawke, 1975; Parodi, 1980; Laakso and Kallio, 1993).

The F band (consisting of two very close together) contained 37% monounsaturated fatty acids, predominantly C18:1 (which accounted for 81% of the total), 10% C18:2 (including 0.8% $C18:2^{c,t-conj}$), and 3.7% C18:3. Thus, the highest theoretical value for triunsaturated TGs is about two-thirds, and this would mean that the other third was trisaturated, which is very unlikely. Other results indicate that this band may contain diunsaturate TGs (Myher et al., 1988; Kemppinen and Kalo, 1933) that would be mainly butyrates. According to that, the molar percentage of butyric acid in band F (6.2%) was higher than in band E (5.0%). On the other hand, as the TG content of the milk fat sample determined by TLC without $AgNO_3$ was 98.1%, other components [mainly mono- (MG) and diglycerides (DG)] are concentrated in band F, because they did not migrate on plates in the experimental conditions of the present work, increasing the saturated fatty acid presence in this band.

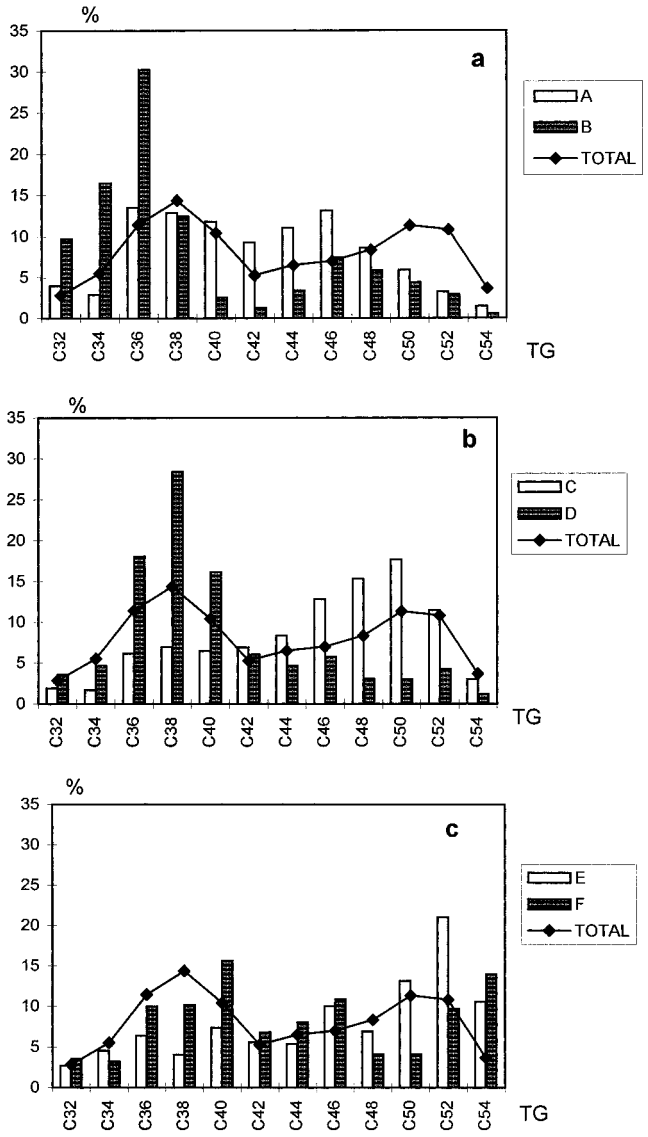

Figure 2. Distribution (mol %) of SSS (a), SSM (b), and other (c) triglycerides of milk fat according to their carbon number.

Saturated, monounsaturated, and diunsaturated TGs accounted, respectively, for 47, 33, and 10% of the total (Table 1), figures that were comparable to those reported by other authors (Parodi, 1981; Laakso and Kallio, 1993; Kemppinen and Kalo, 1993). In the present case [like Myher et al. (1988) and Kemppinen and Kalo (1993) and unlike Parodi (1980) and Laakso and Kallio (1993)], there was no separation between SM^cM^t and SM^cM^c TGs (Figure 1). This would suggest once again that the experimental conditions determine the extent to which the TGs are separated by their geometric configuration.

TG Composition of Fractions Obtained by Ag-NO_3-TLC. Figure 2 shows the distributions (molar percent) of the TGs in the milk fat and in bands A and B (Figure 2a), C and D (Figure 2b), and E and F (Figure

2c), obtained by $AgNO_3$-TLC and calculated from the GC data. Only TGs from C32 upward were considered in view of the very low percentages of C30 and below (close to or less than 1%). Distribution in the milk fat was bimodal, peaking at C38 and C50 (which, respectively, accounted for 14 and 11% of the total TGs considered).

Each of the six classes of TG from C36 to C46 in band A accounted for ~12% of the total, followed by C48 with 8.6% (Figure 2a). None of the rest (C32, C34, C50, C52, and C54) exceeded 4% individually. The distribution of the TGs in band B was quite different. C36 accounted for 30%, followed by C34 with 16.5%. C32 and C38 together accounted for ~22%. In other words, the sum of these four TGs made up 70% of the total, whereas from C40 upward, no individual TG accounted for >7%. Similarly, Myher et al. (1988) reported that the chromatogram for band B displayed no significant peaks beyond C40 (although this author was using a distillate with the more volatile fraction of milk fat).

Analysis of bands A and B together revealed a unimodal distribution of SSSs with a peak at C36 (average = 21.5%), confirming the findings of Laakso and Kallio (1993). There was a low proportion of long-chain SSSs (average = 16.5%) despite the high proportions of fatty acids with 16 and 18 carbon atoms that were detected in these bands (see Table 1). This result links up with the need to obtain TGs with the appropriate melting point (Gresti et al., 1993) to allow the fat to be secreted. For this reason most SSSs contain a short-chain fatty acid: C6, C8, or C10 in the case of band A and butyric acid in the case of band B. Thus, the sum of the fatty acid percentages in the saturated bands from C14 to C20 accounted for an average of 66% (Table 1).

The distribution of the SSMs was slightly bimodal (with peaks at C38 and C50), similar to that reported by Laakso and Kallio (1993). However, when bands C and D were analyzed separately, the distribution was clearly unimodal in band D, with a peak at C38, while there was a more even spread in band C. Both profiles resembled those derived for bands A and B (Figure 2b), which would be related to the importance of chain length in the separation of the SSMs in the given conditions. The proportion of short-chain TGs was lower in band C than in band D (16.7 and 54.7%, respectively). However, where separation of the SSMs is according to the isomeric configuration, the proportions of short-chain TGs in bands C and D were not so different (48 and 65%; Laakso and Kallio, 1993).

In band E (Figure 2c) the percentage of TGs was higher the longer the chain, with long-chain TGs accounting for 51.7% of the total. A higher degree of unsaturation offsets the effect of a longer chain on the melting point of TGs. As for band F, the distribution (Figure 2c) was bimodal, with peaks around C40 (15.6%) and C54 (14%).

Composition of Molecular Species of Main TGs. Figure 3 shows the chromatogram of the milk fat, and Table 2 shows the relative retention times (RRT) and molar percentages corresponding to the different peaks (a total of 113). Also, where possible the table indicates the band that each TG belongs to and provides a tentative identification. In these cases, the molar percentages of the peaks for individual $AgNO_3$-TLC bands are indicated. The unidentified peaks (NI) in each band are indicated but only when their molar

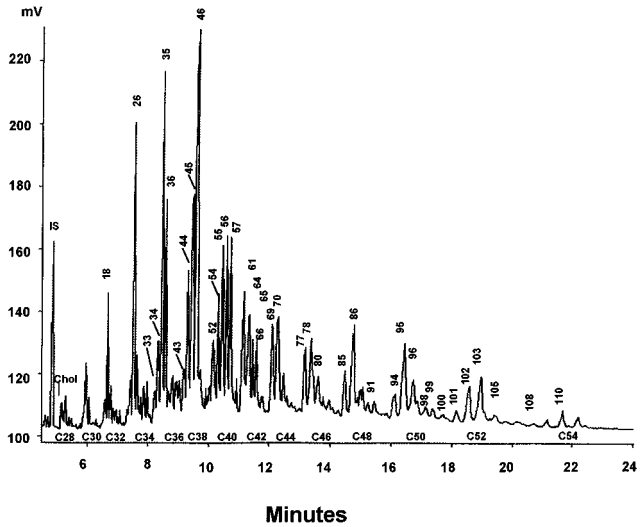

Figure 3. Capillary GC profile of triglycerides in milk fat. Peak numbers are identified in Table 2.

percentages multiplied by the mass proportion of the corresponding band were >0.1%.

The RRT of the majority peaks of each TG class was longer in band B than in band A (Table 2). Similarly, the majority peaks of the other bands exhibited longer RRTs in proportion to the degree of unsaturation of the corresponding TG.

The most important peak (46, C38) in quantitative terms (6.29%) was located predominantly in band D (57%; see Table 2) and hence corresponded to an SSM containing butyric acid: this was identified as 4,16,18:1, the most abundant TG in cow's milk, in agreement with Gresti et al. (1993), although it could contain a smaller proportion of the TG 4,18,16:1 (Myher et al., 1993). Four peaks exceeded 3% of the total content; two, peaks 35 (C36) and 45 (C38), were located in band B, which attain high molar percentages (16.4 and 8.5%, respectively). They were therefore SSSs containing butyric acid and were identified, respectively, as 4,16,-16 + 4,14,18 and 4,16,18. The other two peaks, 95 (C50) and 103 (C52), were located predominantly in bands C and E and were identified, respectively, as 16,16,18:1 and 16,18:1,18:1. This last was the most abundant TG in band E (16.1%).

There were seven peaks of over 2%, three of which were predominantly located in band B or D. They were thus identified as TGs containing butyric acid. Peak 26 (C34) was identified as SSSs (4,14,16 + 4,12,18), and peaks 36 (C36) and 56 (C40) were identified as SSMs (4,14,18:1 + 4,16,16:1 and 4,18,18:1, respectively). The other four, peaks 44 (C38), 55 (C40), 86 (C48), and 102 (C52), were predominantly located in band C and were identified as 8,14,16:1; 6,16,18:1; 14,16,18:1; and 16,-18,18:1, respectively. In these last four, however, there was considerable overlapping with TGs from the nearest saturated bands. Briefly, then, of the 12 most quantitatively important peaks, there were 6 TGs whose molecule contained butyric acid: 3 SSSs and 3 SSMs. The rest, except for peaks 44 and 55, were mainly formed by three long-chain fatty acids.

A further 25 peaks each accounted individually for >1% of the total; 44% of them were located in band A. There is no reference in the literature to symmetric TGs with C4−C12 fatty acids in milk fat, and the percentages of symmetric TGs with C14, C16, and C18 fatty acids were low (Gresti et al., 1993). Hence, peaks 33

Table 2. TG Composition (Molar Percent)[a] of Milk Fat and Location of TGs in the $AgNO_3$-TLC Bands According to Relative Retention Time (RRT) to C27

peak no.	CN[b]	RRT	mol (%)	$AgNO_3$-TLC bands				
				A	B	C and D	E	F
16	32	1.346	0.11	NI[c]				
17		1.360	0.25		NI			
18		1.376	1.12		4,12,16 + 4,14,14 (*4.44*)[d]			
19		1.397	0.36			4,10,18:1[e] + 4,12,16:1[e] (*1.77*)		
20		1.418	0.16					
21		1.435	0.25	NI		NI		
22		1.457	0.20					
23	33	1.481	0.10	NI				
24		1.507	0.22		NI			
25	34	1.529	0.61	NI	NI			
26		1.550	2.55		4,14,16 + 4,12,18 (*9.63*)	6,10,18:1 (*0.96*)		
27		1.575	0.60			4,14,16:1[e] + 4,12,18:1[e] (*2.55*)		
28	35	1.588	0.16	NI				
29		1.602	0.32		NI			
30		1.620	0.49		NI		NI	
31		1.643	0.48		NI			
32		1.665	0.19				NI	
33	36	1.696	0.56	8,14,14 + 8,12,16 + 10,12,14 (*2.46*)				
34		1.718	1.38	6,14,16 + 6,12,18 (*3.19*)	NI			
35		1.753	4.36		4,16,16 + 4,14,18 (*16.43*)	NI		
36		1.772	2.22			4,14,18:1[e] + 4,16,16:1[e] (*10.40*)		
37	37	1.792	0.46	NI		NI	4,16:1,16:1 (*3.27*)	
38		1.805	0.23		NI			NI
39		1.815	0.71		NI			
40		1.839	0.48		NI			NI
41		1.850	0.23			NI		
42		1.863	0.53			NI		
43	38	1.895	1.12	8,14,16 + 10,14,14 + 10,12,16 (*2.81*)				
44		1.921	2.12	6,16,16 + 6,14,18 (*5.41*)		8,14,16:1 (*1.57*)		
45		1.952	3.29		4,16,18 (*8.50*)	6,14,18:1 + 6,16,16:1 (*4.42*)		
46		1.980	6.29			4,16,18:1[e] + 4,18,16:1[e] (*21.4*)		
47		1.992	0.01				4,16:1,18:1 (*1.19*)	
48	39	2.012	0.06	NI				NI
49		2.020	0.09		NI			NI
50		2.045	0.43			NI		
51		2.069	0.67			NI		NI
52	40	2.089	1.09	10,14,16 + 12,14,14 (*3.01*)				NI
53		2.100	0.79	8,16,16 + 8,14,18 (*2.03*)				
54		2.128	1.60	6,16,18 (*2.69*)		8,14,18:1 + 10,14,16:1 (*2.91*)		
55		2.156	2.29	NI	4,18,18 (*1.30*)	6,16,18:1 (*6.78*)		
56		2.186	2.08	NI		4,18,18:1[e] (*7.94*)	NI	NI
57		2.212	1.86				4,18:1,18:1 (*3.17*)	NI
58		2.224	0.40				NI	
59		2.235	0.24					4,18:1,18:2 (*2.20*)
60	41	2.250	0.60	NI				
61	42	2.298	1.70	10,14,18 + 10,16,16 (*5.23*)				
62		2.310	0.44	NI		10,14,18:1 + 12,14,16:1 (*1.94*)		
63		2.324	0.61					
64		2.337	1.15			8,16,18:1 (*3.03*)		
65		2.362	1.02			6,18,18:1 (*1.70*)		NI
66		2.389	1.12				6,18:1,18:1 + 8,16:1,18:1 (*2.62*)	
67	43	2.427	0.57	NI				NI

Table 2. Continued

peak no.	CN[b]	RRT	mol (%)	$AgNO_3$-TLC bands A	B	C and D	E	F
68		2.458	0.24					
69	44	2.480	1.63	10,16,18 + 12,16,16 + 14,14,16 (*4.86*)				
70		2.534	1.40		NI			
71		2.543	0.76			10,16,18:1 (*4.18*)		
72		2.575	0.83				10,16:1,18:1 + 8,18:1,18:1 (*2.14*)	NI
73	45	2.595	0.50	NI	NI		NI	NI
74		2.631	0.46			NI		NI
75		2.652	0.37	NI				
76		2.685	0.39					NI
77	46	2.721	1.47	14,16,16 (*4.53*)				
78		2.762	1.57		NI	12,16,18:1 + 14,14,18:1 (*8.00*)		
79		2.779	0.60			10,18,18:1 (*0.70*)		
80		2.813	1.19				10,18:1,18:1 (*2.97*)	
81	47	2.846	0.65	NI			NI	
82		2.883	0.54	NI	NI	NI		NI
83		2.909	0.40	NI		NI		NI
84		2.950	0.43		NI		NI	
85	48	2.992	1.47	16,16,16 + 14,16,18 (*5.06*)	NI	NI		
86		3.048	2.34		NI	14,16,18:1 (*9.09*)		
87		3.070	0.39				12,18:1,18:1 (*1.95*)	
88		3.091	0.65				16,14:1,18:1 (*1.71*)	
89		3.115	0.85					
90		3.151	0.72					
91	49	3.196	1.11	NI		NI	NI	NI
92		3.252	0.25		NI			
93		3.278	0.38					
94	50	3.331	1.51	16,16,18 (*3.41*)				
95		3.397	3.63		NI	16,16,18:1 (*12.72*)		
96		3.459	1.88				14,18:1,18:1 (*8.20*)	
97		3.486	0.94			NI		NI
98		3.540	1.02					NI
99		3.592	1.05			NI		
100	51	3.658	1.06				15,18:1,18:1 (*1.53*)	
101	52	3.759	1.18	16,18,18 (*2.56*)		NI		
102		3.836	2.89		NI	16,18,18:1 (*6.67*)		
103		3.920	3.81				16,18:1,18:1 (*16.10*)	
104		3.977	0.29					
105		4.025	1.05					16,18:1,18:2 (*6.32*)
106		4.093	0.53					
107		4.177	0.85				NI	
108	54	4.283	0.46	18,18,18 + 16,18,20 (*1.25*)				
109		4.383	0.69			18,18,18:1 (*2.30*)		
110		4.488	1.37				18,18:1,18:1 (*7.92*)	
111		4.593	0.76					18:1,18:1,18:1 (*10.28*)
112		4.633	0.20				NI	
113		4.749	0.12					18,18:1,18:2 (*1.85*)

[a] Mean values of three replicates. [b] Carbon number. [c] NI, no identified TGs (see text). [d] In parentheses are the molar percentages in each band of TGs identified. [e] Recovered mainly in band D.

(C36), 61 (C42), 85 (C48), and 108 (C54) had to include TGs with other fatty acid combinations. The most quantitatively important peaks located predominantly in band E (besides 103, noted above) were 57 (C40), 66 (C42), 96 (C50), 100 (C51), and 110 (C54): these were respectively identified as 4,18:1,18:1; 6,18:1,18:1 + 8,-16:1,18:1; 14,18:1,18:1; 15,18:1,18:1; and 18,18:1,18:1, and their respective molar percentages were 3.2, 2.6,

8.2, 1.5, and 7.9%. Last, there were two peaks of >1% located in band F, 98 (C50) and 105 (C52), the second of which could correspond to 16,18:1,18:2, which was the most abundant TG in band F (10.28%).

The recovery percentages of some molecular species of TGs (deduced from Table 2) were lower than would expected, mainly due to some peaks of the chromatogram of milk fat being located in more than one TLC band. However, some of the peaks in the band F (111 and 113) showed recoveries >100%. These results are a consequence of the presence in this band of other components besides triunsaturated TGs such as MG and DG (discussed above) which increase the relative mass proportion of band F with respect to the others. On the other hand, several peaks of chromatograms of individual bands contained more than one molecular species. Because of this, most of the TGs from the milk fat could not be quantified as molecular species.

In Figure 3, a number of quantitatively small peaks can be seen at the end of each TG molecular class, which were attributed to the presence of odd-chain TGs. These were basically the result of replacement of one of the even-chain fatty acids by C15 or C17, which were the principal odd-chain fatty acids identified in the milk fat (Table 1); these were not detected upward of C51. After each TG with an even number of carbon atoms and after the peaks located in the unsaturated bands in each class of TG, there was a new sequence (Table 2) starting with the location of a peak in one of the saturated bands, which corresponds to TGs with odd numbers of carbon atoms.

According to Table 1, the percentage of odd-chain fatty acids in the milk fat reached molar 4.7%, and hence the proportion of odd-chain TGs could (in theory) be near 14%, which is within the range of values (from 11.6 to 15%) reported by Maniongui et al. (1991). However, the data from Table 2 could not be used to calculate their actual quantitative importance because these peaks generally overlapped with other more unsaturated TGs of the previous class. Only peak 100 (C51), which exceeded 1%, was located solely in band E. This was identified as 15,18:1,18:1.

On the other hand, the odd-chain SSSs accounted for 15.8 and 15.2%, respectively, in bands A and B; these data are close to the theoretical values (18 and 16%, respectively; see Table 1) and confirm the value (16%) reported for the saturated band by Laakso and Kallio (1993). The distribution of odd-chain TGs in the saturated bands was bimodal, with two peaks located at C37 and C47 (32 and 28%, respectively). A peak in the region of C37−C39 has also been reported by Laakso and Kallio (1993), in both the saturated and the monounsaturated bands.

Conclusions. In the experimental conditions, the distribution of TGs in milk fat, classified according to their degree of unsaturation and chain length, was successfully quantified using a combination of the $AgNO_3$-TLC technique and capillary GC. Also, the saturated TGs (and to a lesser extent the monounsaturated TGs) were differentiated according to the length of the short-chain fatty acids into butyrates and all the others. The most important quantitatively were TGs containing butyrate. Odd-chain TGs were also identified, accounting for 15−16% of the total in the case of saturated TGs.

Finally, progress was made with regard to quantification of long-chain TGs. From C44 upward there were 16 peaks of >1%, important among which were those corresponding to polyunsaturated TGs. However, taking into account that, barring exceptional cases, the TGs responsible for the peaks in the milk fat chromatogram contained more than one species, the techniques used need to be complemented to quantify molecular species.

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