Separation of Milk Fat Triacylglycerols by Argentation Thin-Layer Chromatography

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ABSTRACT: Argentation thin-layer chromatography was investigated as a method of obtaining detailed compositional information about milk fat. A modified argentation thin-layer chromatography procedure, developed to optimize the separation of the complex mixture of total milk fat triacylglycerols, provided nine different groups of triacylglycerols, based on both the degree of unsaturation and the total length of fatty acid groups. Fatty acid methyl ester (FAME) analysis was performed to determine the composition of each band. Separation on the basis of chainlength was most pronounced among the fully saturated triacylglycerol groups, as evidenced by the high level of $C_{4:0}$ and $C_{6:0}$ in bands 7 and 8, respectively. For the *cis*-monoenoic triacylglycerols, the separation of $C_{4:0}$ and $C_{6:0}$ was less distinct. The *cis,cis* dienes and other dienoic, trienoic, or tetraenoic species were principally observed in two bands of retention factor <0.08 on the chromatography plate. Minimal cross-contamination of bands was observed, with the exception of the lowest of the trisaturate bands, band 7, in which *trans*-monoenes were found to be present. Three samples from different points of the New Zealand dairy season were separated by argentation thin-layer chromatography, and their FAME distributions were measured. In addition to differences in the masses of band extracts from these samples, levels of $C_{10:0}$ and $C_{12:0}$ in all bands, and levels of monounsaturates in the dienoic and trienoic bands, were found to differ. These changes were generally consistent with a pattern of decreasing fat hardness over the November to March period of a typical dairy season.
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KEY WORDS: Argentation, length separation, milk fat, seasonal variation, thin-layer chromatography, triacylglycerols, unsaturation.

The physical properties of milk fat depend to a large extent on the chemical composition. It is important to have methods that provide detailed information on the chemical composition of milk fat to understand the factors that affect the performance of milk fat products. Unlike many vegetable oils, milk fat has a limited range of unsaturated fatty acids but a wide range of fatty acid chainlengths ($C_{4:0}$–$C_{18:0}$). Thus, both the degree of unsaturation and chainlength are important in determining physical properties. The aim of this study was to develop a method to separate and quantitate the various types of milk fat triacylglycerols.

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Argentation thin-layer chromatography (Ag-TLC) is a technique by which this separation may be achieved [see, for example, Lund (1)]. Triacylglycerols that bear unsaturated fatty acid groups are retarded owing to their interaction with silver ions impregnated in the solid phase. The nature of the silver ion/double bond interaction (2,3) is twofold: π-electrons in a filled olefin orbital overlap with an empty silver *s* orbital, and silver *d*-electrons overlap with vacant antibonding orbitals on the olefin. Some recent data (4) indicated that the carbonyl moiety of an esterified fatty acid also interacts with silver ions.

There are two critical steps in the preparation of an Ag-TLC plate: impregnation of the solid phase with silver ions and choice of mobile phase. Mobile phases need to be varied with the nature of the sample because some samples require a stronger mobile phase if they are high in oleic, linoleic, or linolenic acid groups. Differences in plate manufacture arise largely from researchers' individual preferences or practice. The silver ions are incorporated either when the solid phase is being slurried (5), or postmanufacture by way of plate immersion (6) or spraying (7,8). Each treatment has its advantages, although immersion and spraying techniques appear to be used much less frequently.

Although many published methods in this area have dealt with simple triacylglycerol mixtures and vegetable oils, few have addressed the more challenging complexity of milk fat triacylglycerols. Lund (1), Shehata *et al.* (5), Parodi (9), Myher *et al.* (10), and Taylor and Hawke (11) have reported milk fat separations by Ag-TLC, but these were generally of purified milk fat fractions, not total milk fat. Only Lund (1) reported an analysis of a sample of total milk fat, and in that instance, total carbon number, but not fatty acid methyl ester (FAME), data were acquired from three Ag-TLC bands.

The goal of this study was to establish a single Ag-TLC technique that would give a reliable, distinct, and meaningful separation of triacylglycerol groups from total milk fat, but that did not require prior fractionation into high-, medium-, and low-molecular weight samples (5,9,11). In the establishment of that technique, an Ag-TLC procedure, developed for confectionery fats (6), was found to provide an interesting separation by length and unsaturation of milk fat triacylglycerols. A second goal was to characterize the triacylglycerols from the recovered Ag-TLC bands by profiling their fatty acid groups. Once the technique was established, we sought to

apply it to some samples from distinct points of the Southern Hemisphere dairy season, to test both the reliability of the method and its ability to measure seasonal composition changes.

EXPERIMENTAL PROCEDURES

TLC plates (EM Science, Darmstadt, Germany) were glass-backed and precoated with silica gel 60C, 20 cm × 20 cm × 0.25 mm. All solvents (BDH, Poole, United Kingdom) were of Analar grade, with the exception of chloroform [high-performance liquid chromatography ((HPLC)-grade, alcohol-free], and were used as received. Silver nitrate (BDH Analar), dichloro-(*R*)-fluorescein, cupric sulfate (as the pentahydrate, BDH, 98.5%+) and phosphoric acid (BDH Analar, 85% min) were also used as received. Water was of milli-Q grade, supplied by a New Zealand Dairy Research Institute installation.

Anhydrous milk fat samples were supplied by Anchor Products (Edgecumbe, New Zealand). The fats were from October, November, February, and March of the New Zealand 1993/1994 dairy season. The fat from October was used for all developmental experiments of the Ag-TLC procedure. The other three fats were run in a separate experiment after the Ag-TLC procedure had been developed. The triacylglycerol standards PPB, PStP, OOP, POP and PPO (P, palmitoyl group; B, butyroyl group; St, stearoyl group; O, oleoyl group) were synthesized and proven by FAME analysis. Triolein was obtained from Nu-Chek-Prep Inc. (Elysian, MN). Plates were impregnated according to the procedure of Dallas and Padley (6). Ethanol (100 mL) was added to an aqueous solution (100 mL) of silver nitrate (20.0 g). The solution was poured into a plastic tray (20 cm × 26 cm × 5 cm), and the tray was tilted such that all solution was at one end. While taking care to avoid wetting, a chromatography plate was placed in the tray. The tray was leveled in such a way that the solution quickly and completely immersed the plate. Immersion was maintained for 30 s. The plate was lifted out and allowed to drain to one corner, and then left to stand, vertically, in a darkened fume hood for 1 h. The plate was activated for 1 h at 110°C and stored in a desiccator.

A milk fat sample (1 mg), dissolved in chloroform (0.1 mL), was loaded onto the TLC plate with a Desaga AS 30 TLC sample applicator (Desaga GmbH, Heidelberg, Germany). Triacylglycerol standards were loaded similarly from solutions of 0.5 mg in chloroform (0.1 mL). The eluent, chloroform/toluene (1:1, vol/vol), was charged to the development tank 1 h prior to plate development to ensure vapor equilibration. Development usually took 1 h over a distance of 18 cm. After development, the plate was dried in a well-ventilated fume hood for at least 1 h. Where the bands were to be analyzed later by FAME, a larger quantity of sample, of the order of 8–10 mg, was loaded across the entire plate width.

Plates were visualized by one of two methods. For those instances where recovery of individual bands was required, the plate was sprayed with a solution of dichloro-(*R*)-fluorescein in methanol (0.1%, wt/vol) and inspected under an ultraviolet lamp at 350 nm. For instances where a visible light image was required, the plate was immersed for approximately 1 s in a solution of cupric sulfate (10%, wt/vol) in aqueous phosphoric acid (8%) (12) and heated in a thermal oven for 35 min at 180ºC. As is often the case with charring, minor bands were revealed in addition to the eight bands routinely observed with fluorescein.

The resolved bands, numbered 1 (at the bottom of the plate) to 9 (at the top), were recovered by scraping off the visualized areas with a scalpel, and extracting the silica with a mixture of diethyl ether/hexane (4:1, vol/vol, 2 × 2 mL). The filtered, evaporated residues were weighed to five decimal places of a gram. Addition of the individual band masses to calculate the recovery of the initial sample mass always gave 90% or better. FAME analysis of each band was undertaken according to the procedure of Christopherson and Glass (13) by using sodium methoxide in methanol as the *trans*-methylating agent and an aqueous solution of potassium dihydrogen orthophosphate (10%, wt/vol) and sodium chloride (15%, wt/vol) as the neutralizing agent. Recommendations (14) regarding handling of samples that contain short fatty acids were also followed. Because of the small sample size, quantities of reagents were scaled down in proportion to the amount of fat reported in Reference 13; thus, the mass of band extracts was between 0.6 and 1.6 mg, and 1/50 of the quantities from the original method was typically used.

RESULTS AND DISCUSSION

Plate preparation. A number of published procedures were attempted during the development of an Ag-TLC procedure for the analysis of total milk fat triacylglycerols. Results obtained from plates prepared by the method of Shehata *et al.* (5) and Parodi (9) were acceptable, in that the retention factors (R_f) and band strengths were reproducible, but the overall sharpness of individual bands was inferior. The method of Dallas and Padley (6) gave a total milk fat separation that was superior to other methods. In particular, the impregnation method made a difference to the quality of the results, after a number of other impregnation methods, attempted with the same brand of silica gel plates, gave results that were inferior to those of Dallas and Padley (6). It was also observed that plates prepared according to Reference 6 did not undergo photoreductive darkening, before or after chromatography, as quickly as those prepared by the other methods described.

Mobile phase. The common practice of including acetic acid (up to 1%) and either methanol or ethanol (up to 1%) in the mobile phase was found to be detrimental to the separation. Traces of acetic acid caused bunching of the lowest R_f bands, elevating them to a higher R_f than was desired. Methanol or ethanol promoted all unsaturated bands to a higher R_f, sometimes to the point of overlap with trisaturated bands. The mobile phase of chloroform/toluene (1:1) proved to be superior to other eluent mixtures. A particular feature of the chloroform/toluene solvent mixture was that it accentuated the

separation of the saturated triacylglycerols at the expense of the most unsaturated triacylglycerols. It was subsequently found that a good separation of the most unsaturated triacylglycerols could be obtained, if desired, with the eluent mixture toluene/acetonitrile (99:1, vol/vol). This gave a total of 14 bands, but the eight at lowest R_f were mostly minor in mass, and this separation was achieved at the expense of resolution of the trisaturate bands. Thus, for the purposes of all further experiments, the former mobile phase (chloroform/toluene, 1:1) was employed.

Argentation chromatography results. An Ag-TLC separation is shown in Figure 1. Although it is generally accepted that R_f values are highly variable (4), it was observed here that the R_f of the most mobile bands were reproducible to within 0.04, and the sharpness of individual bands was better than results obtained by other methods.

Charring with cupric sulfate/phosphoric acid, a sensitive visualization reagent (12), revealed 10 bands. Between 3 and 13 bands have been reported elsewhere (1,15). The three bands at highest R_f (numbered 7–9) appeared to be well separated from the next strongest band, 5, and the traces of material between the two was later designated band 6, after it was visualized with cupric sulfate/phosphoric acid. A second pattern of three bands, numbered 3–5, was evident between R_f 0.14 and 0.26. Finally, below R_f 0.14, there was a weak band (not numbered) and two bands of medium intensity. Band 6 and the weak band between bands 2 and 3 were not readily visualized by fluorescein. Thus, the latter weak band was routinely collected as part of band 3, and a reasonable estimate had to be made as to the boundaries of band 6 during recovery, most often by taking all material between bands 5 and 7.

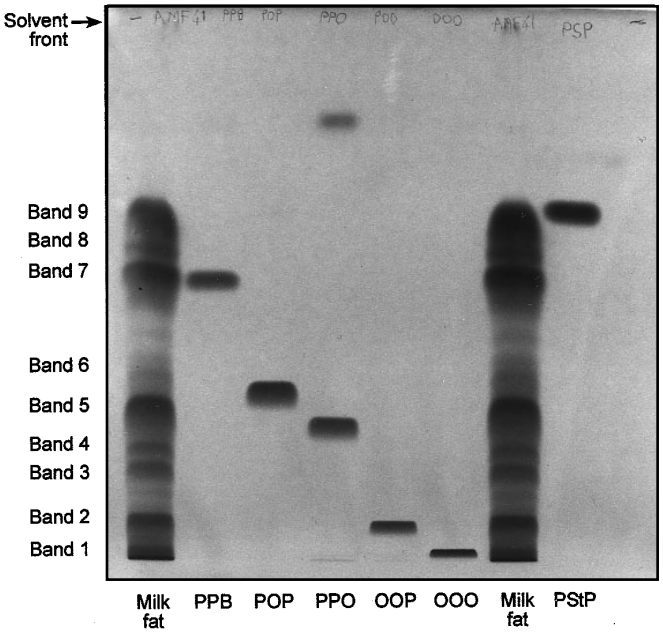

FIG. 1. Argentation thin-layer chromatography separation of October 1993 milk fat, PPB, POP, PPO, OOP, OOO, October milkfat again and PStP (P, palmitoyl group; B, butyroyl group; St, stearoyl group; O, oleoyl group).

The measured masses of each band are provided in Table 1. No band mass was significantly larger than the others, although bands 4 and 6 were consistently the weakest. Of note was the additive mass of the proposed trisaturate bands, over 40%. *cis*-Monoenes accounted for another 40%, and the proposed dienes and other unsaturates accounted for 20%.

Based on a commonly held order of elution (15), it is a reasonable expectation that the uppermost band, or bands, will be trisaturates, and that the remaining bands, in order of descending R_f, will be *trans*-MSS, *cis*-SMS, *cis*-MSS, *cis*,*trans*-MMS, *cis*,*cis*-MMS, DSS, MMM, DMS and, if detected, DMM, DDS, and MDD/TSS (M, monoenoic acyl group; S, saturated acyl group; D, dienoic acyl group; T, trienoic acyl group).

The identity of the triacylglycerol group, or groups, in each band was obtained by the use of some commercial and synthesized standards. From Figure 1, the standards PPB and PStP gave good agreement with bands 7 and 9, respectively. The OOP standard was coincident with band 2, OOO with band 1, and the standards POP and PPO, although well resolved from each other, gave only a fair agreement with bands 6 and 5, respectively.

It was therefore provisionally indicated from visual inspection of the Ag-TLC plate that a reasonable separation had been achieved on the basis of the degree of unsaturation. It also appeared that an interesting separation of short-long-long from long-long-long trisaturates (bands 7–9) may have occurred, of a type similarly described by Myher *et al.* (10) on Ag-TLC plates, or by Adlof (16,17) on dual Ag-HPLC columns, providing a useful differentiation of milk fat. Data from a FAME analysis of each Ag-TLC band of milk fat, expressed as mole percentages, are shown in Table 2.

Trisaturates. Bands 9, 8 and 7, the triacylglycerols at highest R_f on the plate, were almost exclusively composed (>93%) of saturated fatty acid groups. Furthermore, there was a separation on the basis of chainlength between these three bands, as detailed in Table 2 and summarized in Tables 3 and 4. Indeed, the high incidence of $C_{4:0}$ in band 7 (24.0 mol%) and its almost exclusive esterification in milk fat at position *sn*-3 on the glycerol backbone (18) would seem to indicate that band 7 was over 70% composed of triacylglycerols of the type S-S-4:0. Similarly, band 8 was highly enriched in $C_{6:0}$ (19 mol%), indicative of trisaturates of the type S-S-6:0. Band

TABLE 1
Mass Percentage of Individual Bands Recovered from Ag-TLC[a]

Band number	R_f	Mass, as percentage of total fat recovered
1	0.01	10.0
2	0.07	10.7
3	0.15	8.9
4	0.19	8.0
5	0.26	13.6
6	0.33	7.5
7	0.49	16.2
8	0.53	9.5
9	0.60	15.6

[a]Ag-TLC, argentation thin-layer chromatography.

TABLE 2
FAME Analysis (mol%) of Milk Fat Ag-TLC Bands[a]

	Band 1	Band 2	Band 3	Band 4	Band 5	Band 6	Band 7	Band 8	Band 9
$C_{4:0}$	7.1	5.1	19.9	10.2	1.5	11.6	24.0	1.2	0.0
$C_{6:0}$	3.4	3.3	3.5	9.9	5.0	5.5	4.6	19.0	1.9
$C_{8:0}$	0.0	1.8	0.0	3.9	2.3	0.0	1.2	6.0	3.4
$C_{10:0}$	2.8	3.3	2.8	3.8	5.2	3.3	3.0	7.1	9.3
$C_{10:1}$	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$C_{12:0}$	2.7	3.1	3.2	3.1	4.9	3.7	4.5	5.7	8.4
$C_{14:0}$	6.5	7.7	9.1	7.2	11.4	9.4	13.3	13.6	18.0
$C_{14:1}$	2.3	2.1	1.8	3.3	1.2	0.0	0.6	0.0	0.5
$C_{15:0}$	0.0	0.8	0.0	1.1	1.3	1.3	1.4	1.5	2.0
$C_{16:0}$	13.1	16.2	15.2	15.7	23.1	19.5	29.1	27.4	33.9
$C_{16:1}$	3.8	3.6	3.1	2.7	2.8	2.7	0.9	1.0	0.9
$C_{17:0}$	0.0	0.0	0.0	0.0	0.7	0.0	0.8	1.3	1.6
$C_{17:1}$	0.9	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0
$C_{18:0}$	3.7	5.1	3.6	5.1	8.3	8.5	10.2	8.6	14.4
$C_{18:1}$	26.7	33.7	27.8	20.3	24.8	18.7	1.5	0.0	0.0
$C_{18:2}$	4.9	1.9	3.5	4.0	1.7	0.0	1.2	2.0	0.0
$C_{18:3}$	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$C_{18:2c}$[b]	2.3	1.9	2.6	3.9	1.4	4.5	1.3	2.1	0.9
O.S.[c]	3.7	2.6	2.7	4.1	2.8	4.7	1.8	2.0	4.0
O.U.[d]	7.1	7.8	1.2	1.7	1.2	6.6	0.6	1.5	0.8

[a]Results are reported as normalized mol% of band. FAME, fatty acid methyl ester. See Table 1 for other abbreviation.
[b]c, conjugated linoleic acid.
[c]Other unidentified FAME, proposed to be saturates.
[d]Other unidentified FAME, proposed to be unsaturates.

9 contained little of either $C_{4:0}$ (not detected) or $C_{6:0}$ (1.9 mol%) and probably contained the remaining longer trisaturates. To our knowledge, the resolution of S-S-4:0 and S-S-6:0 bands from milk fat represents a degree of separation by Ag-TLC not commonly obtained, although a band of trisaturates containing S-S-4:0 triacylglycerols was obtained by Myher *et al.* (10). There is evidence that a chainlength separation occurs on an untreated silica plate (10,19), and a similar separation is clearly obtained during fractionation on a silica column (9,11,18). On the basis of a report (4) of interactions between carbonyl oxygen atoms of esterified fatty acids and silver ions, it might be proposed that silver ions played a role in the length separation observed here, perhaps enhancing an existing interaction with the uncapped silanol groups of the silica phase (16,17). The extent of separation may be controlled by the greater steric availability of the carbonyl oxygen of a $C_{4:0}$ fatty acid group than that of a $C_{6:0}$ fatty acid group, and both may be more accessible than those of $C_{8:0}$ and higher.

Monoenes. Bands 6, 5, 4, and 3 are similarly summarized in Table 5. The incidence of $C_{18:1}$ and $C_{4:0}$ in band 6, together with the R_f agreement of this band with a POP standard, indicated that it contained regio-isomers of bands 5, 4, and 3; that is, it contained SMS, but with little length separation. The FAME results of bands 3, 4, and 5 indicated a separation of $C_{4:0}$- and $C_{6:0}$-containing *cis*-monoenes from those of greater length, but the separation between $C_{4:0}$ and $C_{6:0}$ was not as distinct as for bands 7–9: band 3 was highly enriched in $C_{4:0}$, but band 4 contained similar amounts of both $C_{4:0}$ and $C_{6:0}$.

Polyenes. Bands 2 and 1 contained more highly unsaturated fatty acid groups, i.e., elevated levels of $C_{18:2}$ and $C_{18:3}$ as well as a high level of $C_{18:1}$. The levels of $C_{18:2c}$ appeared

TABLE 3
FAME Analysis (wt%) of Milk Fat Ag-TLC Bands[a]

	Band 1	Band 2	Band 3	Band 4	Band 5	Band 6	Band 7	Band 8	Band 9
Short[b]	4.2	4.4	9.8	11.8	4.3	7.2	13.9	14.9	3.0
Medium[c]	4.2	4.8	5.1	5.7	7.7	5.7	7.1	11.2	13.9
Long[d]	28.0	33.3	34.7	38.1	49.7	47.9	70.9	65.2	79.7
Total saturated	36.4	42.5	49.6	55.6	61.7	60.8	91.8	91.3	96.6
Total unsaturated	63.6	57.5	50.4	44.4	38.3	39.2	8.2	8.7	3.4
Monounsaturated	39.6	44.1	41.1	32.3	33.3	25.7	3.9	1.2	1.4
Polyunsaturated	15.7	4.4	7.6	9.9	3.6	5.5	3.4	5.5	1.0

[a]Fatty acid group summary of results reported as normalized wt% of band.
[b]Short-chain saturated fatty acid groups were $C_{4:0}$, $C_{6:0}$, and $C_{8:0}$.
[c]Medium-chain saturated fatty acid groups were $C_{10:0}$ and $C_{12:0}$.
[d]Long-chain saturated fatty acid groups were $C_{14:0}$–$C_{18:0}$. See Tables 1 and 2 for other abbreviations.

TABLE 4
Summary of Fatty Acid Groups in Bands 7–9

Fatty acid group(s)	Distribution
$C_{4:0}$	Almost exclusively confined to band 7
$C_{6:0}$	Majority confined to band 8, small amount in band 7
$C_{8:0}$	Band 8 was major band, significant amounts in band 9, less in band 7
$C_{10:0}$–$C_{12:0}$	Band 9 > band 8 > band 7
$C_{14:0}$–$C_{18:0}$	Spread among all three bands

high across all bands, a result that we have been unable to fully explain. Whereas bands 3–5 contained predominantly MSS triacylglycerols, bands 1 and 2 appeared to contain triacylglycerols with two or more double bonds, that is, groups such as MMS and MSM were located in band 2, and groups such as MMM, DMS, DSS, DSM, and TSS were located in band 1.

Band 7 contained a detectable amount of $C_{18:1}$. After further FAME analysis of this band, we found that the $C_{18:1}$ was entirely *trans*-isomers. It was therefore proposed that band 7 was composed of both S-S-4:0 trisaturates and *trans*-monoenes. Subsequent attempts to separate the two groups by taking finer slices of band 7 were unsuccessful.

Seasonal samples. Seasonal changes in the composition of New Zealand dairy fats impact directly on processability and on the ability of fat products to meet customer needs. The New Zealand dairy industry is principally seasonal and pasture-based, with strong grass growth in the spring. Calving is timed to occur just before this strong growth period, and milk flow during a Southern Hemisphere season generally peaks in October/November. To obtain a measure of the seasonal change in fat composition, three samples from different points of the New Zealand season, November 1993, February 1994, and March 1994, were subjected to the Ag-TLC procedure, and the masses and FAME distributions of the resulting bands were determined. The band mass results are shown in Figure 2, indicating changes in the relative concentration of each band as a percentage of the total sample. Of note from Figure 2 was the observation that the most unsaturated bands, 1 and 2, and the main *cis*-monoene band, 5, generally increased in mass from November to March, and that a general decrease was observed for the trisaturate bands, 7–9.

TABLE 5
Summary of Fatty Acid Groups in Bands 3–6

Fatty acid group(s)	Distribution
$C_{18:1}$	Evenly distributed across bands 3–6, slightly more in band 3
$C_{4:0}$	Low in band 5, intermediate in bands 6 and 4, high (20 mol%) in band 3
$C_{6:0}$	Intermediate in bands 3, 5 and 6, higher in band 4
$C_{14:0}$–$C_{18:0}$	Even across bands 3, 4 and 6, but generally higher in band 5

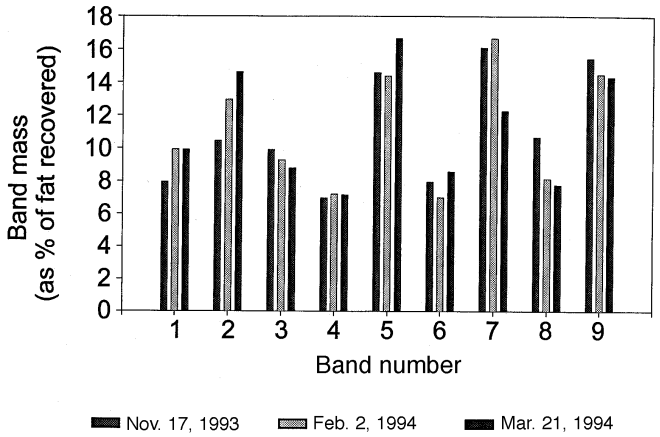

FIG. 2. Mass percentages of individual bands recovered from argentation thin-layer chromatography of seasonal samples.

Some fatty acids that showed a moderate change, when argentation bands from the three seasonal samples were compared, are depicted in Figures 3 and 4. The monounsaturated fatty acid groups ($C_{10:1}$, $C_{14:1}$, $C_{16:1}$, $C_{17:1}$, and $C_{18:1}$) displayed an increasing trend over the November to March period in bands 1–3, whereas the medium-chain saturated fatty acid groups ($C_{10:0}$ and $C_{12:0}$) showed a consistent decrease over the same period for the entire range of bands. This change in $C_{10:0}$ and $C_{12:0}$ appears to have been at the expense of long-chain saturated fatty acid groups. For the other fatty acid groups, either no significant change in FAME distribution between samples occurred, as was seen for the short-chain saturated fatty acid groups (4:0, 6:0, and 8:0), or there was a change with no clear trend, as was seen for the polyunsaturated fatty acids ($C_{18:2}$, $C_{18:3}$, and $C_{18:2c}$). These latter observations suggest that the chromatography had been performed consistently.

With regard to the trends in fatty acid distribution within a band, some fatty acids, such as $C_{4:0}$, clearly played a critical part in band separation, in which case the percentage of that

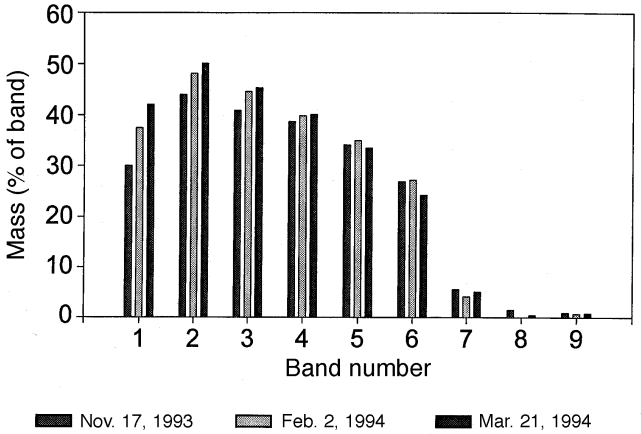

FIG. 3. Fatty acid methyl ester analysis of bands from seasonal milk fats. Monounsaturated fatty acids ($C_{10:1}$, $C_{14:1}$, $C_{16:1}$, $C_{17:1}$, and $C_{18:1}$) reported as normalized mass percentage of band.

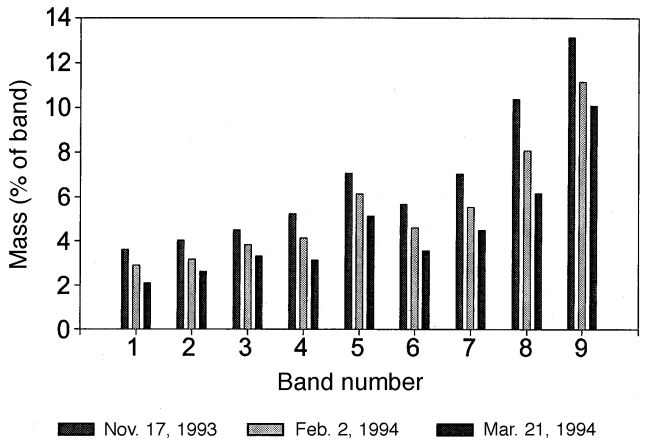

FIG. 4. Fatty acid methyl ester analysis of bands from seasonal milk fats. Medium-chain saturated fatty acids ($C_{10:0}$ and $C_{12:0}$) reported as normalized mass percentage of band.

fatty acid in a band would not be expected to vary between samples. For such a band (band 7 is an example), the percentage of that fatty acid is largely defined by the separation. Other fatty acids, if they do not contribute to the band separation, would show a trend (possibly throughout all bands), and it should be in keeping with that shown in the bulk fat. We propose that this was true for $C_{10:0}$ and $C_{12:0}$. Their decreasing trend from November to March was confirmed by Gray (20) and Taylor and Hawke (11).

Both the band mass and FAME distribution measurements appear to agree with our reasonable expectations of the compositional changes throughout a season. That is, fat hardness peaks around December/January, and from there onward it gradually decreases (21). Concomitant with decreasing hardness, one would expect to see a decrease in fully saturated triacylglycerols, as was observed in the mass percentages of bands 7–9, and an increase in the unsaturated triacylglycerols, as was observed in both the mass percentages of bands 1 and 2, and the higher proportion of monunsaturates in bands 1–3.

In summary, milk fat triacylglycerols were divided into nine bands with compositions indicative of distinct groups. No prior separation or fractionation of the milk fat triacylglycerols was required. Analysis of three seasonal samples indicated increases in dienoic and trienoic triacylglycerols and decreases in trisaturated triacylglycerols that were consistent with decreasing fat hardness throughout the period November to March. The method allows for a high degree of separation prior to other analyses, or it can be used to characterize the type of separation provided by other techniques, such as reversed-phase HPLC.

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REFERENCES

1. Lund, P., Analysis of Butterfat Triglycerides by Capillary Gas Chromatography, *Milchwissenschaft 43*:159–161 (1988).
2. Dewar, M.J.S., A Review of the π-Complex Theory, *Bull. Soc. Chim. Fr. 18*:C71–C79 (1951).
3. Morris, L.J., Separations of Lipids by Silver Ion Chromatography, *J. Lipid Res. 7*:717–732 (1966).
4. Christie, W.W., Silver Ion and Chiral Chromatography in the Analysis of Triacylglycerols, *Prog. Lipid Res. 33*:9–18 (1994).
5. Shehata, A.A.Y., J.M. DeMan, and J.C. Alexander, Triglyceride Composition of Milk Fat: II. Separation of Triglycerides by Argentation-TLC and GLC, *Can. Inst. Food Sci. Technol. J. 5*:13–21 (1972).
6. Dallas, M.S.J., and F. B. Padley, Analysis of Confectionary Fats I—Separation of Triglycerides by Silver Nitrate Thin-Layer Chromatography, *Lebensm. Technol. 10*:328–331 (1977).
7. Wilson, R., and J.R. Sargent, High-Resolution Separation of Polyunsaturated Fatty Acids by Argentation Thin-Layer Chromatography, *J. Chromatogr. 623*:403–407 (1992).
8. Inomata, M., F. Takaku, Y. Nagai, and M. Saito, Assay for Polyunsaturated Fatty Acids by Argentation-Thin-Layer Chromatography Using Commercial Thin-Layer Plates, *Anal. Biochem. 125*:197–202 (1982).
9. Parodi, P.W., Separation of Milk Fat Triglycerides into Classes by Silver Ion Adsorption Thin-Layer Chromatography, *Aust. J. Dairy Technol. 35*:17–22 (1980).
10. Myher, J.J., A. Kuksis, L. Marai, and P. Sandra, Identification of the More Complex Triacylglycerols in Bovine Milk Fat by Gas Chromatography–Mass Spectrometry Using Polar Capillary Columns, *J. Chromatogr. 652*:93–118 (1988).
11. Taylor, M.W., and J.C. Hawke, The Triacylglycerol Compositions of Bovine Milkfats, *N.Z. J. Dairy Sci. Technol. 10*:40–48 (1975).
12. Bitman, J., and D.L. Wood, An Improved Copper Reagent for Quantitative Densitometric Thin-Layer Chromatography of Lipids, *J. Chromatogr. 5*:1155–1162 (1982).
13. Christopherson, S.W., and R.L. Glass, Preparation of Milk Fat Methyl Esters by Alcoholysis in an Essentially Nonalcoholic Solution, *J. Dairy Sci. 52*:1289–1290 (1969).
14. Badings, H.T., and C. de Jong, Glass Capillary Gas Chromatography of Fatty Acid Methyl Esters. A Study of Conditions for the Quantitiative Analysis of Short- and Long-Chain Fatty Acids in Lipids, *J. Chromatogr. 279*:493–506 (1983).
15. Bezard, J., and M. Bugaut, The Component Triglycerides of Rat Adipose Tissue. 1. As Studied After Fractionation into Classes by Silver Ion-Thin Layer Chromatography, *J. Chromatogr. Sci. 10*:451–462 (1972).
16. Adlof, R.O., Analysis of Fatty Acid Mono- and Diacylglycerol Positional Isomers by Silver Ion High-Performance Liquid Chromatography, *J. Chromatogr. A 741*:135–138 (1996).
17. Adlof, R.O., Normal-Phase Separation Effects with Lipids on a Silver Ion High-Performance Liquid Chromatography Column, *J. Chromatogr. A 764*:337–340 (1997).
18. Taylor, M.W., and J.C. Hawke, Structural Analysis of the Triacylglycerols of Bovine Milkfats, *N.Z. J. Dairy Sci. Technol. 10*:49–57 (1975).
19. Steele, W., and W. Banks, Triglyceride Distribution in Hydrogenated Milk Fat and Its Effects on Separation by Thin Layer Chromatography, *Milchwissenschaft 49*:372–376 (1994).
20. Gray, I.K., Seasonal Variations in the Composition and Thermal Properties of New Zealand Milk Fat, *J. Dairy Res. 40*:207–214 (1973).
21. MacGibbon, A.K.H., and W.D. McLennan, Hardness of New Zealand Patted Butter: Seasonal and Regional Variations, *N.Z. J. Dairy Sci. Technol. 22*:143–156 (1987).

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