On the chemometric deconvolution of gas chromatographically unseparated *trans*-7,*cis*-9, *cis*-9,*trans*-11 and *trans*-8,*cis*-10 octadecadienoic acid isomers in ewe and cow milks

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Summary

A generally known problem of gas chromatographic (GC) separation of *trans*-7,*cis*-9, *cis*-9,*trans*-11, and *trans*-8,*cis*-10 conjugated linoleic acid (CLA) isomers was studied by GC on a 200 m capillary column coated with a cyanopropyl silicone phase at 130 °C. The resolution of these CLA isomers obtained at given experimental conditions was not high enough for direct quantitative analysis but it was, however, sufficient for the determination of their peak areas by a commercial deconvolution software. Relative retentions and resolution factors of CLA isomers with overlapped peaks determined by the separation of a commercial CLA standard mixture as well as CLA isomer fractions obtained by the HPLC semi-preparation of ewe milk were used as input data in the deconvolution procedure. The milk of pasture-fed ewes with higher *cis*-9,*trans*-11 isomer contents showed higher contents of *trans*-7,*cis*-9 as well as *trans*-8,*cis*-10 CLA isomers in comparison with milk fat of total mixed rations (TMR)-fed ewes. For cow milk samples showing lower CLA contents, no such trends were evident.

Keywords

ewe milk fat; cow milk fat; conjugated linoleic acid; gas chromatography; chemometric deconvolution

In recent years, consumers are becoming more aware of the link between diet and health. As a consequence, there is an increasing interest of the consumers in functional foods that have beneficial effects on human health besides the nutritional values [1]. Such functional food components are also certain isomers of octadecadienoic acid found in milk fat and in meat of ruminants. Conjugated linoleic acid (CLA) isomers are reported to have anti-carcinogenic, atherogenic, diabetic properties and they also improve the immune system, bone metabolism and body composition [2]. Recent reports suggest that each conjugated fatty acid isomer has different physiological functions [3]. The antitumour activity of CLA is of special interest, since it shows inhibitory effects against multistage carcinogenesis already at relatively low dietary levels [4]. More than 3 100 articles concerning CLA have been published to date with yearly increasing number of articles to 367 in 2006 [5].

The understanding of the biological role of these acids relies on their proper separation, identification and quantitation in complex biological extracts which contain many unsaturated and saturated fatty acids with a number of carbon atoms from C_4 to C_{22}, where the number of 16–20 prevails [2]. There are 14 possible CLA positional isomers counting from carbons 2,4 to carbons 15,17-18:2. Each positional isomer has four geometric isomers *cis*, *trans*; *trans*, *cis*; *cis*, *cis*; *trans*, *trans* for a total of 56 possible isomers. The double bond positions of CLA isomers actually identified in rumen fat range from 6,8- to 12,14-18:2 in most of the possible geometrical configurations for a total of 20 isomers [2]. Data from animal models reportedly suggest that the *cis*-9,*trans*-11 isomer is responsible for CLA anti-carcinogenic properties, and *trans*-10,*cis*-12 isomer is responsible for the re-partitioning of fat to muscle [1, 3, 4, 6, 7]. The *cis*-9,*cis*-11 CLA isomer has been shown to be the

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most effective against breast cancer cells [6], but its presence in milk fat is very low. Dietary supplements enhanced in CLA are being developed and marketed in response to the reported physiological benefits found in animal models. However, these supplements also contain CLA isomers with unknown physiological properties, and do not reflect natural CLA composition. Recent studies which attempted to use relatively pure preparations of single isomers suggest that the effects of CLA may be isomer-specific [7]. The greatest potential for increasing the CLA intake of humans is to consume high-CLA-containing ruminant products (milk, cheese and meat). Milk and dairy products from pasture-fed animals are naturally enriched and have relatively high contents of CLA [8]. Other way of increasing the CLA intake in human diet is based upon improved animal feeding [8-10].

Ruminant milk fat is the richest natural common source of CLA, with levels ranging from 0.2% (w/w) to 5.4% (w/w) [9]. This large range in CLA contents can be attributed to a number of factors. Diet is the most significant factor affecting the CLA contents of milk fat. High values (up to 2.3%) occur with the feeding on fresh pasture [8, 10]. Highest CLA values (up to 5.4%) were determined when suitable total mixed ratios (TMR) including safflower or fish oil were fed or when monensin, an antibiotic food additive, was used in combination with these TMR [11]. However, it should be noted that monensin is harmful for the animal. Large individual animal variation in the CLA contents in milk were observed, whereas effect of breed and lactation number or age can have only a minor influence on CLA levels. Significant seasonal effects on CLA contents in milk were reported as decreased CLA contents during the growing seasons and subsequently as increased CLA contents at the end of the pasture season [12].

The problem of determination of isomeric CLA composition of the ewe milk fat products is a challenging analytical task [2, 9]. Silver-ion high-performance liquid chromatography (Ag^+HPLC) can provide separations of CLA isomers not attainable by other means. However, to obtain reproducible results, the potential sources of errors should be addressed. These were summarized by Collomb et al. [8] and include: batch-to-batch variations in silver loadings of the columns; differences in instrument configuration (number of solvent pumps, mixing chambers, valves); changes in elution volumes and elution orders with sample size, solvent composition and even storage times; lack of internal standards; and control of column temperature. Further, the HPLC separation of a small peak of *trans*-8,*cis*-10 isomer from a large peak of *cis*-9,*trans*-11 isomer is very poor in milk fat samples, and *cis-trans* from *trans-cis* isomers are not distinguished. Analysis of CLA isomers separated by Ag^+HPLC requires the combination with gas chromatography (GC). The CLA peaks are quantified by GC of total fatty acids methylesters (FAME).

It is accepted that the 100 m capillary GC column coated with cyanopropyl polysilicoxane (CP-Sil 88, Varian, Palo Alto, California, USA) as a highly polar stationary phase is mandatory for the analysis of milk lipids, and at best, the 60 m Supelcowax 10 (Supelco, Bellefonte, Pennsylvania, USA) capillary column serves as a complementary GC column to provide different separations in certain fatty acids elution regions based on its intermediate polarity [8, 9]. The CP-Sil 88 column provided better resolution of CLA isomers. The 100 m CP-Sil 88 column resolves five distinguishing peaks in the CLA region on the chromatogram of milk fat: *cis*-9,*trans*-11 + *trans*-7,*cis*-9 + *trans*-8,*cis*-10; *trans*-11,*cis*-13 + *cis*-9,*cis*-11; *trans*-10,*cis*-12; *trans*-11,*trans*-13; and *trans*-9,*cis*-11 CLA isomers. The important *cis,trans* isomers of CLA usually elute in a region of the chromatogram that is free from other fatty acids. However, C21:0 and C20:2 elute in the elution range of the *cis,cis*- and *trans,trans*-CLA isomers. Although the information on CLA isomeric composition provided by GC is incomplete, GC is often the only method used in the analysis of fatty acids for CLA.

In this concern, the most important analytical task is the resolution of GC unseparated triplet of *cis*-9,*trans*-11; *trans*-7,*cis*-9; and *trans*-8,*cis*-10 CLA isomers. The major *cis*-9,*trans*-11 isomer comprises about 75–90% of total CLA in ruminant milk fat [8, 10]. Normally, the *trans*-7,*cis*-9 is the second most abundant CLA isomer in ruminant fat (up to 7% of total CLA). However, under special conditions, this isomer may represent as much as 40% or as little as 1% of total CLA. In milk fat from cows grazing at high altitude, the second most abundant isomer was the *trans*-11,*cis*-13, and therefore this CLA isomer was proposed as a useful indicator of milk products of alpine origin [9, 10]. The contents of other considered CLA isomer, *trans*-8,*cis*-10, in milk fat is low, however, high concentrations of this isomer were determined in synthetic CLA products [9] as well as in products of thermal treatment of butterfat (up to 31%) [13]. From these published results follows that contents ratio of triplet CLA isomers in various CLA products can be very different. Reporting only the contents of the major CLA peak as a *cis*-9,*trans*-11 isomer, which is usually done in GC analyses, one may miss not

only 1% – 40% of two co-eluted CLA isomers, but may also miss a critical information on the correct CLA isomers composition that has a great impact on the understanding and interpretation of CLA contents of milk fat as well as its dietary effects.

In the previous GC study of CLA isomers composition in milk fat of ewes feeding fresh pasture using 100 m CP-Sil 88 column, we determined higher CLA values (up to 3%) in comparison with published data obtained at similar feeding conditions (up to 2.2%) [14]. The confirmation and explanation of this very interesting result requires, for resolution of GC unseparated triplet CLA isomers with major *cis*-9,*trans*-11 isomer, to use a GC system with a higher resolution than achievable using a 100 m capillary column.

For resolution of gas chromatographically unseparated peaks, various chemometric or mass spectrometric deconvolution procedures can generally be used. In principle, most of them require advanced knowledge, so they are usually not used in everyday practice. To give an impression about complexity of this problem, usually a chromatographer is faced with signal de-noising, peak detection, selecting a certain deconvolution method, choosing a peak model, and specifying the range of characteristics for the parameters of the peak model. This makes the routine application of deconvolution to non-experienced users difficult. Many decisions comprising the full process must be made in each step, before the deconvolution itself can be carried out [15-20]. In order to facilitate deconvolution and to encourage chromatographers to use this technique, an automatic program was developed [21, 22]. The main idea at developing this program was to make the task of deconvolution easy for non-experienced users with little knowledge about implementing chemometrics tools. Several deconvolution softwares are commercially accessible for computer-assisted single-channel-detected chromatograms. PeakFit (Systat Software UK, London, United Kingdom) and Peak Fitting (OriginLab Corporation, Northampton, Massachusetts, USA) belong to the most popular. Mass spectral deconvolution technique for the deconvolution of CLA isomers with overlapped peaks could not be used because mass spectra of *trans*-7,*cis*-9 and *trans*-8,*cis*-10 CLA isomers were not available.

The possibilities and limitations of the use of a commercial Peak Fitting Module (PFM) of the Microcal Origin 7.5 software (OriginLab Corporation) for deconvolution of chromatographically unresolved peaks of *trans*-7,*cis*-9, *cis*-9,*trans*-11 and *trans*-8,*cis*-10 CLA isomers in ewe and cow milk products were studied in this paper.

MATERIALS AND METHODS

Commercially unavailable standard reference materials of *trans*-7,*cis*-9 and *trans*-8,*cis*-10 CLA isomers necessary for obtaining gas chromatographic parameters for deconvolution procedures were investigated in case of *trans*-8,*cis*-10 CLA isomer by using commercial CLA isomers mixture (Nu-Chek Prep, Elysian, Minnesota, USA). This standard sample does not contain *trans*-7,*cis*-9 isomer. The sample model with increasing contents of *trans*-7,*cis*-9 CLA isomer was obtained by a semi-preparative HPLC procedure from a milk fat sample.

The milk sample of ewes fed by total mixed rations (TMR) and fed by pasture with lower and higher CLA contents were obtained from Research Institute of Animal Production (Trenčianská Teplá, Slovakia). The winter and summer samples of cow milk were of commercial origin (ARO, Kežmarok, Slovakia; Rajo, Bratislava, Slovakia). Following samples were analysed: sample No. 1 – milk of pasture-fed ewes with higher CLA contents; sample No. 2 – milk of pasture-fed ewes with lower CLA contents; sample No. 3 – milk of TMR-fed ewes with higher CLA contents; sample No. 4 – milk of TMR-fed ewes with lower CLA contents; sample No. 5 – summer cow milk (ARO); sample No. 6 – winter cow milk (Rajo).

The lipids from milk samples were extracted using a chloroform – methanol mixture (2:1), the extracts obtained were filtered through anhydrous sodium sulfate, then dried and stored under nitrogen at –18 °C. GC-MS analyses of the extracts were performed on a 6890 N gas chromatograph with a 5973 Network mass-selective detector (Agilent, Waldbronn, Germany). FAME were separated using a capillary column of 200 m × 0.25 mm I.D. coated with a film of a thickness of 0.2 μm of cyanopropyl polysilicoxane stationary phase (CP-Sil 88, Varian, Palo Alto, California, USA) at an isothermic column temperature of 130 °C. Identification of separated compounds was obtained on the basis of standard reference materials. Heptadecanoic acid was used as the internal standard for quantification. C_4–C_{24} FAME were analysed in milk fat. The measured contents of CLA in ewes milk fat was the total contents of three CLA isomers *cis*-9,*trans*-11 + *trans*-7,*cis*-9 + *trans*-8,*cis*-10 C18:2, which were gas chromatographically unseparated.

A commercial Peak Fitting Module (PFM) of Microcal Origin 7.5 software (OriginLab Corporation, Northampton, Massachusetts, USA) [15] was used for deconvolution of single peak data from chromatographically unresolved peaks of *trans*-7,*cis*-9, *cis*-9,*trans*-11 and *trans*-8,*cis*-10 CLA

isomers. This tool offers the ability to automatically detect the baseline and peak locations and to fit over 100 peaks. PFM contains a set of built-in functions, providing user with ultimate flexibility when performing least squares fitting and peak data set analysis. Individual peaks can even be assigned to use a specific function. User-defined functions with arbitrary baselines can be also created. PFM is operated through a wizard interface. The deconvolution procedure includes data import; specification of a sub-range of the data; filtration of the data (three filters are built in); definition and subtraction of the baseline by one of four methods; specification of a built-in or user-defined function for each peak (22 functions are built in); automatic location of peaks by specifying a threshold height or number of peaks or manual location of the position of peaks; graphical adjustment of peak parameter values; starting the fitting procedure; controlling the peak data on peak parameter display; displaying the plot of residuals or second derivative; preparation of the data for the report; displaying the peak characterization report, and modification of the report.

RESULTS AND DISCUSSION

The procedure for computer-assisted deconvolution of overlapped peaks of *trans*-7,*cis*-9, *cis*-9,*trans*-11 and *trans*-8,*cis*-10 CLA isomers obtained by capillary GC-MS separation of FAME isolated from lipids of ewe milk products started with an import of a chromatogram into PFM. A sub-range of data containing unseparated methyl esters of *trans*-7,*cis*-9, *cis*-9,*trans*-11, and *trans*-8,*cis*-10 CLA isomers were extracted from the entire chromatogram into PFM. Noisy data were filtered with a fast Fourier transform (FFT) filter. Constant baseline was chosen and Gaussian function was used to fit peak shapes. Since the precision of the fitting procedure for highly overlapped peaks increases with a decrease in the number of optimised parameters, predicted peak widths for three expected overlapped peaks were introduced into the deconvolution procedure. The clusters of overlapped peaks of *trans*-7,*cis*-9, *cis*-9,*trans*-11 and *trans*-8,*cis*-10 CLA isomers obtained by the separation of FAME isolated from lipids of ewe and cow milk products under various experimental conditions were deconvoluted to find the perspectives and limitations of this method. Isothermal separations were investigated for the separation of methyl esters of all carboxylic acids isolated from lipids of ewe and cow milk products. As expected, the separation of methyl esters of *trans*-7,*cis*-9, *cis*-9,*trans*-1 and *trans*-8,*cis*-10 CLA isomers required very long capillary columns and low GC column temperatures. This, however, increased the duration of separations and affected the peak symmetry.

The procedure for deconvolution of overlapped peaks using commercial PFM software was used to treat chromatograms obtained by the separation of FAME by capillary GC-MSD on a 200 m long column at 130 °C. Fig. 1 shows the separation of methyl esters of *cis*-9,*trans*-11 and *trans*-8,*cis*-10 octadecadienoic acids in the CLA standard mixture.

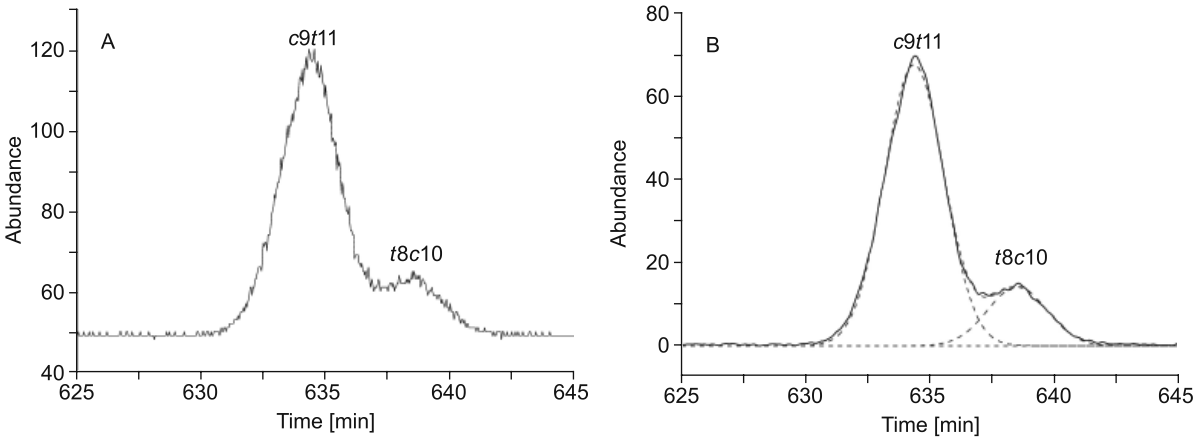

Fig. 1. Separation of methyl esters of *cis*-9,*trans*-11 and *trans*-8,*cis*-10 octadecadienoic acids in the CLA standard mixture on a 200-m capillary column at 130 °C.
Details on experimental conditions are in Materials and methods. A - Part of the experimental chromatogram, B - part of the chromatogram treated with the PFM procedure.

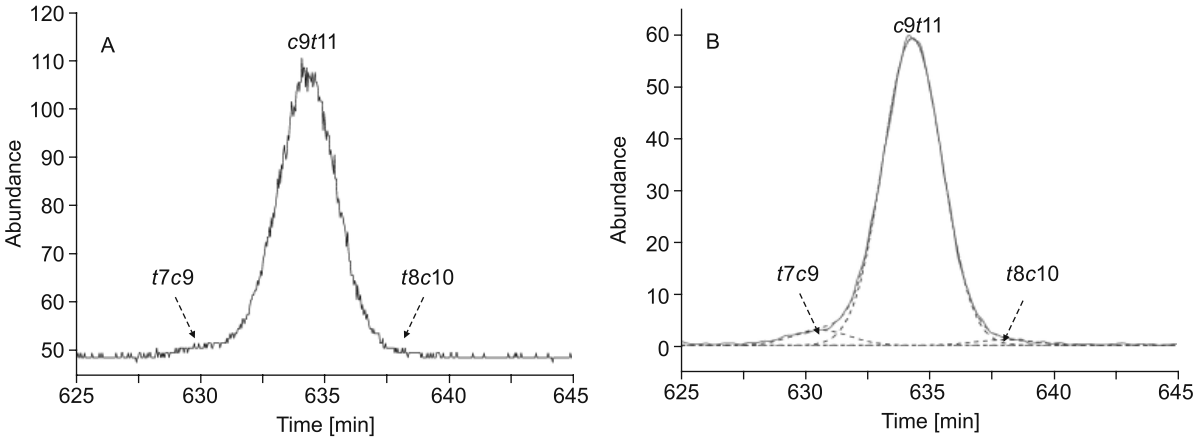

Fig. 2. Separation of methyl esters of *trans*-7,*cis*-9, *cis*-9,*trans*-11 and *trans*-8,*cis*-10-octadecadienoic acids in the sample of milk fat of ewes fed with TMR and with higher CLA contents (No. 3) on a 200-m capillary column at 130 °C.
Details on experimental conditions are in Materials and methods. A - Part of the experimental chromatogram, B - part of the chromatogram treated with the PFM procedure.

The data obtained for both peaks by deconvolution procedure were used for the determination of the relative retention time:

$$r_{t8c10/c9t11} = \frac{t'_{R,t8c10}}{t'_{R,c9t11}}, \quad (1)$$

and the resolution factor:

$$R_{s,t8c10/c9t11} = 1.18\frac{t_{R,t8c10} - t_{R,c9t11}}{w_{h,t8c10} + w_{h,c9t11}}, \quad (2)$$

where t_R is retention time, t'_R – adjusted retention time ($t'_R = t_R - t_M$, where t_M is gas hold-up time), w_h is a half-height peak width, *t*7*c*9 – *trans*-7,*cis*-9; *t*8*c*10 – *trans*-8,*cis*-10; *c*9*t*11 – *cis*-9,*trans*-11 CLA isomer.

Both the relative retention $r_{t8c10/c9t11}$ as well as the resolution factor $R_{s,t8c10/c9t11}$ were used for the determination of the retention time of *trans*-8,*cis*-10 isomer at each analysis of real samples. The part of typical chromatograms with the overlapped peaks of *trans*-7,*cis*-9, *cis*-9,*trans*-11 and *trans*-8,*cis*-10 CLA isomers present in milk of ewes fed TMR with higher CLA contents, as well as in the summer cow milk (ARO) are shown in Fig. 2A and Fig. 3A. Data corresponding to these

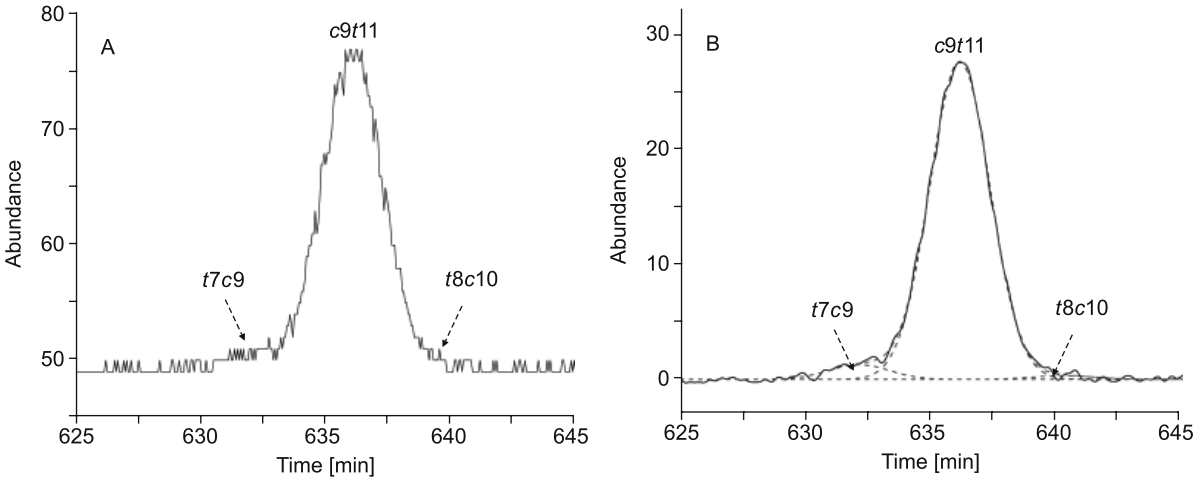

Fig. 3. Separation of methyl esters of *trans*-7,*cis*-9, *cis*-9,*trans*-11 and *trans*-8,*cis*-10 octadecadienoic acids in the sample of summer cow milk (ARO) (No. 5) on 200 m capillary column at 130 °C.
Details on experimental conditions are in Materials and methods. A - Part of experimental chromatogram, B - part of chromatogram treated with PFM procedure.

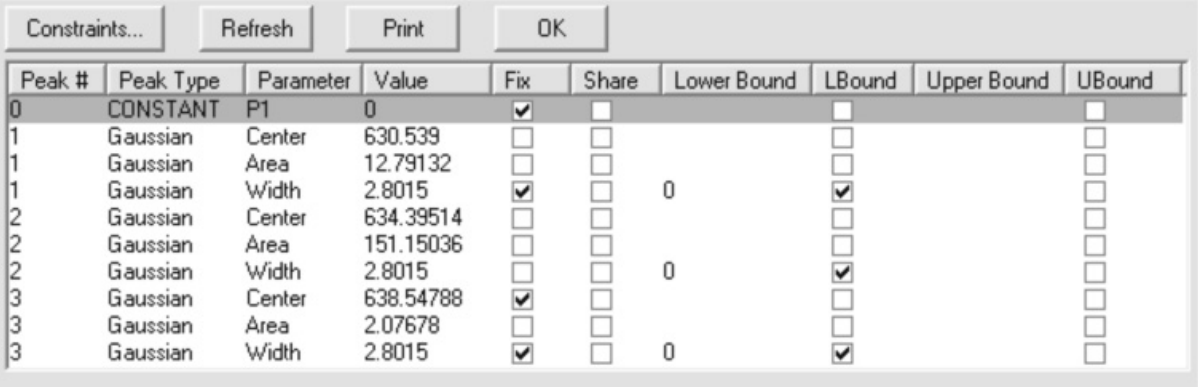

Fig. 4. Display of the modified peak parameters.

Tab. 1. Deconvoluted peak area ratio (in %) of methyl esters of *trans*-7,*cis*-9, *cis*-9,*trans*-11 and *trans*-8,*cis*-10 isomer of octadecadienoic acids and their standard deviation in the gas chromatographically unseparated CLA peak.

Sample*	No. 1 Pasture-fed ewes milk	No. 2 Pasture-fed ewes milk	No. 3 TMR-fed ewes milk	No. 4 TMR-fed ewes milk	No. 5 Summer cow milk (ARO)	No. 6 Winter cow milk (RAJO)
CLA content**	2.5	1.1	1.5	0.5	0.9	0.5
trans-7,*cis*-9	2.3 ± 0.22	3.2 ± 0.21	4.4 ± 0.01	6.6 ± 0.16	4.8 ± 0.36	10.6 ± 0.12
cis-9,*trans*-11	96.2 ± 0.21	95.2 ± 0.32	94.3 ± 0.09	92.0 ± 0.28	94.2 ± 0.84	88.0 ± 0.30
trans-8,*cis*-10	1.5 ± 0.20	1.6 ± 0.11	1.3 ± 0.07	1.4 ± 0.24	1.0 ± 0.49	1.4 ± 0.18

Listed values were determined using the PFM software, as well as contents of CLA determined by GC-MS on a 200-m capillary column at 130 °C in ewe and cow milk samples.
* Samples No. 1-6 are described in Materials and methods. ** CLA contents (sum of *trans*-7,*cis*-9, *cis*-9,*trans*-11 and *trans*-8,*cis*-10 isomers as % of total FAME determined by GC-MS).

chromatograms were exported into PFM and processed by PFM software similarly as described above for standard CLA mixture sample. Fig. 2B and Fig. 3B depict peaks obtained by deconvolution procedure for *trans*-7,*cis*-9, *cis*-9,*trans*-11 and *trans*-8,*cis*-10 CLA isomers. The deconvolution of overlapped peaks in CLA mixture standard on a 200 m capillary column at 130 °C was relatively simple because the peak area ratio of *cis*-9,*trans*-11 and *trans*-8,*cis*-10 isomers was not too high (4.8) in spite of a resolution factor being not very high (R_s = 0.873). Deconvolution procedure was working automatically without any action of the operator. There were, however, problems with the automatic deconvolution of peak clusters in real samples where the peak area ratio of methyl esters of *cis*-9,*trans*-11/*trans*-7,*cis*-9 and *cis*-9,*trans*-11/*trans*-8,*cis*-10 CLA isomers was higher than 10. In these cases, prior to finishing deconvolution procedure, the peak widths and retention time of *trans*-8,*cis*-10 isomer were fixed using the following conditions:

– the peak width of *cis*-9,*trans*-11 isomer was used for all overlapped peaks in the cluster,
– the retention time of *trans-8,cis*-10 peak predicted from the relative retention of *cis*-9,*trans*-11/*trans*-8,*cis*-10 isomers was used.

Fig. 4 shows a table listing the parameters which were used at the PFM deconvolution procedure. The width of the first (*trans*-7,*cis*-9) and third (*trans*-8,*cis*-10) peaks were fixed according to the value found by the software for the second peak (*cis*-9,*trans*-11). The retention time of the third peak (*trans*-8,*cis*-10) was calculated from the relative retention time and fixed, too. Using these fixed values, the optimization in the deconvolution procedure was convergent, giving the peak data for the unseparated CLA cluster. Tab. 1 shows the relative contents of methyl esters of *trans*-7,*cis*-9, *cis*-9,*trans*-11 and *trans*-8,*cis*-10 isomers of octadecadienoic acids in various matrices found by capillary gas chromatographic separation on a 200 m capillary column at 130 °C using this simplification in the PFM software.

The determination of *trans*-8,*cis*-10 isomer us-

ing this procedure was approved by standard addition of simulated peak areas of *trans*-8,*cis*-10 to the chromatogram obtained by the separation of methyl esters of *trans*-7,*cis*-9, *cis*-9,*trans*-11 and *trans*-8,*cis*-10 CLA isomers in sample No. 4. Calibration curve is depicted in Fig. 5. Equation describing the calibration curve is as follows:

$$Y = 2.80 + 0.700X, \quad (3)$$

where Y is the peak area obtained by the PFM procedure from the chromatogram and X is the added peak area of *trans*-8,*cis*-10 isomer. The equation was characterized by the correlation coefficient of $r = 0.99964$. Intercept of the calibration curve with the Y axis (2.80) was in good agreement with the peak area of 2.50 found by an independent PFM procedure.

The PFM deconvolution procedure allowed the determination of peak parameters in unseparated peak clusters at a very low signal-to-noise ratio (SNR). Fig. 6 shows the determination of SNR for the separation of methyl esters of *trans*-7,*cis*-9, *cis*-9,*trans*-11 and *trans*-8,*cis*-10 octadecadienoic acids in the sample of winter cow milk (Rajo) depicted in Fig. 3.

PARK et al. [23] summarized the composition range of isomeric CLA as % of total conjugated linoleic acid determined by silver-ion HPLC in ewe milk fat for *trans*-7,*cis*-9 isomer as 3.3–9.7%, for *trans*-8,*cis*-10 isomer 0.1–0.7%, and in cow milk fat 0.6–6.7% and 0.3–1%, respectively. Considering that *cis*-9,*trans*-11 isomer comprises about 75–90% of total CLA in ruminant milk fat [8, 9], there is a good agreement of our results based on deconvoluted chromatograms (Tab. 1) with these published data.

Taking into account also the CLA contents in samples No. 1-6, the contents of *trans*-7,*cis*-9 isomer in sample No. 1 was 0.058, in No. 2 was 0.035, in No. 3 was 0.066 and in No. 4 was 0.033%. Similarly calculated contents of *trans*-8,*cis*-10 isomer in sample No. 1 was 0.038, in No. 2 was 0.018, in No. 3 was 0.020 and in No. 4 was 0.007%. It is obvious that milk of pasture-fed or TMR-fed ewes with higher CLA contents had higher contents of *trans*-7,*cis*-9 as well as *trans*-8,*cis*-10 CLA isomers. For cow milk samples with lower CLA contents, no such trends are evident.

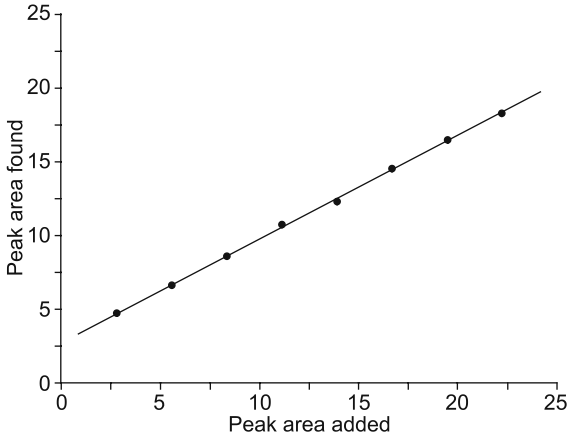

Fig. 5. Calibration curve obtained for standard addition of methyl ester of *trans-8,cis-10* octadecadienoic acid to sample No. 4.

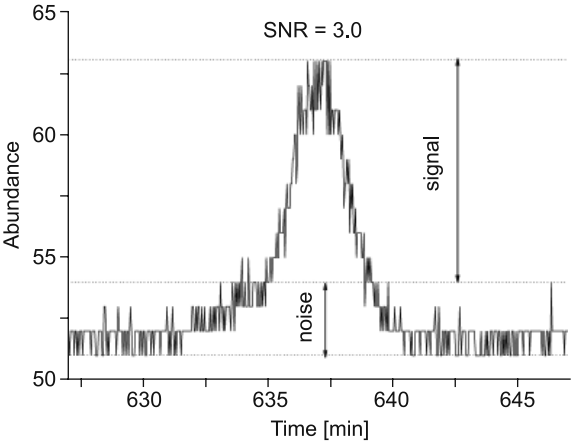

Fig. 6. Determination of signal-to-noise ratio (SNR) at the separation of methyl esters of *trans-7,cis-9*, *cis-9,trans-11* and *trans-8,cis-10* octadecadienoic acid in the sample of winter cow milk (Rajo; No. 6) on a 200-m capillary column at 130 °C.

CONCLUSION

Resolution of *trans*-7,*cis*-9/*cis*-9,*trans*-11 and *cis*-9,*trans*-11/*trans*-8,*cis*-10 CLA isomers with relative retentions of $r_{c9t11/t7c9} = 1.006$ and $r_{t8c10/c9t11} = 1.007$, and resolution factors of $R_{s,c9t11/t7c9} = 0.784$ and $R_{s,t8c10/c9t11} = 0.873$, was obtained by gas chromatography on a 200 m capillary column coated with CP-Sil 88 polar stationary phase at 130 °C. The resolution was not sufficient for direct quantitative analysis but it allowed determination of peak areas by a commercial chemometric deconvolution software. The developed deconvolution procedure allowed the determination of the contents of studied CLA isomers in ewe and cow milks. Determined contents of CLA isomers allowed differentiation of the milk from ewes fed by pasture from that fed by total mixture rations, as well as the differentiation between the summer and winter cow milks.

Acknowledgments

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