The Composition of New Zealand Milk Fat Triacylglycerols by Reversed-Phase High-Performance Liquid Chromatography

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ABSTRACT: A reversed-phase high-performance liquid chromatography (HPLC) method was developed for the purpose of analyzing milk fat triacylglycerols by evaporative light scattering detection. With a binary solvent system, comprising dichloromethane and acetonitrile, the method allowed a separation of 61 distinct peaks, on the basis of chainlength and number of double bonds. Triacylglycerols of differing partition numbers were clearly resolved, and the resolution between peaks of the same partition number was high. An argentation thin-layer chromatography method, separating triacylglycerols on the basis of chainlength and degree of unsaturation, provided nine band extracts that were analyzed by HPLC. By using existing fatty acid methyl ester data of these bands, an identity for each HPLC peak has been proposed.

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KEY WORDS: Argentation, HPLC, milk fat, partition number, triacylglycerols.

Milk fat is one of the most complex naturally occurring fats or oils because of the wide variety of fatty acids it contains. It has been reported to contain almost 400 different fatty acid groups (1,2), and although the distribution of these is not random, it is generally accepted that the number of distinct triacylglycerols arising from such a multitude of possible combinations is of the order of thousands.

It is currently impractical to measure the level of every chemical entity within this mixture, but some forms of chemical analysis, such as high-performance liquid chromatography (HPLC) in the reversed-phase mode, have provided opportunities to investigate the triacylglycerols in more detail.

Several methods involving reversed-phase HPLC have resolved milk fat into 50 or more chromatographic peaks (3–12). Typically, the solvent system contained a weak component, acetonitrile, and a strong component, either chloroform (9), acetone (8), isopropanol (10), propionitrile (12), dichloromethane/dichloroethane (4:1) (7), ethanol/hexane (4), water (13), or *tert*-butyl methyl ether (14). The stationary phase was one or a combination of bonded-phase C_{18} columns. An excellent recent review of reversed-phase methods (15) has provided a summary of the current possibilities. Many of the results have been detailed and informative, particularly if some further analysis followed, such as mass spectrometry (10–12), or gas chromatography. Gresti *et al*. (8) collected 49 peak fractions from HPLC, and measured the relative concentrations of triacylglycerols within a fraction by both fatty acid composition and carbon number.

The separation that occurs during reversed-phase chromatography of triacylglycerols is based on both chainlength and number of double bonds. A common expression that describes the influence of the two factors is

$$PN = CN - (a' \times DB) \quad [1]$$

where PN is the partition number, CN the acyl carbon number, DB the number of double bonds, and a' is 2 (16). The expression arose from the frequent observation (5,6,8,16,17) that a double bond reduces peak retention time by the same amount as the loss of two carbon atoms, thereby grouping in close proximity a trisaturate, its CN + 2 monoenoic analog, its CN + 4 dienoic analog, etc. Where peaks of the same PN are resolved, the separation is often mirrored in other PN groups. This results in a periodicity of peaks that greatly assists identification. It was recently noted (18) that the influence of a double bond is often less than two carbon atoms, but in other reports (4,19), it was clearly more (in the latter, a' was calculated as 2.12 and 2.15 for earlier and later parts of the gradient, respectively). Both the solvent scheme and the column are probable dominant factors in the magnitude of a'. In the past, PN values were integers that applied to individual peaks; with the increased resolution of modern HPLC columns, they must either expand to include nonintegers or be applied to peak groups.

An absolute measure of the concentration of individual triacylglycerols in complex mixtures remains difficult, in part because good separation has not been achieved. No report of HPLC separation of milk fat can yet claim that peaks contain single triacylglycerols in a pure state, and this has been emphasized by previous reports in which some individual signals contained up to 10 distinct triacylglycerols, excluding regioisomers and enantiomers (6,8,12). Even where a high degree of separation has been obtained, some detection methods have fallen short by virtue of being nonlinear, being of low sensitivity, or requiring a wide variety of unavailable standards. Some researchers have tried to address this by modeling the detector response to pure triacylglycerols, followed by deconvolution, and have produced some meaningful absolute concentrations (19,20).

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An alternative approach, especially useful for those peaks that contain a combination of saturates and unsaturates, has been to use a prior separation technique, such as argentation thin-layer chromatography (TLC), followed by HPLC of the resulting fractions. This approach, where argentation HPLC was the prior separation technique, was described by Laakso *et al*. (7) and Laakso and Kallio (11).

The aim of this study was to conduct a detailed investigation into the triacylglycerols of New Zealand milk fat. We report here a new reversed-phase HPLC method with a simple binary solvent system. In addition, an analysis by HPLC of bands from argentation TLC is described, which provides a means to identify the components of many of the peaks of the reversed-phase chromatogram.

EXPERIMENTAL PROCEDURES

Samples and reagents. All solvents (BDH, Poole, United Kingdom) were of HPLC grade and were used as received. Helium and nitrogen (oxygen-free grade) were supplied by BOC Gases (Palmerston North, New Zealand). A monoacid triacylglycerol standard mixture, containing equal masses of tricaprin, trilaurin, trimyristin, tripalmitin and tristearin, and a triolein standard were obtained from Nu-Chek-Prep Inc. (Elysian, MN). The triacylglycerol standards 16:0,18:1,18:1; 16:0,16:0,18:1; 18:0,16:0,18:1; and 16:0,16:0,4:0 were synthesized and were proven by fatty acid methyl ester (FAME) analysis. An anhydrous milk fat (AMF) sample was supplied by Bay Milk Products (Edgecumbe, New Zealand). The fat was a typical October AMF from the 1993–1994 season. A 10-mg/mL solution was prepared by melting the sample (1.00 g) and dissolving it in chloroform (100 mL). Filtration through a cotton plug into an autosampler vial gave a clear, pale yellow solution. The injection volume for a total milk fat sample was 5 µL. Argentation TLC band samples were recovered from a TLC plate after chromatography of an AMF (10 mg) sample by a previously reported method (21). Each band extract was redissolved in hexane (1 mL), filtered through a cotton plug, evaporated to dryness, and reconstituted in chloroform (0.5 mL). The HPLC injection volume of Ag-TLC band samples was 10 µL.

HPLC. Reversed-phase chromatography was performed on an LC Module 1 Plus (Waters Associates, Milford, MA) that included a quaternary gradient pumping system and low-pressure mixer. The chromatography column was a Waters Nova-Pak™ C18, 4 µm, 3.9 × 150 mm, with a precolumn module that contained inserts of the same solid phase. The column temperature was maintained at 20°C by water circulated from a bath to a column jacket. Triacylglycerols were detected with a Varex MkIII evaporative light-scattering detector (ELSD) (Alltech Associates Inc., Deerfield, IL). Nitrogen gas was supplied to the ELSD at a pressure of 80 psi, and the nitrogen flow at the nebulizer was maintained at 2.00 standard L/min (SLPM) to generate a nebulizer gas pressure of 20 psi. The solvent pressure at the nebulizer was typically 2–3 psi. The ELSD drift tube temperature was set at 96°C. Peak data, collected at a sampling rate of 2 points/s, were acquired through a BUS/LACE interface with Millennium 2010 software (Version 2.15, Waters Associates, Milford, MA). The mobile-phase gradient program is shown in Table 1.

TABLE 1
Mobile-Phase Gradient Program Used for Triacylglycerol Separation[a]

Time (min)	Flow (mL/min)	Solvent (%) A	Solvent (%) B
0	1.00	90	10
20	1.00	90	10
100	1.00	65	35
110	1.00	50	50
115	1.00	50	50
115.1	1.00	90	10
125	1.00	90	10

[a]3.9 × 150 mm, 4 µm Nova-Pak™ C18 column (Waters Associates, Milford, MA). A = acetonitrile, B = dichloromethane. The mobile-phase composition changed linearly between the steps listed.

RESULTS AND DISCUSSION

A chromatogram of the reversed-phase HPLC separation of a typical New Zealand milk fat is shown in Figure 1. The trace contains 61 peaks, and the separation showed improvement when compared to recent reports (12,19); the resolution of peaks of the same PN was close to the baseline, and peaks from neighboring PN groups were not obviously overlapping. We also note that in some analogous methods (3,12,19), two columns in series were used, where we have found that one column of 15 cm length was sufficient to obtain the separation shown.

The use of column temperature control, provided by a 20°C recirculating water bath linked to a column jacket, gave excellent reproducibility of peak retention times (Fig. 2). This allowed for more consistent analysis and reduced time wastage during data processing. Changes in ambient conditions can result in retention time drift (18).

Retention time reproducibility was also enhanced by the employment of a simple binary solvent system because neither solvent (acetonitrile or dichloromethane) was altered, mixed, or diluted prior to the HPLC pump mixing chamber; the proportioning of two neat solvents was completely controlled by instrumentation. The volatility problems sometimes encountered with dichloromethane (18) were not a problem if that solvent was continually sparged with helium.

To aid the identification of triacylglycerols in the total milk fat, argentation TLC was performed, and each of the resulting nine bands was recovered and analyzed by reversed-phase HPLC. The Ag-TLC method has previously been described (21) and was based on a report by Dallas and Padley (22). The method gave a new separation of fully saturated and *cis* monoene milk fat triacylglycerols. In particular, those triacylglycerols that contained one $C_{4:0}$ were distinguishable from those containing a $C_{6:0}$, and both groups were separated from the $C_{8:0}$ and higher triacylglycerols. This length separation was very reproducible for the trisaturated species, but less so

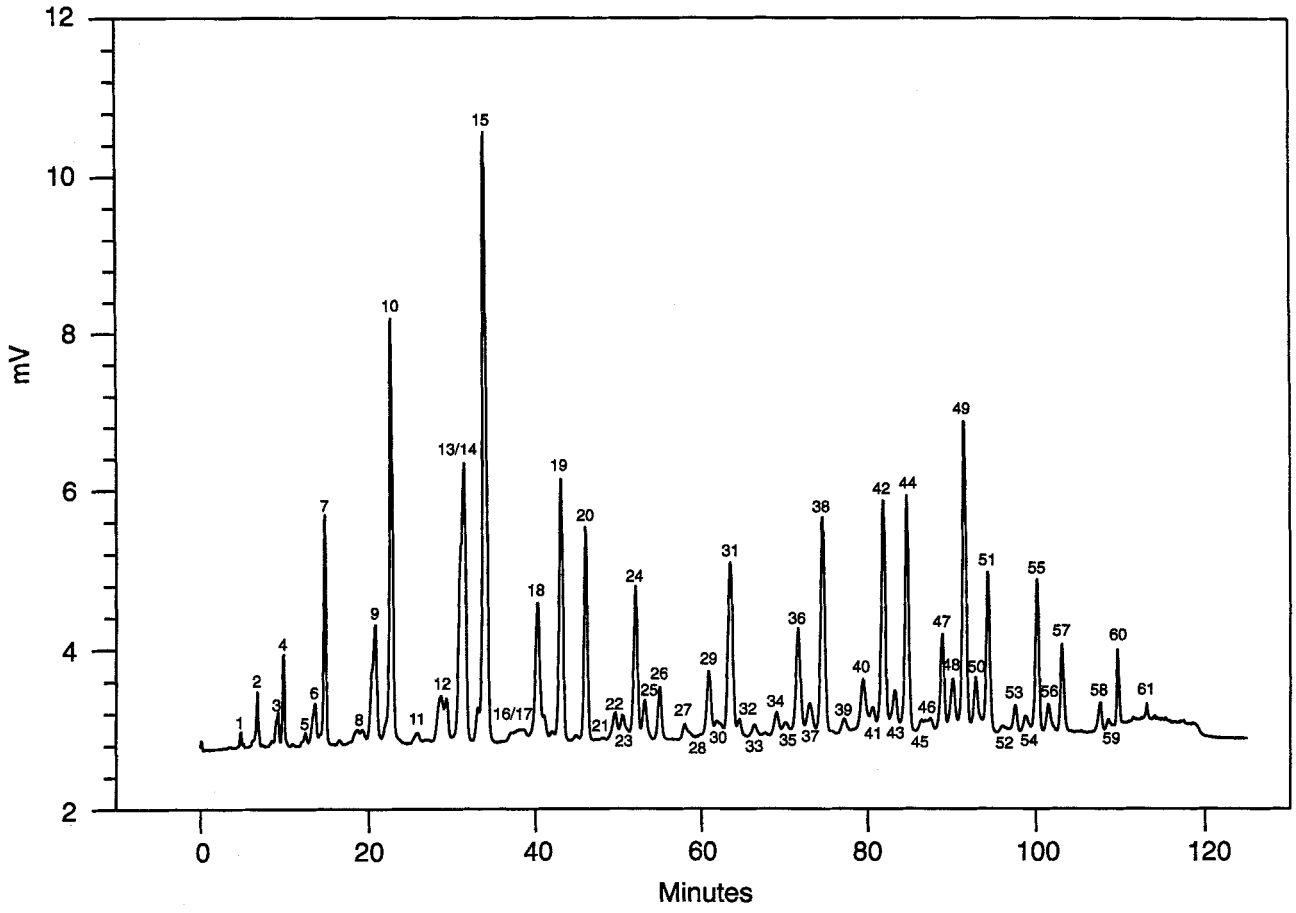

FIG. 1. Reversed-phase high-performance liquid chromatography (HPLC) trace of a typical anhydrous milk fat. Details of the gradient program are described in the Experimental Procedures section.

for the monoenes; the $C_{4:0}/C_{6:0}$ separation was less distinct. For the *cis,cis* dienes, no length separation was observed. To our knowledge, no comparable Ag-TLC separation of total milk fat triacylglycerols has been described elsewhere.

In addition to the injections of Ag-TLC bands, some commercial and synthesized triacylglycerol standards were injected to identify HPLC peaks. An injection of a mixture of the triacylglycerol standards, tricaprin, trilaurin, trimyristin, tripalmitin, and tristearin, is shown in Figure 3. The mixture contained 20% by weight of each triacylglycerol, but it was evident from Figure 3 that the peak areas and peak shapes were not identical (the area ratios were

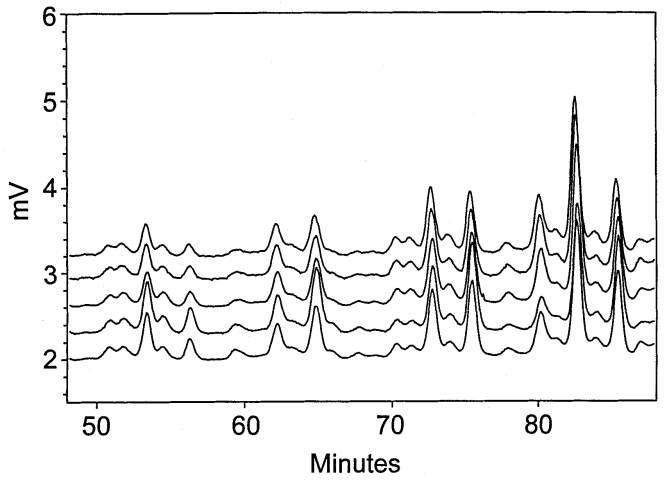

FIG. 2. A portion of the HPLC trace depicting reproducibility of retention times of five different fat samples. See Figure 1 for abbreviation.

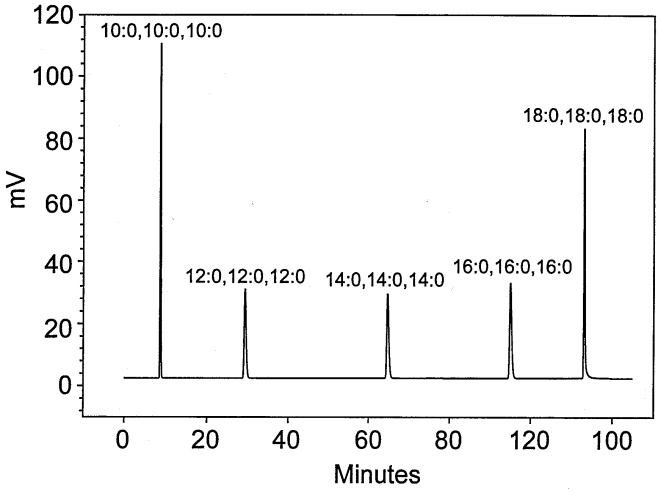

FIG. 3. HPLC chromatogram of Nu-Chek-Prep (Elysian, MN) standard triacylglycerols. For abbreviation see Figure 1.

1.57:0.95:0.93:1.04:1.65), indicating a difference in response factors. A synthesized standard of 16:0,18:1,18:1 coincided with peak 47, 16:0,16:0,18:1 coincided with peak number 49, 18:0,16:0,18:1 with peak number 55, and 16:0,16:0,4:0 showed good agreement with peak number 15 (Fig. 1).

Similarly, injections of all Ag-TLC bands allowed a reasonable assignment of triacylglycerol identities in the reversed-phase HPLC of the total fat. For example, the isolation of an Ag-TLC band (band 7), known to contain trisaturates of the type sat'd, sat'd, 4:0 (21), gave a series of six peaks, the strongest of which was coincident with peak number 15 (Fig. 4). It can reasonably be concluded that the others in the band 7 series are two-carbon additions or subtractions to this dominant triacylglycerol. PN groups appeared to have remained distinct in the reversed-phase separation, and it became clear that considerable separation within each PN group had occurred, even for triacylglycerols of the same CN and number of double bonds. For example, injections of Ag-TLC bands 7 and 8, which contained such compounds as 16:0,16:0,4:0 and 16:0,14:0,6:0, respectively (and other trisaturates of like type), gave two distinguishable series of peaks, despite identical CN and PN (Fig. 4).

Similarly, the triacylglycerols of band 9 were separable in the reversed phase from both bands 7 and 8. The chromatogram of band 9 also indicated that, at lower CN, some triacylglycerols of identical CN containing $C_{8:0}$ and $C_{10:0}$, such as 16:0,14:0,10:0 and 16:0,16:0,8:0, could be separated, as evidenced by peak numbers 24 and 25. In this region of the reversed-phase chromatogram, a consistent order of elution was therefore observed, in which for a given CN and PN, $(x,y)_n$,10:0 eluted before, in ascending order: $(x,y)_{n+2}$,8:0, $(x,y)_{n+4}$,6:0, and $(x,y)_{n+6}$,4:0. However, these separations were mostly lost above $C_{10:0}$; that is, 10:0,16:0,18:0 was not distinguishable from 12:0,16:0,16:0. A similar phenomenon has been reported by Gresti *et al*. (8), although their separation was lost above $C_{8:0}$. For *cis* monoenes in this same region of the trace, a similar pattern was observed, as indicated by peak numbers 22, 23 and 24, all of which were *cis* monoenes of identical carbon number. This separation and the length separation of short *cis* monoenes obtained from Ag-TLC (bands 3 and 5) are shown in Figure 5.

As noted earlier, a double bond reduces the retention time of a given triacylglycerol by about two carbon atoms (16). The magnitude of these factors as observed in the present study was as follows: a trisaturate of total length 50 (16:0,16:0,18:0) eluted at 99.6 min, and the monoene analog 16:0,16:0,18:1 eluted at 88.3 min. The two-carbon analog below 16:0,16:0,18:0, namely, 16:0,16:0,16:0, eluted at 91.0 min. Thus, it appeared that a double bond had a greater effect than the loss of two carbons.

Some bands from Ag-TLC contained more than one set of structurally related triacylglycerols. For example, band 7 contained trisaturates of the type *x,y*,4:0, as noted above, but it also contained a set of signals that coincided with peak numbers 37, 43, 50, 56, and 59. From the higher retention times, these could not have been *x,y*,4:0 trisaturates. Injection of a synthesized *trans* monoene standard (18:0,18:1*t*,18:0) gave a single peak that was coincident with peak number 59, and FAME data from the band indicated that the $C_{18:1}$ content was all *trans* (21): the two results together indicated that the series 37, 43, 50, 56, and 59 was a set of *trans* monoenes. The resolution of a *trans* monoene signal within each partition number group is of particular note, because of the current interest in the nutritional effects of *trans* fatty acids (23).

A similar argument followed for *cis,trans* dienes. In band 3 from Ag-TLC, there occurred a series of peaks that were coincident with peak numbers 35, 41, 48, and 54 and were not related to the other peaks (numbers 3, 6, 9 and 13) of the band. Based on arguments about their higher mobility in Ag-TLC than the *cis,cis* dienes, it was postulated that these were *cis,trans* dienes. A pictorial summary of the general assignment within groups of PN ≥ 44 is shown in Figure 6.

Band 1 from Ag-TLC contained the most unsaturated triacylglycerols and gave only a weak set of signals when ana-

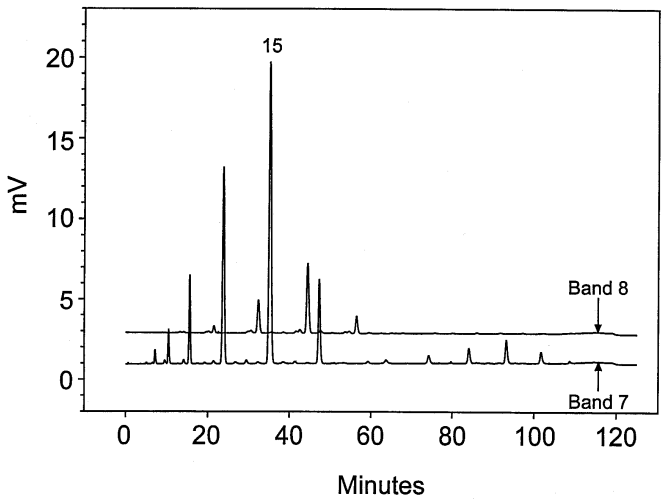

FIG. 4. Overlaid chromatograms of the triacylglycerols of bands 7 and 8 from argentation thin-layer chromatography (Ag-TLC).

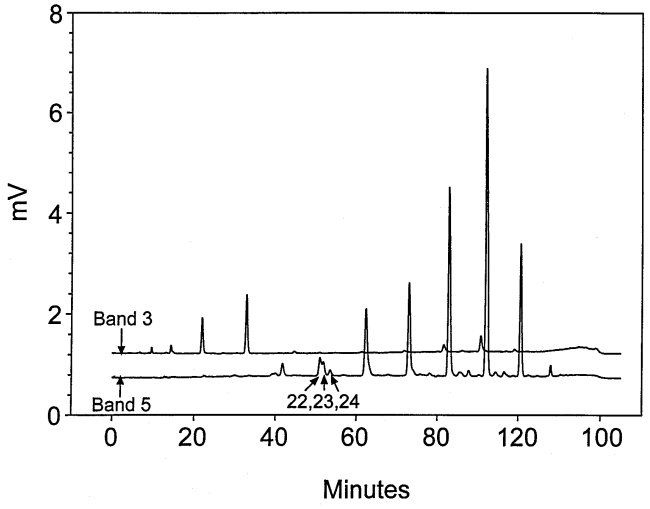

FIG. 5. Overlaid chromatograms of triacylglycerols of Ag-TLC bands 3 and 5. For abbreviation see Figure 4.

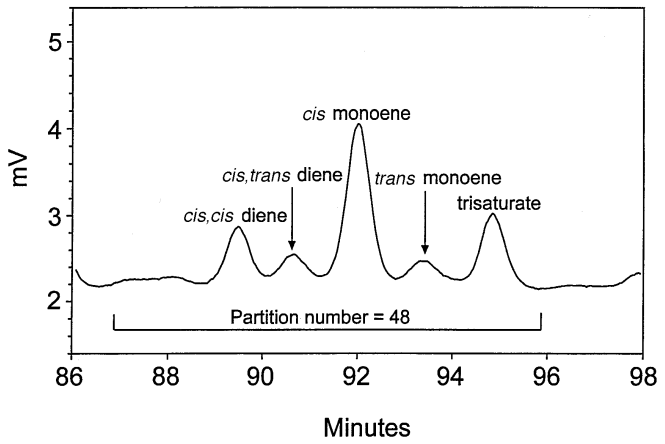

FIG. 6. General assignment of triacylglycerol groups, as applied to partition number of 48.

lyzed by reversed-phase HPLC. Other than peak number 45, which was found to be coincident with a triolein standard, no clear pattern of signals emerged from this band. FAME data (21) indicated that the band was high in $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$, but the observed signals suggested that the triacylglycerols that incorporated these fatty acid groups were minor in relation to the signals from other bands.

A summary of the general groups of triacylglycerols from each Ag-TLC band, as confirmed by reversed-phase HPLC, is given in Table 2. A summary of assignments obtained after injections of all Ag-TLC bands is given in Table 3.

It has been suggested (12) that different retention times for equivalent PN peaks might represent differences in location or geometry of double bonds, rather than in positional distribution of the fatty acid groups. This was also our observation. The less intense series of peaks from band 7, identified as *trans* monoenes, were clearly distinguishable from the *cis* monoenes in a chromatogram of the total fat, but the peaks of bands 5 and 6, separable by Ag-TLC and understood to be regioisomers of *cis* monoenes, were coincident. It is emphasized that although this applies to all the proposed triacylglycerols in Table 3, there is a known positional distribution in milk fat (2), and this is reflected in our description of triacylglycerols such as *x,y*,4:0 and *x,y*,6:0.

The methodology discussed in the present study will provide opportunities to investigate in more detail the structure of milk fat and its variation with season. The ability of the method to differentiate samples to a high level of compositional detail will also lead inevitably to a relationship with physical properties, such as solid fat content or butter hardness. This relationship will ultimately benefit consumers of milk fat, as it will enable better product consistency and functionality.

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REFERENCES

1. Jensen, R.G., A.M. Ferris, and C.J. Lammi-Keefe, The Composition of Milkfat, *J. Dairy Sci. 74*:3228–3243 (1991).
2. Creamer, L.K., and A.K.H. MacGibbon, Some Recent Advances in the Basic Chemistry of Milk Proteins and Lipids, *Int. Dairy J. 6*:539–568 (1996).
3. Stolyhwo, A., H. Colin, and G. Guiochon, Analysis of Triglycerides in Oils and Fats by Liquid Chromatography with the Laser Light Scattering Detector, *Anal. Chem. 57*:1342–1354 (1985).
4. Herslöf, B., and G. Kindmark, HPLC of Triglycerides with Gradient Elution and Mass Detection, *Lipids 20*:783–790 (1985).
5. Maniongui, C., J. Gresti, M. Bugaut, S. Gauthier, and J. Bezard, Determination of Bovine Butterfat Triacylglycerols by Reversed-Phase Liquid Chromatography and Gas Chromatography, *J. Chromatogr. 543*:81–103 (1991).
6. Bornaz, S., G. Novak, and M. Parmentier, Seasonal and Regional Variation in Triglyceride Composition of French Butterfat, *J. Am. Oil Chem. Soc. 69*:1131–1135 (1992).
7. Laakso, P.H., K.V. Nurmela, and D.R. Homer, Composition of the Triacylglycerols of Butterfat and Its Fractions Obtained by an Industrial Melt Crystallization Process, *J. Agric. Food Chem. 40*:2472–2482 (1992).
8. Gresti, J., M. Bugaut, C. Maniongui, and J. Bezard, Composition of Molecular Species of Triacylglycerols in Bovine Milk Fat, *J. Dairy Sci. 76*:1850–1869 (1993).
9. Kermasha, S., S. Kubow, M. Safari, and A. Reid, Determination of the Positional Distribution of Fatty Acids in Butterfat Triacylglycerols, *J. Am. Oil Chem. Soc. 70*:169–173 (1993).
10. Myher, J.J., A. Kuksis, and L. Marai, Identification of the Less Common Isologous Short-Chain Triacylglycerols in the Most

TABLE 2
Groups of Triacylglycerols Present in Bands from Ag-TLC[a]

Band from Ag-TLC	Triacylglycerol groups present, *x, y, z*
1	Trienes and higher unsaturates
2	*cis,cis* Dienes
3	*cis* Monoenes of type $z = C_{4:0}$, $x + y < 36$; *cis,trans* dienes
4	*cis* Monoenes of type $z = C_{4:0}$ or $C_{6:0}$
5	*cis* Monoenes of type *x, y* and $z > 4$; trace of $C_{6:0}$
6	*cis* Monoenes, regioisomers of band 5
7	Trisaturates of type *x* and $y > 8$, $z = C_{4:0}$, $x + y > 20$; *trans* monoenes
8	Trisaturates of type *x* and $y > 8$, $z = C_{6:0}$, $x + y > 24$
9	Trisaturates of type *x, y,* and $z > 6$, CN > 36

[a]Ag-TLC, argentation thin-layer chromatography; CN, acyl carbon number.

TABLE 3
Summary of Proposed Triacylglycerols of Individual Peaks from High-Performance Liquid Chromatography[a]

Peak No.	Typical RT at 20°C (min)	Estimated CN	Estimated PN	Suggested triacylglycerols
1	5.08	26	28	14:0,8:0,4:0; 12:0,10:0,4:0
2	7.15	28	28	16:0,8:0,4:0; 14:0,10:0,4:0
3	9.84	32	30	10:0,18:1,4:0
4	10.55	30	30	18:0,8:0,4:0; 16:0,10:0,4:0; 14:0,12:0,4:0
5	13.37	36?	32	—
6	14.60	34	32	12:0,18:1,4:0
7	15.81	32	32	14:0,14:0,4:0; 16:0,12:0,4:0
8	20.63	38	34	18:2,16:0,4:0?
9	22.22	34 and 36	34	16:0,12:0,6:0; 18:1,14:0,4:0
10	24.24	34	34	16:0,14:0,4:0
11	27.44	42	36	—
12	30.33	40	36	18:1,18:1,4:0; 18:1,14:0,6:0
13/14	33.20	38	36	18:1,16:0,4:0; 16:0,14:0,6:0; 18:0,12:0,6:0
15	35.80	36	36	16:0,16:0,4:0; 18:0,14:0,4:0
16	39.95	40	38	18:1,12:0,10:0
17	40.43	40 and 42	38	18:1,18:1,6:0; 18:1,14:0,8:0
18	42.05	38 and 40	38	16:0,14:0,8:0; 16:0,12:0,10:0; 18:1,16:0,6:0
19	44.87	38 and 40	38	18:0,18:1,4:0; 16:0,16:0,6:0; 18:0,14:0,6:0
20	47.93	38	38	18:0,16:0,4:0
21	49.84	44	40	18:1,18:1,8:0
22	51.45	42	40	18:1,14:0,10:0
23	52.33	42?	40	18:1,16:0,8:0
24	53.93	40 and 42	40	18:1,18:0,6:0; 16:0,14:0,10:0
25	55.03	40	40	16:0,16:0,8:0; 18:0,14:0,8:0
26	56.84	40	40	18:0,16:0,6:0
27	59.98	40	40	18:0,18:0,4:0
28	60.15	46	42	18:1,18:1,10:0
29	62.82	44	42	18:1,14:0,12:0; 18:1,16:0,10:0; 18:1,18:0,8:0
30	63.65	44	42	18:1*t*,16:0,10:0
31	65.45	42	42	16:0,16:0,10:0; 18:0,14:0,10:0; 16:0,14:0,12:0
32	66.44	42	42	18:0,16:0,8:0
33	68.23	42	42	18:0,18:0,6:0
34	70.85	48	44	12:0,18:1,18:1
35	71.83	48	44	12:0,18:1,18:1*t*
36	73.37	46	44	16:0,12:0,18:1; 14:0,14:0,18:1; 10:0,18:0,18:1
37	74.58	46	44	16:0,12:0,18:1*t*; 14:0,14:0,18:1*t*; 10:0,18:0,18:1*t*
38	76.08	44	44	16:0,14:0,14:0; 16:0,16:0,12:0; 18:0,16:0,10:0; 18:0,14:0,12:0
39	78.67	47?	45?	17:0,12:0,18:1?; 15:0,14:0,18:1?
40	80.83	50	46	14:0,18:1,18:1
41	81.88	50	46	14:0,18:1,18:1*t*
42	83.30	48	44 and 46	14:0,16:0,18:1; 16:0,16:0,18:2; 12:0,18:0,18:1
43	84.63	48	46	14:0,16:0,18:1*t*; 12:0,18:0,18:1*t*
44	86.13	46	46	16:0,16:0,14:0; 12:0,16:0,18:0; 14:0,14:0,18:0
45	88.23	54	48	18:1,18:1,18:1
46	88.43	49?	47?	17:0,14:0,18:1?; 15:0,16:0,18:1?
47	90.02	52	48	16:0,18:1,18:1
48	91.23	52	48	16:0,18:1,18:1*t*
49	92.60	50	48	16:0,16:0,18:1; 14:0,18:0,18:1
50	94.03	50	48	16:0,16:0,18:1*t*; 14:0,18:0,18:1*t*
51	95.45	48	48	18:0,16:0,14:0
52	97.08	51?	49?	17:0,16:0,18:1?; 15:0,18:0,18:1?
53	98.48	54	50	18:1,18:1,18:0
54	99.88	54	50	18:1,18:1*t*,18:0
55	101.18	52	50	18:1,18:0,16:0
56	102.48	52	50	18:1*t*,18:0,16:0
57	104.18	50	50	16:0,16:0,18:0; 14:0,18:0,18:0
58	108.23	54	52	18:1,18:0,18:0
59	109.17	54	52	18:0,18:0,18:1*t*
60	110.24	52	52	18:0,18:0,16:0
61	113.63	54	54	18:0,18:0,18:0

[a]RT, retention time; CN, acyl carbon number; PN, partition number.

Volatile 2.5% Molecular Distillate of Butter Oil, *Ibid. 70*:1183–1191 (1993).
11. Laakso, P., and H. Kallio, Triacylglycerols of Winter Butterfat Containing Configurational Isomers of Monoenoic Fatty Acyl Residues. I. Disaturated Monoenoic Triacylglycerols, *Ibid. 70*:1161–1171 (1993).
12. Spanos, G.A., S.J. Schwartz, R.B. van Breemen, and C.-H. Huang, High-Performance Liquid Chromatography with Light-Scattering Detection and Desorption Chemical-Ionization Tandem Mass Spectrometry of Milk Fat Triacylglycerols, *Lipids 30*:85–90 (1995).
13. Lee, T.W., Quantitative Determination of Medium Chain Triglycerides in Infant Formula by Reverse Phase HPLC, *J. Am. Oil Chem. Soc. 63*:317–320 (1986).
14. Barron, L.J.R., M.T.G. Hierro, and G. Santa-Maria, HPLC and GLC Analysis of the Triglyceride Composition of Bovine, Ovine and Caprine Milk Fat, *J. Dairy Res. 57*:517–526 (1990).
15. Laakso, P., Analysis of Triacylglycerols—Approaching the Molecular Composition of Natural Mixtures, *Food Rev. Int. 12*:199–250 (1996).
16. Litchfield, C., *Analysis of Triglycerides,* Academic Press, New York, 1972.
17. Plattner, R.D., G.F. Spencer, and R. Kleiman, Triglyceride Separation by Reverse Phase High Performance Liquid Chromatography, *J. Am. Oil Chem. Soc. 54*:511–515 (1977).
18. Christie, W.W., Reversed-Phase High Performance Liquid Chromatography of Triacylglycerols, *Lipid Technology* (November):140–142 (1996).
19. Ruiz-Sala, P., M.T.G. Hierro, I. Martinez-Castro, and G. Santa-Maria, Triglyceride Composition of Ewe, Cow and Goat Milk Fat, *J. Am. Oil Chem. Soc. 73*:283–293 (1996).
20. Felinger, A., Deconvolution of Overlapping Skewed Peaks, *Anal. Chem. 66*:3066–3072 (1994).
21. Robinson, N.P., and A.K.H. MacGibbon, Separation of Milk Fat Triacylglcerols by Argentation Thin-Layer Chromatography, *J. Am. Oil Chem. Soc. 75*:783–788 (1998).
22. Dallas, M.S., and F.B. Padley, Analysis of Confectionery Fats. I. Separation of Triglycerides by Silver Nitrate Thin-Layer Chromatography, *Lebensm.-Wiss. Technol. 10*:328–331 (1977).
23. Gurr, M., *Trans* Fatty Acids Revisited—Is There a Health Problem? *Lipid Technol.* (September):111–113 (1995).

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