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Original article

Quantitative characterization of unsaturated and trans fatty acids in ewe's milk fat

Hanane GOUDJIL[a], Javier FONTECHA[a], Pilar LUNA[a], Miguel Angel de la FUENTE[a]*, Leocadio ALONSO[b], Manuela JUÁREZ[a]

[a] Instituto del Frío (CSIC), José Antonio Novais 10, Ciudad Universitaria s/n, 28040-Madrid, Spain
[b] Instituto de Productos Lácteos de Asturias (CSIC), 33300-Villaviciosa, Asturias, Spain

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Abstract – Ewe's milk fat obtained from the milk of five different herds were studied to characterize main and minor fatty acids using a combination of gas chromatography and mass spectrometry. In order to assess monounsatured *trans* fatty acids content, fatty acid methyl esters (FAME) were fractionated by silver ion thin-layer chromatography prior to gas chromatography analysis. *Trans* fatty acids (TFA), including conjugated linoleic acid, represented 4.9% of the total FAME; most of them belonged to C18:1 *trans* isomers (2.9%) with a small range of variation (2.5–3.2%). The distribution profile of *trans*-C18:1 molecules indicated that more than half were *trans*-11, whereas the proportion of potentially unhealthy compounds (*trans*-10 and *trans*-9 C18:1) was less than 10%. Mean *trans*-C16:1 content was 0.25% of total FAME. The sum of the different *trans*-C18:2 isomers (excluding conjugated linoleic acid) was 0.88% and C18:3 *trans* isomers represented 0.16%.

Ewe's milk / fatty acid / *trans* fatty acid

Résumé – Caractérisation quantitative des acides gras insaturés et *trans* de la matière grasse du lait de brebis. Les acides gras mineurs et majeurs du lait de brebis provenant de cinq troupeaux différents ont été caractérisés par chromatographie gazeuse associée à la spectrométrie de masse. En vue de connaître le contenu en acides gras monoinsaturés *trans*, les esters méthyliques des acides gras ont été fractionnés en recourant à la chromatographie sur couche mince à l'ion argent suivie d'une analyse par chromatographie gazeuse. Les acides gras *trans*, incluant l'acide linoléique conjugué, représentaient 4,9 % du total des esters méthyliques des acides gras. La plupart d'entre eux appartenaient aux isomères *trans* du C18:1 (2,9 %) avec une variation faible entre les échantillons (2,5–3,2 %). Le profil de distribution des molécules C18:1 *trans* montraient que plus de la moitié étaient des *trans*-11, tandis que les proportions de *trans*-10 et *trans*-9, potentiellement nuisibles à la santé, étaient inférieures à 10 %. La teneur moyenne en C16 :1 *trans* était de l'ordre de 0,25 % du total des esters méthyliques des acides gras. Le total des différents isomères C18:2 *trans* (à l'exception de l'acide linoléique conjugué) et C18:3 représentait respectivement 0,88 % et 0,16 % du total.

Lait de brebis / matière grasse / acide gras *trans*

* Corresponding author: ifraf91@if.csic.es

1. INTRODUCTION

Although the world production of ewe's milk (8 million tonnes, representing 1.3% of the total world milk production) is relatively low compared with the cow's milk market, ewe's milk products are widely consumed in the Mediterranean and Middle East where climatic and geographic conditions are not the most ideal for cattle rearing. Ewe's milk is primarily for cheese production and, in some countries, it is used in the manufacture of yogurts or serum cheeses. The fat/protein ratio is higher in ewe's than cow's milk and therefore cheese yield from ewe's milk is also higher (approximately 15%, compared with 10% for cow's milk). Moreover, ewe's milk lipids are quantitatively more important than in the other ruminant species. This high content in lipids means that ewe's milk is a suitable substrate for the manufacture of cheese due to the characteristic flavor that it lends to these products.

In the lipid fraction of ewe's milk, the most abundant fatty acids are palmitic, oleic, miristic and estearic [12, 15, 19, 24, 26]. Short- and medium-chain fatty acid content (caproic, caprilic and capric) is higher in ewe's than cow's milk. Of the C4-C8 fatty acids present, greater than 95% were specifically attached to the glycerol molecule in the position 3 [12]. These acids are important since they are associated with the organoleptic characteristics of ovine cheese and they can also be used as a criterion for detecting the fraudulent addition of milk from other ruminant species [9, 25]. As regards the minor fatty acids, although some studies mention differences in the C11, C12:1, C14, C14:1 and C15 content between cow's and ewe's milk [25], there is currently not very much data available on these acids in the literature. Information about unsaturated fatty acids, mainly *trans* isomers, is not abundant either [15, 24, 26]. The nutritional importance of *trans* fatty acids (TFA) has been widely stressed on in recent years [6, 17, 18, 27]. Physiological functions of TFA, specially its possible role in atherosclerosis, the level of blood cholesterol, and coronary heart disease is of concern, but still subject to controversy. Conjugated linoleic acid (CLA) and possibly also *trans* vaccenic acid (*trans*-11 C18:1) (TVA), seem to have beneficial effects on different body functions as well as a protective action on some pathologies such as cancer, obesity, cardiovascular disease and diabetes. Although some works have provided data on monounsaturated *trans* fatty acid (MUTFA) isomers in ewe's milk [4, 24, 30] there is hardly any information on polyunsaturated *trans* fatty acid (PUTFA) isomers.

The purpose of this research was to determine the range of variation in the composition of major and minor fatty acids in ewe's milk. We will focus in particular on MUTFA and PUTFA isomers. To achieve this aim, milk fats from five different ovine herds were investigated by capillary column gas chromatography (GC). Analyses of MUTFA were based on the combination of silver ion thin-layer chromatography (Ag^+-TLC) with GC and mass spectrometry (MS) to obtain the contents of MUTFA positional isomers.

2. MATERIALS AND METHODS

2.1. Samples

Milk samples were collected at monthly intervals during the milking period from five ovine herds. They were taken from the storage tanks containing milk from the whole herd. Each herd consisted of a different breed: CH (Churra breed with 130 head), W (Awasi breed with 130 head), AxC (cross between Assaf and Castellana breeds with 170 head), M (Manchega breed with 2200 head) and A (Assaf breed with 480 head). The herds were located in different places in Spain to ensure a wide variety of milk composition. A total of 45 samples was collected over the year. Fats were extracted following a procedure described by ISO [7], exposed to a stream of N_2 and frozen at –20 °C in amber vials until analysis. The mean fat content of the different herds

ranged from 7.5–9.8%. All the ovine milk fat samples extracted from each herd were combined in equal volumes to obtain five mixture samples.

2.2. Standards

An anhydrous milk fat with a certified fatty acid composition (reference material BCR-164, obtained from the Commission of the European Communities, Brussels, Belgium) was used to determine the fatty acid methyl esters' (FAME) response factors. Tentative identification of *trans*-C18:2 and *trans*-C18:3 isomers was done by comparing the equivalent chain-length values of FAME obtained with those of reference oils: partially isomerized linseed oil FAME, refined rapeseed oil (BCR 686), partially hydrogenated sunflower seed oil (BCR-688) and a blend of palm oil and partially hydrogenated sunflower seed oil (BCR-687), which had served as test material in the research project SMT4-CT97-2144 of the European Union.

Besides this test material, FAME pure isomers (C18:1: *cis*-9; *cis*-13; *trans*-9; *trans*-11; *trans*-13) and polyunsaturated (PUFA) mixtures (C18:2 mixture: *trans*-9 *trans*-12 + *cis*-9 *trans*-12 + *trans*-9 *cis*-12 + *cis*-9 *cis*-12; C18:3 mixture: *trans*-9 *trans*-12 *trans*-15 + *trans*-9 *trans*-12 *cis*-15 + *trans*-9 cis-12 *trans*15 + *cis*-9 *trans*-12 *trans*-15 + *cis*-9 *cis*-12 *trans*-15 + *cis*-9 *trans*-12 *cis*-15 + *trans*-9 *cis*-12 *cis*-15 + *cis*-9 *cis*-12 *cis*-15) supplied by Supelco (Bellefonte, USA) were also used as standard.

2.3. Preparation of fatty acid derivatives

FAME were prepared by base-catalyzed methanolysis of the milk fat (2N KOH in methanol) according to ISO [8]. In order to confirm the correctness of *trans*-C18:1 peak assignment positional isomers, FAME were converted to 4,4 dimethyloxazoline (DMOX) derivatives according to Fay and Richli [5] and analyzed by GC-MS.

2.4. Silver ion thin-layer chromatography (Ag^+-TLC)

FAME were fractionated according to the number and geometry of double bonds by Ag^+-TLC following the Precht and Molkentin procedure [20] modified by Alonso et al. [1]. After the chromatographic run, the bands corresponding to the saturated and *trans* monoenoic FAME were scraped, dissolved in heptane and used for GC analysis. To calculate the total content of *trans*-C18:1 isomers, the ratio C18:0 to total *trans*-C18:1 was determined in the saturated plus *trans* monoenoic Ag^+-TLC fraction and was related to the C18:0 content of total FAME.

2.5. Gas chromatography analysis

Analysis of FAME was carried out on an Autosystem GC model (Perkin-Elmer, Co., Beaconsfield, United Kingdom) equipped with a FID on a CP Sil 88 column (100 m X 0.25 mm i.d.) (Chrompack, Middelburg, The Netherlands). The column was held at 100 °C for 6 min after injection, temperature-programed at 5 °C·min^{-1} to 210 °C, and finally held there for 30 min. Helium was the carrier gas with a column inlet pressure set at 31 psi. The detector temperature was 250 °C. For identification purposes these analyses were also performed on a gas chromatograph (Agilent, Palo Alto, USA) model HP 6890 coupled with a 5973 mass spectrometer detector (Agilent) using the same column described above. FAME were identified by standards and by comparing their mass-spectral data to the mass-spectral database in the library Wiley 7.0 (HP Mass Spectral Libraries, Palo Alto, USA).

3. RESULTS AND DISCUSSION

3.1. Fatty acids global profile

The mean values, standard deviation, and minimum and maximum percentages of major and minor fatty acids (>0.01% of total FAME) are shown in Table I. Mean content in short-chain fatty acids

Table I. Mean values, standard deviation and minimum and maximum contents of ewe's milk fatty acids (% in total FAME).

Fatty acid	Mean	Standard Deviation	Minimum	Maximum
C4	3.51	0.31	3.07	3.93
C5	0.02	0.01	0.02	0.03
C6	2.90	0.31	2.68	3.44
C7	0.04	0.02	0.01	0.05
C8	2.64	0.42	2.10	3.27
C9	0.07	0.02	0.03	0.08
C10	7.82	1.49	5.54	9.73
C10:1	0.26	0.03	0.23	0.31
C11	0.09	0.03	0.04	0.14
C12	4.38	0.54	3.48	4.92
C12:1	0.04	0.01	0.03	0.05
C13	0.17	0.03	0.13	0.22
iso-C14	0.11	0.02	0.08	0.14
C14	10.43	0.34	9.85	10.66
iso-C15	0.34	0.08	0.26	0.43
anteiso-C15	0.47	0.11	0.33	0.60
C14:1	0.28	0.13	0.19	0.50
C15	0.99	0.08	0.89	1.11
iso-C16	0.21	0.04	0.17	0.26
C16	25.93	2.18	22.47	28.17
iso-C17	0.53	0.07	0.44	0.59
anteiso-C17	0.30	0.04	0.26	0.36
C16:1	1.03	0.20	0.74	1.27
C17	0.63	0.06	0.58	0.70
C17:1	0.20	0.02	0.17	0.22
C18	9.57	0.92	8.51	11.04
C18:1 (total)	21.10	1.98	17.77	23.02
C18:2 (total)	3.21	0.12	2.89	3.57
C20	0.45	0.07	0.36	0.52
C18:3 (total)	0.80	0.21	0.52	1.04
C20:1	0.06	0.01	0.05	0.08
C18:2 conjugated (total)	0.74	0.21	0.56	0.97
C22	0.20	0.05	0.14	0.26
C23	0.16	0.04	0.11	0.22
C20:4	0.06	0.02	0.03	0.08
C24	0.03	0.02	0.00	0.06
No identified (total)	0.15	0.05	0.10	0.22

(C4-C7) was 6.5%, medium-chain (C8-C15) 28.1% and long-chain (C16-C24) 65.4%. Most of the saturated and monounsaturated fatty acids identified in the present research, mainly the major ones, have already been detected in cow's [10], goat's [1, 13] and ewe's milk [19, 24, 25, 30]. Nevertheless, the presence of some minor long-chain fatty acids (C20:1, C22, C23 and C24) has not been documented in ewe's milk.

The five most significant fatty acids in quantitative terms (C16, C18:1, C14, C18 and C10, respectively) accounted for around 75% of total FAME (Tab. I). Individually, the percentages of major fatty acids were within the range of variation of those reported by other authors [24, 30]. The concentration of branched-chain fatty acids was 1.96% and this percentage was made up of six different acids: *iso*-C14, *iso*- and *anteiso*-C15, *iso*- and *anteiso*-C17, and *iso*-C16. Unidentified peaks represented an average 0.15% of total FAME (Tab. I). Four of them were observed in the five herds studied: one eluting between C12 and C12:1 (0.03%), probably a C13 isomer; another between C15 and *iso*-C16, (0.06%) probably a C15 unsaturated fatty acid; another peak between C17 and C17:1 (0.03%), likely a C18 isomer and finally, the fourth between C22 and C23 (0.03%), for which MS did not provide any information.

In this work the mean value of the C12/C10 ratio (0.56), commonly used to test milk fat authenticity, was very similar to that reported by Iverson and Sheppard [9] and Wolff [30] (0.58) in ovine milk and cheese samples, respectively, and it was also the same as that (0.57) more recently observed by Precht et al. [24]. These authors reported a very different value in cow's milk fat for this ratio (1.1–1.2). Another of the ratios used for assessing the authenticity of the milk is C15/C14:1; in the present work this ratio was 3.54. When the C15/C14:1 ratio for cow's milk is compared, the differences are considerable, because values reported for this ratio are much lower (0.8–1.6; Ramos and Juárez [25]). However, differences in the C15/C14:1 ratio cannot be established for ewe's and goat's milk fat because the data on this ratio for goat's milk fat are very different ((3.8) Alonso et al. [1]; (6.6) Wolff [30]).

3.2. MUTFA content and profile

MUTFA content represented an average 3.15% of the total FAME. Most of them belonged to *trans*-C18:1 isomers (2.9%) with a small range of variation between the different samples (2.5–3.2%) (Tab. II). Almost 14% of C18:1 geometric isomers were *trans*. The total percentage of *trans*-C18:1 isomers measured in this study was much higher than the percentage found in goat's milk [1, 13]. In general terms, ewe's milk has been highlighted as having the highest levels of *trans* C18:1 in all the ruminants [24, 30].

Table II shows the relative distribution of *trans*-positional C18:1 isomers in ewe's milk fat fractionated by Ag^+-TLC prior to GC analysis. The pattern is qualitatively identical to that presented by *trans*-C18:1 isomers in cow's [10] and goat's milk [1, 13, 24]. TVA is the main constituent acid and varies between 45 and 57%. The second main *trans*-C18:1 isomer is the unresolved *trans*-13/*trans*-14 pair (20%). It has been discovered that it is only under low-temperature conditions and using long isothermal GC programs [24] that these isomers can be separated and that their concentrations are similar in cow's milk fat. Elaidic acid (*trans*-9 C18:1) is only present in considerably low amounts, as it represents on average only 2.6% (2.3–2.8%), whereas minor isomers (*trans*-5 and *trans*-4 C18:1) were not quantified.

Despite the fact that the *trans*-6 to *trans*-8 group (2.98%) remains unresolved, we would suggest that *trans*-8 C18:1 could be the major isomer in that peak. C18:1 DMOX derivatives possess mass spectra, easily recognizable for determining the double bond position. Figure 1 shows the DMOX derivative mass spectrum of the *trans*-6 to

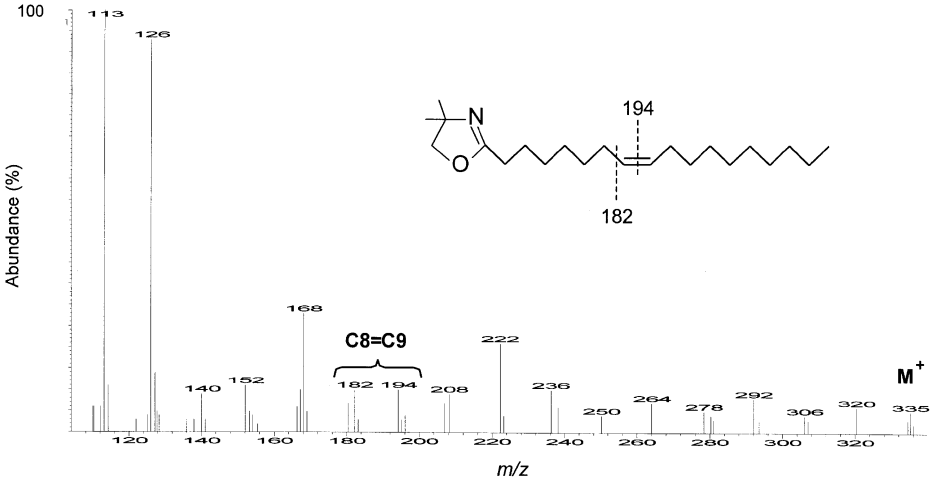

Figure 1. Electron impact mass spectrum of 6- to 8-*trans* C18:1 DMOX derivative peak showing a double bond between atomic mass unit *m/z* 182 and 194. M^+ corresponds to molecular ion.

Table II. Total content of *trans*-C18:1 and *trans*-C16:1 (wt % of total methyl esters) in ewe's milk fat and proportions of the different *trans*-C18:1 isomers (wt % of total *trans*-C18:1) isolated by silver thin-layer chromatography and analyzed by gas chromatography.

Item	*trans*-C18:1 Isomer	Mean	Standard deviation	Minimum	Maximum
Total *trans*-C16:1		0.25	0.05	0.17	0.31
Total *trans*-C18:1		2.90	0.28	2.45	3.21
	Trans-6 to *trans*-8	2.92	0.29	2.44	3.20
	trans-9	2.61	0.20	2.32	2.79
	trans-10	6.28	0.63	5.49	6.95
	trans-11	51.23	5.19	44.76	56.71
	trans-12	7.11	0.44	6.38	7.56
	Trans-13 + *trans*-14	20.01	0.83	19.29	21.43
	trans-15	3.63	0.28	3.32	3.97
	trans-16	6.21	0.50	5.56	6.74

trans-8 C18:1 peak. This spectrum forms the pattern predicted from the work of Zhang et al. [31] for *trans*-8 C18:1 and is nearly identical to that reported for this isomer by Mossoba et al. [14], with two prominent ions (*m/z* 168 and 222) and a gap of 12 atomic mass units between *m/z* =182–194 to locate the double bond. Spectra from *trans*-6 and *trans*-7 C18:1 DMOX exhibit different ion profiles [3, 14]. The pattern observed in Figure 1 was similar for the same peak in the five ovine herds studied. Other evidence in this direction comes from previous observations on cow's milk fat: Parodi [16] found five times as much *trans*-8 C18:1 as *trans*-6 or *trans*-7 C18:1 acids in butter fat using ozonolysis. Furthermore, Aro et al. [2] did not detect these isomers in butter but reported a small percentage of *trans*-8 C18:1.

The implications of *trans*-C18:1 isomer distribution could be important from a nutritional point of view. Wolff [30] noted that ewe's milk fat could be important as a source of dietary *trans*-C18:1 acid in countries where cattle rearing is not really feasible, particularly the Mediterranean region. It has been recommended that for people who wish to limit their *trans* intake, goat's cheese should thus be preferred to bovine or ovine dairy products. However, although a high intake of MUTFA has been associated with the risk of coronary heart disease and myocardial infarction, other aspects should also be highlighted. It has been suggested that TFA isomers from dairy products and those from partially hydrogenated vegetable oils may have different effects on the risk of coronary heart disease. *Trans*-9 C18:1 isomer, the component most widely studied in relation to the intake of TFA and the risk of coronary disease, represents a very small fraction (2.6%) of the total *trans*-C18:1 with not very much variability between the herds (Tab. II). In contrast with the majority of hydrogenated vegetable oils enriched in *trans*-10 and *trans*-9 C18:1 acids, consumption of food derived from ovine milk would represent a very low intake.

On the other hand, *trans*-11 C18:1 was the most abundant *trans* isomer in ewe's milk fat, comprising 51% (variability between 45 and 57%) of total *trans*-C18:1 isomers (Tab. II). The importance of TVA lies in its role as a precursor of rumenic acid (RA) (*cis*-9 *trans*-11 C18:2) synthesis in the mammary gland and other tissues. The literature increasingly describes the anti-carcinogenic and anti-atherosclerotic effects of RA, the biologically active form of CLA. Recent studies [10] have found that most of the RA in ruminant milk fat is synthesized endogenously from 11-*trans* C18:1 by desaturases. Furthermore, TVA has also been found as an RA precursor in human tissues [28]. If this evidence were confirmed, remarkable benefits could be obtained from ewe's milk fat product consumption.

As for the *trans*-C18:1 isomers, accurate and reliable *trans*-C16:1 determination can only be obtained by using Ag^+-TLC/GC combination [1]. Ag^+-TLC allows the removal of interfering branched C17:0 acids and *cis*-C16:1. The *trans*-C16:1 total content found in this work was 0.25% (Tab. II), similar to levels previously reported in ewe's cheese and milk and slightly higher than in cow's and goat's milk [1, 4, 30]. Quantitatively, the *trans*-C16:1 only represent 8% of the sum of the *trans*-C16:1 plus *trans*-C18:1 isomers, therefore they do not seem to be of great nutritional importance.

3.3. Octadecadienoic isomers

Mean linoleic acid content (*cis*-9 *cis*-12 C18:2) accounts for 73% of the total C18:2, whereas the *trans*-C18:2 isomer group, excluding CLA, represents slightly more than a quarter of this fraction and 0.88% of the total FAME. The data for *trans*-C18:2 reported for cow's milk are similar or slightly higher, although the ranges of variation are large (0.6–1.44%) because they vary according to diet [21–23].

Leaving aside linoleic acid, four peaks including five isomers were separated and quantified (Tab. III): *trans*-9 *trans*-12, *cis*-9 *trans*-13, *cis*-9 *trans*-12 and *trans*-9 *cis*-12 coeluting with *trans*-11 *cis*-15. These isomers were identified by comparing the relative retention times of the chromatographic peaks of the ewe's milk fat samples with the certified vegetable fats and with the milk fat chromatograms analyzed in similar chromatographic conditions in the literature [11, 23, 29].

In contrast to CLA, the other *trans*-C18:2 isomers are partly related to negative effects. The *trans*-9 *trans*-12 C18:2 isomer is a competitive inhibitor for the conversion of linoleic to arachidonic acid and a completely blocking C20:5 ω-3 formation. It has been suggested that the other *trans*-C18:2 could affect the essential fatty acid metabolism as well. There are no data in the literature on the *trans*-C18:2 isomer content in ewe's milk. Although different authors

Table III. Contents of octadecadienoic acids, linoleic acid (*cis*-9 *cis*-12 C18:2) and *trans*-18:2 isomers (excluding conjugated linoleic acid) in ewe's milk fat (wt % total methyl esters).

Item	Identification	Mean	Standard deviation	Minimum	Maximum
Total C18:2		3.21	0.12	2.89	3.57
Total *trans* C18:2		0.88	0.34	0.49	1.35
	cis-9 *cis*-12 C18:2	2.33	0.31	1.93	2.51
	trans-9 *trans*-12	0.03	0.01	0.01	0.04
	cis-9 *trans*-13	0.31	0.11	0.19	0.47
	cis-9 *trans*-12	0.25	0.06	0.18	0.34
	trans-9 *cis*-12 + *trans*-11 *cis*-15	0.29	0.19	0.11	0.50

Table IV. Total contents of octadecatrienoic acids, linolenic acid (*cis*-9 *cis*-12 *cis*-15 C18:3) and *trans*-C18:3 isomers (wt % total methyl esters).

Items	Identification	Mean	Standard deviation
Total C18:3		0.80	0.21
Total *trans*-C18:3		0.16	0.02
	cis-9 *cis*-12 *cis*-15 18:3	0.63	0.25
	trans-9 *trans*-12 *trans*-15	0.03	0.01
	trans-9 *cis*-12 *trans*-15 + *cis*-9 *trans*-12 *trans*-15 + *cis*-9 *cis*-12 *trans*-15 + *trans*-9 *trans*-12 *cis*-15	0.09	0.02
	cis-9 *trans*-12 *cis*-15	0.04	0.03

[21, 23] quantify some of these isomers in cow's milk, it is difficult to establish comparisons with the present work, since in the articles mentioned the *cis*-9 *trans*-13 and *trans*-9 *trans*-12 coelute in just one peak. Conversely, in our study, the major isomer *trans*-11 *cis*-15 could not be resolved from the *trans*-9 *cis*-12 isomer. The data show (Tab. III) that the *trans*-9 *trans*-12 isomer assessed as negative from a physiological point of view is negligible in ewe's milk fat (0.03% of total FAME).

The total CLA content in the samples studied is 0.74% (Tab. I), most of which was RA (over 80%). The other corresponds to positional and geometric isomers which are found in low concentrations. The CLA levels measured in this work were slightly lower than those in other studies on ovine cheese [30]. We should, however, bear in mind that the CLA levels may increase during the manufacturing process. The great variability observed between herds (0.56–0.97%) could be related to the large range of variation in the *trans*-11 C18:1 content (Tab. II) which, as mentioned in the previous section, is the RA precursor.

3.4. Octadecatrienoic isomers

The linolenic acid content (*cis*-9 *cis*-12 *cis*-15 C18:3) accounts for 79% of the total C18:3 fraction (Tab. IV). This value (0.63% of total FAME) is slightly lower than that given by Wolff [30] for ovine cheese (0.93%, with a range of variation of 0.60–1.18%), although this author does not indicate whether this percentage includes isomers

other than linolenic acid. The data on linolenic acid content in cow's milk found in the literature [10], as in the case of linoleic acid, are slightly lower (0.25–0.60%).

The *trans*-C18:3 isomers hardly represent 0.16% of the total FAME and they correspond to 6 species grouped in 3 peaks and only two isomers quantified individually (*trans*-9 *trans*-12 *trans*-15 and *cis*-9 *trans*-12 *cis*-15). As in the case of the *trans*-C18:2 isomers, there are no data in the literature on ewe's milk for these acids to establish comparisons.

The data of this study, generated from the milk of five ewe herds of different regions, did not show important differences in the content of fatty acids. Concerning the total TFA content, the levels found (4.9% of total FAME, including CLA) did not differ substantially from those reported in previous studies for other ruminant milk fat. This research also quantified octadecadienoic and octadecatrienoic *trans* acids, isomers not previously reported in ewe's milk. It is important to know the total TFA content, but it is even more important to know the *trans* isomer composition of TFA composition. Elaidic and other TFA labeled as unhealthy were found in low concentrations. On the other hand, these results confirm that ewe's milk fat exhibits the highest CLA content and TVA in ruminant milk.

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