Potential of Near Infrared Spectroscopy for differentiation of organically and conventionally produced milk

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Abstract

Increasing sales of organic milk require powerful and reliable methods to test the authenticity of milk. The composition of milk, especially the contents of ω3-fatty acids (FA), is fundamentally influenced by feeding. Therefore, near infrared reflectance spectroscopy (NIRS) was evaluated as a possible alternative to gas chromatography (GC) for the quantitative analysis of FA. Furthermore, the applicability of NIRS for discrimination between organically and conventionally produced milk was examined.

To record the variable effect of feeding, including seasonal effects, two conventional brands and one organic brand of retail milk were purchased biweekly during a period of 18 months. Additionally, one organically produced milk sample was collected in the same intervals directly from a farm.

FA contents were determined by GC, and NIRS calibrations were developed with these reference data by PLS regression. As a result, a standard error of prediction (SEP) of 0.099 % for C18:3ω3 and a regression coefficient (Rval) of 0.9 were obtained. For the prediction of C20:5ω3, the SEP was 0.014 %, and the Rval 0.83. The contents of ω3-FA predicted by NIRS reflected seasonal differences, with higher levels in organic milk samples. The average amount of C18:3ω3 content in organic milk fat was 0.73 ± 0.16 %, and in conventional milk fat 0.42 ± 0.1 %. The average amount of C20:5ω3 content in organic milk fat was 0.12 ± 0.02 % and in conventional milk fat 0.08 ± 0.01 %. Under time resolved analysis of milk samples from both production systems, the differentiation was successful.

The results indicated that NIRS could be used to predict ω3-FA in milk samples and had the potential to be used as fast method to discriminate between the milk production systems.

Keywords: milk authentication, differentiation, organic milk, fatty acids, NIRS

Zusammenfassung

Potential der Nahinfrarotspektroskopie (NIRS) zur Differenzierung ökologisch und konventionell produzierter Milch

Der ständig wachsende Marktanteil ökologisch erzeugter Milch erfordert leistungsstarke und zuverlässige Methoden zur Herkunftssicherung der Milch. Die Zusammensetzung der Milch, speziell die Gehalte an ω3-Fettsäuren (FS) werden im Wesentlichen durch die Fütterung beeinflusst. Deshalb wurde die NIRS als mögliche Alternative zur Gaschromatographie (GC) für die quantitative Analyse der FS bewertet und ihre Eignung für die Unterscheidung von ökologisch und konventionell produzierter Milch geprüft. Um variable Effekte der Fütterung einschließlich saisonaler Effekte aufzuzeigen, wurden zwei konventionell und eine ökologisch erzeugte Markenmilch im zweiwöchigen Abstand über 18 Monate im Einzelhandel gekauft. Zusätzlich wurde im gleichen Intervall eine ökologisch erzeugte Milch direkt ab Hof bezogen. Die FS-Gehalte wurden mittels GC bestimmt und für die Entwicklung von NIRS Kalibrationen verwendet. Für die Vorhersage der Gehalte an C18:3ω3 wurde ein Standardfehler (SEP) von 0,099 % und ein Regressionskoeffizient (R) von 0,9 erhalten, für C20:5ω3 betrug der SEP 0,014 %, der R 0,83. Die geschätzten Gehalte an ω3-FS spiegelten die jahreszeitlichen Schwankungen wider mit höheren Werten in den ökologischen Milchproben. Der Durchschnittsgehalt an C18:3ω3 im Fett ökologisch erzeugter Milch (öM) betrug 0,73 ± 0,16 %, der im Fett konventionell erzeugter Milch (kM) 0,42 ± 0,1 %. Für C20:5ω3 betrug der Durchschnittsgehalt in öM 0,12 ± 0,02 %, in kM 0,08 ± 0,01 %. Unter jahreszeitlicher Auflösung der Ergebnisse beider Produktionsweisen war eine Differenzierung erfolgreich. Die NIRS kann zur Schätzung der ω3-FS in Milchproben eingesetzt werden und bietet Potential, als schnelle Methode zur Unterscheidung der Produktionsweise von Milch genutzt zu werden.

Schlüsselwörter: Authentifizierung, Differenzierung, ökologisch erzeugte Milch, Fettsäuren, NIRS

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Introduction

The market for organically produced foodstuffs has been growing continuously over recent years. The organic foodstuff market was, with 10 % growth in 2008, the most dynamic market within the whole foodstuff market in Germany (BÖLW, 2009). The milk market recorded real growth as well. In Germany, 11 % of fresh drinking milk sold in food retailing was organically produced (Behr et al., 2009). In contrast, the proportion of organic UHT-milk amounted to only 1 %. Altogether the share of organic milk in food retailing was 4.1 % in 2008 (BÖLW, 2009).

The consumption of organic milk is, on the one hand, associated with potential benefits to human health resulting from the increased amounts of ω3-fatty acids (FA) and, largely, of the main conjugated linoleic acid (CLA) isomer C18:2 cis-9, trans-11. In fact, ω3-FA have been linked to protection against coronary heart disease (Hu et al., 2002) and as a preventive agent against breast cancer (Saadatian-Elahi et al., 2004). Consumers are willing to pay higher prices for organic products if they are sure of the organic source and convinced, furthermore, of the positive effects of organic production on ecological, ethical and social factors (Rippin, 2008). To assure the authenticity of organic products, and therefore to protect the consumer from wrongly labelled products, there is a continuing demand for new, rapid and relatively cheap methods for direct measurements of food and food ingredients.

There have been previous promising investigations for authentication of organic milk based on gas-chromatographic analysis of FA, or stable isotope analysis of carbon in milk fat, or a combination of both (Molkentin et al., 2007; Molkentin, 2009). It's a matter of common knowledge that the FA profile of bovine milk depends both on the FA profile of the feed and the biohydrogenation process in the rumen. The rules for organic milk production in the EU (EG, 2007; EG, 2008) require a high proportion of roughage in rations (60 %) during the whole year, and specify pasturing. The content of concentrates is also limited to 40 % (EG, 2007; EG, 2008). Several investigations reported higher levels of ω3-FA in milk fat depending on pasture periods or on the intake of roughage, fresh grass or clover, respectively (Butler et al., 2008; Ellis et al., 2006; Jahreis et al., 1996; Kraft et al., 2003). The positive association of the amount of pasture with the CLA content was also investigated in numerous studies. However, the results differ with regard to the composition of organic milk. Some authors reported a higher CLA content in organic milk (Bergamo et al., 2003; Butler et al., 2008; Jahreis et al., 1996; Kraft et al., 2003), whereas others reported no significant overall differences (Ellis et al., 2006; Molkentin, 2009; Toledo et al., 2002). All results in the cited studies were obtained by sophisticated analytical methods and special analytical equipment, requiring skilled operators and large amounts of time. Therefore, the applicability of near infrared spectroscopy (NIRS) should be examined as a tool for authentication of organic milk.

NIRS is widely used in the food and feed industries, and many applications in agricultural and food analysis exist, as recently summarized (Karoui et al., 2007; Wang et al., 2004). For instance, NIRS was used to predict the FA composition of cheeses (Lucas et al., 2008) and for determination of the geographic origin of Emmental cheeses (Pillonel et al., 2003). It has also been used to quantify FA in forages (Foster et al., 2006), or to predict FA in rabbit meat with the aim of discriminating between conventional and organic production (Pla et al., 2007). The possibilities of using NIRS are enormous, and the main advantages of NIR techniques are their rapidity, no need for sample preparation and therefore the low costs.

The objective of this study was to evaluate the ability of NIRS to differentiate between organically and conventionally produced milk based on the prediction of ω3-FA and CLA-contents in milk fat. Considering the known seasonal variations of FA-contents, the study was conducted over 18 months. With regard to the high share of fresh drinking milk in retail food sales, the focus was retail milk, but compared with organic farm milk as directly sold milk.

Materials and methods

Samples

Milk samples were collected between December 2005 and May 2007 in biweekly intervals. On each sampling day two conventionally and one organically produced brands of whole milk were purchased in retail stores in Kiel, Germany. Conventional milk originated from Schleswig-Holstein as well as Mecklenburg-Western Pomerania and organic milk from North Rhine-Westphalia. Additionally one organically produced milk sample was collected at the same intervals directly from a farm located in the north of Germany, near Kiel. Directly after collection, the farm-milk was pasteurized. Subsequent to this biweekly collection, the samples were subdivided into two sub-samples. One sub-sample was directly transferred to the laboratory in Trenthorst and subjected to spectral analysis by NIR. The other sub-sample was used for extraction of the milk fat according to the method of Roese-Gottlieb with the modification that the solvents were removed by rotary evaporation at a maximum water bath of 45 °C instead of 102 °C to prevent fat from heat-induced changes. The extracted fat samples were stored at -18 °C until further analysis of fatty acids by gas chromatography. Altogether a sample set of 144 milk samples, 72 organically and 72 conventionally produced, could be used for the development and validation of the NIR calibrations.

Reference analysis of fatty acids

The fatty acid composition of milk fat was determined by gas chromatography (GC) after transesterification into fatty acid methyl esters using sodium methylate, as described by (Molkentin, 2009). The results were expressed as weight percentage (g/100 g of fatty acids).

NIR Analysis

Near infrared spectra were obtained by the Fourier-Transform NIR-Spectrometer NIRLab N-200 (Büchi, Essen, Germany). Approximately 20 ml of the fresh milk, tempered to 20 °C, were placed into a glass Petri dish, covered with a plate and the spectrum was measured by diffuse reflection in the spectral range from 1.000 to 2.500 nm with a spectral resolution of 1 nm. Each sample was scanned in triplicate and than the spectra were averaged. Spectral data were exported to the NIRCal software (Büchi, Essen, Germany), and different mathematical pre-treatments were performed.

Calibration and statistics. Calibrations were developed using the chemometric software NIRCal version 4.21 (Büchi, Essen, Germany). The samples were divided into calibration (n = 96) and validation sets (n = 48). Spectral data were regressed against GC data. The partial least square (PLS) regression method was used for the individual fatty acids, whereas the main focus was given to the polyunsaturated n-3 fatty acids α-linolenic acid (C18:3ω3) and eicosapentaenoic acid (C20:5ω3) and to the CLA (C18:2c9, t11). To optimize the accuracy of calibration, the data were subjected to different scattering corrections and mathematical treatments (especially Savitzky-Golay smoothing, normalisation between 0 and 1 and derivations of first order). The best treatment was selected for each fatty acid on the basis of the highest coefficient of determination in calibration (R_{cal}) and validation (R_{val}) and the lowest standard error of calibration and validation (SEE and SEP), respectively.

Results and discussion

NIRS calibration equations were developed for α-linolenic acid (C18:3ω3), eicosapentaenoic acid (C20:5ω3) and the main conjugated linoleic isomer C18:2 c9, t11 after testing different mathematical treatments. The statistical summary of the best calibration and prediction for the above mentioned fatty acids is shown in table 1. C18:3ω3, C20:5ω3 and CLA were satisfactorily predicted with values ranging from 0.98 to 0.83 for R_{val} values and 0.014 to 0.099 for standard error of prediction.

Table 1

Fatty acid composition in weight percentage (g/100 g fat) within the calibration and validation sets

	Calibration set				Validation set			
Fatty acid	Range	Mean	R_{cal}	SEE	Range	Mean	R_{val}	SEP
C18:2c9, t11	0.28-1.32	0.55	0.98	0.035	0.30-0.92	0.50	0.98	0.035
C18:3ω3	0.1-1.19	0.58	0.91	0.098	0.31-1.16	0.54	0.88	0.099
C20:5ω3	0.06-0.16	0.098	0.84	0.014	0.06-0.15	0.099	0.83	0.014

The successful prediction of the ω3-fatty acids and the main CLA was the basis for the investigations on the applicability of NIRS for differentiation between conventionally and organically produced milk. The main question of the investigation was whether the NIRS-derived contents of specific fatty acids in milk from both production systems vary enough to show differentiation over the whole year. The seasonal variations in FA composition, documented by various authors (Ellis et al., 2006; Molkentin, 2009; Toledo et al., 2002), were included in the study and therefore the sampling period lasted 18 months. The FA composition is mainly influenced by the feed. It is well known that the higher the percentage of roughage in organic feeding, the higher the level of polyunsaturated ω3-FA in milk fat (Collomb et al., 2008; Jahreis et al., 1996; Molkentin et al., 2007). Another factor influencing the elevated ω3-FA levels in milk fat have been related to the limited use of concentrates in organic farming (EG, 2007; EG, 2008).

Figure 1 shows the seasonal variations of the C18:3ω3 content in milk fat of organically (OM) and conventionally (CM) produced milk predicted by NIRS. As expected, the amounts of C18:3ω3 varied between the seasons independently of the production system, but the amounts were always higher in organically produced milk, with the average value of 0.73 ± 0.16 % versus 0.42 ± 0.10 % in conventionally produced milk. The higher C18:3ω3 content in organic milk corresponds well with the guidelines for organic milk production where permanent access to pasture is regulated (EG, 2007; EG, 2008). The seasonally resolved values (Figure 1) showed differences between the amounts of C18:3ω3 in organically and conventionally produced milk on each individual sampling date except for one sampling date (20/02/2006) in the winter season. The difference averaged 0.32 ± 0.17 %. The differences in evidence were higher between the farm-milk samples and the retail conventional milk samples, but unfortunately conventional farm-milk samples were not included in the actual study. Except for the first winter season 2005/2006, the minimum difference was 0.1 % but extended up to 0.88 % during the grazing period.

Recently a C18:3ω3 content of >0.50 % (analyzed by GC) of total milk fat was proposed (Molkentin, 2009) as an all-year criterion for the authentication of organic milk. However, with respect to grazing periods in conventional production systems restricted to certain regions, such as highlands or mountain regions, this limit is too tight to exclude any conventional milk. In addition, special feeding programs within the scope of conventional production (in the meaning of not being certified organic) aiming at producing a so-called pasture milk with a higher content of ω3-fatty acids and CLA result in an independent niche product and will not be competitive compared to organic milk in terms of a lower price. However, a different limit for summer and winter milk could be a solution and should be further discussed in the scientific community and examined in additional investigations. Even though the C18:3ω3 content is not suitable for a complete distinction of organic and conventional milk, a minimum content set up for organic milk allows for the exclusion of the vast majority of conventionally produced German milk as non-organic.

The direct comparison of the all-year variation and the variation during the grazing period is shown in figure 2. In detail, the average amount of C18:3ω3 in organic farm-milk samples was 0.77 ± 0.19 % versus 0.70 ± 0.11 % in organic retail milk samples and 0.42 ± 0.10 % in conventional retail samples (Figure 2, white filled columns). Marked differences between organic farm and retail milk, shown respectively in the grey columns in figure 2, were observed during the grazing period from April to October with 0.94 ± 0.13 % versus 0.72 ± 0.08 % for the C18:3ω3 content. In contrast, the amount of C18:3ω3 in conventional milk samples during the grazing period increased marginally to 0.46 ± 0.09 %. NIRS-analyses have to be continued on a higher number of samples to establish representative variations. However, the distinction between organic and conventional milk on the basis of C18:3ω3 contents have been demonstrated on principle also using a greater collection of German milk (Molkentin, 2009).

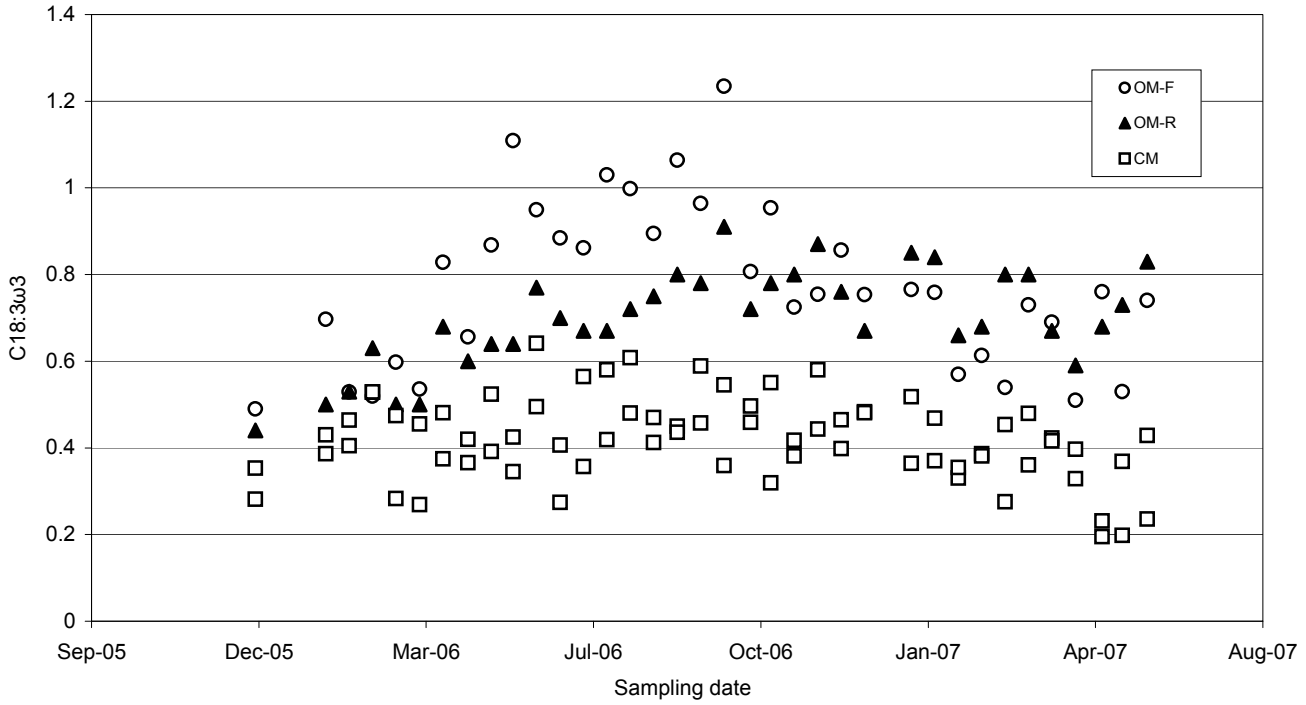

Figure 1:

Seasonal variation of the C18:3ω3 content in milk fat of retail and farm milk (OM-F: organic farm milk, OM-R: organic retail milk, CM: conventional retail milk) predicted by NIRS

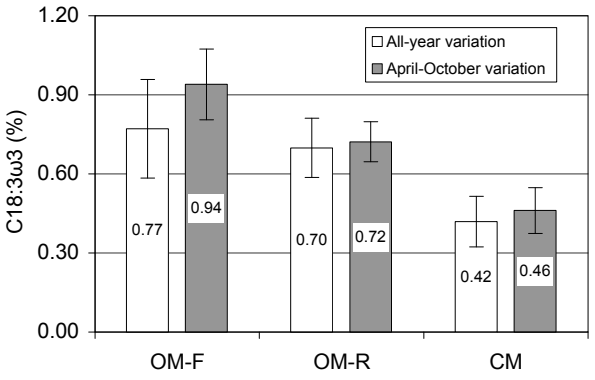

Figure 2:

All-year variation in contrast to April to October variation of the C18:3ω3 content in milk fat (means and standard deviations for: OM-F: organic farm milk, OM-R: organic retail milk, CM: conventional retail milk) predicted by NIRS

The seasonal differences, described above for C18:3ω3 were also observed for C20:5ω3 (Figure 3), but the variations are not as definitive. As expected, the average amount of C20:5ω3 in organically produced milk fat was greater than in conventionally produced milk fat, with 0.12 ± 0.02 % compared with 0.08 ± 0.01 %. On each individual sampling day, the minimum difference between organic and conventional milk samples accounted for 0.01 %. The differences were detectable by NIRS except for one sampling day (20/02/2006). One considers only the contents in organically produced milk, it is evident that the contents of C20:5ω3 in organic farm milk were always higher than in organic retail milk. This is confirmed by examination of the average values, both over the whole year and during the grazing period from April to October (see Figure 4).

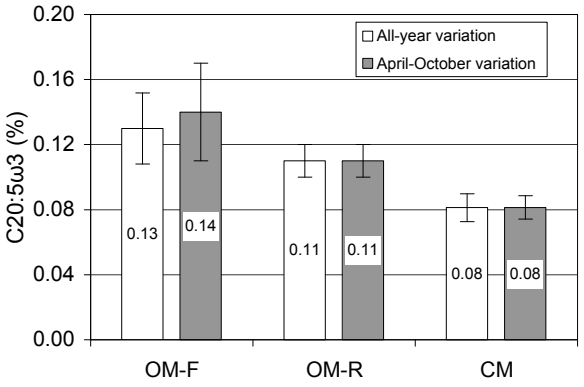

Figure 4:

All-year variation in contrast to April to October variation of the C20:5ω3 content in milk fat (means and standard deviations for: OM-F: organic farm milk, OM-R: organic retail milk, CM: conventional retail milk) predicted by NIRS

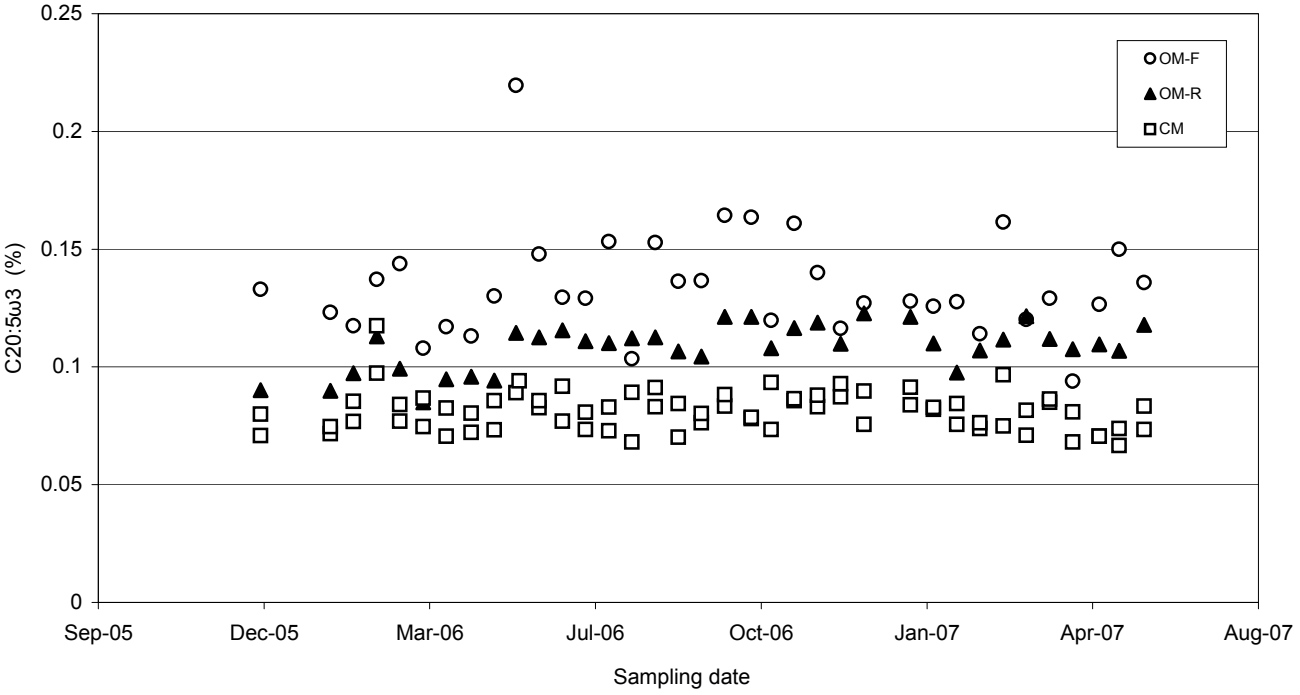

Figure 3:

Seasonal variation of the C20:5ω3 content in milk fat of retail and farm milk (OM-F: organic farm milk, OM-R: organic retail milk, CM: conventional retail milk) predicted by NIRS

The separate evaluation (Figure 4) of the contents of C20:5ω3 in organic farm-milk samples resulted in 0.13 ± 0.02 % versus 0.11 ± 0.01 % in organic retail samples. In contrast, in conventional milk samples 0.08 ± 0.01 % C20:5ω3 were analyzed. During the grazing period between April to October, the content of C20:5ω3 changed only marginally for the farm milk samples to 0.14 ± 0.03 %.

Different authors describe elevated amounts of CLA in milk as characteristic of organic production systems (Bergamo et al., 2003; Jahreis et al., 1996; Kraft et al., 2003). Our data show that CLA contents were higher during the grazing period, but in both production systems (Figure 5). A differentiation between organically and conventionally produced milk, especially during the winter season, was not possible with NIRS. These findings are in accordance with other published data by several investigators (Bergamo et al., 2003; Ellis et al., 2006; Molkentin et al., 2007; Molkentin, 2009; Toledo et al., 2002) using classical methods of fatty acid analysis. The differing results of the cited studies illustrate that CLA is not an appropriate parameter for differentiation of the milk production system.

Conclusions

The question of authentication of organic products remains a challenge for chemical analysts especially given the consideration of general consumer protection.

With regard to its rapidity and ease of handling, the NIRS has enormous potential for product authentication and differentiation of products from various systems.

The presented data indicated the possible application of NIRS to predict ω3-FA in milk and the possibility of using the NIR results as a fast and easy method to differentiate milk production systems. Especially with due consideration to seasonal variations in the contents of the ω3-FA C18:3ω3 and C20:5ω3, the NIRS could be used as screening method and therefore the quantity of extensive and costly gas-chromatographic analysis could be reduced. The prediction of CLA contents was also successful with NIRS, but the applicability of this parameter for differentiation appears not to be reasonable. Further investigations are necessary to prove the results for milk from other production regions, especially for imported milk.

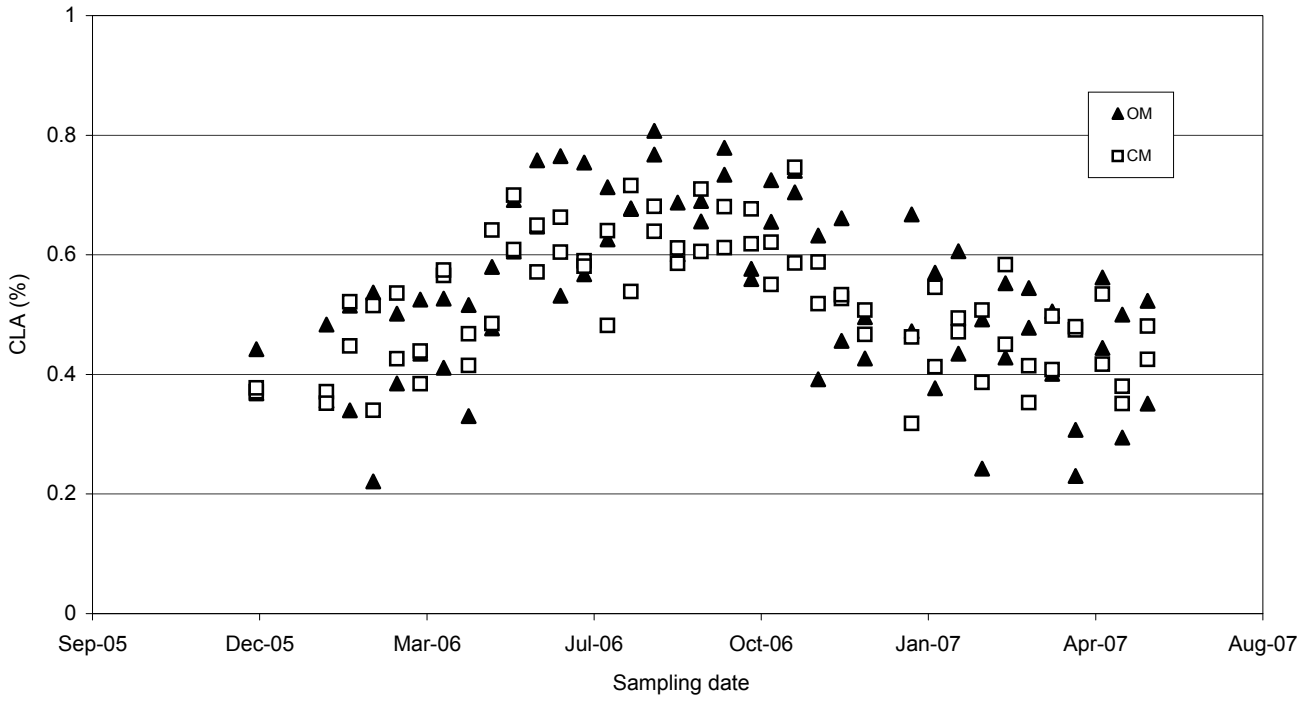

Figure 5:

Seasonal variation of the CLA (C18:2 c9 t11) content in milk fat (OM: organically produced milk, CM: conventionally produced milk) predicted by NIRS

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