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Analytical Methods

Applicability of organic milk indicators to the authentication of processed products

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Abstract

The validity of established threshold values for the analytical authentication (stable isotopes and fatty acids) of organic drinking milk in Germany was determined for more strongly processed organic dairy products (n = 56). Milk fat extracted from both soft and semi-hard cheeses, butter, cream, sour cream, buttermilk, yoghurt and low-fat milk always possessed an α-linolenic acid (C18:3ω3) content above the minimum level of 0.50% and a stable isotope ratio of carbon ($\delta^{13}C$) below the maximum level of -26.5‰ required for organic milk. Noncompliant results were obtained for whey as well as both Italian ice creams and cheeses. Analyses of German cream cheese and curd lipids revealed that 7 out of 39 samples did not comply with the two thresholds. An additional analysis of δ^{13} C in the defatted dry matter showed that these reconstituted products apparently contained a combination of organic skim milk and conventional or imported organic cream. The inherent correlation between $\delta^{13}C$ in the fat and defatted dry matter indicates their different origins, which may provide evidence of fraud. This study showed that the previous C18:3ω3 and $\delta^{13}C$ thresholds are generally applicable to processed dairy products from Germany. An analysis of $\delta^{15}N$ in defatted dry matter confirmed the recently proposed threshold of ≤5.5‰ for organic dairy products.

Keywords: Fatty acid, α-Linolenic acid, Stable isotopes, Carbon, Organic milk products, Authentication

1. Introduction

Procedures for authenticating organic milk continue to be of interest because of its high price and the limitations of available natural resources. The risk of conventional milk fraudulently labelled as organic can be countered with adequate controls to protect consumers and secure fair trade. An obvious starting point for potential procedures is the milk composition, which can vary greatly depending on differences in the diets of cows.

Any specific characteristics from feeding reflected in organic and conventional milk must be distinctive to allow for differentiation despite the seasonal influences on milk composition generally observed. Some studies have reported higher α-tocopherol and ß-carotene contents in organic milk than in conventional milk (Bergamo, Fedele, Iannibelli, & Marzillo, 2003; Slots, Sorensen, & Nielsen, 2008). An analysis of phytanic acid indicated an increased minimum level in organic milk fat; however, it was insufficient to distinguish organic milk from conventional (Vetter & Schröder, 2010). More promising were reports of elevated levels of ą-linolenic acid (C18:3ω3) in organic milk fat (Bergamo et al., 2003; Butler, Stergiadis, Seal, Eyre, & Leifert, 2011; Ellis et al., 2006; Jahreis, Fritsche, & Steinhart, 1996; Slots et al., 2008), which is in agreement with our previous work (Molkentin, 2009; Molkentin & Giesemann, 2007). Furthermore, we demonstrated that the stable isotope ratio of carbon ($\delta^{13}C$) in milk fat is an interesting variable for organic milk authentication (Molkentin, 2009; Molkentin & Giesemann, 2007), which is related to the varying ratio of C_3 to C_4 plants in the cow's diet (Metges, Kempe, & Schmidt, 1990).

Therefore, in 2009, we proposed threshold values for C18:3ω3 and $\delta^{13}C$ in milk fat that may be used to identify German organic retail milk (Molkentin, 2009). According to our studies, which systematically included seasonal variability in bulk milk, organic milk always possessed a minimum C18:3ω3 content of 0.50% and a maximum $\delta^{13}C$ of -26.5‰. Although conventional milk can sometimes exceed these limits, this procedure differentiates the vast majority of conventional milk from organic milk.

The aim of the present study was to determine whether these established milk fat limits also apply to processed dairy products. Such products may offer a challenge for the food chemist because they are not always produced directly from fresh milk but are often made from previously isolated individual milk components, such as skim milk, cream or casein. During these previous technological treatments, the composition of the milk components may be inadvertently changed, which may impair the authentication of organic milk. Moreover, beyond lipid analysis the authentication of reconstituted dairy products may necessitate an additional analysis of non-lipid components to exclude fraud. Therefore, our investigations into validating fatty acids and stable isotopes as indicators for organic dairy products focus on reconstituted products.

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2. Materials and methods

2.1. Samples

Dairy products were purchased between August 2007 and September 2009 from retail stores in Kiel, Germany. All products were made exclusively from cow's milk and were certified as organic according to European Council Regulation (EEC) No. 2092/91 (Council Regulation, 1991) and its amendments. Samples primarily comprised cheeses, most notably curd and cream cheese. Additionally, scattered samples of other dairy products were obtained. More detailed information on the 56 samples is given in Table 1.

Milk fat was extracted from cheese using the method of Schmid-Bondzynski-Ratzlaff (International Standard ISO 1735 | IDF 005:2004, 2004). Fat was extracted from all other dairy products according to the method of Roese-Gottlieb (International Standard ISO 1211 | IDF 001:2010, 2010; International Standard ISO 2450 | IDF 016:2008, 2008; International Standard ISO 7208 | IDF 022:2008, 2008; International Standard ISO 7328 | IDF 116:2008, 2008). As a basic principle, residual solvents were removed from the lipids by rotary vacuum evaporation with a maximum water bath temperature of 45 °C rather than drying at 102 °C to avoid heat-induced changes in the fat composition. Additionally, milk fat was obtained from butter by melting and filtering the fat layer at 50 °C in an oven.

Defatted dry matter was prepared from curd and cream cheese via the following procedure: 12.5 g of cheese was thoroughly mixed with 20 mL of 2-propanol and 25 mL of cyclohexane in a 120 mL centrifuge tube for 2 min using an Ultra-Turrax. After adding either 17.8 mL (curd cheese) or 21 mL (cream cheese) of water, the mixture was ultra-turraxed again for 1 min and centrifuged at 2000*g* for 5 min. The top layer was discarded, and the remainder was ultra-turraxed with 25 mL of a cyclohexane/2-propanol (87:13) mixture for 1 min and centrifuged for 5 min (2000*g*). The upper layer was removed, and the remainder was lyophilized and ground in a mortar.

2.2. Gas chromatography of fatty acids

Fatty acid methyl esters (FAME) were obtained from the extracted milk fat as described in (Molkentin & Giesemann, 2007) but using potassium hydroxide instead of sodium methylate for the trans-esterification. FAME analyses were performed on a 50 m capillary column (i.d.: 0.25 mm) coated with a 0.20]m film of CP-Sil 88 (Varian, Palo Alto, CA) as described in (Molkentin & Giesemann, 2007). Calibration of the major fatty acids was accomplished using the reference milk fat CRM 164 (IRMM, Geel, Belgium). Fatty acids ranging from C4 to C24 were determined and calculated as a weight percentage (g/100 g of total fatty acids).

Table 1
Origin and number of analysed dairy products.

Dairy product	Produced in	Samples
Butter	Germany	1
Cream	Germany	1
Sour cream	Germany	1
Ice cream	Italy	2
Yoghurt	Germany	1
Low fat milk (1.5%)	Germany	1
Buttermilk	Germany	3
Whey	Germany	2
Camembert cheese	Germany	1
Blue mold cheese	Denmark	1
Gouda cheese	The Netherlands	1
Parmesan cheese	Italy	2
Cream cheese	Germany	18
Curd	Germany	21

2.3. Isotope ratio mass spectrometry (IRMS)

2.3.1. Sample preparation

The samples were combusted in tin capsules, and the resulting gases were separated using a Thermo Scientific Flash EA 1112 elemental analyser (Waltham, MA) as described previously (Molkentin & Giesemann, 2007). A 0.46 mg sample was used for the carbon isotope analysis in milk fat while a mass of 0.75 mg (curd cheese) or 0.85 mg (cream cheese) was simultaneously analysed for both carbon and nitrogen isotopes in the defatted dry matter.

2.3.2. Stable isotope analysis and calibration

Analyses of the stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were performed using a Delta^{plus} XL isotope-ratio mass spectrometer (Thermo Scientific) with Isodat 1.5 software (Thermo Scientific). These isotope ratios are given in ‰ on a d-scale and refer to the international standards VPDB and AIR for carbon and nitrogen, respectively. For carbon, d-values were calculated as follows:

$$d^{13}C[‰] = \frac{R_{Sample} - R_{Standard}}{R_{Standard}} \cdot 1000 \qquad R = \frac{^{13}C}{^{12}C}$$

Values for $\delta^{15}N$ were calculated in the same way. The standard deviation of the measurements (n = 9) was 60.05‰ for both carbon and nitrogen, using the respective reference gases. To take any inhomogeneity into account and thus obtain representative data for the material, the mean value of three analyses was determined for each sample. The standard deviations of these analyses were <0.15‰ for both carbon (median 0.03‰) and nitrogen (median 0.06‰).

Urea and sucrose (Merck, Darmstadt, Germany) were calibrated as working standards using the following international standards: IAEA-N1 ($\delta^{15}N_{Air}$ = 0.4‰) and IAEA-N2 ($\delta^{15}N_{Air}$ = 20.3‰) for nitrogen; IAEA-CH-6 ($\delta^{13}C_{VPDB}$ = -10.4‰), IAEA-CH-7 ($\delta^{13}C_{VPDB}$ = -31.8‰) and NBS 22 ($\delta^{13}C_{VPDB}$ = -29.8‰) for carbon. Even though the official reference values of the international carbon standards changed slightly in 2008, the previous values were used to ensure consistency with the preceding studies (Molkentin, 2009; Molkentin & Giesemann, 2007, 2010). The working standards were analysed regularly during each sequence to monitor the measurement repeatability and calibrate both the nitrogen and carbon dioxide reference gases (Air Liquide, Düsseldorf, Germany).

3. Results and discussion

Some abnormalities with respect to previous threshold values for C18:3ω3 and $\delta^{13}C$ in German milk fat (Molkentin, 2009) occurred among the first samples of organic cream cheese and curd analysed in this study, which required more detailed investigations. Therefore, cream cheese and curd samples were subsequently purchased repeatedly over a period of roughly two years, which explains why these reconstituted products represent the majority of analysed samples (Table 1), and stable isotopes were also analysed in the defatted dry matter. Because the findings obtained for other organic dairy products were less ambiguous, fewer of these samples were investigated, and they are discussed first.

3.1. Dairy products other than cream cheese and curd

Most of the organic products shown in Fig. 1a had a C18:3ω3 content meeting the minimum threshold value of 0.50% (Molkentin, 2009). Milk fat extracted from both different soft and semi-hard cheeses as well as other high-fat products such as butter, cream and sour cream showed C18:3ω3 contents greater than

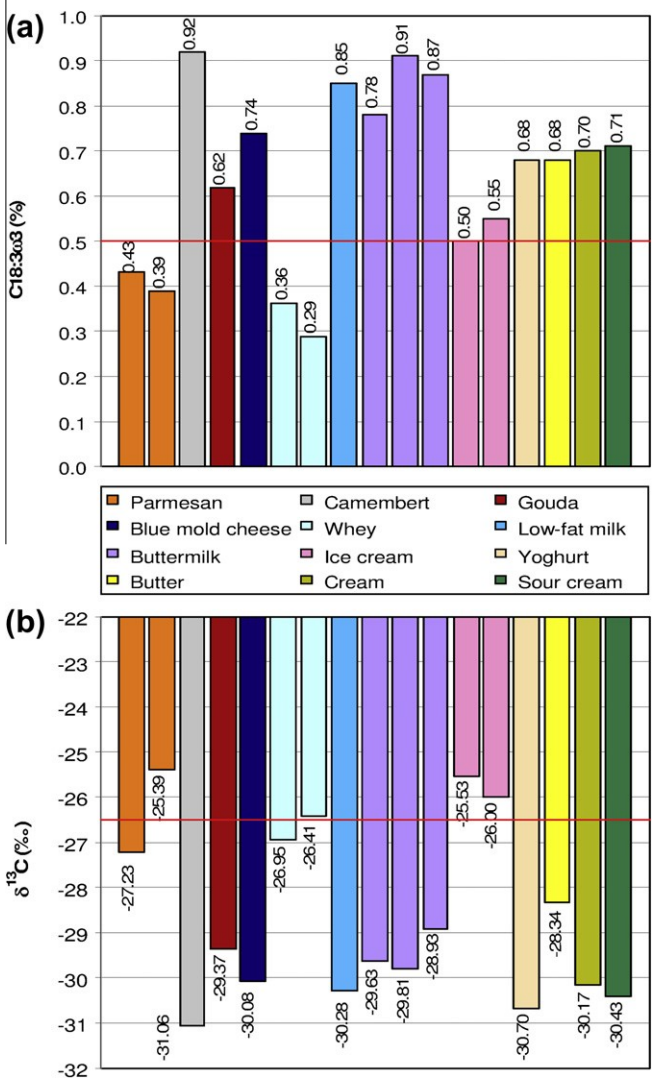

Fig. 1. (a) Content of α-linolenic acid (organic threshold P0.50%) and (b) $\delta^{13}C_{Fat}$ (organic threshold 6-26.5‰) in processed dairy products.

0.60%. Moreover, both yoghurt and low-fat milk (1.5% fat) were confirmed as organic.

Previous studies (Molkentin, 2006) have revealed that total lipid composition of dairy products with a very low fat content can greatly deviate from that of butterfat because minor lipids, such as phospholipids or lipoproteins, compose a higher relative portion. Therefore, the low C18:3ω3 content found in whey samples with a fat content of 0.1–0.2% can be explained as a special fat composition rather than improper labelling. Although a particular lipid composition is also typical for buttermilk, its elevated phospholipid content is known to increase the C18:3ω3 content by at least 25% (Molkentin, 2006). Because C18:3ω3 possessed contents ranging from 0.8% to 0.9% in the three analysed buttermilk samples, the 0.50% minimum would still be exceeded even without this additional 25%.

It is somewhat striking that the organic Parmesan cheese samples possessed C18:3ω3 contents of 0.43% and 0.39%, which fall below the actual limit. However, these values do not necessarily indicate a fraudulent product because this cheese was produced in Italy where organic milk production conditions may differ from those in Germany. The minimum threshold value of 0.50% C18:3ω3 was initially established using milk produced in Germany and may not be valid in other countries (Molkentin, 2009). Moreover, the C18:3ω3 results obtained for Italian ice cream were only just within the organic range. Another explanation for the lower C18:3ω3 content in Parmesan may be a decrease from oxidation during its long ripening process. However, a more detailed investigation would be required to confirm either explanation.

In all, Fig. 1a demonstrates that these organic dairy products generally comply with the C18:3ω3 limit derived from German whole milk. Deviations only arise from milk products not originating from Germany or with a known atypical lipid composition.

The $\delta^{13}C$ results of milk fat, presented in Fig. 1b, generally correspond to those from the C18:3ω3 levels (Fig. 1a) with respect to authenticating the organic products. All high-fat products, including soft and semi-hard cheeses, butter, cream and sour cream, exhibited $\delta^{13}C$ values well below the maximum threshold of -26.5‰ (Molkentin, 2009). Moreover, $\delta^{13}C$ in yoghurt, buttermilk and low-fat milk (1.5%) allowed for the unambiguous identification of organic dairy products. Both whey samples were close to the limit with one slightly above. However, the amount the threshold was exceeded is considerably smaller than the deviation observed for the C18:3ω3 content. Therefore, the elevated level of minor lipids in low-fat products seems to influence $\delta^{13}C$ less than the fatty acid composition.

With respect to living organisms it is well known that lipids have a lower $\delta^{13}C$ value than proteins or carbohydrates because of isotopic fractionation during the synthesis of lipids (DeNiro & Epstein, 1977). While this has as well been reported for milk (Wilson, Mackenzie, & Brookes, 1988), major and minor lipid constituents probably have a similar $\delta^{13}C$ provided that their carbon fraction is mainly composed of fatty acid material, such as in triacylglycerols and phospholipids. However, the elevated $\delta^{13}C$ in whey as compared to buttermilk may be caused by a higher proportion of lipoproteins, while at the same time its decreased C18:3ω3 content indicates a considerably lower percentage ofphospholipids.

Once again, a Parmesan cheese sample was striking with a $\delta^{13}C$ significantly above the threshold (Fig. 1b). Because this higher $\delta^{13}C$ correlates to the lower C18:3ω3 content of both hard cheeses (Fig. 1a), the deviation from both organic threshold values seems more likely to be caused by feeding differences rather than the ripening process. The oxidation of C18:3ω3 hypothesised above should not have as strong an influence on $\delta^{13}C$ in total lipids. Moreover, the negative correlation between C18:3ω3 and $\delta^{13}C$ in milk fat has already been described as a typical interaction between the varying portions of pasture feed, concentrates and maize silage in the diet of cows (Molkentin, 2009).

Consequently, it must be assumed that feeding practices for organic milk cows in Italy differ from those in Germany. This conclusion is confirmed by the two Italian ice cream samples, which both have a $\delta^{13}C$ outside the organic range. These findings most likely result from an increased utilisation of the C_4 plant maize. It can be concluded that the $\delta^{13}C$ threshold of -26.5‰ is widely applicable to German organic dairy products but not necessarily for samples with other geographic origins.

3.2. Cream cheese and curd

In Fig. 2a, the analysed C18:3ω3 contents for 39 organic cream cheese and curd samples are shown in chronological order of their purchase, although some individual data points overlap. From August 2007 to April 2008, 6 out of 14 samples exhibited a C18:3ω3 content below the threshold value of 0.5%. These suspicious samples always originated from the same 2 production plants out of the 7 analysed. Between April 2008 and September 2009, 25 further samples were analysed and always possessed C18:3ω3 contents clearly above the 0.5% threshold. These samples also included 8 from the 2 suspicious plants, but irregular samples

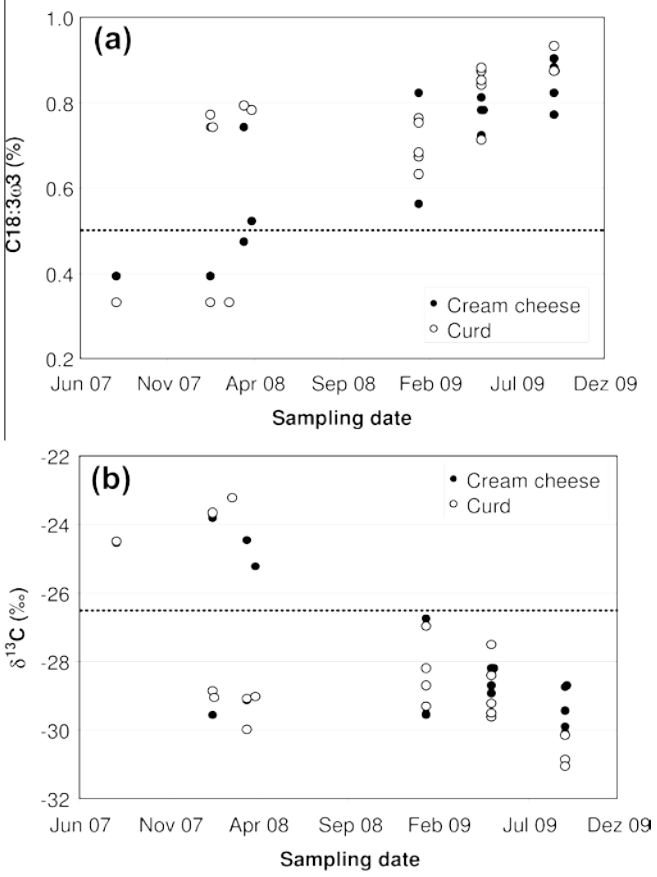

Fig. 2. (a) Content of α-linolenic acid (organic threshold P0.50%) and (b) $\delta^{13}C_{Fat}$ (organic threshold 6-26.5‰) in cream cheese and curd in chronological order of purchase.

never occurred again. Products from the other 5 producers never resulted in C18:3ω3 concentrations below the 0.5% threshold during the entire sampling period. The lowest C18:3ω3 content obtained above the threshold in April 2008 belonged to a cream cheese from one of the suspicious plants. As will be seen later, this sample also showed deviations with respect to $\delta^{13}C$ in milk fat.

These striking results corresponded to the equivalent $\delta^{13}C$ analyses of milk lipids from the organic cream cheeses and curds (Fig. 2b). The same 6 samples that had too low C18:3ω3 contents and the one lying barely above the minimum threshold of 0.5% (Fig. 2a), all possessed a $\delta^{13}C$ clearly above the maximum threshold of -26.5‰. Just as with C18:3ω3, no other samples exceeded the $\delta^{13}C$ limit between April 2008 and September 2009. Therefore, all but 7 samples could be identified as organic by their $\delta^{13}C$. Because both the C18:3ω3 and $\delta^{13}C$ analyses identified the same samples as noncompliant and basically both are very closely correlated (Molkentin, 2009), the deviation is thought to actually result from a special molecular as well as isotopic composition of lipids in these samples.

The first step in the manufacturing process of both cream cheese and curd involves the separation of raw milk into cream and skim milk. To adjust the fat content to the level intended for the final product, the cream is added to the skim milk either before (cream cheese) or after (curd) coagulation, and there is no obligation to use cream from the same raw milk as the skim milk. However, to justify the organic label on the processed dairy product, all components must originate from organic milk. Nevertheless, because of the manufacturing process, the milk fat and protein may legally come from different farms or even geographic regions. Moreover, this process provides the opportunity for the fraudulent addition of cream from conventionally produced milk to organic skim milk.

To check the conformity of both the milk fat and protein to organic labelling we analysed $\delta^{13}C$ in the defatted dry matter (DDM) of 17 organic cream cheese and curd samples. These samples included both the 7 suspicious samples and 10 other samples that covered a representative selection of production dates and manufacturers. As published previously, there is a close correlation ($r = 0.99$) between $\delta^{13}C$ of native milk lipids and proteins (Molkentin & Giesemann, 2010), with a higher value in proteins. The average difference between $\delta^{13}C_{Protein}$ and $\delta^{13}C_{Fat}$, $\Delta\delta^{13}C$, was reported to be 2.87 ± 0.30‰ for organic milk and 2.38 ± 0.36‰ for conventional milk. While there is a strong depletion of $\delta^{13}C$ in milk lipids relative to proteins, $\delta^{13}C$ of total milk protein and casein are assumed to almost be isotopically equivalent. Because the influence of residual lactose in cheese DDM is neglectable, the DDM analyses performed in the current study should yield $\delta^{13}C$ results similar to milk protein.

The results for $\delta^{13}C_{DDM}$ obtained for the selected cream cheese and curd samples are shown in Table 2. Although the first 7 samples, which did not comply with the $\delta^{13}C_{Fat}$ limit of -26.5‰, had

Table 2
Composition of selected cream cheese and curd samples.

No.	Purchase	Product	Compliance [a]	$\delta^{13}C_{DDM}$ (‰) [b]	$\delta^{13}C_{Fat}$ (‰)	$\Delta D\delta^{13}C_{DDM\text{-}Fat}$ (‰)	$\delta^{15}N_{DDM}$ (‰)	C18:3ω3 (%)
1	08/2007	Curd	No	-25.73	-24.51	-1.22	5.52	0.33
2	08/2007	Cream cheese	No	-24.22	-24.57	0.35	4.97	0.39
3	01/2008	Curd	No	-24.69	-23.69	-1.00	5.61	0.33
4	01/2008	Cream cheese	No	-23.78	-23.83	0.05	4.75	0.39
5	03/2008	Curd	No	-24.30	-23.25	-1.05	5.37	0.33
6	03/2008	Cream cheese	No	-24.66	-24.48	-0.18	4.75	0.47
7	04/2008	Cream cheese	No	-24.61	-25.23	0.62	4.91	0.52
Mean of No. 1–7			No	-24.57	-24.22	-0.35	5.12	0.39
8	01/2008	Curd	Yes	-25.27	-28.89	3.63	5.39	0.77
9	03/2008	Cream cheese	Yes	-25.33	-29.18	3.85	4.94	0.74
10	01/2009	Curd	Yes	-25.13	-28.72	3.60	5.47	0.67
11	01/2009	Curd	Yes	-25.54	-26.98	1.44	5.42	0.63
12	01/2009	Cream cheese	Yes	-25.00	-26.76	1.75	4.75	0.56
13	05/2009	Curd	Yes	-24.51	-28.46	3.95	5.23	0.84
14	05/2009	Cream cheese	Yes	-24.84	-28.22	3.38	4.92	0.81
15	09/2009	Curd	Yes	-25.98	-30.20	4.22	4.98	0.87
16	09/2009	Curd	Yes	-25.69	-31.08	5.39	5.64	0.93
17	09/2009	Cream cheese	Yes	-25.55	-28.75	3.20	4.60	0.88
Mean of No. 8–17			Yes	-25.28	-28.72	3.44	5.13	0.77

[a] Compliance with $\delta^{13}C_{Fat}$ limit of -26.5‰.
[b] DDM: defatted dry matter.

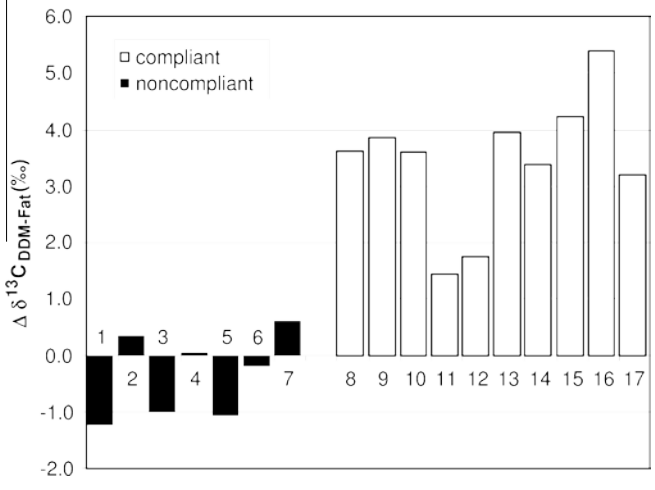

Fig. 3. Difference ($\Delta\delta^{13}$C) between $\delta^{13}C_{DDM}$ and $\delta^{13}C_{Fat}$ in the samples listed in Table 2.

somewhat higher $\delta^{13}C_{DDM}$ values than the other samples on average, all of the products showed a $\delta^{13}C_{DDM}$ below the maximum limit of -23.5‰ for organic milk suggested recently (Molkentin & Giesemann, 2010). Therefore, all samples are apparently from organic skim milk and should thus also have $\delta^{13}C_{Fat}$ values below -26.5‰. Nevertheless, the first 7 samples yielded results that were too high.

Fig. 3 shows the $\Delta\delta^{13}$C values for organic cream cheese and curd. Samples 8–17 (white columns) exhibited distinctly positive differences between $\delta^{13}C_{DDM}$ and $\delta^{13}C_{Fat}$ of 1.4–5.4‰ (mean 3.4‰). The highest differences were found for the samples with the lowest $\delta^{13}C_{Fat}$ values, so the organic $\delta^{13}C_{DDM}$ maximum never was exceeded. In contrast, the suspicious samples, 1 through 7 (black columns), possessed a $\Delta\delta^{13}$C either close to zero or negative
(-1.2 to 0.6‰; mean -0.35‰), which means that $\delta^{13}C_{Fat}$ was sometimes even higher than $\delta^{13}C_{DDM}$ and indicates that the milk fat present in these products did not originate from the same raw milk as the underlying skim milk. Because the higher $\delta^{13}C_{Fat}$ in the added cream fraction is associated with a higher δ^{13}C in the cream-based protein as well, the slightly elevated $\delta^{13}C_{DDM}$ in samples 1 through 7 can be explained.

The elevated $\delta^{13}C_{Fat}$ of samples 1 through 7 (Table 2) may be caused by the addition of conventionally produced rather than organic cream when adjusting the fat content. Despite the natural variation in $\Delta\delta^{13}$C in milk of 1.9–3.5‰ (Molkentin & Giesemann, 2010), which can be somewhat higher in reconstituted products containing milk components from different sources (Fig. 3, samples 8–17), a threshold of <1.0‰ can be derived from our data for identifying suspicious samples with potentially incorrect organic labelling. This threshold is suggested to apply to dairy products made from German bulk milk.

In feeding experiments performed in northern Italy, that underutilized varying amounts of maize, $\Delta\delta^{13}$C values of 2.5–3.0‰ on one farm and 0.7–1.4‰ on another farm were found (Camin, Perini, Colombari, Bontempo, & Versini, 2008). The lowest $\Delta\delta^{13}$C corresponded to the highest percentage of maize (63%) in the diet. However, looking at the δ^{13}C levels reveals that the lowest $\delta^{13}C_{Fat}$ of -23.1‰ in these experiments was still slightly above the maximum $\delta^{13}C_{Fat}$ of -23.2‰ established in our previous study comprising 286 German retail milk samples (Molkentin, 2009). So, the composition of commercial milk in Germany seems to be considerably different.

Another feeding study performed in New Zealand reported $\Delta\delta^{13}$C values of 3.0–4.2‰ during exclusive feeding of C_3-plant material (Wilson et al., 1988). Results for exclusive feeding of C_4-plants decreased from 4.5–5.2‰ during early lactation to 0.8–1.6‰ during late lactation. However, the latter conditions are far away from the reality in German milk production. So, generally speaking $\Delta\delta^{13}$C values decrease with increasing δ^{13}C level of milk components, which is equivalent to increasing maize proportions. Nevertheless, typical feeding conditions in Germany do not reach such experimental extremes.

Because the C18:3ω3 and δ^{13}C threshold values for organic products were established using German retail milk (Molkentin, 2009), organic milk from certain foreign countries may have a deviating composition. Therefore, the striking results for samples 1 through 7 (Table 2) also may be caused by the legal addition of imported organic cream with an altered composition, which is associated with feed containing a maize silage content atypical of organic milk production in Germany but not prohibited by EEC regulations (Council Regulation, 1991).

Cream can be economically transported over longer distances than milk; therefore, it cannot be determined whether samples 1 through 7 (Table 2) exhibited an irregular composition because of fraud or the addition of imported cream. However, it should be noted that such ambiguous samples were only detected between August 2007 and April 2008 and not again before sampling was completed in September 2009.

A $\Delta\delta^{13}$C value of <1.0‰ can be used to clearly detect whether reconstituted dairy products contain protein and fat components from milk sources with different carbon isotopic composition. Provided that all of the processed milk used in the analysed cream cheese and curd samples complied with the abovementioned German δ^{13}C thresholds (Molkentin & Giesemann, 2010), the correlation between $\delta^{13}C_{Fat}$ and $\delta^{13}C_{DDM}$ (Fig. 4) allowed for the identification of dairy products reconstituted from both organic and conventional sources.

All native milk samples will be located, more or less, on a straight line running from the bottom left to the top right corners (Molkentin & Giesemann, 2010) with organic samples occurring in quadrant III and conventional samples in quadrant I of Fig. 4. While dairy products reconstituted from exclusively organic components are still in quadrant III (samples 8–17 of Table 2), they may deviate more strongly from the native-milk line. The combination of organic protein with conventional lipids causes the samples to appear in quadrant IV (samples 1–7 of Table 2), and conventional milk proteins combined with organic lipids appear in quadrant II. Therefore, the analysis of δ^{13}C in both milk protein and fat allows for the rapid classification of the origin of the milk ingredients in processed dairy foods.

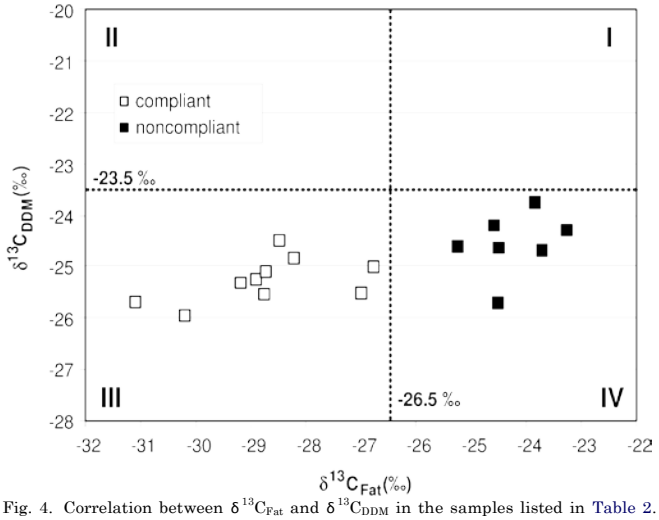

Fig. 4. Correlation between $\delta^{13}C_{Fat}$ and $\delta^{13}C_{DDM}$ in the samples listed in Table 2.

Moreover, $\delta^{15}N$ of selected cream cheese and curd samples (Table 2) has been analysed. Although the stable isotopes of nitrogen do not clearly distinguish between organic and conventional milk, our recent investigations found that conventional milk tends to have a higher $\delta^{15}N$ (Molkentin & Giesemann, 2010). In that study, organic whole milk never exceeded a maximum $\delta^{15}N$ threshold of 5.5‰. As was determined above by $\delta^{13}C_{DDM}$, all of the samples listed in Table 2 were apparently made from organic skim milk. Because the predominant portion of nitrogen in the samples originates from the skim milk rather than the cream fraction, all samples should also adhere to the tentative $\delta^{15}N$ limit. According to Table 2, only 2 samples even slightly exceed the threshold of 5.5‰, and only by 0.1‰. Therefore, the present data on cream cheese and curd confirms the $\delta^{15}N$ threshold previously obtained for organic drinking milk.

4. Conclusions

The C18:3ω3 and $\delta^{13}C$ threshold values previously established for identifying German organic retail milk via lipid analysis are widely applicable to processed dairy products. Noncompliant results may be obtained for certain low-fat organic products or those made from milk produced in countries other than Germany. To confirm the authenticity of reconstituted dairy products, $\delta^{13}C$ must be analysed in both the defatted dry matter and lipids. A resulting $\Delta\delta^{13}C$ value of <1.0‰ indicates different origins for the protein and fat, which may be evidence of fraud. Analysing $\delta^{15}N$ of the defatted dry matter usually results in values of ≤ 5.5‰ for organic dairy products.

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