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ORIGINAL PAPER

GC/EI-MS Determination of the Diastereomer Distribution of Phytanic Acid in Food Samples

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Abstract Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) is a branched-chain fatty acid, produced by bacteria by means of oxidation and biohydrogenation of the chlorophyll side chain phytol (3,7*R*,11*R*,15-tetramethylhexadec-2-en-1-ol). The later reaction generates to a new stereogenic center on C-3 which can be both 3*R*- or 3*S*-configured. Thus, two diastereomers (3*S*,7*R*,11*R*,15- and 3*R*,7*R*,11*R*,15-phytanic acid) are naturally produced. In this study we examined the diastereomer composition of phytanic acid in terrestrial and marine food samples. Phytanic acid was transferred into its methyl ester which was analyzed by GC/MS in the selected ion monitoring mode. The first eluted diastereomer in the samples was tentatively identified as 3*S*,7*R*,11*R*,15-phytanic acid. The marine samples were clearly dominated by 3*S*,7*R*,11*R*,15-phytanic acid whose abundance was higher in marine mammals than in fish. Milk from one organic cow collected over a period of 30 days showed lower proportions of 3*S*,7*R*,11*R*,15-phytanic acid than milk from one cow raised with conventional feed. The difference between organic and conventional dairy products (cheese and butter) was not as pronounced as in milk. Milk samples from other mammals (goat, sheep, mare, camel, moose, and human) also showed an excess of 3*S*,7*R*,11*R*,15-phytanic acid except for camel and moose milk.

Keywords Multi-branched fatty acids · Methyl-branched fatty acids · Phytanic acid · Diastereomers · Organic milk · Dairy products · Marine oils · GC/MS-SIM · Food authentication

Introduction

Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) is a fatty acid formed during the degradation of isoprenoids. The isomer of arachidic acid ($C_{20}H_{40}O_2$) has a longest chain of 16 carbons along with four methyl substituents on C-3, C-7, C-11, and C-15. Phytanic acid cannot be de novo synthesized by humans, and thus is largely derived from food [1]. Primary dietary sources of phytanic acid are milk and dairy products as well as seafood [2, 3]. In either way, the initial compound used for the biosynthesis of phytanic acid is chlorophyll a, b, and d. These are substituted with the alcohol moiety phytol (3,7*R*,11*R*,15-tetramethylhexadec-2-en-1-ol, Fig. 1a). In ruminants, the primary alcohol phytol is released from chlorophyll by different bacteria in the rumen. The two asymmetric carbons on phytol are *R*-configured [4]. The released alcohol is oxidized to 2-phytenic acid [5]. Subsequent saturation of the double bond (biohydrogenation) of 2-phytenic acid leads to phytanic acid (Fig. 1b). Owing to this biosynthetic pathway, the configurations of C-7 and C-11 on phytanic acid resemble the *R*-configurations of phytol. However, biohydrogenation of the double bond leads to a new stereogenic center on C-3 [6].

Typical phytanic acid contents of milk fat were reported to be ~0.28%, whereas, the concentration in human milk fat was ~0.11% [2]. On the other hand, phytanic acid is virtually absent in vegetables [7]. Due to the exclusive precursor chlorophyll/phytol it was found that lipid-based

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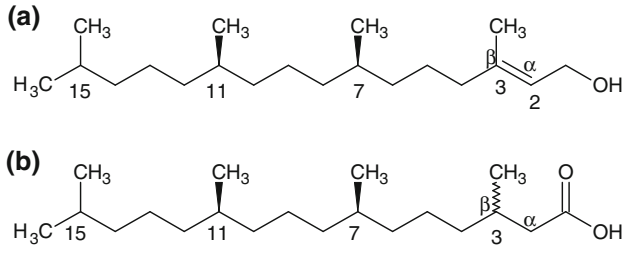

Fig. 1 Chemical structures of **a** chlorophyll-derived 3,7*R*,11*R*,15-tetramethylhexadec-2-en-1-ol (phytol) and **b** biogenic 3*RS*,7*R*,11*R*,15-tetramethylhexadecanoic acid (phytanic acid)

concentrations of phytanic acid were significantly higher in organic milk than in conventionally produced milk [8]. Data on the diastereomer distribution of phytanic acid are scarce. The diastereomers could be resolved after conversion into (*R*)-1-phenylethylamine derivatives [9]. However, a sufficient resolution of the phytanic acid diastereomers has also been obtained in form of methyl esters [10–12]. In most occasions, the multi-branched fatty acid had to be enriched from the fatty acid fraction due to its low concentrations in lipids. This has been achieved by a combination of hydration of unsaturated fatty acids followed by the separation of existing and formed saturated fatty acids from branched-chain fatty acids by urea complexation or by bromination of unsaturated fatty acids [10, 13]. Such cumbersome sample pre-treatments assuredly hampered a thorough investigation of the diastereomer distribution of phytanic acid in food. Historic investigations indicated that both 3*S*,7*R*,11*R*,15- (hereafter "SRR") and 3*R*,7*R*,11*R*,15-phytanic acid (hereafter "RRR") are present in food [14]. On one hand, a clear dominance of SRR (in their paper denoted as LDD-phytanic acid) was reported for marine samples while samples from terrestrial mammals usually contained an excess of RRR [13]. On the other hand, higher proportions of SRR were determined in humans from Europe whereas RRR was the prevailing diastereomer in one human from New Zealand [15]. It was proposed that different abundances of RRR relative to SRR in humans are owing to different ratios in the diet [15]. For instance, a sample of butter fat from New Zealand also contained higher proportions of RRR.

In this study we attempted to determine the phytanic acid diastereomer distribution in a larger set of samples of milk, dairy products and marine oils. Emphasis was also put on milk samples from cows raised organically or conventionally. We chose to substitute the rather complex procedures previously used by taking advantage of the fatty acids transesterified into methyl esters, separation on a polar GC stationary phase and detection by means of electron ionization mass spectrometry operated in the selected ion monitoring (GC/EI-MS-SIM) mode [8].

Experimental Procedures

Food Samples

Milk from a conventionally-raised cow (Deutsche Holstein Schwarzbunt, born 2004, late in the second lactation) was taken on 15 subsequent days both in the morning and evening on the Hohenheim campus (Stuttgart, Germany). The feed consisted of 20.5% corn silage, 24% grass silage, 10.3% clover silage, 3.4% hay, 10.3% after grass, 1.3% mineral feed, and 30.2% concentrate (22% corn, 20% wheat, 20% defatted rape shred, 17.5% barley, 15% field beans, 4.5% corn gluten, and 1% rapeseed oil). Analysis of the feed of the conventional cow: 38.9% dry matter consisting of 136 g/kg crude protein, 125 g/kg ruminally degradable protein, 32 g/kg raw fat, and 220 g/kg raw fiber. No fish meal was used. Organic milk was obtained from a cow (Holstein–Friesian Rotbunt, born 2002, late in the fourth or fifth lactation) raised at an organic farm in the proximity of Stuttgart (Germany). Milk was taken manually in the morning over a period of 30 days. The feed was composed of ground forage (~28.6% hay, ~57.1% grass/clover silage and ~14.3% concentrate of oat, peas, and clover). No fish meal was used. Both milk samples were neither defatted nor pasteurized. Milk samples were manually homogenized before and after freeze drying [8]. In addition we analyzed three further milk samples, i.e. organic whole milk ("Bio-Vollmilch", pasteurized, not homogenized, with a natural fat content of at least 3.8% fat, according to label), organic low fat milk ("fettarme Bio-Milch", pasteurized, not homogenized, fat content 1.5%), and alpine milk ("Alpenmilch") from the German market. We also studied 14 organic cheeses (Emmental cheese (2×), Gouda cheese (2×), butter cheese (2×), Edam cheese, camembert, cow mozzarella, sliced cheese, brie, Bavaria blu, Romadur and mountain cheese) and ten conventional cheeses (Gouda cheese, Edam cheese, Harzer cheese, hand cheese, Emmental cheese, Romadur cheese, Limburger cheese, butter cheese, cow milk feta and cow mozzarella) as well as further dairy products (organic cream, organic sweet cream butter, organic sour cream butter, organic curd cheese, organic yoghurt, and one conventionally produced butter sample) and meat samples. All samples were from the German market and details are given below and in the paper by Vetter and Schröder [8] except for organic curd cheese (Söbekke, DE-NI-059, 29.06.2008, 006) and organic yoghurt (Andechser, DE-BY-117, 10.07.2008, 001). Additional milk samples (goat, mare, sheep, moose, two humans, and three camels) and one moose cheese were from the following sources. The camel milk was sampled in spring 2009 at a camel farm in Rotfelden (Southern Germany). Moose milk and cheese were obtained in June 2009 from a farm in Bjurholm

(Sweden). The mare milk was from a health store (sampled in Germany, 2004). Human milk samples were from two women living close to Stuttgart, Germany (sampled in 2008). The following marine products were analyzed as well: lipids from a sample of trout fillet (fished in a river in Southern Germany), gilthead seabream (local market in Croatia), squid (from the East Indian Ocean), a green turtle from Queensland/Australia (green turtles feed exclusively on seagrass and algae [16]), as well as blubber of a sea lion (King George Island, Antarctica) and four harbor porpoises from Iceland.

Chemicals and Standards

A racemic standard of phytanic acid (>96% in ethanol, mixture of isomers) was ordered from Cayman Chemical (Ann Arbor, MI, USA). Origin of other fatty acids used as reference standards is presented elsewhere [8]. Cyclohexane (purest, VWR, Darmstadt, Germany) and ethyl acetate (purest, Acros Organics, Geel, Belgium) were distilled to give the azeotrope (46:54, w/w). Methanol and *n*-hexane (both HPLC gradient grade) were from Fluka (Sigma–Aldrich Chemie, Seelze, Germany). Isooctane (analytical reagent grade) was from Fisher Scientific (Ulm, Germany), and isolute-HM-N was from Separtis (Grenzlach–Wyhlen, Germany). Boron trifluoride–methanol complex solution (13–15% BF_3 in methanol) was from Riedel-de-Haën (Taufkirchen, Germany) and ethanolic BF_3 ($\sim$10%, $\sim$1.3 M, purris) was from Fluka (Taufkirchen, Germany).

Sample Preparation

Details of the sample preparation were reported elsewhere [8, 17]. In brief, food samples except oils were lyophilized, and lipids were gained by accelerated solvent extraction (ASE 200, Dionex, Idstein, Germany). Formation of methyl esters (transesterification) was performed with 0.5 mL methanolic KOH (0.5 M) followed by 1 mL methanolic BF_3. The internal standard phytanic acid ethyl ester was prepared from phytanic acid by using ethanolic KOH and ethanolic BF_3 according to Thurnhofer and Vetter [18].

Gas Chromatography Coupled to Electron Ionization Mass Spectrometry (GC/EI-MS)

Analyses were performed with a 5890 series II gas chromatograph interfaced to a 5971A mass selective detector (Hewlett-Packard/Agilent, Waldbronn, Germany) [8]. Initially, a 50 m × 0.25 mm i.d. fused-silica capillary column coated with 0.20 μm d_f 100% biscyanopropyl polysiloxane (CP-Sil 88, Chrompack, Middelburg, The Netherlands) was installed in the GC oven [8]. However, improved diastereomer resolution was obtained with two 30 m × 0.25 mm i.d. fused-silica capillary column coated with 0.10 μm d_f 10% cyanopropylphenyl and 90% biscyanopropyl polysiloxane columns (Rtx-2330, Restek, Bellefonte, PA, USA) serially linking by means of a press fit connection (A–Z Analytik Zubehör, Langen, Germany). Different GC oven programs were evaluated and the best diastereomer resolution of phytanic acid was obtained as follows; after 1 min at 50 °C, the oven was heated at 13 °C/min to 115 °C, at 0.5 °C/min to 140 °C (hold time 1 min), and finally at 15 °C/min to 250 °C (hold time 10 min). The total run time was 74.33 min. In the SIM mode, the ions *m/z* 74, *m/z* 88, *m/z* 101, *m/z* 157, *m/z* 171, *m/z* 312, *m/z* 326 were recorded after a solvent delay of 8 min.

Linearity of the GC/EI-MS response ($R^2 = 0.9992$) was given throughout the measured range of standards and samples. The detection limit (signal-to-noise = 10) of phytanic acid was calculated for the less abundant second eluted diastereomer peak (sample Edam cheese, 16.5%). Under these conditions the detection limit of this diastereomer was 26 pg (injection 1 μL) which corresponds with a phytanic acid content of 13 mg/100 g milk fat. Fat of a sample of organic milk was used to determine the precision. The precision ($n = 6$) was 45.5 ± 0.51%. Because there was a lack of a standard with known diastereomer composition, the accuracy was determined by the preparation of a 1:1 mix of a sample of organic Romadur cheese (RRR = 64.5%) with low fat milk (RRR = 49.3%) which gave 57.4% compared to 57.1% in theory. The diastereomer distribution of phytanic acid was evaluated by one-way ANOVA as implemented in Excel. Additional statistic tests were performed in form of the Nalimov outlier test and the *t*-test, and the level of significance was $\alpha = 0.001$.

Results and Discussion

Gas Chromatography and Mass Spectrometry of Phytanic Acid Methyl Ester

The determination of phytanic acid methyl ester by GC/EI-MS-SIM was based on a previously described method using *m/z* 74 and *m/z* 101 [8]. In order to improve the diastereomer resolution we linked two 30 m columns to obtain a total length of 60 m. With this system, the best diastereomer resolution of phytanic acid was achieved by application of a slow heating rate in the elution range of phytanic acid [8]. For this purpose, the GC oven temperature was raised at 0.5 °C/min starting $\sim$25 °C below the elution temperature of phytanic acid (140 °C). GC/EI-MS analysis of the methyl ester of a commercial phytanic acid standard produced the peak pattern shown in Fig. 2a.

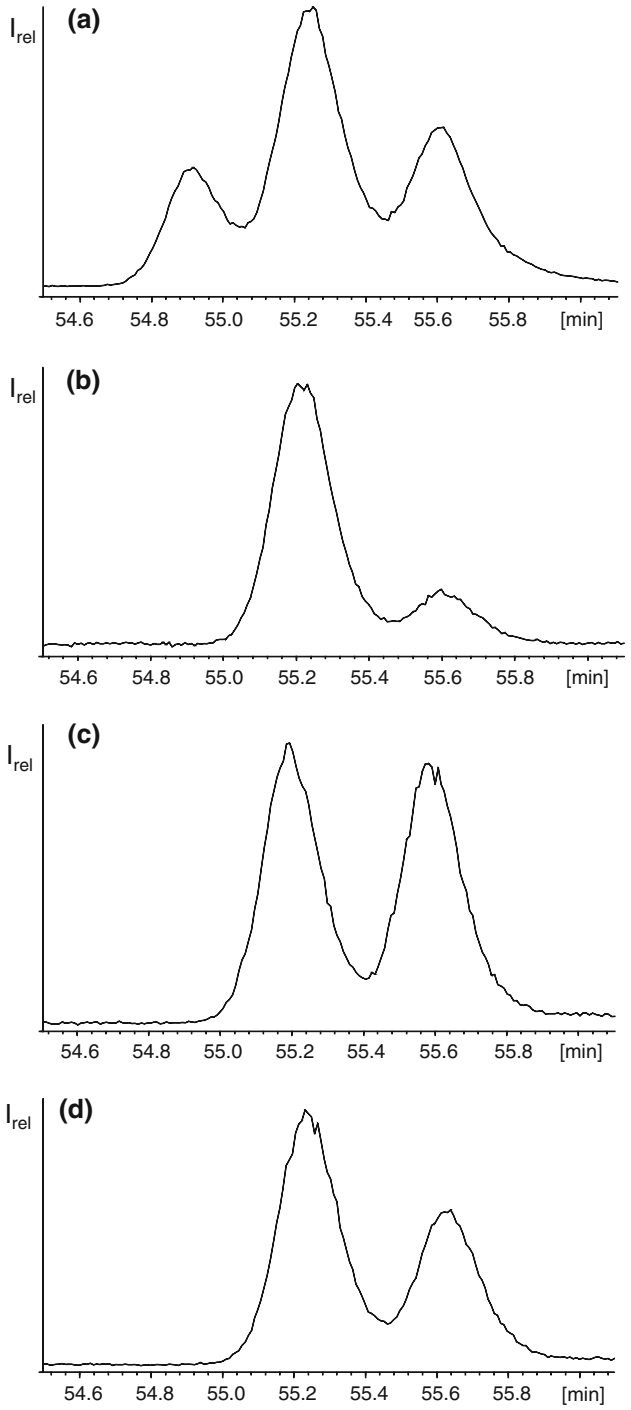

Fig. 2 GC/EI-MS-SIM chromatograms (60 m Rtx-2330; *m/z* 101) of samples containing phytanic acid after conversion into its methyl ester. **a** Synthetic standard, **b** blubber of a harbor porpoise (*Phocoena phocoena*) from Iceland, **c** organic milk, **d** conventionally produced milk

The dominant central peak was flanked by two well resolved peaks. Thus, more diastereomers than the naturally-occurring RRR- and SRR-forms were present in this standard. Due to the three centers of chirality, 2^3 stereoisomers of phytanic acid can be formed, four of which may be separated on a non-chiral GC stationary phase as applied in this study (Table ESM1, Electronic supplementary material). Since the area of the central peak was about two-fold compared to the areas of the neighbored peaks, it was assumed that the central peak was composed of two unresolved diastereomers. Hence, the synthetic standard was likely racemic on the three centers of chirality. A similar peak pattern albeit with smaller valleys between the three peaks was obtained by Ackman for synthetic methyl phytanate on a packed column [19]. A constant ratio of the fragment ions screened (*m/z* 74, *m/z* 101, *m/z* 171 and M^+ at *m/z* 326) was obtained over the whole peaks, which was used for peak authentication [20]. The *m/z* 101 was slightly more abundant than the McLafferty ion *m/z* 74 which is prominent in all conventional saturated and monoenoic fatty acid methyl esters (FAME). By contrast, *m/z* 101 represents cleavage between C-3 and C-4. This fragmentation leads to *m/z* 87 in the mass spectra of other FAME. Thus, abundance of *m/z* 101 is exclusive to FAME with a methyl branch on C-3 as in the case of phytanic acid methyl ester (or on C-2 in the case of pristanic acid methyl ester). Therefore, *m/z* 74 and *m/z* 101 as well as the ratio of these two abundant fragment ions can be used for the verification of the correct determination of phytanic acid in food samples [20]. The low detection limit of this method of 13 mg/100 g phytanic acid (see Experimental procedures) was exceeded by all samples analyzed.

In agreement with literature reports, two peaks originating from phytanic acid were detected in samples (Fig. 2b–d), The diastereomer elution order was assigned following the approach of Ackman and Hansen [14, 19]. They reported that marine samples have an excess of SRR; for instance, fin whale blubber was reported to contain ~66% SRR and 63–91% SRR in typical fish samples [14, 21]. Only one marine sample analyzed 40 years ago was reported to contain less SRR than RRR [21]. We thus analyzed several marine samples and the first eluting diastereomer was prominent in each case (Figs. 2b, 3). This pointed toward SRR being eluted first from the GC column used. The retention times of SRR and RRR matched those of peak 2 and peak 3 of the all-racemic phytanic acid (Fig. 2), and this pattern was also reported by Ackman [19]. The supposable diastereomer resolution of the commercial racemic standard and samples is shown in Table ESM1 and Fig. ESM1 (see Electronic supplementary materials). In the present samples the dominance of SRR was more pronounced in marine top predators than in fish or squid (Fig. 3).

Diastereomer Distribution of Phytanic Acid in Conventional and Organic Milk

Cows are milked twice a day, and we first tested if there was a difference in the ratio of the phytanic acid

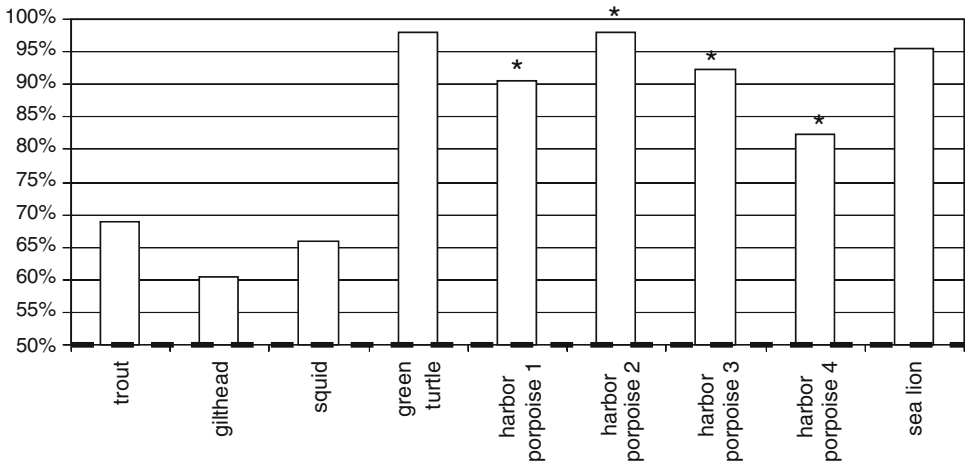

Fig. 3 Percentage contribution of 3*S*,7*R*,11*R*,15-phytanic acid (SRR) in marine samples. The *dotted line* (50%) represents the case when both diastereomers have the same concentration. * The mean value with standard deviation and the median of the percentage of SRR-phytanic acid in the four harbor porpoises analyzed was 90.8 ± 6.4 and 91.4%; the precision tested with replicate samples ($n = 6$) was 0.51%

diastereomers in morning and evening milk. Morning milk is taken after resting of the cows while evening milk follows a period of activity and eating. Analysis of samples taken from the same (conventionally raised) cow over a period of 15 days clarified that the diastereomer distribution was virtually identical at both milking points of time (one-way ANOVA, $F_{1,28}$, $p = 0.5$, Fig. 4; no outlier identified according to Nalimov; *t*-test no variance between both groups). SRR was dominant and generally contributed with >80% to the phytanic acid content. Thus, the time of milking had no influence on the phytanic acid diastereomer distribution in the present cow. The diastereomer distribution was very constant throughout the measurements (Fig. ESM2, Electronic supplementary materials).

Interestingly, the diastereomer composition was significantly different in samples from an organic cow (one-way ANOVA, $F_{1,58}$, $p \ll 0.001$, no outliers according to Nalimov; *t*-test variances between organic and conventional milk samples confirmed). Again, the proportion was constant over a period of 30 days but SRR was slightly less abundant than RRR (Fig. 4; Fig. ESM2, Electronic supplementary material). Two further organic milk samples from the German market confirmed this finding (Fig. 4).

Due to the distinct difference in the phytanic acid diastereomer distribution, this parameter could be a suitable marker for the authentication of organic milk. However, more data needs to be analyzed in future for a more thorough statistic evaluation. We suggest that the different diastereomer distributions in cow milk originate from the complex composition of the rumen bacteria. Hundreds of kinds of microbes are present in the anaerobic ecosystem of the rumen with concentrations in the range of 10^{10} bacteria per mL [22]. It is well known that the microbial populations in the rumen change with the diet of the host animal [22, 23]. Owing to the larger proportion of grass (high in detergent fiber, virtually no triacylglycerides) in the forage of organic cows the rumen bacteria dominant under this condition seem to favor the production of RRR-phytanic acid whereas those required for the digestion of concentrate seem to generate more SRR-phytanic acid. The validity of this hypothesis needs to be verified in future.

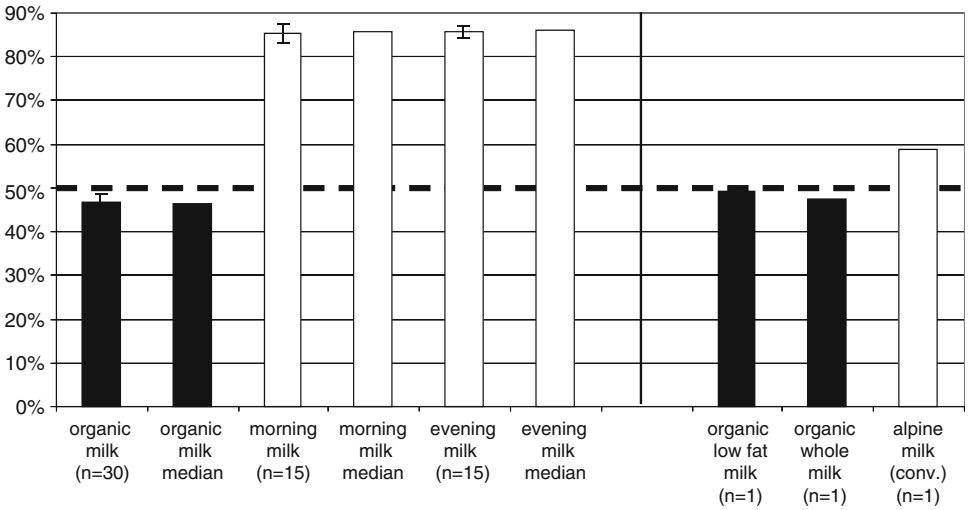

Fig. 4 Percentage contribution of 3*S*,7*R*,11*R*,15-phytanic acid (SRR) in organic milk and conventionally produced milk (mean values with bars for standard deviation as well median values). Samples on the *left* are from milk samples from one organic and one conventionally-raised cow taken daily whereas samples on the *right* are from samples from the German market. Organic samples are shown with *black bars* whereas conventional products have *white bars*. The *dotted line* (50%) represents the case when both diastereomers have the same concentration

Phytanic Acid Diastereomers in Organic and Conventional Cheeses, Dairy Products and Meat

Twenty-four cheeses from the German market including 14 organic products from retail outlets were also analyzed. These samples are difficult to compare in detail because of varying technologies, the use of bacterial starters and molds. In fact, the relative abundance of SRR varied more than in milk and also in dependence of the type of cheese (Fig. 5). SRR was generally higher abundant in conventional products but also in most organic products which is different to the milk samples. For this reason the difference between organic and conventional cheeses were less pronounced compared to milk and both groups could not be differentiated by statistic methods (one-way ANOVA, $F_{1,22}$, $p = 0.06$; Nalimov no outliers; *t*-test no variation statable). The highest proportions of SRR were found in conventionally produced cheeses whereas SRR was lowest in two organic cheeses. When the same types of cheeses (Fig. 5, samples labeled A–F) were compared, the SRR diastereomer was higher in conventional than in organic cheese which confirms the results obtained from milk. An exception formed organic Emmental cheese 1 and organic Romadur (Fig. 5). Organic Emmental 2 was also conspicuous in that it did not contain the high concentrations of phytanic acid typical for organic cheeses [8]. Of all cheeses analyzed, this sample contained the lowest concentration of phytanic acid [8]. However, the high proportion of SRR in organic Emmental 2 could not be explained. Aside from this point, our data suggest that the differences in cheeses compared to milk most likely originate from post-milking changes which might include changes during storage (including different periods) by partial degradation of phytanic acid diastereomers at different velocity and/or the impact of microorganisms used in the processing of cheese. Thus, the diastereomer composition of phytanic acid was not suitable as a marker of organic cheese. The same was found for other dairy products (Fig. 6). While SSR was more prominent in conventional butter than in organic butter, a very high SRR proportion was found in curd cheese (Fig. 6). These results are in contrast to Ackman who reported an excess of RRR in two butter samples (SRR only 32.4–34.7%) [14]. These SRR percentages are much lower than any diastereomer distributions measured in the present milk and dairy product samples.

Samples of conventional beef liver and ground beef matched the value obtained in conventional milk but the SRR proportion in organic ground beef was only slightly lower compared to the conventional sample. Similarly to the cheeses, these differences compared to milk seem to originate from "post-rumen" changes. Once phytanic acid has left the rumen, it cannot be produced any more in the organs or food sources. This feature has also to be taken into account for the human milk samples. Phytanic acid can be subjected to biotransformation which in a first step leads to pristanic acid (2,6,10,14-tetramethylpentadecanoic acid) [24, 25]. Due to this methyl-group on C-3, phytanic acid cannot be degraded by conventional β-oxidation. However,

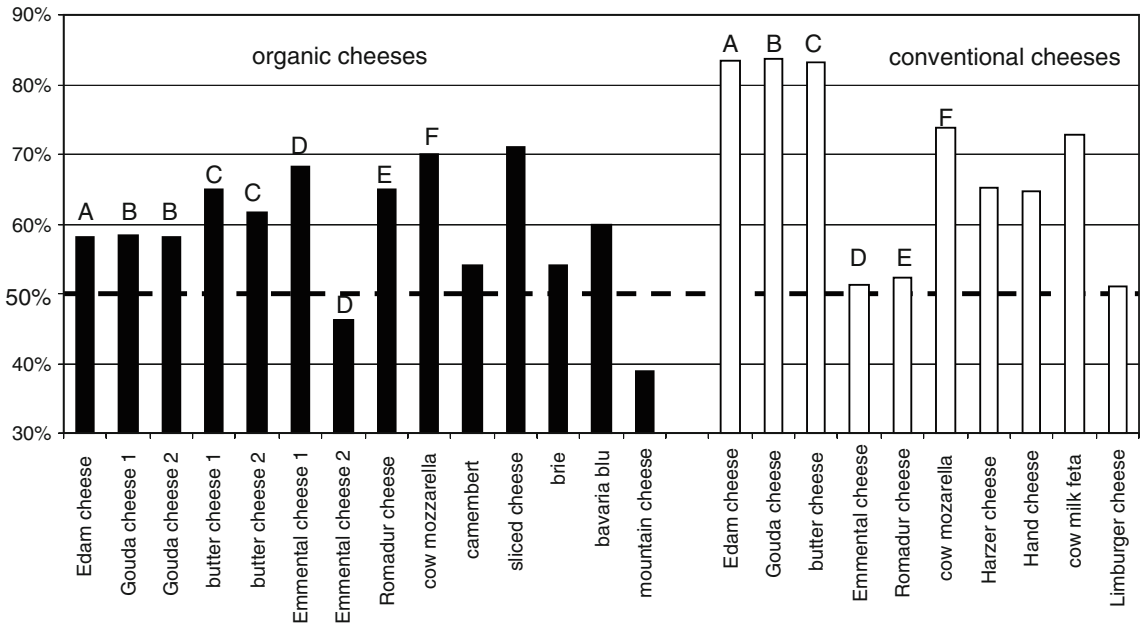

Fig. 5 Percentage contribution of 3*S*,7*R*,11*R*,15-phytanic acid (SRR) in organic cheeses and conventionally produced cheeses. *Letters A–F* refer to the same type of cheese. Organic samples are shown with *black bars* whereas conventional products have *white bars*. The *dotted line* (50%) represents the case when both diastereomers have the same concentration

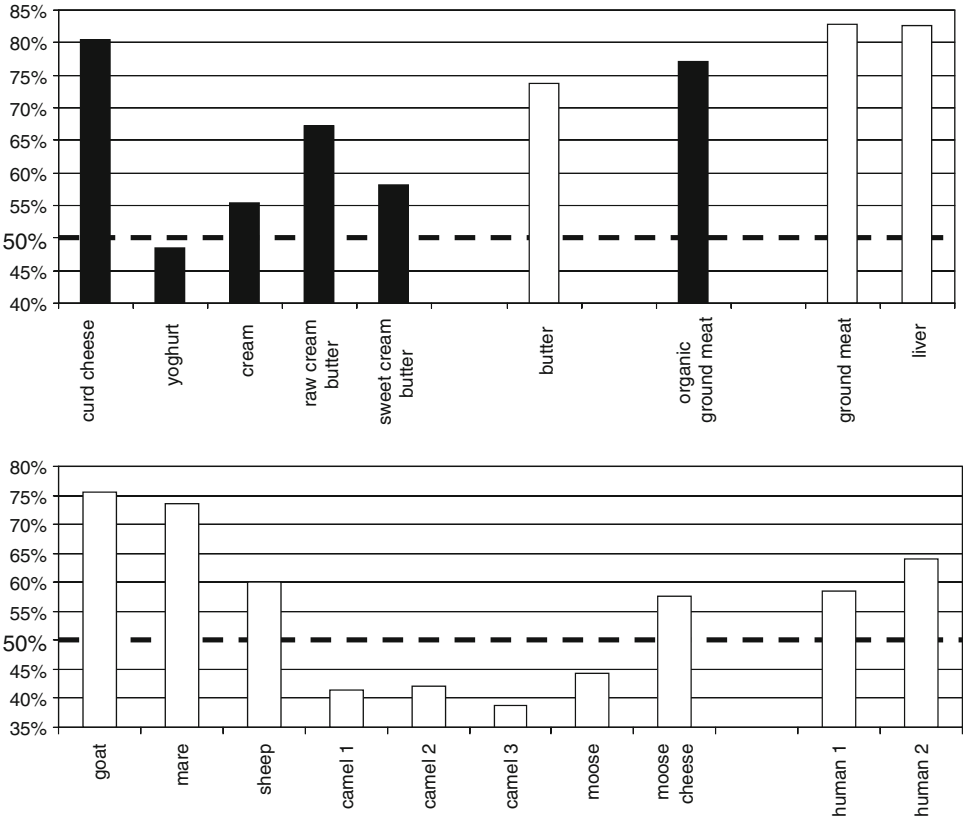

Fig. 6 Percentage contribution of 3*S*,7*R*,11*R*,15-phytanic acid (SRR) in selected terrestrial samples (*upper panel*) as well as milk and one cheese from other mammals than cows (*lower panel*). Organic samples are shown with *black bars* whereas conventional products have *white bars*. The *dotted line* (50%) represents the case when both diastereomers have the same concentration

α-oxidation inside the peroxisome eventually leads to pristanic acid [10]. Peroxisomal α-oxidation is not a stereoselective process [9], so that α-oxidation of phytanic acid leads to both (2*R*,6*R*,10*R*,14)- and (2*S*,6*R*,10*R*,14)-pristanic acid. Our data suggest that SRR- and RRR-phytanic acid were biotransformed with different velocity and that biotransformation seems to favor the abundance of SRR.

Phytanic Acid Diastereomers in Milk from Other Mammals than Cows

Analysis of milk samples from mammals other than cows resulted in a varied picture (Fig. 6, lower panel). These animal milk samples were from farms not certified as being organic. While SRR dominated in goat, mare, and sheep milk, the RRR diastereomer was higher abundant in camel and moose milk (Fig. 6). The later were rather similar to organic (cow) milk with camel milk having the lowest SRR content of any sample analyzed. The three different samples of camel milk taken over a time span of >1 month had similar SRR contributions (39–42%). The SRR content of moose milk was at the low end of organic (cow) milk (compare Figs. 4 and 6). Interestingly, a sample of moose cheese from the same farm contained an excess of SRR and thus a much higher proportion of this diastereomer compared to milk (Fig. 6). An increase of SRR from organic milk to organic cheese was also observed with cows (see above). By contrast, the values in the milk samples with higher proportions of SRR (goat, sheep, mare) were only slightly below the lowest value obtained in conventional cow milk but much higher than in organic cow milk.

Two samples of human milk showed a slight predominance of SRR and were thus located in the middle between organic and conventional cow milk (Fig. 6). Since humans cannot produce phytanic acid, the diastereomer distribution of phytanic acid represents a mixture of food intake (from different sources with different SRR/RRR ratio) which is overlaid by the stereochemical effects during transformation in the human body.

Conclusion

The key to this study marked the quite good diastereomer resolution of phytanic acid methyl ester achieved after a standard clean-up procedure for fatty acids on standard equipment. In combination with GC/EI-MS-SIM the diastereomers of phytanic acid could be determined directly from the fatty acid methyl ester fraction without additional efforts. This method could be used to establish a first data base for phytanic acid diastereomer distributions. On one hand our data confirm the few results from samples published in the 1960s; on the other hand several conclusions drawn in these historic works could not be verified. For

instance reversed ratios of SRR/RRR in terrestrial and marine samples tentatively predicted some 40 years ago were not confirmed in our study. The good resolution of SRR and RRR along with the sensitivity of GC/MS-SIM may be helpful as well in investigations with regard to the metabolism in man [26]. The stereochemical effects reported by Ackman and Hansen [13] might be an interesting option in clinical research on the relevance of phytanic acid.

Our data indicate that the diastereomer composition in milk depends, at least to a certain degree, on the diet which in turn will have an influence on the composition of rumen bacteria composition. The rumen bacteria are responsible for the production of the phytanic acid and its diastereomeric composition. In addition, post milking processes (e.g. diastereoselective degradation during storage and processing of microorganisms) further alter the SRR/RRR distribution. It further appears that higher organisms (and food processing) put their mark on the SRR/RRR distribution with the food items playing only a minor role (compare also marine mammals and fish). Beyond this, the phytanic acid diastereomers may also be stereoselectively transferred to milk. However, these uncertainties need to be explored more in detail. The method presented in the manuscript seems to be a valuable tool for future studies.

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