Journal of Food Composition and Analysis 24 (2011) 1004–1008

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Quantitative determination of conjugated linoleic acid isomers by silver ion HPLC in ewe milk fat

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ARTICLE INFO

Article history:
Received 14 May 2010
Received in revised form 24 September 2010
Accepted 25 October 2010
Available online 15 December 2010

Keywords:
Conjugated linoleic acid isomers (CLA)
Ag + HPLC
Ewe milk
CLA *cis*-9,*trans*-11 triglyceride
Quantitative determination
Analytical validation
Food analysis
Food composition

ABSTRACT

The aim of this research was to validate a method for the quantification of conjugated linoleic acid (CLA) isomers in the fat of ewe's milk. The isomers in the form of methyl ester (FAME) are separated by silver ion high performance liquid chromatography (Ag^+HPLC) and the quantification was based on the measurement of the integrated area under the 231 nm peaks, using external CLA reference standards. The response linearity is maintained in a wide range of concentrations and the quantification limit estimated was of 0.005 μg/injection for the *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers. Referred to the samples this limit would be 0.008 mg/g of fat in the usual working conditions. In the precision assay an RSDr of 2.14% for the isomer *cis*-9,*trans*-11 was calculated and for the rest of the CLA isomers varied between 2.08 and 7.5%. The recovery assays were performed adding CLA *cis*-9,*trans*-11 triglyceride and the mean recovery was 96%. The method proposed allows the determination of the individual content of different CLA isomers using one single technique, HPLC and supposes saving time and resources.

1. Introduction

Conjugated linoleic acid (CLA) refers to a group of positional (6–8 to 13–15) and geometric (*cis*–*cis*, *trans*–*trans*, *cis*–*trans* and *trans*–*cis*) isomers of octadecadienoic acid with a conjugated double bond system. The interest in CLA isomers has increased considerably over the past few years because of their potential beneficial effects on health as they have been linked to a multitude of metabolic effects (Benjamin and Spener, 2009; Park, 2009). However, most of the evidence in relation to the health effects of CLA has been derived from *in vitro* and animal studies and the challenge is to define the health effects of CLA in human subjects. The biological activities of CLA are fundamentally related with two isomers, the *cis*-9,*trans*-11 and *trans*-10,*cis*-12. For other isomers, such as the *cis*-9,*cis*-11 and the *trans*-9,*trans*-11, evidence is still limited (Beppu et al., 2006; Tanmahasamut et al., 2004).

CLA isomers, naturally occurring in foods derived from ruminants, are produced as intermediates of biohydrogenation of polyunsaturated fatty acids, specifically linoleic (*cis*-9,*cis*-12; C18:2) and α-linolenic (*cis*-9,*cis*-12,*cis*-15; C18:3), by rumen bacteria. Endogenous synthesis of *cis*-9,*trans*-11 CLA is the predominant production pathway in the mammary gland and also occurs in other adipose tissue by the action of Δ^9-desaturase enzyme on *trans*-vaccenic acid (C18:1 *trans*-11), which is another intermediate in ruminal biohydrogenation (Collomb et al., 2006).

Milk and dairy products are the richest source of CLA and it is recognised that the fat of ewes' milk has the highest content, followed by those of cow and goat. This last statement needs refining given that there are many factors that condition the content of the CLA isomers in these types of milk (Gómez-Cortés et al., 2008; Luna et al., 2005a; Park et al., 2007; Sanz Sampelayo et al., 2007). Ewe's milk is usually destined for cheese making, but at present it is commercialized in the form of liquid milk for direct consumption, after being heat treated. This commercialization in Spain is relatively recent and this makes interest in knowing its content of CLA isomers greater given that the data collected in the bibliography correspond to raw milk. Moreover, the consumption of liquid ewe's milk could contribute to increase CLA intake.

Analysis of CLA isomers in a foodstuff is resolved combining different techniques and at present gas chromatography–mass spectrometry (GC–MS), gas chromatography with a flame-ionization detector (GC-FID) and high performance liquid chromatography (HPLC) with photodiode detector are usually used (Adlof, 2003; Aldai et al., 2006; Christie et al., 2007; Cruz-Hernández et al., 2004; de la Fuente et al., 2006; Kramer et al., 2008; Roach et al., 2002). The classic procedure for determination of fatty acids in the form of methyl esters by GC-FID allows the determination of the total CLA content in different foods, but it only permits the

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doi:10.1016/j.jfca.2010.10.009

assignation of contents to the some isomers. Nonetheless, silver-ion HPLC (Ag^+HPLC) achieves a good separation of them. Therefore, a combination of GC and multi-column Ag^+HPLC is usually used with GC providing the total content and Ag^+HPLC the isomeric distribution.

The aim of this work is to refine and validate a method which permits the knowledge of the quantity of individual CLA isomers in ewe's milk using only Ag^+HPLC analysis. Fritsche et al. (2000) have described a similar procedure for the determination of isomers in beef fat and this method has later been used, with slight modifications, to establish the content of CLA isomers in ruminant-derived foods (Martins et al., 2007; Melle et al., 2006; Nuernberg et al., 2007). The UV detection does not provide any differentiation between geometric isomers *cis*,*trans* and *trans*,*cis*; thus, for their identification, it has been necessary to seek support in the works of other authors.

2. Materials and methods

2.1. Chemicals

Isomers of CLA methyl esters C18:2 *cis*-9,*trans*-11; C18:2 *trans*-10,*cis*-12 and a mixture (*cis*-9,*trans*-11; *trans*-8, *cis*-10; *cis*-11,*trans*-13; *trans*-10,*cis*-12 C18:2 and small amounts of some *trans*,*trans* C18:2 isomers) were purchased from Nu-Chek Prep. Inc. (Elysian, MN, USA). C18:2 *cis*-9,*trans*-11 triglyceride was acquired at Larodan Chemicals (Sweden). All solvents used were HPLC grade.

2.2. Samples

Semi-skimmed UHT ewe's milk (fat 1.6%) in packed TetraBrick® was supplied by GAZA S.L. a dairy products company (Zamora, Spain).

2.3. Lipid extraction and fatty acid methylation

Milk fat extraction was carried out in accordance with the procedure described in ISO-IDF (2001) for raw and homogenized milk. The fat extract was stored at −20 °C before fatty acid methylation. Fatty acid methyl esters (FAME) were prepared by base-catalysed methanolysis of the glycerides (KOH in methanol) in accordance with ISO-IDF (2002). Between 25 and 30 weighted mg of thawed and homogenized fat were used and the quantities of reactives were adapted in proportion to that amount of fat.

2.4. Silver ion high-performance liquid chromatography

Ag^+HPLC separation of the CLA methyl esters was performed using an HPLC system (Agilent Technologies 1100) equipped with a photodiode array detector (Hewlett-Packard 1040M) and an autosampler injector (Waters™717 Plus). Three Chromospher 5 lipids analytical silver impregnated columns (each 4.6 mm i.d. × 250 mm Stainless steel; 5 μm particle size, Varian Inc.) were used in series and were maintained at temperature 23 ± 1 °C. The isocratic mobile phase was 0.1% acetonitrile in n-hexane with a flow rate of 1 mL/min and was prepared fresh every day. The column was pre-treated daily by eluting with 1% acetonitrile/hexane for 60 min prior to sample analysis (Fritsche et al., 2000; Luna et al., 2005b). The injection volume was 10 μL. The quantification of CLA isomers was based on the measurement of integrated area under the 231 nm peaks.

Identification of CLA isomers was based on co-injection with commercial reference material as well as comparison of the elution order of the CLA with the findings published in the literature. Moreover, for all the chromatographic peaks the UV spectra have been obtained in a range of 190–350 nm with the objective of checking the characteristic maximum of absorption of the conjugated dienes.

The external calibration plots of the standard solutions were adapted to the different concentration levels of the individual CLA isomers in the lipid extracts.

Three calibration lines were prepared from standard methyl esters (FAME). One of them, with concentrations of 0.05–1.2 μg/μL of the standard *cis*-9,*trans*-11 was used to quantify the majority isomer. Another, with the same standard, with quantities of 0.001–0.015 μg/μL was used to quantify the rest of the isomers except *trans*-10,*cis*-12. For the latter a line was elaborated from the standard *trans*-10,*cis*-12 and with concentrations of 0.001–0.015 μg/μL.

Method validation included establishment detector response linearity, quantification limits, precision and recovery of the analytical procedure.

3. Results and discussion

3.1. Identification of the CLA isomers

CLA FAMEs are selectively detected by their characteristic UV absorbance at 231 nm and the identification of isomers in HPLC chromatograms are based on co-injection of known reference materials.

In this way the isomers can be identified with the aid of previously reported results (Adlof, 2003; Cruz-Hernández et al., 2004; Eulitz et al., 1999; Luna et al., 2005b). Fig. 1 presents the chromatograms corresponding to a standard CLA FAME mixture (A), CLA FAME of ewe milk fat (B) and co-injected mixture of standard plus FAME ewe milk fat (C). Observing this figure and bearing in mind the chromatograms given by Luna et al. (2005b) 12 isomers can be identified. The spectra of all the peaks are characteristic of conjugated dienes. Toluene is added to all the test samples to be able to use the relative retention volume (RRV) for identification in the case of modifications being produced in the volume of retention (RV) (Delmonte et al., 2004). These modifications occur over time and cause difficulties in the identification that are avoided working in this way.

Under our conditions it is not possible to differentiate the isomers *cis*,*trans* from *trans*,*cis*. Nonetheless, from the results presented in the bibliography (Eulitz et al., 1999; Delmonte et al., 2005; Luna et al., 2005b) it can be assumed that 9,11 corresponds in the majority to *cis*-9,*trans*-11 and in the case of 7,9, the second most abundant, this would be *trans*-7,*cis*-9. For the same reasons it would be admissible that the peaks named as 11,13 and 10,12 are the isomers *trans*-11,*cis*-13 and *trans*-10,*cis*-12, although the latter is found in very small quantities.

The peak which elutes after the isomer *cis*-9,*trans*-11 corresponds to 8,10 is not well separated and can be appreciated in the chromatogram of the sample with added standards (Fig. 1C), which if the quantity was increased would cause difficulties in the quantification of the *trans*-7,*cis*-9. Normally in the samples of milk this does not occur given that the isomer 8,10 is a minority one. Having in mind the results published by Eulitz et al. (1999), it would be *trans*-8,*cis*-10, identification which is confirmed by calculating the RRV with respect to the *cis*-9,*trans*-11 C18:2. For the aforementioned RRV we have obtained and average value of 1.054 ± 0.004 from 30 samples of ewe's milk analysed in our laboratory. This value is in accordance which the one registered by Delmonte et al. (2005) for *trans*-8,*cis*-10 isomer.

3.2. Calibration lines

Three calibration lines were built and the least square method was used to calculate the regression equations. Table 1 presents

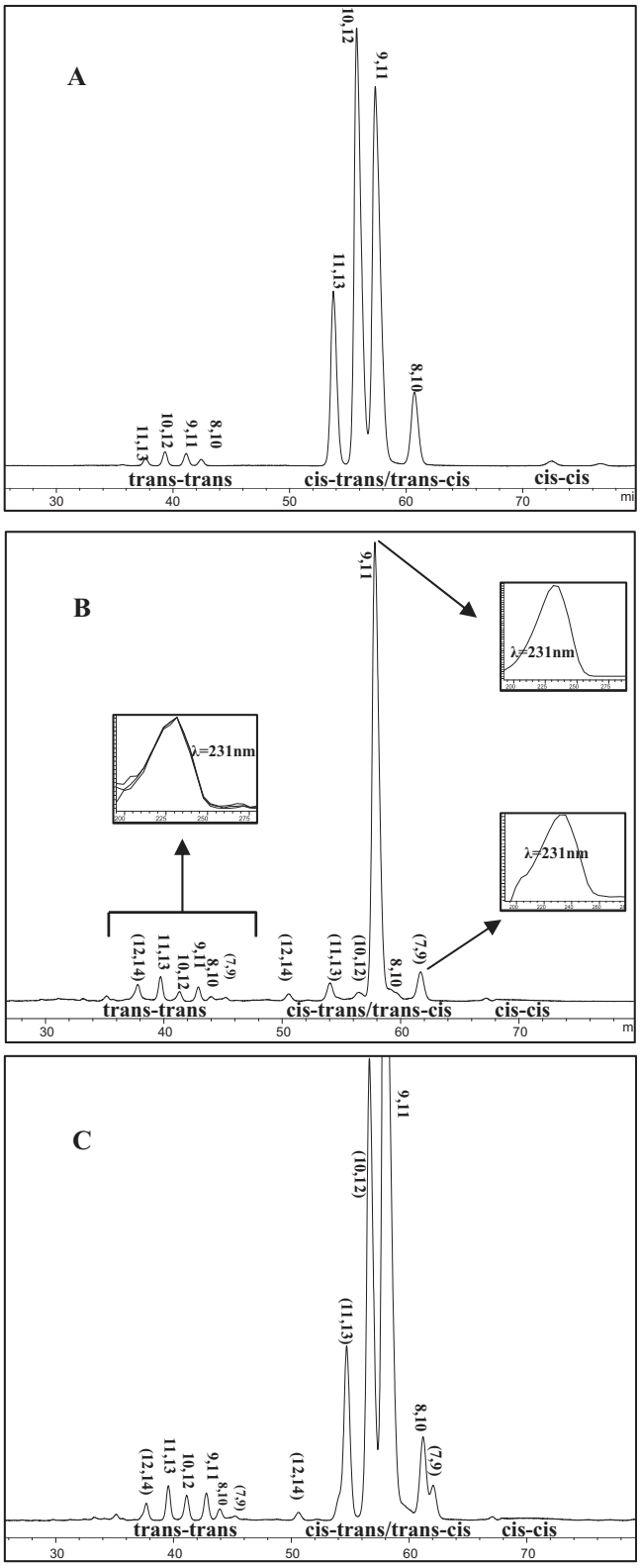


Fig. 1. Ag^+HPLC chromatograms of FAME from: (A) standard mixture, (B) ewe milk fat, (C) co-injected standard mixture plus ewe milk fat, with inserted UV spectra for selected CLA peaks. The numbers on each peak correspond to the CLA double bond position.

Table 1
Estimated regression parameters for the selected standard FAME in the linearity study ($n = 4$).

Isomer	Range (μg/μl[a])	Slope[b]	Intercept[b]	R^2
cis-9,*trans*-11	0.05–1.2	27.2 ± 0.1	0.07 ± 0.06	0.9996
cis-9,*trans*-11	0.001–0.015	27.0 ± 0.2	0.001 ± 0.001	0.9991
trans-10,*cis*-12	0.001–0.015	27.6 ± 0.2	0.004 ± 0.001	0.9996

R^2 = correlation coefficient.
[a] Expressed as FAs (fatty acids).
[b] Mean ± standard error.

the parameters of the response linearity for the FAME standard used. The slopes of the regression lines were similar and for the isomer *cis*-9,*trans*-11 were practically equal in the two intervals of the assay. An adequate linearity was obtained with R^2-values higher than 0.99.

3.3. Quantification limit

The quantification limit was determined in accordance with the standard deviation response and slope method (Dolan, 2009) and a value of 0.0005 μg/μL was obtained in the injected solution both for the isomer *cis*-9,*trans*-11 and for the *trans*-10,*cis*-12, which would correspond to 0.005 μg on the column. In the confirmation assay values of RSD ($n = 4$) of 3.2% and 4.8% were obtained, respectively, for each isomer.

The determination limit (5-fold signal/noise ratio) estimated by Fritsche et al. (2000) could be considered practically of the same order of magnitude as that calculated in our assays although different criteria have been employed.

Referred to the samples the limit of quantification would be of 0.008 mg/g of fat in the habitual working conditions. Although the limit has been established for two isomers it can be assumed that for the remainder it would be practically the same. Therefore it can be considered that the isomers *cis,cis* would be found in the samples studied below this value, as they have not been observed in the chromatograms in the zone in which they should elute.

3.4. Precision

The repeatability assay was performed in a sample which was analysed twelve times, on different days. In Table 2 the mean values for each isomer, the standard deviation and the RSDr are presented. The latter varies between 2.08 and 7.5% and for the *cis*-9,*trans*-11 isomer is 2.14%. The highest deviation corresponds to the isomer 8,10 (*cis,trans* + *trans,cis*), which as previously mentioned presents worse resolution. The contents of the isomers *trans*,8-*trans*,10 and *trans*-7,*trans*-9 were very low in the sample used in this assay and thus have not been included in Table 2. The HORRATr values comply with the criteria specified by the

Table 2
Repeatability of the method for CLA analysis in milk fat ($n = 12$).

	$\bar{X}$ (mg/g)	SD	RSDr (%)
trans-12,*trans*-14	0.081	0.002	2.47
trans-11,*trans*-13	0.077	0.002	2.60
trans-10,*trans*-12	0.039	0.001	2.56
trans-9,*trans*-11	0.113	0.003	2.65
12,14 (ct + tc)	0.043	0.002	4.65
trans-11,*cis*-13	0.048	0.001	2.08
trans-10,*cis*-12	0.056	0.002	3.57
cis-9,*trans*-11	4.491	0.096	2.14
trans-8,*cis*-10	0.133	0.010	7.50
trans-7,*cis*-9	0.379	0.008	2.11

$\bar{X}$ = mean value; SD = standard deviation; RSDr = relative standard deviation.

Table 3
Recovery of *cis*-9,*trans*-11 CLA added as C18:2 *cis*-9,*trans*-11 triglyceride to milk fat, three spiking levels and four samples for each spiking level.

Quantity spiked (mg/g[a])				Quantity found after the addition (mg/g[a])				Recovery[b] (%)
2.562	2.355	2.543	2.477	6.894	6.814	7.016	6.884	97 ± 2.7
4.032	3.869	4.316	3.734	8.341	8.249	8.976	8.286	99 ± 3.7
9.041	9.000	9.209	9.124	12.919	12.695	12.891	12.742	91 ± 1.3
								96 ± 4.2

Content unspiked sample = 4.511 ± 0.094 mg CLA *cis*-9,*trans*-11/g of milk fat.
[a] Expressed as mg of CLA *cis*-9,*trans*-11/g milk fat.
[b] Mean ± standard deviation.

Association of Analytical Communities (AOAC, 2002) and therefore it can be considered that the method has good precision.

In human and rat milk the RSD values for the isomer *cis*-9,*trans*-11 were 2.51 and 1.53, respectively for the within-day precision and 3.27 and 3.08 for the between-day precision assays (Moltó-Puigmartí et al., 2007). In this case the determination was made by GC and if the results for other isomers are considered it is evident that the precision is similar to that of the method used in our laboratory.

3.5. Recovery

These assays were performed adding a standard of C18:2 *cis*-9,*trans*-11 triglyceride, of a purity of 90% determined by GC. 200 mg of the triglyceride were weighted and dissolved in 10 mL of hexane and from this solution the necessary dilutions were prepared to add different quantities to the sample. The determination was performed on four samples for each spiking level and the results obtained are presented in Table 3. The quantities of triglyceride added are expressed as mg of CLA *cis*-9,*trans*-11. The samples used in this assay were analysed on distinct days and the mean value (n = 12) 4.511 ± 0.094 mg CLA *cis*-9,*trans*-11/g of fat was considered as the quantity initially present.

The recovery ranged between 91 and 99% with a mean value of 96%. This indicates complete methylation of the CLA triglycerides, which constitute the majority fraction in the milk (Contarini et al., 2009). The percentages of recovery can be considered good in accordance with the intervals established in function of the concentrations determined (AOAC, 2002).

Methylation in alkaline medium was chosen to avoid CLA isomerization. However, KOH in methanol does not react with free fatty acids (FA) and does not completely methylate phospholipids, which makes this method unsuitable for matrices, such as ruminal liquid or for tissues with high contents in those compounds. In general, this drawback is not an obstacle in dairy-fat studies, except in research in milks with low bacteriological quality (de la Fuente et al., 2006; Lee and Tweed, 2008).

It must be indicated that the data available regarding studies of recovery in the analysis of CLA are very scarce.

Nuernberg et al. (2007) study different methods for the methylation of CLA isomers in beef lipids and find similar recoveries (89–93%), although in this study the conditions are different. They add free CLA *cis*-9,*trans*-11 to a reference material (BCR-163 beef/pig fat). If we take into account the quantity of CLA *cis*-9,*trans*-11 added and the conditions of the study, this quantity would correspond to a level of addition close to the intermediate level of the assays presented here.

In beef meat samples fortified with the selected pure FAs the recovery was 93.67% for the isomer *cis*-9,*trans*-11, determined by gas–liquid chromatography (Aldai et al., 2006).

4. Conclusions

The proposed method permits the quantitative determination of different CLA isomers in milk; keeping in mind their validation parameters, this method could be a good alternative to the conventional determination methods, which combine GC and HPLC, making an important saving of time and resources possible. These aspects are of great interest, from a practical point of view, for the evaluation of the intake of the distinct isomers and the studies whose objective is to relate biological activities with specific quantities of individual isomers. Moreover, the method described can also be used for the analysis of CLA isomers in dairy products and in the routine analysis of commercial mixtures obtained by synthesis.

Acknowledgements

We express our gratitude to the dairy products company GAZA S.L. (Zamora, Spain) for the samples supplied. This work was financed by the Junta de Castilla y León, reference project: SA099A07. José Luis Rodríguez-Castañedas held a fellowship from the Programa de Formación Universidad de Salamanca-Banco Santander Central Hispano. Concepción García-Moreno wishes to express her gratitude to Dra. Manuela Juárez, to Dr. Javier Fontecha and their research teams (Instituto del Frío-CSIC. Madrid, Spain) for allowing her to learn the analysis of CLA with them.

References

Adlof, R., 2003. Application of silver-ion chromatography to the separation of conjugated linoleic acid isomers. In: Sébédio, J.L., Christie, W.W., Adlof, R. (Eds.), Advances in Conjugated Linoleic Acid Research, vol. 2. American Oil Chemists' Society, Champaign, pp. 37–55.
Aldai, N., Osoro, K., Barrón, L.J.R., Nájera, A.I., 2006. Gas–liquid chromatographic method for analysing complex mixtures of fatty acids including conjugated linoleic acids (cis9trans11 and trans10cis12 isomers) and long-chain (n-3 or n-6) polyunsaturated fatty acids. Application to the intramuscular fat of beef meat. J. Chromatogr. A. 1110, 133–139.
AOAC, 2002. Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals. Association of Analytical Communities, p. 38 http://www.aoac.org/dietsupp6/Dieatary-Supplement-web-site/slv_guidelines.pdf.
Benjamin, S., Spener, F., 2009. Conjugated linoleic acids as functional food: an insight into their health benefits. Nutr. Metab. 6, 1–13.
Beppu, F., Hosokawa, M., Tanaka, L., Kohno, H., Tanaka, T., Miyashita, K., 2006. Potent inhibitory effect of trans9, trans11 isomer of conjugated linoleic acid on the growth of human colon cancer cells. J. Nutr. Biochem. 17, 830–836.
Christie, W.W., Dobson, G., Adlof, R.O., 2007. A practical guide to the isolation, analysis and identification of conjugated linoleic acid. Lipids 42, 1073–1084.
Collomb, M., Schmid, A., Sieber, R., Wechsler, D., Ryhänen, E.-L., 2006. Conjugated linoleic acids in milk fat: variation and physiological effects. Int. Dairy J. 16, 1347–1361.
Contarini, G., Pelizzola, V., Povolo, M., 2009. Content of conjugated linoleic acid in neutral and polar lipid fractions of milk of different ruminant species. Int. Dairy J. 19, 342–344.
Cruz-Hernández, C., Deng, Z., Zhou, J., Hill, A.R., Yurawecz, M.P., Delmonte, P., Mossoba, M.M., Dugan, M.E.R., Kramer, J.K.G., 2004. Methods for analysis of conjugated linoleic acids and trans-18:1 isomers in diary fats by using a combination of gas chromatography, silver-ion thin-layer chromatography/gas chromatography, and silver-ion liquid chromatography. J. AOAC Int. 87, 545–562.
de la Fuente, M.A., Luna, P., Juárez, M., 2006. Chromatographic techniques to determine conjugated linoleic acid isomers. Trends Anal. Chem. 25, 917–926.
Delmonte, P., Yurawecz, M.P., Mossoba, M.M., 2004. Improved identification of conjugated linoleic acid isomers using silver-ion HPLC separations. J. AOAC Int. 87, 563–568.

Delmonte, P., Kataoka, A., Corl, B.A., Bauman, D.E., Yurawecz, M.P., 2005. Relative retention order of all isomers of cis/trans conjugated linoleic acid FAME from the 6,8- to 13,15-positions using silver ion HPLC with two elution systems. Lipids 40, 509–514.

Dolan, J.W., 2009. Calibration curves. 2. What are the limits? LC GC Eur. 22, 244–247.

Eulitz, K., Yurawecz, M.P., Sehat, N., Fritsche, J., Roach, J.A.G., Mossoba, M.M., Kramer, J.K.G., Adlof, R.O., Ku, Y., 1999. Preparation, separation, and confirmation of the eight geometrical cis/trans conjugated linoleic acid isomers 8,10- through 11,13-18:2. Lipids 34, 873–877.

Fritsche, J., Fritsche, S., Solomon, M.B., Mossoba, M.M., Yurawecz, M.P., Morehouse, K., Ku, Y., 2000. Quantitative determination of conjugated linoleic acid isomers in beef fat. Eur. J. Lipid Sci. Technol. 102, 667–672.

Gómez-Cortés, P., Hervás, G., Mantecón, A.R., Juárez, M., de la Fuente, M.A., Frutos, P., 2008. Milk production, CLA content and in vitro ruminal fermentation in response to high levels of soybean oil in dairy ewe diet. J. Dairy Sci. 91, 1560–1569.

ISO, 2001. Milk and milk products-extraction methods for lipids and liposoluble compounds. International Standard ISO 14156-IDF 172.

ISO, 2002. Milk fat-Preparation of fatty acid methyl esters. International Standard ISO 15884-IDF 182.

Kramer, J.K.G., Hernández, M., Cruz-Hernández, C., Kraft, J., Dunga, M.E.R., 2008. Combining results of two GC separations partly achieves determination of all cis and trans 16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstrated using Ag-ion SPE fractionation. Lipids 43, 259–273.

Lee, M.R.F., Tweed, J.K.S., 2008. Isomerisation of cis-9 trans-11 conjugated linoleic acid (CLA) to trans-9 trans-11 CLA during acidic methylation can be avoided by a rapid base catalysed methylation of milk fat. J. Dairy Res. 75, 354–356.

Luna, P., Fontecha, J., Juárez, M., de la Fuente, M.A., 2005a. Changes in the milk and cheese fat composition of ewes fed commercial supplements containing linseed with special reference to the CLA content and isomer composition. Lipids 40, 445–454.

Luna, P., Fontecha, J., Juárez, M., de la Fuente, M.A., 2005b. Conjugated linoleic acid in ewe milk fat. J. Dairy Res. 72, 415–424.

Martins, S.V., Lopes, P.A., Alfaia, C.M., Ribeiro, V.S., Guerreiro, T.V., Fontes, C.M.G.A., Castro, M.F., Soveral, G., Prates, J.A.M., 2007. Contents of conjugated linoleic acid isomers in ruminant-derived foods and estimation of their contribution to daily intake in Portugal. Br. J. Nutr. 98, 1206–1213.

Melle, M., Buccioni, A., Petacchi, F., Serra, A., Banni, S., Antongiovanni, M., Secchiari, P., 2006. Effect of forage/concentrate ratio and soybean oil supplementation on milk yield, and composition from Sarda ewes. Anim. Res. 55, 273–285.

Moltó-Puigmartí, C., Castellote, A.I., López-Sabater, M.C., 2007. Conjugated linoleic acid determination in human milk by fast-gas chromatography. Anal. Chim. Acta 602, 122–130.

Nuernberg, K., Dannenberger, D., Ender, K., Nuernberg, G., 2007. Comparison of different methylation methods for the analysis of conjugated linoleic acid isomers by silver ion HPLC in beef lipids. J. Agric. Food Chem. 55, 598–602.

Park, Y., 2009. Conjugated linoleic acid (CLA): good or bad trans fat? J Food Compos. Anal. 22S, S4–S12.

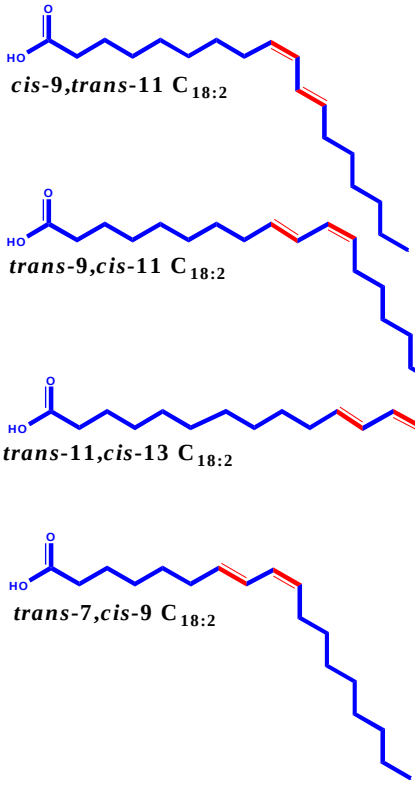
Park, Y.W., Juárez, M., Ramos, M., Haenlein, G.F.W., 2007. Physico-chemical characteristics of goat and sheep milk. Small Rumin. Res. 68, 88–113.

Roach, J.A.G., Mossoba, M.M., Yurawecz, M.P., Kramer, J.K.G., 2002. Chromatography separation and identification of conjugated linoleic acid isomers. Anal. Chim. Acta 465, 207–226.

Sanz Sampelayo, M.R., Chilliard, Y., Schmidely, Ph., Boza, J., 2007. Influence of type of diet on the fat constituents of goat and sheep milk. Small Rumin. Res. 68, 42–63.

Tanmahasamut, P., Liu, J., Hendry, L.B., Sidell, N., 2004. Conjugated linoleic acid blocks estrogen signaling in human breast cancer cells. J. Nutr. 134, 674–680.

Estructura de los isómeros CLA

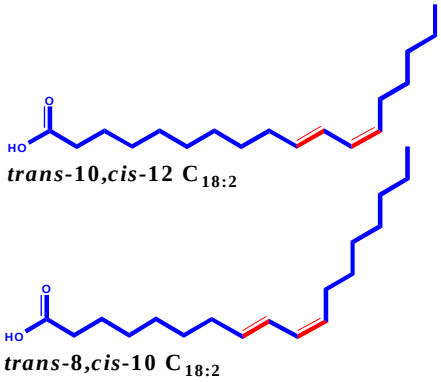


cis-9,*trans*-11 $C_{18:2}$

trans-9,*cis*-11 $C_{18:2}$

trans-11,*cis*-13 $C_{18:2}$

trans-7,*cis*-9 $C_{18:2}$

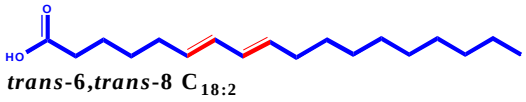


trans-10,*cis*-12 $C_{18:2}$

trans-8,*cis*-10 $C_{18:2}$

trans-12,*cis*-14 $C_{18:2}$

cis-12,*trans*-14 $C_{18:2}$

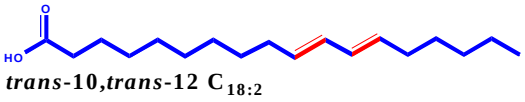


trans-6,*trans*-8 $C_{18:2}$

trans-7,*trans*-9 $C_{18:2}$

trans-8,*trans*-10 $C_{18:2}$

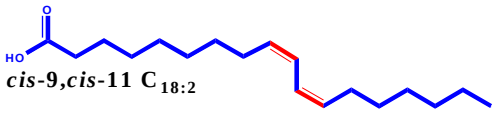
trans-9,*trans*-11 $C_{18:2}$



trans-10,*trans*-12 $C_{18:2}$

trans-11,*trans*-13 $C_{18:2}$

trans-12,*trans*-14 $C_{18:2}$



cis-9,*cis*-11 $C_{18:2}$