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Concentrations of phytanic acid and pristanic acid are higher in organic than in conventional dairy products from the German market

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ABSTRACT

Differentiation of organic and conventional dairy products is an important, yet difficult task in food authentication. In this study it was tested whether phytanic acid and pristanic acid can be used as markers for this purpose. Phytanic and pristanic acid cannot be *de novo* synthesised by mammals, and the predominant source for uptake is chlorophyll in food. Highest concentrations (but still in the sub-gram per 100 g lipids range) are found in ruminants because rumen bacteria are able to release phytol from chlorophyll and transforming it into phytanic acid. Degradation of phytanic acid leads to pristanic acid, and both fatty acids usually co-exist in biota. Owing to the unique source of grass-based feedstuffs in organic farming it was tested whether organic cheeses (n = 13) and other organic dairy products (n = 5) are higher in phytanic and pristanic acid concentrations than conventional products (n = 12). For this purpose, a sensitive gas chromatography–mass spectrometry method in the selected ion monitoring mode was developed.

Organic cheeses contained on average 50% more phytanic acid and 30% more pristanic acid, while concentrations of 14-methylhexadecanoic acid (a17:0), i.e. another minor fatty acid in ruminants, were only slightly increased by 8%. A target value of at least 200 mg/100 g phytanic acid was suggested for the verification of grass-fed, organic dairy products. Some conventional products also reached the target value, and this indicated that the cows that gave the milk were also fed with grass-silage. On the other hand, only one organic cheese fell below this target value. It is recommended to control manufacturers whose organic dairy products show concentrations of below the target value of 200 mg phytanic acid per 100 gram lipids in order to confirm the application of organic principles.

1. Introduction

During the past decade, organically-grown food products have received increasing popularity among consumers. Organic food is usually sold at a higher price, and the organic segment is one of the fastest growing food sectors (Bourn & Prescott, 2002). To maintain this quality and to protect the required standards for organic food, it is important to identify parameters or compounds which allow for controls in order to distinguish organic from conventional products and to identify falsely labelled products (i.e. foodstuff sold as organic without having the typical feature). One potential way of distinguishing organic from conventional products is screening samples for markers which are different in the food sources of both production ways.

Repeatedly, it was shown that cattle feed in form of fresh grass or grass-silage increases the content of phytanic acid in both plasma lipid and milk (Leiber, Kreuzer, Nigg, Wettstein, & Scheeder, 2005; Lough, 1977; Vlaeminck, Lourenço, Bruinenberg, Demeyer, & Fievez, 2004). Since cows used for the production of organic milk have to be fed with organic food, it could be possible that organic cows are taking up a higher amount of phytanic acid via food.

Phytanic acid ($C_{20}H_{40}O_2$) is a fatty acid with 16 carbons on the longest chain along with four methyl substituents on C-3, C-7, C-11, and C-15. Phytanic acid cannot be *de novo* synthesised by mammals, and thus is largely derived from food (Steinberg et al., 1967). Highest concentrations of phytanic acid are found in marine organisms and in the tissue and milk of ruminants (Lough, 1975). In either case, the initial source for phytanic acid is chlorophyll, and more precisely, the alcohol moiety phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol) of chlorophyll a, b, and d. In ruminants, the primary alcohol phytol is released from chlorophyll by different bacteria in the rumen, followed by saturation of the double bond along with oxidation of the resulting alcohol dihydrophytol to phytanic acid (Patton & Benson, 1966). The order of reduction of the double bond and oxidation of the alcohol might be reversed in humans (van den Brink & Wanders, 2006). Typical phytanic acid content of milk fat was reported to be ~0.28% whereas the concentration in human milk fat was found to be ~0.11% (Lough, 1975). Milk and dairy products are thought to be the most relevant source

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for phytanic acid in humans (Allen et al., 2008). On the other hand, no significant amounts of phytanic acid were found in vegetables (Brown et al., 1993). Due to the methyl-group on C-3, phytanic acid cannot be degraded by β-oxidation, i.e. the normal breakdown process for other fatty acids. Thus, transformation has to start with α-oxidation, which – over different steps – leads to pristanic acid. Pristanic acid usually co-exists with its primary natural source phytanic acid in food and humans (Lough, 1975; Verhoeven & Jacobs, 2001). However, pristanic acid concentrations tended to be 3–4-fold lower than those of phytanic acid (Lough, 1975).

Previous investigations as to the differentiation of milk from conventional and alternative/organic production in respect of its content of desirable ingredients resulted in no major differences (Woese, Lange, Boess, & Bögl, 1997). However, Molkentin and Giesemann (2007) showed that stable isotope analysis and in particular, the content of 18:3*n*−3 are significantly higher in organic than in conventional milk. In addition, milk from organically raised alpine cows was higher in *n*−3-fatty acids, conjugated linoleic acids (CLA) and methyl-branched fatty acids (Collomb et al., 2008). While organic milk contained at least 0.56% 18:3*n*−3, the content in conventional milk never reached more than 0.53% (Molkentin & Giesemann, 2007). While these markers proved to be valuable, it is obvious that addition of rather small amounts of vegetable oils rich in 18:3*n*−3 (e.g. linseed oil) to the feed of conventional cows would mimic the content of organic milk. In such a case, authenticity control would become cumbersome. Thus, a second parallel or additive marker would be desirable. Owing to the unique source of grass-derived chlorophyll for phytanic and pristanic acids in ruminants, we investigated whether organic dairy products contained higher concentrations of these fatty acids than conventionally produced dairy products. For reasons of comparison we also determined 14-methylhexadecanoic acid (a17:0) which is also found in the sub-% level in the lipids of dairy products (Thurnhofer, Lehnert, & Vetter, 2008). Owing to these low concentrations in bovine milk lipids, we developed a method based on gas chromatography–mass spectrometry used in the selected ion monitoring mode (GC/MS–SIM) for quantification (Thurnhofer & Vetter, 2005).

2. Materials and methods

2.1. Samples, chemicals, and standards

Samples were collected from the local markets around Stuttgart (Germany). Origin of samples will be presented in details in Section 3.

Cyclohexane (purest; VWR, Darmstadt, Germany) and ethyl acetate (purest, Acros Organics, Geel, Belgium) were combined (1:1, v/v) and distilled to obtain the azeotropic mixture (54:46, v/v). Methanol and *n*-hexane (both HPLC gradient grade) were from Fluka (Taufkirchen, Germany). Ethanol (Carl Roth, Karlsruhe, Germany) was distilled prior to use. 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). BF_3 ethyl etherate (purum, dist.) and ethanolic BF_3 (∼10%, ∼1.3 M, purris) were from Fluka (Taufkirchen, Germany). Potassium hydroxide,>85% was from Carl Roth (Karlsruhe, Germany).

Phytanic acid (>96% in ethanol, mixture of isomers) was from Cayman Chemical (Ann Arbor MI, USA) and pristanic acid (98% in ethanol, mixture of isomers was from Sigma–Aldrich (Seelze, Germany). 10,11-Dichloroundecanoic acid (DC-11:0) was previously synthesised in our group (Thurnhofer et al., 2008) and 14-methylhexadecanoic acid (a17:0) was from Larodan (Malmö, Sweden). A Supelco 37 component FAME "37c-FAME" mix (Sigma–Aldrich, Taufkirchen, Germany) as well as additional free fatty acids and FAME (Larodan, Malmö, Sweden) were used as reference standards.

2.2. Sample preparation

Food samples except oils were lyophilised (Lyovac GT 2 system, Leybold–Heraeus, Hürth, Germany; pressure applied ∼0.1 mbar) prior to extraction. Lipids were gained by accelerated solvent extraction (ASE, Dionex) with the azeotropic mixture of ethylacetate/cyclohexane (54:46, v/v) as the solvent (Weichbrodt, Vetter, & Luckas, 2000). The volume was adjusted, an aliquot was taken for gravimetric lipid determination, and the remaining solvent was removed. For transesterification, about 1 mg of the lipids (namely the fatty acid glycerides) was accurately weighed into a derivatisation vial, and a solution of 86.5 μg DC-11:0 was added. The solvent was evaporated and 0.5 mL methanolic KOH (0.5 M) was added and heated for 5 min to 80 °C. After cooling (ice bath), 1 mL BF_3 solution (see below) was added and heated for additional 5 min at 80 °C. After cooling, 0.75 mL saturated sodium chloride solution and the resulting fatty acid methyl esters were extracted with 2 mL *n*-hexane. The *n*-hexane layer was separated, 300 μL were taken, 3 μL (1.5 μg) of phytanic acid ethyl ester (prepared from phytanic acid by using ethanolic KOH and ethanolic BF_3 (Thurnhofer & Vetter, 2006)) was added as (second) internal standard and subjected to GC/MS in full scan and SIM modes (Thurnhofer et al., 2008).

2.3. Gas chromatography coupled to electron ionisation mass spectrometry (GC/EI–MS)

Samples and standards were analysed with a 5890 series II gas chromatograph interfaced to a 5971A mass selective detector (Hewlett–Packard/Agilent, Waldbronn, Germany). One microliter of sample solutions was injected with a 7673A autosampler (split less mode, split opened after 2 min). The injector and transfer line temperatures were set at 250 °C and 280 °C. The temperature of the ion source was 165 °C. Helium (purity 5.0) was used as the carrier gas at a constant flow rate of 1 mL/min. A 50 m × 0.25 mm i.d. fused-silica capillary column coated with 0.20 μm d_f CP-Sil 88 (Chrompack, Middelburg/The Netherlands) was installed in the GC oven. The GC oven was programmed as follows: after 1 min at 60 °C, the oven was heated at 16 °C/min to 136 °C (hold time 1 min), at 0.5 °C/min to 144 °C (hold time 2 min), and finally at 12 °C/min to 225 °C (hold time 7 min). The total run time was 38.5 min. In the full scan mode, *m/z* 50 to *m/z* 450 was recorded after a solvent delay of 8 min. In the SIM mode, *m/z* 74, *m/z* 87, *m/z* 88, *m/z* 101, *m/z* 102, *m/z* 270, *m/z* 312 were recorded from 8 to 22 min and thereafter (22–38.5 min) we recorded *m/z* 74, *m/z* 87, *m/z* 88, *m/z* 101, *m/z* 115, *m/z* 284, *m/z* 326.

2.4. Evaluation of data

Contents of phytanic acid, pristanic acid, and a17:0 in organic and conventional products were studied by one-way ANOVA as implemented in EXCEL.

3. Results and discussion

3.1. Gas chromatography and mass spectrometry of the methyl esters of phytanic and pristanic acid

Determination of the fatty acids as methyl esters was based on a GC/EI–MS–SIM method previously described (Thurnhofer & Vetter, 2005; Thurnhofer et al., 2008). For saturated long chain and monomethyl-branched FAMEs, this method is based on screening the

abundant fragment ions corresponding to the McLafferty ion (cleavage between C-2 and C-3 including H transfer from C-4 to the carbonyl group) and γ-cleavage (Fig. 1). These abundant ions are found at *m/z* 74 and *m/z* 87 for conventional fatty acid methyl esters (Thurnhofer & Vetter, 2005). However, due to the methyl-branch on C-2 of pristanic acid, the respective ions are shifted by 14 u to higher mass, i.e. *m/z* 88 (McLafferty ion) and *m/z* 101 (γ-cleavage). These *m/z* values are the same as found for ethyl esters of conventional fatty acids (Thurnhofer & Vetter, 2006). On phytanic acid, the first methyl-branch is located on C-3, and thus the McLafferty ion is unchanged found at *m/z* 74 while the γ-cleavage is shifted by 14 u to *m/z* 101.

Initial experiments with standard compounds showed that phytanic acid eluted in the GC range of heptadecanoic acid methyl ester isomers (17:0, a17:0, i17:0) as well as 16:1*n*−7-ME. Since these fatty acids are forming abundant ions at *m/z* 74, phytanic acid was determined with *m/z* 101. A good separation was obtained (t_R 16:1*n*−7 < a17:0 < phytanic acid) by a slow heating rate in the elution range of phytanic acid (see Section 2.3). Under these GC conditions, pristanic acid eluted slightly after i15:0 (18.6 vs. 18.5 min). Due to the selective ions monitored for pristanic acid (*m/z* 88 and *m/z* 101), quantitative concentrations could be established using the conditions applied.

3.2. Validation of the sample clean up

Organic Gouda was chosen for method validation. The lipid content of 43.4–45.2% ($n = 5$, 44.1 ± 0.7%) was slightly lower than according to label. For the intercomparison of data, results from five replicate samples (individually processed from the start) were normalised to the area of the syringe standard phytanic acid ethyl ester as determined in sample 1 as well as the recovery of the internal standard DC-11:0. Finally, sample weights were corrected to a general accurate sample weight of 1000 μg. Each of the five samples was analysed three times by GC/MS (Table 1). Involving identical sample weights, calibration of the GC/MS system with the syringe standard phytanic acid EE, as well as the recovery rate of the internal standard DC-11:0 resulted in a high reproducibility of the results (Table 1). The standard deviation of the three fatty acids was 1.6–2.5%. Note that these standard deviations also include potential variations in the fatty acid distribution in sample aliquots. Based on these results, the method was considered suitable for the quantification of phytanic acid, pristanic acid, and a17:0. Duplicate samples analysed by routine throughout the study have confirmed this.

3.3. Quantification of phytanic acid, pristanic acid and a17:0 in conventional and organic milk products

The mean concentration of pristanic acid in conventional and organic dairy products was 43 mg/100 g and 56 mg/100 g lipids, respectively (Table 2). The median (44 mg/100 g vs. 57 mg/100 g lipids) was almost identical. Thus, the content of this fatty acid was significantly higher in organic than in conventional products (>30%; one-way ANOVA, $p \ll 0.1$, Table 2). This difference was even higher for phytanic acid, where the mean concentration in organic products (270 mg/100 g lipids; median: 265 mg/100 g lipids) exceeded the one in conventional products by 50% (one-way ANOVA, $p \ll 0.01$, Table 2). In contrast, a17:0 was only slightly higher concentrated in organic food (~8%).

The concentration of phytanic acid was on average five fold higher than pristanic acid but lower than a17:0 (Table 2). Based on the mean concentrations of phytanic and pristinic acid, organic products could be distinguished from conventional products (one-way ANOVA, Table 2). However, the concentration ranges of both product classes overlapped for the three fatty acids under investigation (Table 2). The highest content in conventional products surpassed the lowest concentration determined in organic products. Thus, a dairy product could not be directly assigned to the way of production (see below).

3.4. Quantification of phytanic and pristanic acid in conventional and organic cheeses

Concentrations of pristanic acid in organic cheeses ranged from 42 to 71 mg/100 g lipids whereas phytanic acid was significantly higher (170–390 mg/100 g lipids). The amounts in conventional products were 26–56 mg/100 g lipids pristanic acid and 75–

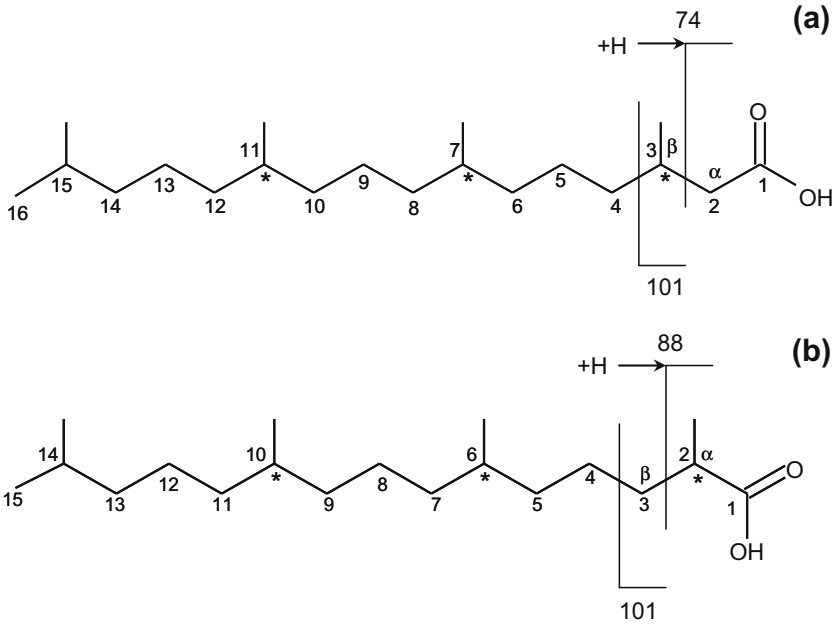

Fig. 1. Chemical structure of (a) 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid) and (b) 2,6,10,14-tetramethylpentadecanoic acid (pristanic acid). Masses labelled show characteristic fragmentations of the corresponding methyl esters in GC/EI–MS spectra.

Table 1
Normalised areas* of methyl esters of phytanic acid, pristanic acid and a17:0 from five samples of organic Gouda each analysed three times.

Sample no.	1	2	3	4	5	Mean ± STDEV**
Pristanic acid-ME (*m/z* 88)***	270 (268, 268, 273)	273 (269, 281, 272)	273 (266, 278, 274)	281 (279, 281, 282)	276 (274, 271, 272)	274 ± 4.5
a17:0-ME (*m/z* 74)***	1120 (1120, 1100, 1130)	1130 (1120, 1150, 1120)	1130 (1130, 1140, 1130)	1170 (1180, 1170, 1170)	1150 (1140, 1170, 1130)	1140 ± 20
Phytanic acid-ME (*m/z* 101)***	990 (970, 990, 1010)	1000 (980, 1040, 970)	980 (950, 990, 990)	1010 (1010, 1000, 1010)	1040 (1000, 1080, 1010)	1000 ± 24.5

* Inclusion of the recovery of the internal standard 10,11-DC-11:0, the variation of the syringe standard phytanic acid ethyl ester, and adjustment to a sample weight of 1000 μg.
** Mean value of the area in arbitrary units and standard deviation.
*** Ion used for quantification.

Table 2
Concentrations (mg/100 g lipids) and ratios of pristanic acid, phytanic acid, and a17:0 as well as the contribution of the second eluting phytanic acid isomer in organic and conventional dairy products as well as a sample of human milk.

Sample	Producer, producer code, best before date (day.month.year), and organic control authority*	Pristanic acid	Phytanic acid	a17:0	Pr/Ph [%]**
Organic cheeses					
Organic Emmental 1	ÖMA, DE-BW-331, 09.05.2008, 006	59	303	381	19.5
Organic Emmental 2	ÖMA, DE-BW-448, 04.07.2008, 006	42	167	442	25.2
Organic Edam	Söbbeke, DE-BY-136, 06.06.2008, 003	61	288	415	21.2
Organic Gouda 1	Söbbeke, DE-BY-136, 19.06.2008, 003	59	265	413	22.3
Organic Gouda 2	Blauer Engel, AT-M-S-0044, 13.08.2008, AT-O-02-BIO	53	256	397	20.7
Organic Camembert	ÖMA, DE-BW-077, 16.06.2008, 001	59	279	430	21.2
Organic butter cheese 1	Andechser, DE-BY-117, 12.07.2008, 001	71	389	368	18.3
Organic butter cheese 2	Blauer Engel, AT-M-S-0044, 27.07.2008, AT-O-02-BIO	62	302	360	20.5
Organic brie	ÖMA, DE-BW-077, 17.06.2008, 001	60	287	443	20.9
Organic cow mozzarella	Brodowin, DE-BB-016, 24.06.2008, 037	47	178	430	26.4
Organic sliced cheese	Käserei Bayreuth, DE-BY-402, 06.08.2008, 037	52	241	384	21.6
Organic mountain cheese	ÖMA, DE-BW-331, 04.07.2008, 006	49	244	374	20.1
Organic romadur	Andechser, DE-BY-117, 28.06.2008, 001	67	363	353	18.5
Organic bavaria blue	Andechser, AT-M-ST-04, 29.07.2008, AT-N-01-BIO	64	318	411	20.1
Conventional cheeses					
Edam	DE-SN-009, 29.05.2008	26	75	374	34.7
Cow mozzarella	I-03/001, 07.12.2005	31	102	382	30.4
Butter cheese	DE-MV-026, 15.09.2008	31	89	384	34.8
Emmental cheese	CH (details unknown), unknown	43	192	356	22.4
Gouda	NL-Z-0829 E, 18.06.2008	45	229	369	19.7
Harzer	D-SN-016, 28.09.2006	48	170	383	28.2
Hand cheese	DE-HE-036, 17.09.2006	41	156	329	26.3
Cow-feta	D-NI-057 EWG, 25.03.2006	51	248	375	20.6
Limburg cheese	DE-BY-709, 23.11.2006	54	290	366	18.6
Romadur cheese	03.11.2006, DE-BY-709, 15.11.2006	56	291	382	19.2
Organic dairy products					
Organic low-fat milk	Dennree, DE-BY-110, 13.06.2008, 001	57	289	413	19.7
Organic whole milk	Dennree, DE-BY-110, 15.06.2008, 001	58	296	418	19.6
Organic cream	Söbbeke, DE-NW-501, 28.06.2008, 006	53	238	418	22.3
Organic raw cream butter	Schwarzwälder, DE-BW-020, 10.04.2008, 003	47	211	366	22.3
Organic sweet cream butter	Schrozberg, DE-BY-514, 06.07.2008, 007	47	200	396	23.5
Conventional dairy products					
Alpine milk	Weihenstephan/Freising, unknown	44	176	362	25.0
Butter	Midgesäuert, DE-NW-204, 13.07.2008	40	141	392	28.4
Human milk	Anonymous donor from Stuttgart, Germany	16	30	280	53.3
Sum of all organic samples		38–71 ∅56	140–390 ∅270	350–440 ∅400	18.3–26.4% ∅21.5%
Sum of all conventional samples		26–56 ∅43	75–290 ∅180	330–390 ∅370	18.6–34.8% ∅25.7%
One-way ANOVA (cheeses only), $F_{1,22}$ (P)***		15.86 (0.0006)	10.51 (0.004)	7.92 (0.01)	–
One-way ANOVA (all dairy products), $F_{1,29}$ (P)***		19.78 (0.0001)	14.35 (0.0007)	10.78 (0.003)	–

* Applies only for organic products.
** Ratio of pristanic acid to phytanic acid in [%].
*** $F_{a,b}$: *F*-distribution with degrees of freedom; *P* highly significant for <0.01.

290 mg/100 g lipids phytanic acid (Table 2). It is evident that organic cheeses contained higher and more constant levels of phytanic and pristanic acid than conventional products. Among the organic cheeses the sample of organic Emmental 2 contained the lowest concentration of phytanic acid which was about half the value only determined in organic Emmental 1 (Fig. 2). Therefore, the lower content in Emmental 2 unlikely due to different production processes of Emmental cheese. The concentrations of phytanic acid varied in all types of cheeses obtained from different manufacturers, but not to that content (see organic Gouda 1 + 2 and organic butter cheese 1 + 2, Table 2). It is rather likely, that the cows offering the milk for organic Emmental 2 were fed with higher proportions of non-grass feed or, less likely, with non-organic feed. According to EU regulation, up to 25% of the dry weight

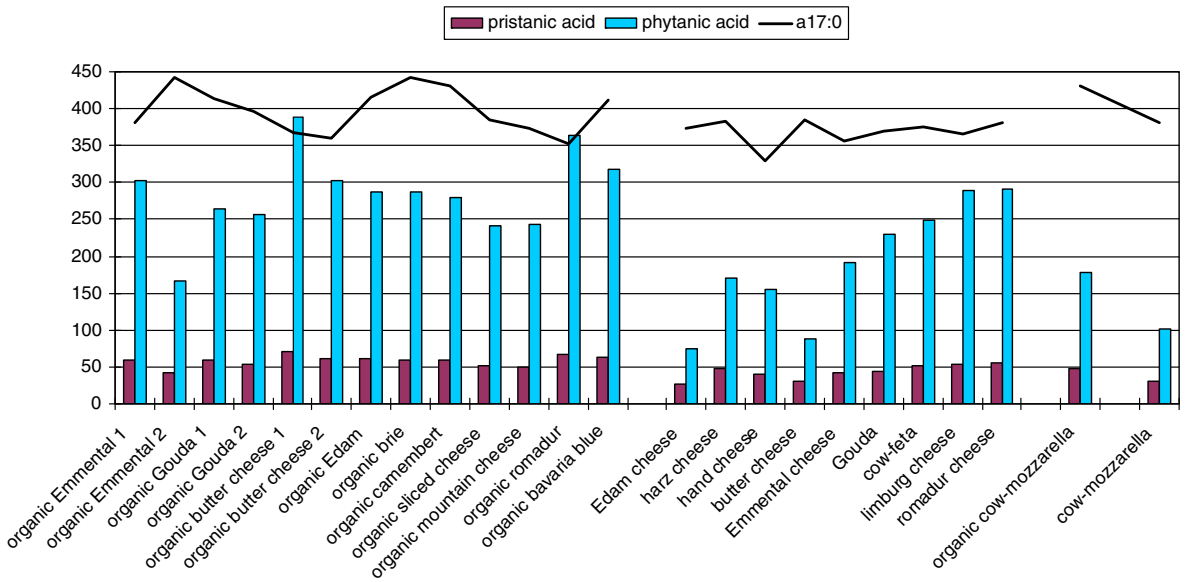

Fig. 2. Concentrations (mg/100 g lipids) of phytanic acid, pristanic acid, and a17:0 in organic and conventionally produced cheeses.

of a daily ration can be added in form of non-organic food, but over the whole year the amount must be not exceed 5% (European Union, 2004). It would thus be in agreement with organic principles that the manufacturers have used high proportions of conventional food in the short period which was reflected by the lower phytanic acid content of the milk. Except for fish, most conventional feed items (e.g. cereals, canola, soy, sun flowers, olives) and leguminoses do not contain significant amounts of phytanic acid (Brown et al., 1993). Likewise, organic feed other than grass-based from the producer, for instance in form of grain, would result in lower content of phytanic acid. While this is in accordance with regulations for organic products, a fodder mix as close as possible to the natural feed would be desirable. Thus, grass and grass-silage will match this best from an ecological point of view. Obviously, this was not the case in the sampling period of organic Emmental 2. Remarkably as well, both organic and conventional mozzarella contained lower amounts of phytanic acid and pristanic acid than the classic cheese samples. Although the concentration in organic mozzarella was higher (Fig. 2) it appeared likely that these differences may originate from the way of production of the cheese. Due to the low sample size this problem could not be explored in details.

After exclusion of mozzarella (suspected to be subject to alterations in the phytanic acid concentrations of cheese lipids during processing) and Emmental 2 (suspected to be non-grass-based fed) from the data set, all further organic cheeses contained >200 mg/100 g phytanic acid. This value was chosen as target value for an authenticity assessment of organic dairy products with high grass-based feed. Moreover, the mean value of pristanic acid in this set of organic cheeses was 60 mg/100 g lipids while phytanic acid on average amounted to 295 mg/100 g lipids. This mean value of phytanic acid was higher than the highest concentrations determined in conventional products (Table 2). Moreover, it was almost identical with concentrations reported in butter fat analysed in the 1970s (Lough, 1975) and also higher than in literature data published for conventional cheeses (Brown et al., 1993). Noteworthy, conventional products occasionally contained higher amounts of phytanic acid and pristanic acid. This indicated that producers of conventional milk also applied grass-based fodder which is linked with a higher level of phytanic acid and pristanic acid. Higher concentrations of phytanic and pristanic acid in milk and dairy products may also be obtained by feeding fish meal which contains similar amounts of phytanic acid as milk fat (Brown et al., 1993). However, feeding lifestock with fish meal would also be associated with a higher content of $n-3$-fatty acids which are richly found in fish.

In all samples, phytanic acid was much higher concentrated than pristanic acid. The ratio of pristanic acid to phytanic acid in organic cheese was 18–22% except for two samples with >25% (Table 2). Conventional cheeses showed similar ratios, and three samples even reached 30% of pristanic acid (Table 2). However, high relative amounts of pristanic acid were only observed in samples with phytanic acid concentrations of <200 mg/100 g lipids (Table 2).

3.5. Concentrations of phytanic acid and pristanic acid in organic and conventional milk, additional dairy products and one sample of human milk

In all organic milk, cream, and butter samples, phytanic acid reached the target value of 200 mg/100 g lipids while those in conventional dairy products were generally below this boundary (Fig. 3). In either case, organic milk and butter was also richer in pristanic acid than the respective conventional products. As observed in organic cheeses, pristanic acid amounted to 20–22% of phytanic acid. Higher relative amounts of pristanic acid were again only observed in samples with phytanic acid <200 mg/100 g lipids (Table 2). The concentrations of a17:0 were about 50% higher than in a recent study of alpine cows (Collomb et al., 2008), i.e. 0.41 vs. 0.24 mg/100 g milk fat in organic and 0.36 vs. 0.23 mg/100 g milk fat in conventional milk.

Content of the analytes in organic sweet cream butter and organic raw cream butter from different producers contained phytanic acid at 200 or 210 mg/100 g lipids and were thus at the lower end of the proposed target value of 200 mg/100 g lipids for grass-fed organic dairy products. However, the concentration in conventional butter was significantly lower (Fig. 3). Pristanic acid was also higher concentrated in the organic butter samples. The ratio of pristanic acid to phytanic acid was >20% in organic butter and significantly higher in conventional products as evidenced by the proposed target value.

The lowest concentrations of both phytanic and pristanic acid as well as a17:0 were determined in human milk. Exempli gratia, the content of phytanic acid in human milk was ten fold lower than in bovine milk and dairy products, and lower concentrations were

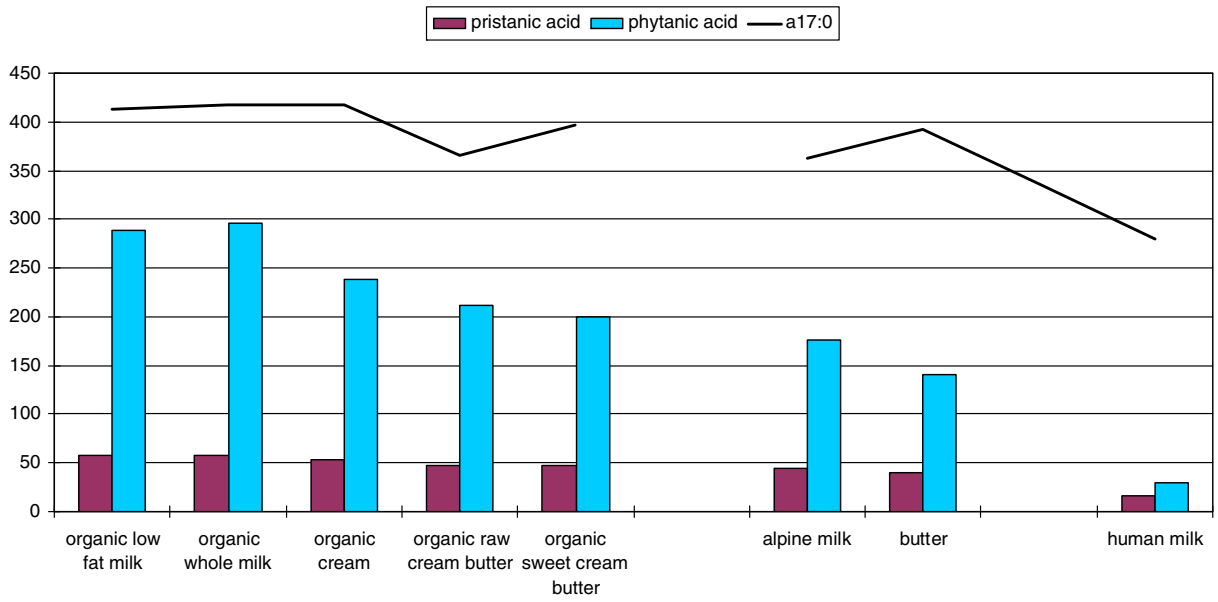

Fig. 3. Concentrations (mg/100 g lipids) of phytanic acid, pristanic acid, and a17:0 in organic and conventionally produced bovine milk, butter, milk, and cream, as well as human milk.

also obtained for pristanic acid and a17:0 (Table 2). Of all samples analysed, human milk showed the highest ratio of pristanic acid to phytanic acid. Phytanic acid is not produced in significant amounts by humans (Verhoeven & Jacobs, 2001). Thus, only metabolism of phytanic acid can take place, and pristanic acid is an intermediate of this process (Verhoeven & Jacobs, 2001). As found for cheeses, it appears to be a rather general fact that the relevance of pristanic acid increased with decreasing amounts of phytanic acid. The concentration of phytanic acid was less than 1/3 of the value reported for one human milk fat sample (0.11%) from the 1970s (Lough, 1975). A decrease in average concentration of phytanic acid in humans would fit well with a lower uptake from dairy products due to lower amounts of grass fodder in conventionally-raised milk cows. However, this hypothesis cannot be proven due to the low number of data in humans from 30 or more years ago.

4. Conclusions

The results in this study demonstrate that phytanic acid and pristanic acid were significantly higher concentrated in organic dairy products from the German market than in conventional. The difference was due to higher levels in grass-based fodder which is the natural primary feed of cows and thus the best choice from an ecological point of view. Nevertheless, samples with particularly high content of these fatty acids in conventional dairy products could reach the level of organic samples with the lowest concentrations. Phytanic acid was found to be a better marker for the assessment of organic dairy products. A target value of 200 mg/100 g lipids was suggested for control measures of organic dairy products. Manufacturers of organic dairy products with phytanic acid below this boundary of 200 mg phytanic acid per 100 g lipids should be inspected more in details. This should also be in the producer's interests because short-term surpassing of the target value is acceptable by regulations but on the long term, low concentrations of phytanic acid would indicate the use of non-grass-based feed which is undesirable. Our results suggest that phytanic acid would be an interesting marker similar as was found for 18:3$n-3$ (Molkentin & Giesemann, 2007). The availability of different markers will surely make it more difficult to falsify organic dairy products.

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