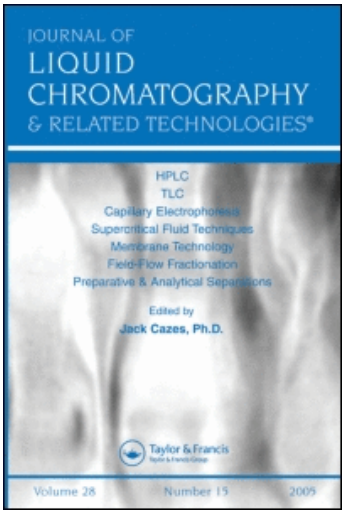

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Fatty Acid Composition and Seasonal Variation of *trans* Fatty Acid Content in Bulgarian Butter Fats by Silver Ion TLC and GC

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Abstract: Ninety three commercial samples of Bulgarian butter fats manufactured evenly through the year were subjected to quantitative silver nitrate-thin layer chromatography of the component fatty acids (as isopropyl esters), with particular attention to *trans* monoenoic fatty acid (Mt) content. The average annual content of Mt was $4.04 \pm 1.52\%$ of the total with a minimal value of 1.3% and a maximal value of 7.3%. Winter butters, comprising 50 samples, contained an average of $2.85 \pm 0.66\%$ of Mt, while the summer group (43 samples) contained $5.43 \pm 0.91\%$ of Mt. In addition, the fatty acid composition of eight Bulgarian butter fats and that of ten samples of foreign origin was determined by gas-liquid chromatography of the isopropyl esters. In general, the conjugated dienes content in Bulgarian samples was higher than that in the foreign samples; so were *trans* monoenoic and *cis,cis* dienoic acids. These could be attributed to the longer period of pasture feeding of cows in Bulgaria.

Keywords: Gas liquid chromatography, Milk fat, Seasonal variation, Silver ion thin layer chromatography, *trans* Fatty acids

[†]This paper is dedicated to the memory of Roumyana Tarandjiiska who initiated and encouraged this study.

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INTRODUCTION

Since the earliest times, mankind has used the milk of goats, sheep, and cows as food. Milk contains nearly all the nutritional components necessary to sustain life. Today, the term "milk" is generally synonymous with cow milk. Bovine milk fat is regarded as one of the most complex naturally-occurring fats containing approximately 400 distinct fatty acids.[1] Besides the saturated (S) and unsaturated FA with *cis* configuration, milk contains a relatively low amount (2–8%) of *trans* fatty acids (TFA).

Trans fatty acids in milk are produced by biohydrogenation of dietary unsaturated fatty acids in the stomach of ruminant animals. The mandatory requirement in many countries to declare the amount of *trans* fat present in food products and dietary supplements, has led to the need for validated official methods that are both sensitive and accurate for the rapid quantitation of total *trans* fatty acids. All aspects of bovine fat *trans* FA occurrence, analysis, and health effects have been reviewed in recent books.[2–6]

Historically, gas chromatography (GC) has established itself as the most widely used method for detailed fatty acids analysis and methyl esters are the universally used derivative. Generally, direct GC, using 30 to 50 m capillary columns without preliminary separation of the sample has been applied. When overlap of positional isomer peaks occurs, the results are variable and inconsistent, and correction factors are often needed to obtain correct data. Use of longer (100 m) highly polar cyanosiloxane capillary columns with an appropriate column temperature program can improve the resolution significantly and, depending on sample composition, just minor overlaps may occur. If, however, greater accuracy and complete separation are required, prior to GC, fatty acids should be fractionated according to their unsaturation by Ag-TLC or Ag-HPLC.

The detailed analysis of TFA content in milk fat should also take into account the inevitable seasonal variations caused by feeding conditions, milk output, breed, and stage of lactation. Evidently, only data derived by precise analysis of a large number of samples could represent the composition of butter fats in a particular country. This approach has been employed by Precht et al.[7–9] and Wollf et al.[10,11] who studied the positional isomers of *trans* monoenoic, Mt, fatty acids in such detail that butter samples could be used as reference standards for FA identification in the GLC analysis.

Despite the intensive use of dairy products in the Bulgarian diet, data on the FA composition of commercial Bulgarian butter fats are still missing. The aim of this study was, on the base of recently developed in our laboratory procedure for rapid quantitation of *trans* fatty acids in butter

fats, to determine the seasonal variations of total TFA content in a large number of commercial butter samples from the local market. Samples were chosen according to a specific scheme allowing to obtain representative picture for the whole country. To minimize the loss of volatile short-chained fatty acids, samples were analysed as isopropyl esters. In addition, the individual FA composition of Bulgarian butter fats studied by GLC was compared with that of imported samples.

EXPERIMENTAL

Samples and Reagents

Samples from all existing brands of Bulgarian butter were purchased from the local supermarkets during the period June 2004 to March 2006. A total of 93 samples were collected covering (according to the date of manufacturing) a whole year period with at least one sample representing every week. In addition, 5 samples of imported and another 5 samples of butter fats purchased in the local markets in Austria and Switzerland, all manufactured in winter, were analysed.

All solvents, reagents, and sorbents were of analytical grade or better and were purchased from Merck (Darmstadt, Germany). All individual fatty acids used for reference mixtures were purchased from Sigma-Aldrich (Taufkirchen, Germany).

Isopropyl Esters of FA (FAIPE)

The procedure described by Wolff[11,12] was employed. Briefly, portions of 500 mg of the sample were dispersed in 5 mL isopropanol, the solution was dried with anhydrous Na_2SO_4 and 8 mL of hexane was added. The mixture was dispersed for a second time and allowed to settle. An aliquot of the solution (2.5 mL, containing about 100 mg) was withdrawn with a Pasteur pipette, filtered into a vial, and 1.8 mL isopropanol and 0.25 mL concentrated H_2SO_4 were added. The vial was tightly capped, shaken for 30 sec, and then heated at 100°C for 1 hour. Then, the vial was cooled, 5 mL of distilled water were added, and the sample was vortexed for 30 sec. After settling, the upper phase was withdrawn and an equal volume of hexane was added. Two more extractions with hexane were performed in the same manner. The combined hexane extracts were subjected to further analysis. The completion of the derivatization reaction was followed by silica gel TLC by single development with petroleum ether/acetone, 100:10 v/v.

Analysis of FAIPE by TLC-$AgNO_3$ and Densitometry

FAIPE were purified by preparative TLC on 20×20 cm, 1 mm layer, silica gel G plates by single development with petroleum ether/acetone, 100:10 v/v; sample load 80 mg. The pure FAIPE were then dissolved in n-heptane to give 0.1%, 0.2%, or 0.5% solutions. Preliminary identification of the FA, separated into groups according to the numbers and configurations of double bonds, followed by densitometric quantification, was performed according to the TLC-$AgNO_3$-densitometric procedure developed in our laboratory,[13] adapted later to butter fat.[14] The inevitable loss of short chain FA during derivatization and analysis steps was thoroughly discussed and a conversion coefficient was proposed.[14] FAIPE groups differing in unsaturation were separated on 19×4 cm glass plates, coated with ca 0.2 mm silica gel G layer and impregnated by dipping into a 0.5% (or 1%, when needed) methanolic solution of silver nitrate. Continuous ascending development with a specified volume of the mobile phase in open cylindrical tanks (24 cm × 5 cm i.d) was performed, allowing the whole mobile phase volume to pass through the plate. The plate was then dried (1 hour at 110°C), and treated consecutively with bromine and sulfuryl chloride vapors (30 min each, closed tanks, fume cupboard) to ensure the correct quantitative charring (at 180–200°C) of the separated FA groups. Recording of Ag-TLC chromatograms and quantitative measurement of peak areas were performed with a CS-930 densitometer, (Shimadzu Corporation, Kyoto, Japan) equipped with a DR-2 Shimadzu integrator. Scanning was performed in the zigzag reflection mode at 450 nm, beam-slit size 1.2×1.2 mm and stage step 0.2 mm.

Comments on the Analytical Procedure (Ag-TLC Procedure)

Three different sets of chromatographic conditions (sample load, mobile phase composition) were used for correct quantification. Analyses were done in triplicate and the values were the mean of three densitograms, percentage error for S was 0.1%, for Mt–3.3%, and under 15% for minor classes. Typical densitograms are shown in Figure 1.[14]

The quantity of each spot was presented as relative area percent, as derived from the integrator. The values of saturated fatty acids (S) were multiplied by the previously proposed by us[14] correction coefficient of 1.16 in order to compensate for the loss of short chain FA. Simple recalculation of all values was then performed.

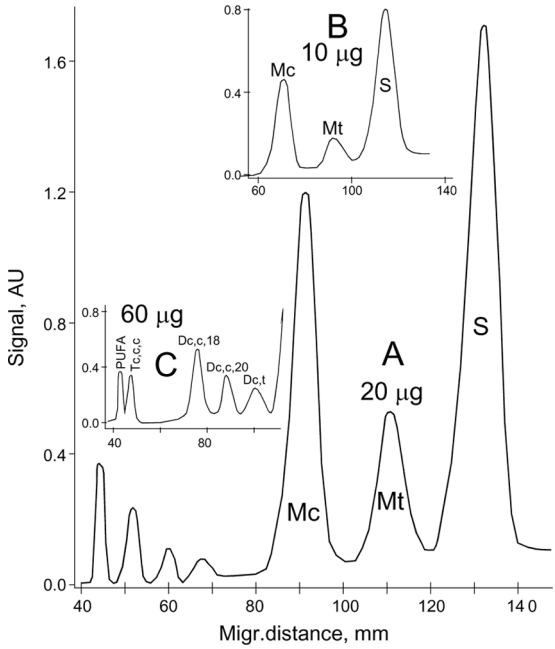

Figure 1. Densitograms of Bulgarian butter sample. Plates were impregnated with 0.5% $AgNO_3$. Mobile phase: **A** and **B** – petroleum ether: acetone 100:2 (v/v), 3.5 mL; **C** – petroleum ether:acetone 100:6 (v/v)/3 mL, S – saturated, M – monoenoic, D – dienoic, T – trienoic fatty acids; c – *cis*; t – *trans*; con – conjugated. (Reproduced from Ref. [14] with kind permission of the Journal of Planar Chromatography – Modern TLC).

Analysis of FAIPE by GLC

GLC was performed on a Hewlett-Packard 5890A (Hewlett-Packard, Cambridge, England) gas chromatograph equipped with a flame-ionization detector (FID), split/splitless injector, 30 m × 0.25 mm CP Wax 52 fused silica capillary column (Chrompack, Middelburg, The Netherlands), and a Chromatopac CR-3A integrator (Shimadzu Corporation, Kyoto, Japan). An aliquot of the combined hexane extracts was injected into the chromatograph exactly as described by Wolff.[10,11] The temperature gradient was from 65°C (4 min), to 245°C (20 min) at 5°C/min. The temperature of the detector and the injector was 260°C. Nitrogen was the carrier gas, inlet pressure 85 kPa. As shown previously,[10] converting FA into isopropyl esters eliminates the necessity of correction factors (for shorter chain acids, mainly) to transform peak area percentages into weight percents and the results derived from the

integrator directly correspond to FAIPE weight percents. Results were the mean of 2 injections. Fatty acids were identified using relevant reference mixtures. These comprised even numbered saturated fatty acids from 4:0 to 26:0 and the odd numbered 13:0, 15:0 and 17:0; series of isomeric *cis*- monoenoic - 9–16:1; 6-, 7-, 9-, 12-, 13-, and 15–18:1; and *cis,cis*- dienoic fatty acids - 9, 12–18:2; 11, 14–20:2; 13, 16–22:2, and *cis,cis,cis*-9, 12, 15–18-3, and conjugated linoleic acids (isolated from alkali isomerised sunlower oil).

RESULTS AND DISCUSSION

Seasonal Variation of *trans* Monoenoic Fatty Acids Content in Butter

There are two difficulties faced when dealing with milk fat samples. The first is linked to the seasonal variations in TFA content caused by the different conditions of animal feeding throughout a year. An adequately large pool of samples, manufactured evenly throughout the year, minimizes the effects of animal feeding. The second challenge is to avoid the loss of short chain fatty acids during sample derivatization and analysis[15] and to ensure exact and reproducible quantification of the low levels of TFA present.[16,17]

The long-term experience of our group in using silver ion TLC/densitometry as a method for direct quantitative analysis of triacylglycerols[18] and geometrically isomeric FA in PHVO[13] was the basis for developing a rapid procedure for quantitative analysis of geometrically isomeric FA in milk fats. It allowed for baseline separation and direct measurement of all FA groups differing by degree of unsaturation and double bond geometry as follows: Tccc, Tcct, Dcc, Dct, (Dtt + Mc + Dct/tc con), Mt, SFA (ordered according to decreased retention), where S denotes saturated; M – monoenoic; D – dienoic; T – trienoic; c – *cis*; t – *trans* fatty acids. The composition of the mixed zone (Dtt + Mc + Dct/tc con) and that of the Mt zone was established on a second TLC plate (1% Ag, chloroform as a mobile phase) with an appropriate reference standard mixture applied alongside. Since GLC analysis revealed that Mc dominates in the mixed zone (Dtt + Mc + Dct/tc con), further in the text it will be designated as Mc only. Additional, and undesired, separation of positional isomers within a given FA group does not occur under the experimental conditions applied. This enabled the determination of low levels (down to 0.1–0.2%) of TFA with sufficient precision.

Fatty acid classes, and not individual fatty acids, are separated and quantified under the proposed Ag-TLC procedure, but the approach is direct, without intermediate stages of fractionation and is sufficiently informative and convenient for rapid screening of a great number of samples.

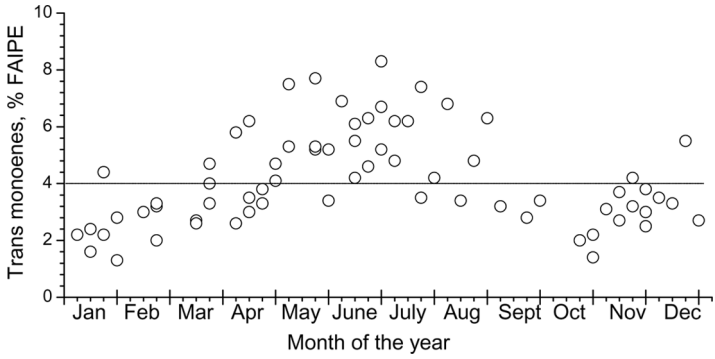

Figure 2. Weekly variation in the content of *trans*-monoenoic fatty acids in Bulgarian butterfats (weight % of total FAIPE) as determined by Ag-TLC/densitometry.

Figure 2 represents the seasonal variation in Mt content in Bulgarian commercial butter samples as determined by TLC-$AgNO_3$/densitometry. A total of 93 samples from different regions of the country, have been analyzed; each week is represented by at least one sample, each point being the mean of three densitograms.

The Mt values represent the summary value of all *trans* monoenoic FA which were determined as a group, i.e., t18:1 + t16:1. However, by a close approximation, it could be assumed that Mt comprises 18:1 TFA only, since the average content of 16:1 TFA reported in literature does not exceed 0.13% of the total.[10,19] The average annual content of Mt found in this study was $4.04 \pm 1.52\%$ with a minimal value of 1.3% and a maximal value of 7.3%. This value is in agreement to that reported for a similar number of German butter fat samples.[8,9] The somewhat higher Mt levels of Bulgarian butter fat is probably due to different climate. When relating the Mt content of the butter fat samples studied in the present work to the average Mt value of 4%, two groups are outlined – a "summer" group of 43 samples with Mt value of $5.43 \pm 0.91\%$, and a "winter" group consisting of 50 samples with $Mt = 2.85 \pm 0.66\%$. The allocation of the points in Figure 2 shows a clear trend of increased Mt content during the spring-summer period. Comparison with similar profile for German butter fats[6,8] shows that the values found in the present work are more scattered. The spring increase of Mt in Bulgarian samples begins by the end of March, i.e., about a month earlier than reported for Germany, and the seasonal variation is smoother. This is probably due to the specific features of the Bulgarian climate and terrain. Also, animal husbandry in Bulgaria is small and sporadic and the period of pasture feeding is much longer, compared with those in Germany.

Fatty Acid Composition by GLC

In order to confirm the Ag-TLC-densitometry quantification and to compare the content of the individual FA between Bulgarian and foreign butter fats, 18 samples were selected and subjected to GLC. Ten of these were of foreign origin, all manufactured in the winter. Four pairs from the Bulgarian butter fats were selected in such a manner that each pair was manufactured by the same producer, originated from the same region, representing one summer and one winter butter fat. The fatty acids are listed in Table 1 according to their elution order. Of course, data in Table 1 cannot be representative for FA composition of foreign butter fats; these were just the available ones on Bulgarian market. Due to the limited resolution of the 30 m GLC column, identification of C18:2 isomers was tentative, based on comparison with literature data. Using a medium polarity 30 m column (CP Wax52) under routine GC conditions, no clear separation between *cis* and *trans* isomers was observed. The broad group of peaks in the C18:1 region was assigned to the sum of *cis* and *trans*-18:1 isomers. The value of *cis*-18:1 given in Table 1 was calculated by subtracting from the sum the value for total Mt content as determined by Ag-TLC-densitometry.

Pure fractions of *cis*-monoenes and *trans*-monoenes were isolated by preparative TLC-$AgNO_3$ and solutions with known concentrations were prepared. The fractions and mixtures of each fraction with butter samples were subjected to GLC under identical conditions. The results proved that the 18:1 isomer with most distant double bond, t16–18:1, eluted together with c9, c12–18:2, thus increasing its value with about 0.2%.[6] The content of *cis,trans*/*trans,cis* dienoic fatty acids (ct/tc-18:2) detected directly by TLC-densitometry varied between 0.1% and 0.7% and corresponded to the values reported by others.[20–22] Linoleic acid positional isomers were determined directly by GLC (see footnote C to Table 1).[23,24] Linolenic acid produced a single peak. Ag-TLC revealed that there were no mono-*trans* trienoic isomers present in any of the butter samples.

Under GLC Dct/tc con formed a single peak with values between 0.40 and 1.20% of total FA content. The result corresponded to the data reported elsewhere[11,21,22,25–29] and was confirmed by GLC analysis of the compounds isolated by preparative TLC-$AgNO_3$ mixed zone, Dtt +Mc +Dct/tc con, in which conjugated dienes accounted for an average content of 2.24%.

Trans,trans-dienes were found to be in the range of 0.03–0.12% of the Mc fraction, which corresponds to a value of about 0.01–0.03% of the total FA content, a value that was much lower than the average value of 0.09% of the total in German milk fats.[22] Evidently, under TLC-$AgNO_3$/densitometry these minor FA classes represent a negligible

Table 1. Fatty acid composition (Mean ± SD, FAIPE wt %) of Bulgarian and foreign butter samples

	Origin (number of samples)							
	Winter							Summer
Fatty acids	Germany (2)	Austria (2)	Switzerland (3)	Denmark (1)	France (1)	Sweden (1)	Bulgaria (4)	Bulgaria (4)
4:0	4.12 ± 0.03	3.94 ± 0.42	4.52 ± 0.12	4.02	3.92	3.35	4.18 ± 0.38	4.24 ± 0.39
6:0	2.38 ± 0.01	2.46 ± 0.08	2.52 ± 0.06	2.28	2.57	2.29	2.32 ± 0.26	2.45 ± 0.27
8:0	1.34 ± 0.05	1.45 ± 0.09	1.59 ± 0.03	1.42	1.38	1.31	1.44 ± 0.15	1.52 ± 0.22
10:0	3.18 ± 0.19	3.27 ± 0.25	3.42 ± 0.11	3.10	3.24	2.87	3.09 ± 0.31	3.35 ± 0.57
10:1	0.25 ± 0.01	0.30 ± 0.01	0.33 ± 0.01	0.30	0.27	0.20	0.23 ± 0.03	0.24 ± 0.05
12:0	3.76 ± 0.06	3.75 ± 0.22	3.92 ± 0.15	3.68	3.42	3.14	3.22 ± 0.24	3.68 ± 0.50
12:1	0.05 ± 0.01	0.11 ± 0.03	0.14 ± 0.01	0.10	0.09	0.07	0.07 ± 0.02	0.09 ± 0.02
13:0	0.14 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08	0.09	0.05	0.07 ± 0.01	0.10 ± 0.02
i14:0[a]	0.12 ± 0.03	0.14 ± 0.01	0.14 ± 0.00	0.09	0.10	0.07	0.13 ± 0.06	0.15 ± 0.02
14:0	10.92 ± 0.73	10.85 ± 0.13	11.04 ± 0.27	9.87	10.37	10.68	10.20 ± 0.64	9.33 ± 0.64
14:1	1.06 ± 0.17	1.01 ± 0.03	1.07 ± 0.05	1.16	1.01	0.79	0.86 ± 0.09	0.79 ± 0.15
i15:0	0.30 ± 0.03	0.28 ± 0.01	0.30 ± 0.01	0.27	0.28	0.20	0.33 ± 0.02	0.32 ± 0.06
ai15:0	0.52 ± 0.03	0.44 ± 0.01	0.50 ± 0.01	0.50	0.44	0.36	0.57 ± 0.05	0.63 ± 0.10
15:0	1.08 ± 0.03	1.25 ± 0.08	1.11 ± 0.03	0.99	1.03	0.77	1.04 ± 0.12	1.21 ± 0.07
i16:0	0.24 ± 0.08	0.28 ± 0.01	0.26 ± 0.01	0.21	0.25	0.18	0.30 ± 0.05	0.31 ± 0.01
16:0	30.10 ± 0.95	31.91 ± 1.55	28.89 ± 0.50	28.67	28.26	28.46	28.75 ± 1.52	24.81 ± 2.83
16:1	2.08 ± 0.34	1.91 ± 0.07	1.88 ± 0.04	1.96	2.02	1.79	1.73 ± 0.09	1.82 ± 0.11
i17:0	0.54 ± 0.07	0.47 ± 0.01	0.44 ± 0.02	0.47	0.50	0.40	0.48 ± 0.06	0.52 ± 0.02
ai17:0	0.54 ± 0.13	0.42 ± 0.05	0.42 ± 0.01	0.46	0.45	0.39	0.49 ± 0.06	0.54 ± 0.03
17:0	0.58 ± 0.06	0.62 ± 0.08	0.59 ± 0.01	0.48	0.54	0.39	0.60 ± 0.06	0.69 ± 0.09

(*Continued*)

Table 1. Continued

	Origin (number of samples)							
	Winter							Summer
Fatty acids	Germany (2)	Austria (2)	Switzerland (3)	Denmark (1)	France (1)	Sweden (1)	Bulgaria (4)	Bulgaria (4)
17:1	0.28 ± 0.03	0.32 ± 0.01	0.28 ± 0.07	0.25	0.36	0.23	0.31 ± 0.04	0.31 ± 0.03
18:0	9.13 ± 0.69	8.87 ± 0.04	8.57 ± 0.39	9.23	10.35	11.61	10.23 ± 1.90	10.59 ± 1.92
18:1 t[b]	2.45 ± 0.35	1.80 ± 0.56	1.93 ± 0.11	3.00	2.80	3.06	2.72 ± 0.64	5.18 ± 1.98
18:1c	20.45 ± 1.94	18.87 ± 0.81	20.87 ± 0.34	22.42	21.03	20.89	19.74 ± 0.76	20.71 ± 0.68
18:2ct/tc	0.48 ± 0.18	0.15 ± 0.05	0.27 ± 0.04	0.20	0.55	0.22	0.26 ± 0.09	0.35 ± 0.10
18:2 (n-6)	1.35 ± 0.09	1.37 ± 0.04	1.47 ± 0.08	1.79	1.04	1.69	1.61 ± 0.19	1.77 ± 0.11
18:2p.isom[c]	0.25 ± 0.05	0.45 ± 0.02	0.35 ± 0.10	0.33	0.26	0.54	0.46 ± 0.13	0.56 ± 0.32
19:0	0.10 ± 0.00	0.12 ± 0.00	0.12 ± 0.03	0.07	0.10	0.17	0.24 ± 0.11	0.16 ± 0.08
18:3 (n-3)	0.42 ± 0.03	0.74 ± 0.08	0.70 ± 0.06	0.52	0.52	0.55	0.63 ± 0.14	0.71 ± 0.22
18:2ct/tc con	0.40 ± 0.02	0.63 ± 0.03	0.65 ± 0.21	0.70	0.60	0.70	0.96 ± 0.16	1.30 ± 0.23
20:0	0.20 ± 0.02	0.27 ± 0.08	0.20 ± 0.06	0.18	0.24	0.56	0.56 ± 0.18	0.33 ± 0.13
20:1	0.15 ± 0.01	0.20 ± 0.13	0.17 ± 0.14	0.10	0.10	0.43	0.16 ± 0.04	0.12 ± 0.09
20:2	0.05 ± 0.00	0.03 ± 0.00	0.16 ± 0.01	0.03	0.04	0.36	0.31 ± 0.17	0.10 ± 0.11
22:0	0.08 ± 0.03	0.09 ± 0.01	0.07 ± 0.02	0.09	0.08	0.10	0.25 ± 0.09	0.14 ± 0.02
24:0	0.02 ± 0.00	0.08 ± 0.01	0.05 ± 0.01	0.03	0.04	–	0.05 ± 0.03	0.06 ± 0.02
Others	0.89	1.06	0.98	0.97	1.66	1.11	1.41	0.82

[a]abbreviations: i – iso; ai – anteiso; con – conjugated.
[b]18:1 t – determined by Ag-TLC/densitometry.
[c]Sum of peaks which elute immediately after the linoleic acid (c9, c12–18:2) peak and presumably represents positional isomers of linoleic acid having double bonds with *cis* configuration and/or being shifted to the terminal (methyl) end of the molecule.[24,25]

part of the mixed zone which may be, thus, considered as comprising of Mc only.

In general, the fatty acid composition of the studied samples was in agreement with the data reported for other countries.[9,11,20,21] The values of linoleic acid and its positional isomers with *cis* double bonds were higher than that reported by others, but the values presented here were confirmed by TLC. Also, the conjugated dienes content in Bulgarian samples was slightly higher than in foreign samples, 20:0, 22:0, and 20:2 acids were also higher, being similar to the respective levels in the Swedish sample.

CONCLUSION

The Ag-TLC procedure proposed here is direct, without intermediate stages of fractionation, and is sufficiently informative and convenient for rapid screening of a great number of samples. The mean values and the trends found for seasonal variation of *trans* monoenoic fatty acids content in Bulgarian butter fat are similar to the reported data for French and German samples. The results characterize Bulgarian butter FA composition close to butter imported from European countries. The somewhat higher content of Mt and Dcc acids in Bulgarian samples could be attributed to the longer period of pasture feeding of cows in the country. Our results supplement to the knowledge base of milk fat compositions in Europe and can be used for reliable nutritional and epidemiological conclusions.

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REFERENCES

1. Jensen, R.G. The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. **2002**, *85*, 295–350.
2. Aro, A. Epidemiological studies of trans fatty acids and cardiovascular disease, in *Trans Fatty Acids in Human Nutrition*; Sébédio, J.L., Christie, W.W., Eds.; Oily Press: Dundee, UK, 1998; 235–260.
3. Dijkstra, A.J.; Hamilton, R.J.; Hamm, W. *Trans Fatty Acids*; Blackwell Publishing: Oxford, UK, 2008.
4. Palmquist, D.L.; Jensen, R.G. Fatty Acids in Milk Fat, in *Fatty Acids in Foods and their health implications*, 3rd Ed., Chapter 6; Ching Kuang

Chow, Ed.; CRC Press, Taylor & Francis Group, Boca Raton, FL, USA, 2008; 109–125.
5. Massoba, M.M.; McDonald, R.E. Methods for trans fatty acid analysis, in *Food Lipids: Chemistry, Nutrition, and Biotechnology*, 3rd Ed.; Akoh, C.C.; Min, D.B., Eds.; CRC Press, Taylor & Francis Group: Boca Raton, FL, USA, 2008; 157–188.
6. Wolff, R.L.; Precht, D.; Molkentin, J. Occurrence and distribution profiles of *trans*-18:1 acids in edible fats of natural origin, in *Trans Fatty Acids in Human Nutrition*; Sébédio, J.L.; Christie, W.W., Eds.; Oily Press: Dundee, 1998; 1–33.
7. Precht, D.; Molkentin, J. *Trans*-octadecenoic acids in milk fat and margarine (in German). Kieler Milchwirtsch. Forshch. **1994**, *46*, 249–261.
8. Precht, D. Variation of *trans* fatty acids in milk fats, Z. Ernahrungswiss. **1995**, *34*, 27–29.
9. Precht, D.; Molkentin, J. Comparison of the fatty acids and the isomeric distribution of *trans*-C18:1 fatty acids of milk fat, margarine, shortenings, cooking and dietetic fats (in German). Kieler Milchwirtsch. Forshchungsber. **1997**, *49*, 17–34.
10. Wolff, R.L. Contribution of *trans*-C18:1 acids from dairy fat to European diets. J. Am. Oil Chem. Soc. **1994**, *71*, 277–283.
11. Wolff, R.L.; Bayard, C.C.; Fabien, R.J. Evaluation of sequential methods for the determination of butterfat fatty acid composition with emphasis on *trans*-18:1 acids. Application to the study of seasonal variations in French butters. J. Am. Oil Chem. Soc. **1995**, *72*, 1471–1483.
12. Wolff, R.L.; Fabien, R.J. The use of isopropanol for fat extraction from dairy products and for the subsequent esterification of fatty acids (in French). Le Lait. **1989**, *69*, 33–46.
13. Chobanov, D.; Tarandjiiska, R.; Nikolova-Damyanova, B. Quantitation of isomeric fatty acids by argentation TLC. J. Planar Chromatogr. **1992**, *5*, 157–163.
14. Marekov, I.; Tarandjiiska, R.; Panayotova, Sv.; Nikolova, N. Evaluation of argentation TLC-densitometry for the determination of *trans* fatty acids in butterfat. J. Planar Chromatogr. **2001**, *14*, 384–390.
15. Christie, W.W. *Gas Chromatography and Lipids: a Practical Guide*, The Oily Press: Ayr, UK, 1989; 71–72; 112–114.
16. Precht, D.; Molkentin, J. *Trans* unsaturated fatty acids in bovine milk fat and dairy products. Eur. J. Lipid Sci. Technol. **2000**, *102*, 635–640.
17. Buchgraber, M.; Ulberth, F. Determination of *trans* octadecenoic acids by silver-ion chromatography-gas liquid chromatography: An intercomparison of methods. J. Assoc. Off. Anal. Chem. Intl. **2001**, *84*, 1490–1498.
18. Chobanov, D.; Tarandjiiska, R.; Chobanova, R. Rapid densitometric determination of triglyceride groups by argentation thin layer chromatography. J Am. Oil Chem. Soc. **1976**, *53*, 48–51.
19. Molkentin, J.; Precht, D. Occurence of *trans*-C16:1 acids in bovine milkfats and partially hydrogenated edible fats. Milchwissenschaft. **1997**, *52*, 380–385.
20. Ulberth, F.; Henninger, M. Quantitation of *trans* fatty acids in milk fat using spectroscopic and chromatographic methods. J. Dairy Res. **1994**, *61*, 517–527.

21. Henninger, M.; Ulberth, F. *Trans* fatty acid content of bovine milk fat. Milchwissenschaft. **1994**, *49*, 555–558.
22. Precht, D.; Molkentin, J. *Trans*-geometrical and positional isomers of linoleic acid including conjugated linoleic acid (CLA) in german milk and vegetable fats. Fett Lipid. **1997**, *99*, 319–326.
23. Christie, W.W. Equivalent chain-lengths of methyl ester derivatives of fatty acids on gas chromatography - a reappraisal. J. Chromatogr. **1988**, *447*, 305–314.
24. Wolff, R.L.; Nour, M.; Bayard, C.C. Participation of the *cis*-12 ethylenic bond to *cis-trans* isomerization of the *cis*-9 and *cis*-15 ethylenic bonds in heated alpha-linolenic acid. J. Am. Oil Chem. Soc. **1996**, *73*, 327–332.
25. Chin, S.F.; Liu, W.; Storkson, J.M.; Ha, Y.L.; Pariza, M.W. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognised class of anticarcinogens. J. Food Comp. Anal. **1992**, *5*, 185–197.
26. Ulberth, F. Fatty acids in Austrian milk fat. Tejgazdaság – Hungar. Dairy J. **1995**, *1*, 34–38.
27. Banni, S.; Carta, G.; Contini, M.S.; Angioni, E.; Deiana, M.; Dessi, M.A.; Melis, M.P.; Corongiu, F.P. Characterization of conjugated diene fatty acids in milk, dairy products, and lamb tissues. J. Nutr. Biochem. **1996**, *7*, 150–155.
28. Ruiz-Sala, P.; Hierro, M.T.G.; Martinez-Castro, I.; Santa-Maria, G. Triglyceride composition of ewe, cow and goat milk fat. J. Am. Oil Chem. Soc. **1996**, *73*, 283–293.
29. Fritsche, J.; Steinhart, H. Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. Z. Lebensm. Unters. Forsch. A. **1998**, *206*, 77–82.

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