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Quantitation of *cis*- and *trans*-Monounsaturated Fatty Acids in Dairy Products and Cod Liver Oil by Mass Spectrometry in the Selected Ion Monitoring Mode

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Monounsaturated fatty acids (MUFAs) are important constituents of dietary fats. While *cis*-configurated isomers belong to the classic fatty acids of food, *trans*-fatty acids are suspected to pose a risk to human health. In either case, a thorough assessment of both positional and geometrical isomers of MUFAs is an important task in food and life sciences. For this purpose, a method suitable for routine analysis was developed. After lipid extraction and conversion of fatty acids into corresponding fatty acid methyl esters, *cis* and *trans* isomers of MUFAs were separated on silver-ion-impregnated cartridges. Fractions containing either *cis*- or *trans*-MUFAs were determined by gas chromatography−electron ionization mass spectrometry in the selected ion monitoring (GC/EI−MS−SIM) mode using $[M-32]^+$ as quantification ions and $[M-74]^+$ as well as M^+ as qualifier ions. A total of 14 MUFAs were available as reference standards, but a total of 40 MUFAs (22 *cis* and 18 *trans* isomers) were identified with high selectivity in samples of cheese, goat fat, human milk, and cod liver oil. The 18:1 and 16:1 isomers contributed most to both the *cis*- and *trans*-MUFAs. Application of internal standards allowed for the quantification of MUFAs only in the food samples. The amount of *trans*-fatty acids was determined to be 0.9− 4.3 g/100 g, with the lowest levels in human milk fat and the highest levels in Roquefort cheese. After exclusion of oleic acid, the concentrations of *trans*- and *cis*-MUFAs were at the same level in samples from ruminants and human milk fat.

KEYWORDS: Monoenoic fatty acids; *trans*-fatty acids; dairy products; milk; fish oil

INTRODUCTION

Monounsaturated fatty acids (MUFAs) are important constituents of vegetable and animal lipids. The double bond is usually found in *cis* (or *Z*) configuration, with oleic acid [18:1(9)] and palmitoleic acid [16:1(9)] being the most important members of this class of fatty acids. Concentrations of these and other abundant *cis*-configurated MUFAs are determined in routine food analysis. However, a range of *trans* (or *E*) isomers (*trans*-fatty acids, TFAs) has also been reported at low but significant amounts in treated oils and ruminant fats (*1*, *2*). The TFAs are more and more the focus of interest because of their negative influence on diet-related diseases, such as cardiovascular and coronary diseases (*3*). Furthermore, the association between the intake of dietary TFAs and the risk of insulin resistance and diabetes type 2 was recently discussed (*4*). Hence, U.S. food laws require labels to include the total TFA content on food and dietary supplements (*5*, *6*). As a consequence, quantification of TFAs is an important task in life and food sciences. The 11-*trans*-octadecenoic acid [vaccenic acid, 18:1(11tr)], elaidic acid [18:1(9tr)], and 18:1 (10tr) are the most common *trans* isomers in food (*7*, *8*). The latter two are important markers for industrial fats, especially formed by the partial hydrogenation of vegetable and fish oils (*9*). The 18:1(11tr) isomer is the dominating TFA in ruminants, which is generated by rumen bacteria via the biohydrogenation of polyunsaturated FAs (*10*). However, additional minor 16:1 [e.g., 16:1(4tr)−16:1(14tr); (*11*)] and 18:1 [18:1(2tr)−18:1(16tr); (*2*, *5*, *12*, *13*)] isomers were occasionally described in ruminants, shortenings and margarines, hydrogenated fish oils, as well as fats of human origin (*2*, *14*).

Gas chromatographic determination of MUFAs as methyl esters (MUFAMEs) requires the application of polar capillary columns coated with 70% (BPX-70) to 100% (CP-Sil 88, SP-2560) *bis*-cyanopropyl polysiloxane, because of their unique ability to separate the isomers of both MUFAs and polyunsaturated fatty acids (PUFAs) (*5*, *15*−*17*). Although these stationary phases allow for a good separation of positional and geometrical FAME isomers (*2*, *13*), a few overlaps of *cis* and *trans* isomers were still obtained in food samples (*1*, *2*, *11*, *17*). Furthermore, co-elutions of MUFAMEs with methyl esters of branched-chain fatty acids have been reported (*11*, *18*). As a consequence, intralaboratory exercises resulted in high relative standard deviations even when 100 m columns were used (*17*). Unfortunately, most of the data available concentrates on the investigation of the MUFA pattern, while detailed quantitative results are scarcely available (*1*, *2*, *19*−*23*).

This study was aimed at developing a method for the routine quantification of *cis* and *trans* isomers in dairy

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products and other food samples. After extraction and conversion of food fatty acids into FAMEs, solid-phase extraction (SPE) cartridges coated with silver ions were used for the fractioning into (i) saturated and *trans*-monoenoic FAMEs, (ii) *cis*-monoenoic FAMEs, and (iii) di- to hexaenoic FAMEs (*20*, *24*). For the quantification of MUFAMEs, the high sensitivity and selectivity of gas chromatography coupled with electron ionization mass spectrometry performed in the selected ion monitoring (GC/EI−MS−SIM) mode using selective fragment ions was used for quantification and verification.

MATERIALS AND METHODS

Chemicals, Standards and Samples. Cyclohexane (purest, VWR, Darmstadt, Germany) and ethyl acetate (purest, Acros Organics, Geel, Belgium) were combined and distilled to obtain the azeotropic mixture (46:54, v/v). Methanol and *n*-hexane (both HPLC gradient grade) were from Fluka (Taufkirchen, Germany). Boron trifluoride−methanol complex solution (13−15% BF_3 in methanol) was from Riedel-de-Haën (Taufkirchen, Germany). A 37-component FAME mix as well as other additional FA standards were from Sigma-Aldrich (Taufkirchen, Germany) or Larodan (Malmö, Sweden). Silver ion SPE cartridges (750 mg/6 mL) were from Supelco, Inc. (Bellefonte, PA).

The following food samples were analyzed: goat tissue fat (purchased from a local farm), human milk (anonymous donor from Stuttgart, Germany), Bavaria blue (68% fat in dry matter, Bergader Privatkäserei, Waging, Germany; made from pasteurized bovine milk), Roquefort (52% fat in dry matter, made from untreated ewe's milk), cod liver oil (Rügen Fisch, Sassnitz, Germany), and organically produced butter (Demeter, Darmstadt, Germany).

Gas Chromatography in Combination with Electron Ionization Mass Spectrometry (GC/EI−MS). An HP 5890 series II GC equipped with an HP 5971A mass selective detector (Hewlett-Packard, Waldbronn, Germany) was used for FAME analysis. A total of 1 μL of sample solution was injected by an HP 7673A autosampler (Hewlett-Packard). The injector was operated in the splitless mode (open after 2 min). Injector and the transfer line temperatures were set to 250 and 280 °C, respectively. A 50 m × 0.25 mm inner diameter fused silica capillary column coated with 0.20 μm 100% cyanopropyl polysiloxane (CP-Sil 88, Chrompack, Middelburg, The Netherlands) was installed in the GC oven. Helium (purity 99.9990%, Sauerstoffwerke, Friedrichshafen, Germany) was used as the carrier gas, at a constant flow rate of 1.0 mL/min. The ion source temperature was set at 165 °C, and the ionization energy was 70 eV. The GC oven program started at 60 °C (held for 1 min), and then the oven was heated at 3 °C/min to 230 °C (held for 7 min). A solvent delay of 8 min was used in all analyses. In the GC/EI−MS full-scan mode, *m*/*z* 50−450 were recorded at a rate of 1.8 scans/s. In the selected ion monitoring (SIM) mode, 12 ions including *m*/*z* 74, *m*/*z* 87 (for saturated and monoenoic FAMEs), *m*/*z* 81 and *m*/*z* 79 for polyunsaturated FAMEs (*25*), as well as *m*/*z* 101 and *m*/*z* 88 for fatty acid ethyl esters (FAEEs), used as internal standards (*26*), were recorded, as shown in **Table 1**.

Extraction of Food Samples. Samples were processed by accelerated solvent extraction (ASE 200, Dionex, Idstein, Germany) according to Weichbrodt et al. (*27*). In brief, ∼1 g was extracted with 3× 40 mL of ethyl acetate/cyclohexane (54:46, v/v) in 11 mL extraction cells filled to the brim with ∼2.0 g of diatomaceous earth (isolute-HM-N, Separtis, Grenzlach-Wyhlen, Germany). The three extracts were combined, condensed in a rotary evaporator (180 mbar and 30 °C water bath temperature), and made up with ethylacetate/cyclohexane to 5.0 mL. Aliquots taken for gravimetric determination of the lipid content verified the amounts listed on the label of the dairy products. Further aliquots of the remaining lipids were taken for the following procedures.

Table 1. Time Windows and Fragment Ions Screened for the Determination of MUFAMEs by GC/EI−MS−SIM[a]

time window	range (min)	isomer	M^+	$[M-32]^+$	$[M-74]^+$
1	8−18.1	8:1	156	124	82
		9:1	170	138	96
2	18.1−23.85	10:1	184	152	110
		11:1	198	166	124
3	23.85−29.2	12:1	212	180	138
		13:1	226	194	152
4	29.2−34.1	14:1	240	208	166
		15:1	254	222	180
5	34.1−38.5	16:1	268	236	194
		17:1	282	250	208
6	38.5−42.5	18:1	296	264	222
		19:1	310	278	236
7	42.5−46.3	20:1	324	292	250
		21:1	338	306	264
8	46.3−49.75	22:1	352	320	278
		23:1	366	334	292
9	49.75−64.7	24:1	380	348	306
		25:1	394	362	320

[a] *m*/*z* 74, *m*/*z* 87, *m*/*z* 79, and *m*/*z* 81 (for FAMEs) (*25*) as well as *m*/*z* 88 and *m*/*z* 101 for (FAEEs) (*26*) were measured throughout the run.

Formation of Alkyl Esters of Fatty Acids. FAMEs were prepared using the "DFG Einheitsmethode" as previously described (*25*). Accordingly, the internal standards 14:0 ethyl ester (14:0 EE), 16:0 EE, 18:0 EE, and 20:0 EE were prepared using (i) ethanolic 0.5 M KOH and (ii) ethanolic BF_3 (*26*). The final volume of both methyl esters and ethyl esters was adjusted to 2 mL (*n*-hexane).

Silver Ion Chromatography. The silver ion (Ag^+) chromatography was carried out with SPE cartridges (750 mg/6 mL) preconditioned with both 4 mL of acetone and *n*-hexane (*28*). Then, 1 mL of *n*-hexane containing approximately 1 mg of FAMEs derived from sample lipids (described above) was placed on the column. Elution of both saturated and *trans*-monoenoic FAMEs was accomplished with 6 mL of *n*-hexane/acetone (96:4). The *cis*-monoenoic FAMEs were targeted with 4 mL of *n*-hexane/acetone (90:10) into a second fraction, and finally, methyl esters of PUFA were eluted into a third fraction with 4 mL of acetone (*28*). The individual fractions were each evaporated to dryness by a gentle stream of nitrogen and were finally filled up with exactly 1 mL of *n*-hexane. Owing to the smearing of significant amounts of vaccenic acid into the subsequent second fraction (*cis* fraction) (see also below), the second fraction was evaporated, made up with 1 mL of *n*-hexane, and refractionated on a second silver ion SPE cartridge.

Quantification Procedure. An aliquot of 100 μL of the FAMEs (10%) was taken from the silver ion SPE fractions, and 10 μL of FAEE mixture (2−2.5 μg of 14:0 EE, 16:0 EE, 18:0 EE, as well as 20:0 EE) was added as internal standards (ISs) to give a final volume of 110 μL. The final concentrations of the ISs were each ∼18.2−22.7 ng/μL.

As a reference standard, an aliquot of 100 μL of a 37-component FAME mix (1 mg/mL) containing nine MUFAMEs in the range of 14:1−24:1 as well as the single standard of 12:1(7) ME was used and spiked with the IS in the same way. MUFAMEs were quantified by GC/EI−MS−SIM using $[M-32]^+$ as a quantifier as well as M^+ and $[M-74]^+$ as qualifier ions, whereas the FAEEs were determined with *m*/*z* 101 (quantification ion) and *m*/*z* 88 (qualifier ion) (*26*).

RESULTS AND DISCUSSION

Identification of Characteristic Fragment Ions for the Determination of MUFAMEs. GC/EI−MS spectra of FAMEs show characteristic patterns, mainly in the low mass range, which are different for saturated, monoenoic, dienoic, and polyenoic FAMEs (*25*). In the high mass range, the number

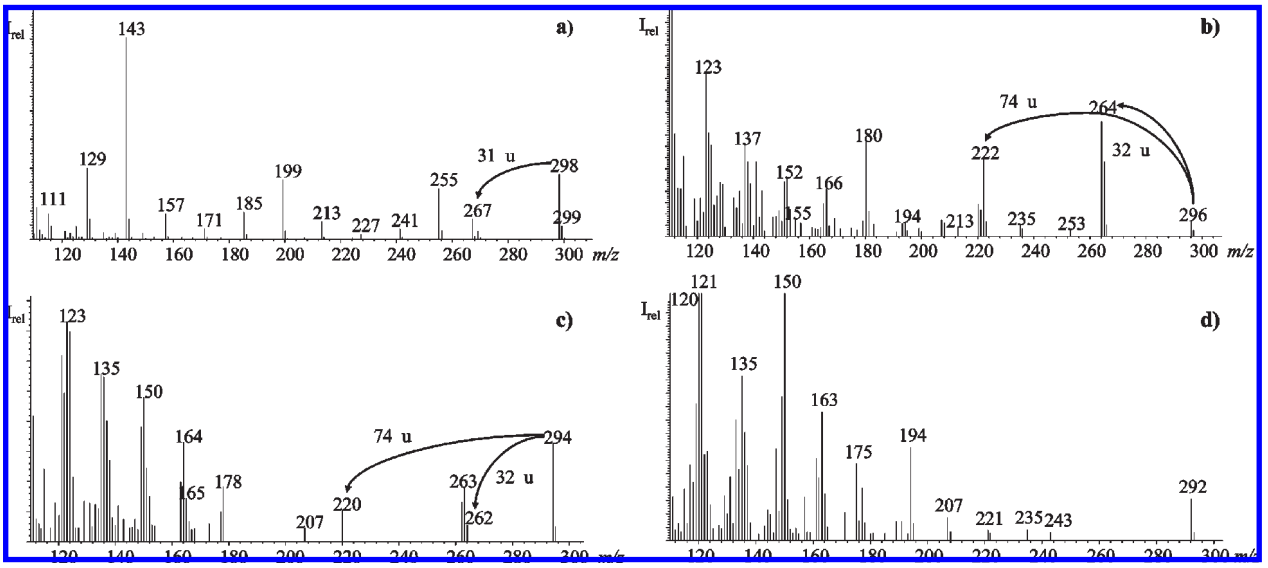

Figure 1. GC/EI mass spectra of the methyl esters of (a) stearic acid, (b) oleic acid, (c) linoleic acid, and (d) α-linolenic acid.

of double bonds can be derived from the molecular ion, but this was only abundant in low quantities (< 2%) (parts **a**−**d** of **Figure 1**). However, GC/EI−MS spectra of MUFAMEs contain diagnostic abundant fragment ions in the form of $[M-32]^+$ and $[M-74]^+$ (**Figure 1b**) (*2*, *29*).

In the case of 18:1 isomers, $[M-32]^+$ and $[M-74]^+$ are found at *m*/*z* 264 and *m*/*z* 222, respectively (**Figure 1b**). The corresponding eliminations are not found in saturated FAMEs, which only form $[M-31]^+$ fragment ions (*m*/*z* 267 in the case of 18:0 ME) (**Figure 1a**). Thus, fragment ions of saturated FAMEs do not interfere with $[M-32]^+$ fragment ions of MUFAMEs. Interfering fragment ions were not found in either of the mass spectra of methyl esters of polyunsaturated fatty acids (**Figure 1d**). However, dienoic fatty acids, such as 18:2(9,12) ME, also produced $[M-32]^+$ (*m*/*z* 262) (**Figure 1c**) and $[M-74]^+$ at *m*/*z* 220. While these do not overlap with the respective $[M-32]^+$ and $[M-74]^+$ fragment ions of MUFAMEs, the EI−MS spectra of 18:2(9,12) ME also contained *m*/*z* 264 ($[M-30]^+$). Thus, for an unequivocal identification of MUFAMEs, we additionally screened *m*/*z* 263 ($[M-31]^+$) and *m*/*z* 262 ($[M-32]^+$) along with *m*/*z* 264. In the case of 18:1 ME isomers, *m*/*z* 264 was predominant (only traces of *m*/*z* 263 and *m*/*z* 262), whereas higher abundances of both *m*/*z* 263 and *m*/*z* 262 compared to *m*/*z* 264 proved the presence of an 18:2 isomer (*m*/*z* 263 > *m*/*z* 262 > *m*/*z* 264; **Figure 2a**) (*29*). Note the overlap of the retention range of 18:1 and 18:2 isomers (**Figure 2b**).

Establishing a GC/EI−MS−SIM Method for the Quantification of MUFAMEs. The characteristic M^+ for MUFAMEs as well as the diagnostic fragment ions $[M-32]^+$ and $[M-74]^+$ (see previous section) along with the low-mass fragments *m*/*z* 74 and *m*/*z* 87 (*25*) were chosen as potential candidates for GC/EI−MS−SIM of MUFAMEs. Owing to a presumable different fragmentation of homologues and/or isomers, 14 MUFAMEs ranging from 12:1 to 24:1 and including 11 *cis* and 3 *trans* isomers were analyzed, all of which could be unambiguously identified by application of a comparably slow GC−oven temperature program (parts **a**−**c** of **Figure 3**). For instance, the co-elutions of 20:1(11) and 18:3(9,12,15) as well as 22:1(13) and 20:3(11,14,17) (**Figure 3a**) did not prevent determination of MUFAMEs when $[M-32]^+$ and $[M-74]^+$ were used in GC/EI−MS−SIM (parts **b** and **c** of **Figure 3**). Thus, $[M-74]^+$ and

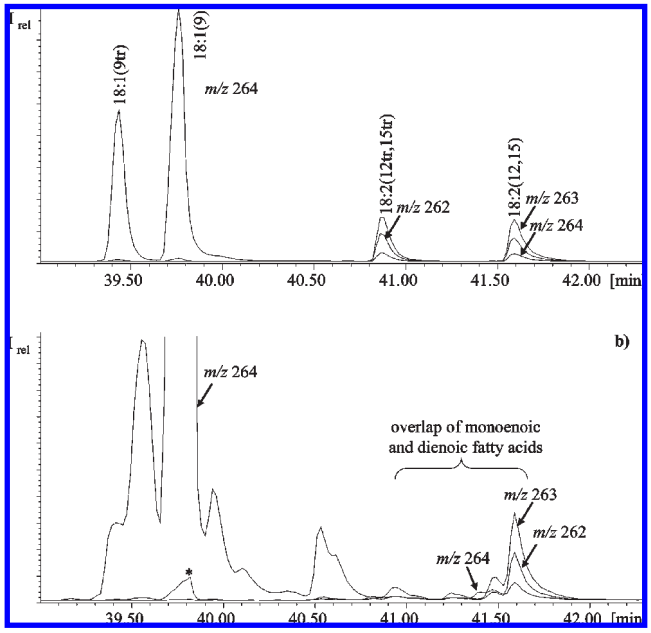

Figure 2. GC/EI−MS−SIM chromatograms [*m*/*z* 262 (gray line), *m*/*z* 263 (dotted line), and *m*/*z* 264 (black line)] extracted from total ion chromatogram) of (a) a 37-component FAME mix and (b) a detail of an unfractionated cheese sample (Bavaria Blue). (∗) Traces of *m*/*z* 263 were visible, because of the high abundance of oleic acid.

$[M-32]^+$ proved to be characteristic for the identification of MUFAMEs (*2*). However, $[M-32]^+$ of MUFAMEs also gave a response for dienoic FAMEs because the $[M-30]^+$ fragment ion and $[M-32]^+$ of MUFAMEs are isobar (i.e., they share the same nominal mass). Furthermore, trienoic FAMEs also gave a response because their molecular ions corresponded to the $[M-32]^+$ of MUFAMEs with two carbons less (**Figure 3b**). For instance, M^+ of 18:3 ME (*m*/*z* 292) corresponded to $[M-32]^+$ of 20:1 isomers (**Figure 3b**). In contrast, MUFAMEs could selectively be detected using $[M-74]^+$ (**Figure 3c**). Moreover, a 20-fold zoom into the ordinate of a FAME mix containing four 18:1 isomers (**Figure 3d**) verified that extraction of M^+, $[M-32]^+$, and $[M-74]^+$ gave smaller peaks compared to the dominating low-mass fragment ions *m*/*z* 74 or *m*/*z* 87. This

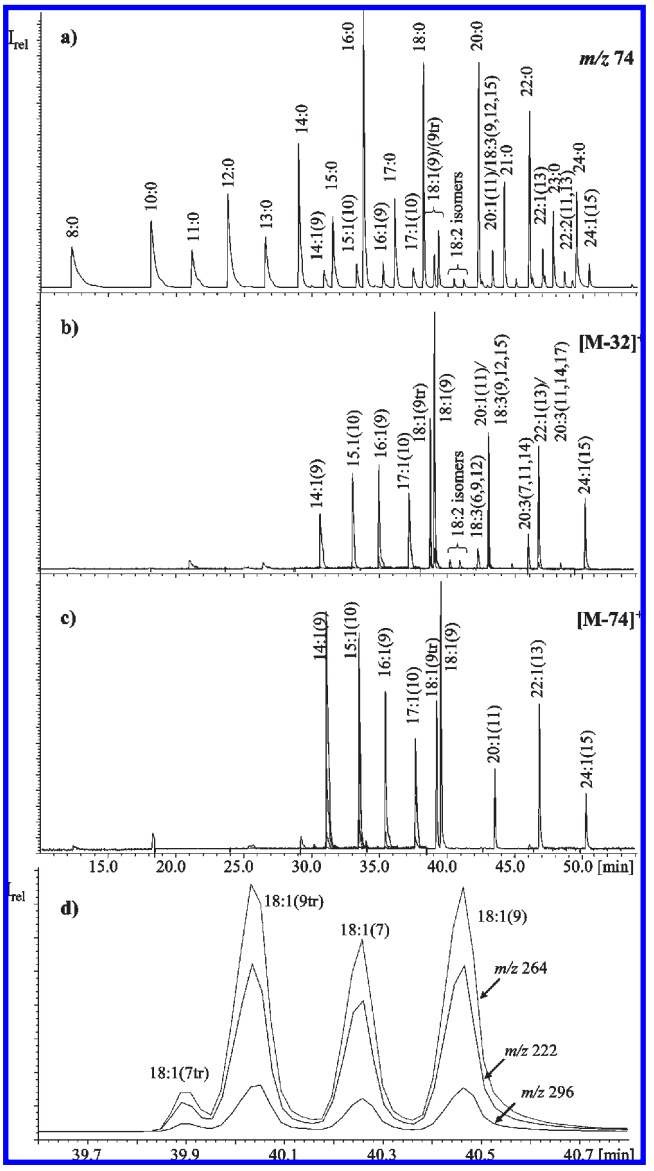

Figure 3. GC/EI−MS−SIM chromatograms of a 37-component FAME mix used for identification and quantification of MUFAMEs. (a) *m/z* 74, (b) $[M-32]^+$, (c) $[M-74]^+$, and (d) a 20-fold zoom into the ordinate of a mix of four 18:1 isomers [18:1(11)/(11tr) and 18:1(9)/(9tr)] [*m/z* 296 (black line), *m/z* 264 (dotted line), and *m/z* 222 (gray line)].

thus improved the peak resolution (especially in the usual case of a clear dominance of oleic acid), while the sensitivity proved to be sufficient for quantitative analysis.

To expand the GC/EI−MS−SIM method on MUFAMEs present in samples but not available as reference standards, the relative abundances of $[M-32]^+$ and $[M-74]^+$ in the mass spectra of MUFAME standards were studied and compared to each other (**Figure 4**). The relative contribution of *m/z* 74 to the total ion current was nearly constant (SD < 5%) for the methyl esters of 12:1−24:1 (*25*). Moreover, variations in the relative intensity of *m/z* 74 from injection to injection were negligible (SD = 0.01−0.6%, $n = 5$; data not shown). Likewise, the relative abundance of $[M-32]^+$ was relatively constant, except for 18:1 isomers, whose intensity was higher compared to other MUFAMEs (5.21 ± 0.18 for 18:1 isomers versus 4.21 ± 0.46 for all other MUFAMEs). Variations subject to multiple injections were ≪ 1.3%, expect for *cis*-vaccenic acid ME and 20:1 ME (SD = 1.6%) (data not shown). On the other hand, the abundance of $[M-74]^+$

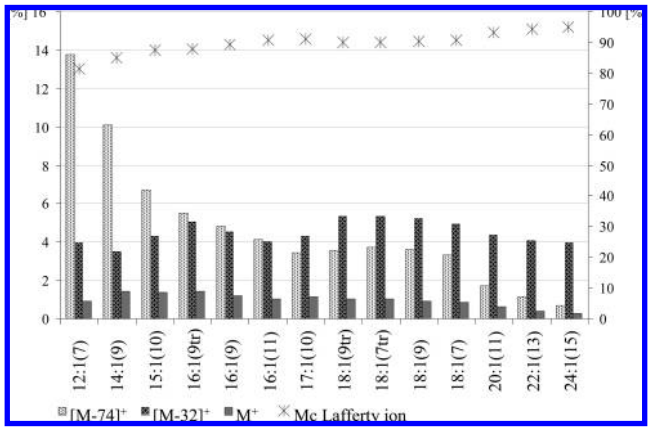

Figure 4. Relative distribution of M^+, $[M-32]^+$, $[M-74]^+$, and *m/z* 74 in the range of 12:1−24:1 isomers.

decreased with an increasing chain length (**Figure 4**). Hence, for quantitative analyses, $[M-32]^+$ was used as a quantification ion and $[M-74]^+$ was used as a qualifier ion. MUFAMEs not available as a standard were determined with the mean response of the respective ions (except for $[M-32]^+$ of 18:1 isomers, which were calculated using the factor 5.2, and all other MUFAMEs using the factor 4.2). Using this GC/EI−MS−SIM method, MUFAMEs could be unequivocally identified, even in the case of co-elutions with methyl esters of branched-chain fatty acids (*iso*/*anteiso* and phytanic acid). However, several *cis* and *trans* isomers could not be distinguished from each other. Because these cannot even be GC-resolved on 100 m CP-Sil 88 columns (*2*), a separation of *cis* and *trans* isomers was carried out prior to GC/EI−MS−SIM quantification of MUFAMEs.

Separation of *cis*- and *trans*-MUFAMEs via Silver Ion Chromatography Using SPE Cartridges. Fractionation was achieved on commercial silver ion SPE cartridges (*28*). The *cis*-MUFAME fraction was fractionated a second time (see the Materials and Methods) because one fractionation step only did not provide a full separation of *cis*- and *trans*-MUFAMEs in either case. Repeatedly, significant amounts of the dominant vaccenic acid in the *trans* fraction smeared into the *cis* fraction (Figure S1 in the Supporting Information). After a second fractionation of the *cis* fraction, the *cis* fraction was generally free of *trans* isomers (Figure S1 in the Supporting Information). Spiking experiments with 16:1tr and 18:1tr standards resulted in recovery rates of 95−106% ($n = 4$) for the silver ion SPE. In the present fractionation, we focused on *cis*- and *trans*-MUFAMEs only. Although a third fraction was collected for polyenoic fatty acids, the solvent used (acetone) does not allow for a quantitative elution of trienoic through hexaenoic fatty acids into this fraction 3 (see the Materials and Methods). However, these PUFAs can be obtained quantitatively by the use of acetonitrile in addition to acetone in the third fraction (*20*, *24*).

While an unfractionated sample of goat fat allowed for the identification of nine peaks from 18:1 isomers (**Figure 5a**), the silver ion chromatography fractions contained nine peaks (from up to 12) from *trans*-18:1 isomers (**Figure 5b**) and five peaks (from up to seven isomers) from *cis*-18:1 isomers (**Figure 5c**). Noteworthy as well, additional peaks that did not originate from 18:1 and 16:1 isomers were detected in the "*cis*-MUFAME fractions" because of the wrong abundance ratios of the SIM ions (parts **c** and **f** of **Figure 5**). Tentative peak assignment of isomers not available as standards was carried out by a comparison of the FA

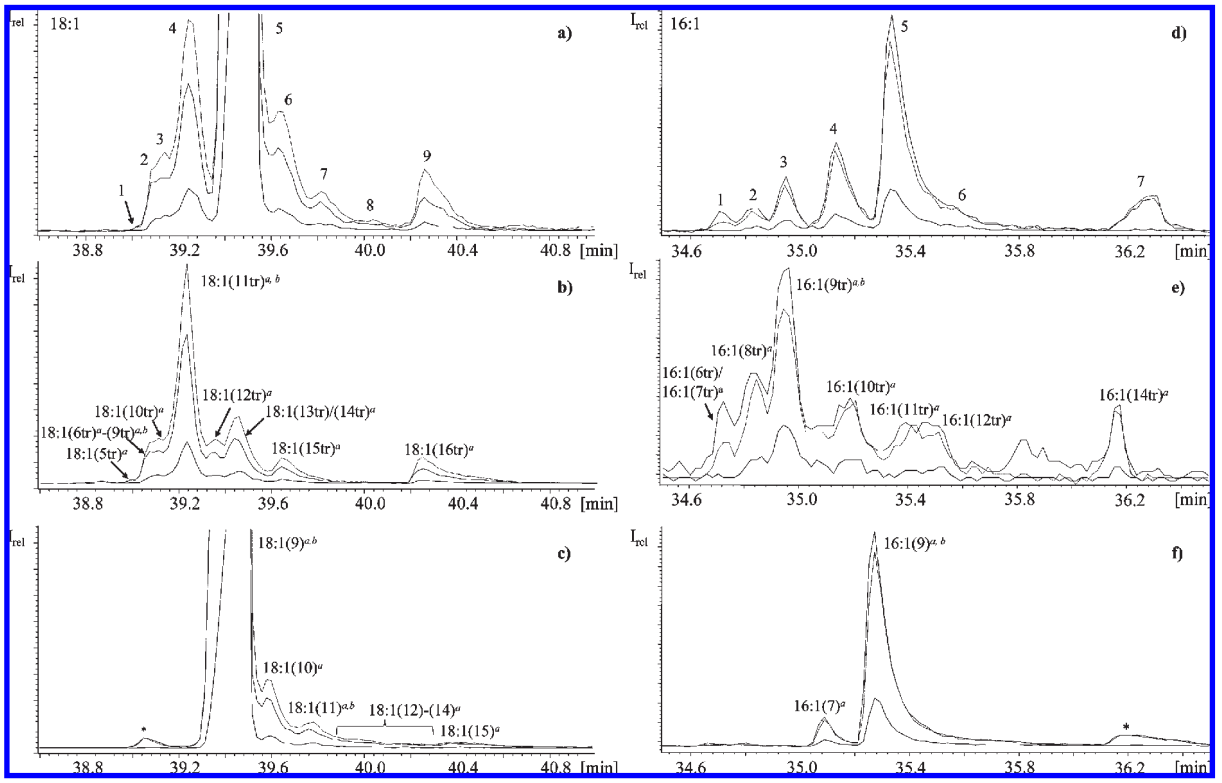

Figure 5. GC/EI−MS−SIM chromatograms [M^+ (black line), $[M-32]^+$ (dotted line), and $[M-74]^+$ (gray line)] of the retention time range of (a−c) 18:1 isomers from goat fat tissue and (d−f) 16:1 isomers from a cheese sample before and after silver ion chromatography. (a and d) Unfractionated samples, (b and e) *trans* isomer fractions, and (c and f) *cis* isomer fractions obtained after silver ion chromatography. (∗) Peak identified as a non-MUFA because of its wrong ratios of SIM-ion traces. (*a*) Peak tentatively identified according to refs (*2*, *16*, *20*, and *30*). (*b*) Peak identification by the 37-component FAME mix.

pattern obtained by Alonso et al., using similar chromatographic conditions (*30*). The distribution of *trans*-18:1 isomers in goat tissue fat was almost identical to the pattern described in goat's milk fat (*16*, *30*). Likewise, peak assignment of 16:1 isomers was based on the pattern reported by Kramer et al. (*20*) and additional standards in the form of 16:1(9tr) and 16:1(11). In agreement with Precht and Molkentin (*2*), the isomeric pattern of 18:1 isomers was very similar to the 16:1 isomers (parts **d**−**f** of **Figure 5**). For instance, the differences in retention times between the known peaks of main compounds [e.g., 16:1(9) and 18:1(11)] and neighboring isomers were almost identical (*2*). For example, Δt_R between 18:1(16) and 18:1(11) and 16:1(14) and 16:1(9) was nearly the same. Thus, all peaks could be tentatively assigned to isomer structures. However, in the case of poor resolution of close eluting isomers, some *trans* and also some *cis* isomers were quantified together (see **Table 2**).

Concentrations of *cis*- and *trans*-MUFA in Food Samples. A total of 22 *cis*- and 18 *trans*-fatty acids could be identified in the six food samples of ruminant (ewe, cow, and goat), human, and marine (cod) origin. In either case, the *cis*-MUFA content (sum of *cis* isomers) was clearly dominant. However, when the concentrations of 18:1(9) were subtracted from the *cis*-MUFA content, the concentration of the remaining *cis*-MUFAs [sum of *cis* isomers without 18:1 (9)] was comparable to the *trans*-MUFA content in the samples, except for cod liver oil (**Table 2**).

cis-MUFAs. A total of 20 even-chained *cis*-MUFAs in the range of 12:1−24:1 along with 17:1(10) and one 19:1 isomer were detected in the samples (**Table 2**). The highest total amount of *cis*-MUFAs was found in cod liver oil (38.1 g/100 g), followed by goat fat (30.4 g/100 g), whereas human milk contained the lowest amount of *cis*-MUFAs (**Table 2**). In either case, 18:1(9) was the dominating MUFA, which contributed >87% to the MUFA content, with the exception of cod liver oil (∼32% of *cis*-MUFAs). Similar results for oleic acid were also reported in other dairy products (*1*, *19*). In cod liver oil, cetoleic acid [22:1(11)] was the second most relevant MUFA, whereas 16:1(9) and 18:1(11) were the second and third most relevant MUFAs in dairy products (**Table 2**). Concentrations in the range of 0.7 mg/100 g for 18:1(11) are in excellent agreement with data reported in dairy products and human milk (*21*). The concentration of 18:1(11) was significantly lower in the organic butter sample than data reported for conventional butter (*21*). This is, however, in agreement with Collomb et al., who determined a lower content of *cis*-18:1 isomers in organic than in conventional milk fat (*31*). Comparably, high concentrations (>0.2%) were determined for 18:1(12) (*13*) and 17:1(10) (*30*) in the samples from ruminants. In addition, 12:1(7) was high in Bavaria Blue (>1% of oleic acid), whereas 14:1(9) was >1.1% of oleic acid in Bavaria Blue and organic butter (**Table 2**). Additionally, 18:1 isomers in cheese [18:1(16), 18:1(15), 18:1(14/13), and 18:1(12)] were found in the 0.02−0.15 g/100 g range.

The pattern of the 16:1 isomers was the same for all ruminant samples as well as human milk fat. In all cases, the concentration of 16:1(7) was 3−10 times lower than that of 16:1(9) (∼0.5−1 g/100 g). Both organic butter and Bavaria Blue contained the same amounts of 20:1(11) (0.06 g/100 g) and 20:1(13) (0.03 g/100 g). Finally, the terrestrial samples contained neither 22:1 nor 24:1 isomers. In contrast to ruminant and human milk fats, cod liver oil contained a wide spectrum of abundant long-chain MUFAs (even-chained 20:1−24:1 isomers). Three 20:1 isomers were

Table 2. Concentrations (g/100 g of Lipids) and Variety of *cis*- and *trans*-MUFA in Food Samples

	Bavaria Blue (g/100 g)	Roquefort (g/100 g)	organic butter (g/100 g)	goat tissue (g/100 g)	human milk (g/100 g)	cod liver oil (g/100 g)
			cis Isomers			
12:1(7)[a]	0.26	nd[b]	nd	0.06	nd	nd
14:1(9)[a]	0.26	nd	0.36	0.15	nd	nd
16:1(7)[c]	0.09	0.13	0.10	0.20	0.06	0.15
16:1(9)[a,c]	1.02	0.59	0.97	0.66	0.46	5.44
16:1(10)[c]	nd	nd	nd	nd	nd	0.39
17:1(10)[a]	0.27	0.22	0.28	0.41	0.03	0.20
18:1(6)/(7)[c]	nd	nd	nd	nd	nd	0.61
18:1(8)/(9)[a,c]	23.94	13.48	18.77	27.88	10.10	12.05
18:1(11)[a,c]	0.67	0.70	0.65	0.61	0.83	2.93
18:1(12)[c]	0.29	0.29	0.41	0.41	0.05	nd
18:1(13)[c]	nd	0.00	nd	nd	nd	0.24
18:1(14)[c]	0.12	0.03	nd	0.01	nd	nd
18:1(15)[c]	0.15	0.11	nd	0.03	nd	nd
18:1(16)[c]	0.05	0.06	nd	nd	nd	nd
19:1[d]	0.12	nd	nd	nd	nd	nd
20:1(11)[a]	0.07	nd	0.06	nd	0.01	1.49
20:1(13)[e]	0.03	0.02	0.03	nd	0.07	6.24
20:1(15)[e]	nd	nd	nd	nd	nd	0.29
22:1(11)[a]	nd	nd	nd	nd	nd	7.25
22:1(13)[e]	nd	nd	nd	nd	nd	0.73
24:1(15)[a]	nd	nd	nd	nd	nd	0.05
24:1(17)[e]	nd	nd	nd	nd	nd	0.01
number of *cis* isomers	15	13	11	11	10	16
$\sum$18:1 (*cis* isomers)	25.24	14.68	19.82	28.94	10.98	15.83
$\sum$16:1 (*cis* isomers)	1.11	0.73	1.07	0.86	0.51	5.97
sum of *cis*-MUFA	27.35	15.64	21.63	30.41	11.60	38.05
sum of *cis*-MUFA without 18:1(9)	3.41	2.16	2.86	2.51	1.51	26.02
			trans Isomers			
12:1(7tr)[a]	nd	nd	0.05	0.07	nd	nd
14:1(9tr)[a]	nd	nd	nd	0.01	nd	nd
15:1(10tr)[a]	nd	nd	0.03	0.04	nd	nd
16:1(6tr)/(7tr)[c]	0.01	0.04	0.02	0.01	0.01	0.02
16:1(8tr)[c]	0.03	0.05	0.03	0.03	0.01	0.20
16:1(9tr)[a,c]	0.06	0.10	0.07	0.05	0.01	0.02
16:1(10tr)[c]	0.02	0.04	0.02	0.02	0.01	nd
16:1(11tr)[c]	0.02	0.03	0.02	0.01	nd	nd
16:1(12tr)[c]	0.02	0.03	0.02	0.01	nd	nd
16:1(14tr)[c]	0.02	0.02	0.02	0.02	0.01	nd
18:1(5tr)[c]	0.03	0.03	0.03	0.01	0.02	nd
18:1(6tr)−(9tr)[a,c]	0.27	0.38	0.28	0.18	0.15	0.02
18:1(10tr)[c]	0.30	0.41	0.29	0.23	0.18	nd
18:1(11tr)[a,c]	1.41	1.88	1.32	0.77	0.34	0.05
18:1(12tr)[c]	0.15	0.36	0.28	0.18	0.08	0.01
18:1(13tr)/(14tr)[c]	0.42	0.66	0.49	0.22	0.10	0.01
18:1(15tr)[c]	0.15	0.19	0.15	0.08	0.03	0.01
18:1(16tr)[c]	0.15	0.12	0.10	0.07	0.04	nd
number of *trans* isomers	15	15	17	18	13	8
$\sum$*trans*-18:1 isomers	2.89	4.03	2.94	1.74	0.94	0.11
$\sum$*trans*-16:1 isomers	0.18	0.30	0.19	0.14	0.05	0.24
sum of *trans*-MUFA	3.07	4.34	3.22	2.01	0.99	0.35

[a] Peak identification by the 37-component FAME mix. [b] nd = not detected (limit of detection = 0.006 g/100 g of lipid). [c] Peak was tentatively identified according to refs (*2*, *16*, *20*, and *30*). [d] Peak identification was not possible because the reference standard was lacking. [e] Peak was tentatively identified by evaluation of Δt_R.

detected, with the most abundant being 20:1(13) (6.2 g/100 g). Furthermore, two 22:1 isomers amounting to ~8 g/100 g [including 0.7 g/100 g for 22:1(13), erucic acid] were detected in the fish oil. Concentrations of 16:1(9) (5.5 g/100 g), 18:1(9) (12 g/100 g), and 18:1(11) (3 g/100 g) were comparable to those reported in other marine food samples (*21*). Short-chain MUFAs were not detected in this cod liver oil.

trans-MUFAs. The total content of TFAs in the sample ranged from 0.35 g/100 g (cod liver oil) to 4.3 g/100 g in the raw ewe's milk cheese Roquefort (*13*, *32*). Owing to the unique pattern in the cod liver oil, this sample will be discussed separately below. In the terrestrial samples, 18:1tr isomers contributed >92% to the total *trans*-MUFA content. In addition, the samples contained a wide range of *trans*-16:1 isomers (<10% of *trans*-18:1 isomers), whereas organic butter also contained 12:1(7tr) and 15:1(10tr) and goat fat contained the latter two as well as 14:1(9tr) (**Table 2**).

The isomer composition in milk is influenced by the feed and season in which the samples are taken (*31*, *33*). Nevertheless, both the dominance of 18:1(11tr) and its

concentrations (~1.3−1.9 g/100 g) in dairy products (>45% of 18:1tr, >42% of total TFA, respectively) and human milk (>35% of 18:1tr, >34% of the TFA content) agreed well with data published by other authors (*2*, *32*, *34*). The 18:1(9tr), 18:1(8tr), 18:1(7tr), and 18:1(6tr) were quantified together, but 18:1(9tr) was likely to be the most prominent isomer of this peak pattern. The contributions of both the 18:1(9tr) peak and 18:1(10tr) to the total *trans*-18:1 content were nearly constant in all ruminant products (10%), which is in agreement with literature reports (*21*). Higher proportions of 18:1(9tr) and 18:1(10tr) were determined in human milk (>15%, respectively). All samples, except for cod liver oil, showed a relative identical pattern of 18:1 isomers in the range of 18:1(16tr) (3−5%) to 18:1(5tr) (0.8−2.3%), which are well-matched with those obtained in the literature (*32*).

The *trans*-16:1 pattern in the terrestrial samples was very similar and dominated by 16:1(9tr) (28−36%) > 16:1(8tr) (16−26%) > 16:1(10tr) (11−14%) (**Table 2**). The total amounts of 16:1tr (0.18−0.3 g/100 g) as well as their ratio to total *trans*-MUFA (6−7%) obtained for dairy products was nearly the same as in dairy products analyzed by Destaillats et al. (*11*). While seven *trans*-16:1 isomers were detected in the ruminant samples, the human milk sample contained only five *trans*-16:1 isomers. The 12:1(7tr) and 15:1(10tr) were also observed in organic butter and goat fat.

Only cod liver oil contained very low *trans*-MUFA amounts (0.045 g/100 g), and the *trans*-16:1 isomers were more than double-fold as abundant as *trans*-18:1 isomers. The highest TFA amounts originated from 16:1(8tr), which contributed 83% to *trans*-16:1 isomers and 57% to the total TFA content of the fish oil. The cod liver oil only contained 18:1(9tr) (17.9% to total 18:1tr) and not 18:1(10tr). Noticeably, cod liver oil had both the lowest total TFA contents as well as the least variety of TFAs.

Potential of the Quantitative GC/EI−MS Method for Application in Food Analysis. The results obtained with the present method matched those determined with other methods (see above). However, most previous results were obtained using very long columns (100 m) (*1*, *13*) in combination with isothermal oven programming. This resulted in a good resolution of nearly all *cis*- and *trans*-18:1 and 16:1 isomers but at the price of a long analysis time (>3 h) (*1*). Faster GC programming using 100 m columns (run times < 2 h) (*5*, *20*, *34*) allowed for the determination of one, at most two, isomer groups (*2*), whereas MUFAs of other chain length could not be studied. In contrast, the shorter 50 m column used in this study in combination with GC/EI−MS−SIM produced similar results for 18:1 and 16:1 isomers within approximately 1 h. This compensated for the extra time required for the application of a second fractionation step using the silver ion SPE applied in this study, to warrant a complete fractionation of *cis*- and *trans*-MUFAMEs (see above).

The high sensitive SIM mode allowed for the quantification of MUFAMEs irrespective of the chain lengths at very low concentrations (limits of detection in the range of 0.006 g/100 g of lipid; **Table 2**) and even the identification of unknown or unusual MUFAMEs. Lastly, in combination with the use of IS, we were able to quantify absolute concentrations of MUFAMEs in food lipids without carrying out a cumbersome quantitation of all fatty acids and determination of the MUFA content (also known as a "100% method"). Determination of MUFAMEs in this way is a highly automatable method for fatty acid determination, for not only the content but also the isomer distribution.

Supporting Information Available: GC/EI−MS−SIM chromatograms of the *cis* and *trans*-18:1 region of a sample of butter fat obtained after single and repeated silver ion fractionation. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Precht, D.; Molkentin, J.; Destaillats, F.; Wolff, R. L. Comparative studies on individual isomeric 18:1 acids in cow, goat, and ewe milk fats by low-temperature high-resolution capillary gas−liquid chromatography. *Lipids* **2001**, *36*, 827–832.

(2) Precht, D.; Molkentin, J. Identification and quantitation of *cis*/*trans* C16:1 and C17:1 fatty acid positional isomers in German human milk lipids by thin-layer chromatography and gas chromatography/mass spectrometry. *Eur. J. Lipid Sci. Technol.* **2000**, *102*, 102–113.

(3) Aro, A.; Kosmeijer-Schuil, T.; van de Bovenkamp, P.; Hulshof, P.; Zock, P.; Katan, M. B. Analysis of C18:1 *cis* and *trans* fatty acid isomers by the combination of gas−liquid chromatography of 4,4-dimethyloxazoline derivatives and methyl esters. *J. Am. Oil Chem. Soc.* **1998**, *75*, 977–985.

(4) Odegaard, A. O.; Pereira, M. A. *trans*-Fatty acids, insulin resistance, and type 2 diabetes. *Nutr. Rev.* **2006**, *64* 364–372.

(5) Ratnayake, W. M. N.; Hansen, S. L.; Kennedy, M. P. Evaluation of the CP-Sil 88 and SP-2560 GC columns used in the recently approved AOCS official method Ce 1h-05: Determination of *cis*-, *trans*-, saturated, monounsaturated, and polyunsaturated fatty acids in vegetable or non-ruminant animal oils and fats by capillary GLC method. *J. Am. Oil Chem. Soc.* **2006**, *83*, 475–488.

(6) Food and Drug Administration. Food labeling: *trans*-Fatty acids in nutrition labeling, nutrient content claims, and health claims. *Fed. Regist.* **2003**, *68*, 41434–41506.

(7) Wolff, R. L.; Combe, N. A.; Destaillats, F.; Boue, C.; Precht, D.; Molkentin, J.; Entressangles, B. Follow-up of the Δ4 to Δ16 *trans*-18:1 isomer profile and content in French processed foods containing partially hydrogenated vegetable oils during the period 1995−1999. Analytical and nutritional implications. *Lipids* **2000**, *35*, 815–825.

(8) Ratnayake, W. M. N. Overview of methods for the determination of *trans* fatty acids by gas chromatography, silver-ion thin-layer chromatography, silver-ion liquid chromatography, and gas chromatography/mass spectrometry. *J. AOAC Int.* **2004**, *87*, 523–539.

(9) Hay, J. D.; Morrison, W. R. Isomeric monoenoic fatty acids in bovine milk fat. *Biochim. Biophys. Acta, Lipids Lipid Metab.* **1970**, *202*, 237–243.

(10) Ledoux, M.; Juaneda, P.; Sebedio, J. L. *trans*-Fatty acids: Definition and occurrence in foods. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 891–900.

(11) Destaillats, F.; Wolff, R. L.; Precht, D.; Molkentin, J. Study of individual *trans*- and *cis*-16:1 isomers in cow, goat, and ewe cheese fats by gas−liquid chromatography with emphasis on the *trans*-Δ3 isomer. *Lipids* **2000**, *35*, 1027–1032.

(12) Jensen, R. G. The composition of bovine milk lipids: January 1995 to December 2000. *J. Dairy Sci.* **2002**, *85*, 295–350.

(13) Precht, D.; Molkentin, J. Rapid analysis of the isomers of *trans*-octadecenoic acid in milk fat. *Int. Dairy J.* **1996**, *6* 791–809.

(14) Bysted, A.; Cold, S.; Holmer, G. An optimized method for fatty acid analysis, including quantification of *trans*-fatty acids, in human adipose tissue by gas−liquid chromatography. *Scand. J. Clin. Lab. Invest.* **1999**, *59*, 205–214.

(15) Ratnayake, W. M. N.; Plouffe, L. J.; Pasquier, E.; Gagnon, C. Temperature-sensitive resolution of *cis*- and *trans*-fatty acid isomers of partially hydrogenated vegetable oils on SP-2560

and CP-Sil 88 capillary columns. *J. AOAC Int*. **2002**, *85* 1112–1118.
(16) Wolff, R. L.; Combe, N. A.; Precht, D.; Molkentin, J.; Ratnayake, W. M. N. Accurate determination of *trans*-18:1 isomers by capillary gas−liquid chromatography on cyanoalkyl polysiloxane stationary phases. *Ol., Corps Gras, Lipides* **1998**, *5*, 295–300.
(17) Delmonte, P.; Rader, J. I. Evaluation of gas chromatographic methods for the determination of *trans* fat. *Anal. Bioanal. Chem*. **2007**, *389*, 77–85.
(18) Molkentin, J.; Precht, D. Occurrence of *trans*-C16:1 acids in bovine milk fats and partially hydrogenated edible fats. *Milchwissenschaft* **1997**, *52*, 380–385.
(19) Chilliard, Y.; Ferlay, A.; Rouel, J.; Lamberet, G. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. *J. Dairy Sci*. **2003**, *86*, 1751–1770.
(20) Kramer, J. K. G.; Hernandez, M.; Cruz-Hernandez, C.; Kraft, J.; Dugan, M. E. R. Combining results of two GC separations partly achieves determination of all *cis*- and *trans*-16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstrated using Ag-ion SPE fractionation. *Lipids* **2008**, *43*, 259–273.
(21) Thurnhofer, S.; Lehnert, K.; Vetter, W. Exclusive quantification of methyl-branched fatty acids and minor 18:1-isomers in foodstuff by GC/MS in the SIM mode using 10,11-dichloroundecanoic acid and fatty acid ethyl esters as internal standards. *Eur. Food Res. Technol*. **2008**, *226*, 975–983.
(22) Mendis, S.; Cruz-Hernandez, C.; Ratnayake, W. M. N. Fatty acid profile of Canadian dairy products with special attention to the *trans*-octadecenoic acid and conjugated linoleic acid isomers. *J. AOAC Int*. **2008**, *91*, 811–819.
(23) Wolff, R. L. Content and distribution of *trans*-18:1 acids in ruminant milk and meat fats. Their importance in European diets and their effect on human milk. *J. Am. Oil Chem. Soc*. **1995**, *72*, 259–272.
(24) Christie, W. W. Silver ion chromatography using solid-phase extraction columns packed with a bonded sulfonic acid phase. *J. Lipid Res*. **1989**, *30*, 1471–1473.
(25) Thurnhofer, S.; Vetter, W. A gas chromatography/electron ionization−mass spectrometry−selected ion monitoring method for determining the fatty acid pattern in food after formation of fatty acid methyl esters. *J. Agric. Food Chem*. **2005**, *53*, 8896–8903.
(26) Thurnhofer, S.; Vetter, W. Application of ethyl esters and d_3-methyl esters as internal standards for the gas chromatographic quantification of transesterified fatty acid methyl esters in food. *J. Agric. Food Chem*. **2006**, *54*, 3209–3214.
(27) Weichbrodt, M.; Vetter, W.; Luckas, B. Microwave-assisted extraction and accelerated solvent extraction with ethyl acetate−cyclohexane before determination of organochlorines in fish tissue by gas chromatography with electron-capture detection. *J. AOAC Int*. **2000**, *83*, 1334–1343.
(28) Shimelis, O.; Aurand, C.; Trinh, A. Fractionation of *cis*/*trans* FAMEs using Discovery Ag-ION SPE. *LCGC North Am*. **2006**, *24* (9 Supplement), 48.
(29) Hallgren, B.; Ryhage, R.; Stenhagen, E. Mass spectra of methyl oleate, methyl linoleate, and methyl linolenate. *Acta Chem. Scand*. **1959**, *13*, 845–847.
(30) Alonso, L.; Fontecha, J.; Lozada, L.; Fraga, M. J.; Juarez, M. Fatty acid composition of caprine milk: Major, branched-chain, and *trans*-fatty acids. *J. Dairy Sci*. **1999**, *82*, 878–884.
(31) Collomb, M.; Bisig, W.; Butikofer, U.; Sieber, R.; Bregy, M.; Etter, L. Fatty acid composition of mountain milk from Switzerland: Comparison of organic and integrated farming systems. *Int. Dairy J*. **2008**, *18*, 976–982.
(32) Destaillats, F.; Golay, P. A.; Joffre, F.; de Wispelaere, M.; Hug, B.; Giuffrida, F.; Fauconnot, L.; Dionisi, F. Comparison of available analytical methods to measure *trans*-octadecenoic acid isomeric profile and content by gas−liquid chromatography in milk fat. *J. Chromatogr., A* **2007**, *1145*, 222–228.
(33) Cruz-Hernandez, C.; Kramer, J. K. G.; Kennelly, J. J.; Glimm, D. R.; Sorensen, B. M.; Okine, E. K.; Goonewardene, L. A.; Weselake, R. J. Evaluating the conjugated linoleic acid and *trans*-18:1 isomers in milk fat of dairy cows fed increasing amounts of sunflower oil and a constant level of fish oil. *J. Dairy Sci*. **2007**, *90*, 3786–3801.
(34) Precht, D.; Molkentin, J. C18:1, C18:2 and C18:3 *trans*- and *cis*-fatty acid isomers including conjugated *cis*-Δ9, *trans*-Δ11 linoleic acid (CLA) as well as total fat composition of German human milk lipids. *Nahrung* **1999**, *43*, 233–244.

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