TAG Composition of Ewe's Milk Fat. Detection of Foreign Fats

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ABSTRACT: The TAG composition of 45 samples of ewe's milk, collected throughout the year from five Spanish breeds, was analyzed according to their carbon number by using short capillary column GC. The TAG content had a bimodal distribution with maxima at C_{38} (12.8%) and C_{52} (8.4%). The TAG composition did not vary significantly with respect to the time of year of sampling but was affected by the breed. Multiple regression equations based on TAG content are proposed to detect foreign fats in ewe's milk fat. Analysis of known mixtures of lard, palm oil, and cow's milk fat with ewe's milk fat have experimentally confirmed the accuracy of the equations.

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KEY WORDS: Ewe's milk fat, foreign fat, short capillary column GC, triacylglycerols.

The lipid fraction of milk and the products of its degradation, mainly volatile FFA, play an important role in the flavor of cheeses. Different studies have indicated that lipolysis depends on the composition of the TAG of ewe's milk fat, i.e., chain length and degree of unsaturation (1,2). Thus, Batelli and Peregrino (3) observed that lipolysis had a pronounced effect on TAG composition within a low M.W. range during prolonged ripening.

The determination of TAG classes according to their carbon number (CN) has been reported as an effective criterion for assessing milk origin (4–6). TAG determination also has been proposed as a means to detect mixtures of foreign fats in milk fat and by the European Community (EC) as an official method for assessing cow milk fat purity (7).

TAG composition has been studied in cow's and goat's milk but less so in ewe's milk, where most of the published data refer to a limited number of samples (2,8–11). The exception is a study carried out by Muir *et al.* (12) where samples were taken from ewes, mainly of the Frisone breed, for the duration of 1 yr.

FA composition of milk fats varies considerably in response to factors such as feed. It has also been reported that the composition of TAG changes with lactation period (13) or season (12).

The objective of this work was, first, to study the range of variation in the composition of TAG, using GC, in ewe's milk fat from five breeds located in different regions and collected throughout the year and, second, to propose multiple regression equations based on the TAG content to detect foreign fat in ewe's milk fat.

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MATERIAL AND METHODS

Samples. Forty-five 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 (Awassi breed with 2,500 head), M (Manchega breed with 2200 head), A (Assaf breed with 480 head), A × C (cross between Assaf and Castellana breeds with 170 head). The herds were located in different parts of Spain. For statistical analysis, the samples were distributed in four groups or seasons.

Samples from the herds M and W were taken through the year between January and December (12 samples/herd). The number of samples per herd for the other breeds was adjusted to the duration of lactation (7 mon on average), because births were clustered.

In addition, nine mixtures prepared with ewe's milk fat and different amounts of cow's milk fat (10, 20, and 30%), lard, and palm oil (5, 10, and 15%) were analyzed to test the accuracy of proposed equations.

TAG analysis. Fat was extracted following a procedure described by Alonso *et al*. (14) and frozen at −20°C in amber vials until analysis.

GC analysis. For analysis of TAG, 0.5 µL of a solution of ewe's milk fat in hexane (5 mg/mL) was injected into the gas chromatograph. Duplicate analyses were performed for each sample. TAG analyses were performed on a gas chromatograph, equipped with an automatic injector split/splitless (split ratio 1:20) and programmed temperature. A capillary column 2.5 m long [Rtx-65 TG (35% dimethyl, 65% diphenyl polysiloxane) d_f = 0.1 µm], supplied by Restek (Bellefonte, PA), was used. Experimental chromatography conditions were as in a previous work (6).

Statistical analysis. The results were analyzed using SPSS 11.0 (Chicago, IL) on Windows XP. Sources of variation in ANOVA were ewe origin and seasonal period. Multiple linear regression analyses were made to determine the most accurate relationships between TAG as a means of discriminating between ewe's milk fat and other fats.

RESULTS AND DISCUSSION

In the chromatograms obtained from the samples of ewe's milk, 16 peaks were distinguished, corresponding to TAG with CN from 24 (including cholesterol) to 54. Figure 1 shows the chromatographic profile of the TAG from ewe's milk fat. Table 1 presents the numerical data for the average

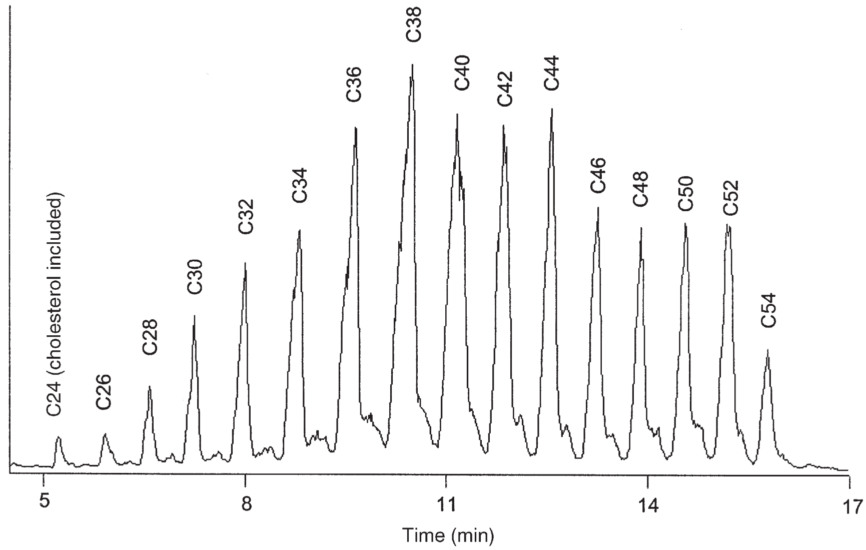

FIG. 1. Chromatographic profile of ewe's milk fat TAG.

composition and the range of variation in the classes of TAG in ewe's milk obtained in this study. The TAG content found in ewe's milk has a bimodal distribution with maxima at C_{38} (12.8%) and C_{52} (8.4%). The values obtained by other authors (2,9,10) for ewe's milk show a similar profile, with two maxima in the ranges C_{36}–C_{38} and C_{50}–C_{52}.

The chromatographic profile of TAG shows similarities to that reported for cow's milk. Breckenridge and Kuksis (8) established that the milk fat of sheep and goats contain proportionally more of the C_{54} TAG component than cow's milk fat; however, this observation was not supported by either the present work or a study of goat's milk by Fontecha *et al.* (6) when those data were compared with the data for cow's milk given by Precht (15). Also, Barrón *et al.* (16), using HPLC–GC, found substantial quantitative differences among the milks from the three species. The major relative differences found here, as compared to cow's milk fat, were found for the TAG C_{42}, C_{50}, and C_{52}.

TABLE 1
TAG Composition of Ewe's Milk Fat Samples (wt%)

	Range of variation			
CN[a]	Min	Max	Mean value	CV (%)
26	0.30	1.09	0.72	30.98
28	0.67	2.52	1.60	28.38
30	1.04	3.9	2.52	27.27
32	1.78	5.5	3.63	23.08
34	4.02	8.22	6.03	14.70
36	7.33	11.69	9.64	10.14
38	10.65	14.12	12.82	5.83
40	8.65	13.23	11.98	8.41
42	6.54	10.45	9.02	11.40
44	6.24	9.60	8.08	10.29
46	5.72	8.98	6.77	9.14
48	5.26	11.06	6.67	19.37
50	4.61	12.44	7.63	23.54
52	3.85	14.97	8.43	27.68
54	1.58	8.14	4.48	39.04

[a]CN, carbon number.

To estimate how representative the samples were, the CV were compared with those obtained by Fontecha *et al.* (6) using 35 samples of goat's milk fat, all from the same breed taken at different times during the year. The values recorded in our work were higher than those given by Fontecha *et al.* (6) and slightly higher than those reported by Precht (17) for 775 cow milk samples, as these latter covered a greater variety in the origin of samples (five breeds for a period of 12 mon). The CV of the TAG with 52 and 54 carbon atoms were high in both species (17.7 and 30.3% in goat and 27.7 and 39.1% in ewe). This could be explained by the fact that some samples were taken from ewes that were using their fat reserves at the beginning of the lactation period. In ewes, as in goats, the TAG from C_{38} to C_{46} exhibited the lowest CV.

The mean distribution of the TAG by chain length (short-, medium-, and long-chain: C_{26}–C_{34}, C_{36}–C_{44}, and C_{46}–C_{54}, respectively) obtained in this study (14.9, 51.8, and 33.3%, respectively) was similar to that found by Fontecha *et al.* (6) for goat's milk (14.6, 58.2, and 26.7%, respectively). The data reported by Precht (15) for the same groups of TAG in cow's milk (10.5, 46.4, and 42.8%, respectively) were different due to corresponding differences in the FA composition, with smaller concentrations of short- and medium-chain FA. Ruiz-Sala *et al.* (18), in comparing milk fat from three species, observed lower concentrations of short-chain TAG in cow's milk (10.8%) than in ewe's and goat's milk (18.2 and 15.2%, respectively). This distribution favors an appropiate m.p. because the lower degree of unsaturation reported in these species will offset the effect of a longer chain on the m.p. of TAG.

Effect on milk TAG composition of the period of sample collection. The composition of TAG in ewe's milk did not significantly change in relation to the time of year, even when the samples were grouped by season.

Variation in TAG composition according to season was reported by Muir *et al.* (12), who observed differences in the proportions of TAG with carbon atoms 38, 40, and 52. Other authors also found variations in milk TAG content in samples coming from the same herd and fed on the same diet (19). In cow's milk, Zegarska and Jaworski (13) found that the content of short- and medium-chain TAG decreased as lactation progressed, and the reverse was true for long-chain TAG. The same authors also reported that milk fat in animals that grazed contained more long-chain TAG than animals fed commercial feed.

In this study, the absence of variation may perhaps be masked by the differences in milk yield, diet, and stage of lactation among the breeds at different periods of the year.

Effect on milk TAG composition of the breed. Some of the quantitatively more important TAG (C_{40}, C_{42}, C_{44}, C_{48}, and C_{50}), which together constitute about 45% of the total TAG, did vary according to breed ($P < 0.05$). The largest quantitative differences were found for TAG C_{50} (up to 30%). The Churra breed presented more differences than some of the other breeds studied (Table 2). Short-chain TAG were not affected by the breed factor. There are no data with which to compare our results.

Detection of foreign fat in ewe's milk fat. Different methods have been proposed for detecting foreign fats in milk fat samples using TAG content as a variable (17,20,21). The official method recently adopted by the EC (7) for the detection of foreign fats in milk fat is based on equations proposed by Precht (22) using as variables the concentrations of certain TAG from milk obtained by GC in packed short columns. When the general mean values and the individual mean values for the different breeds were applied to the overall formula suggested by Precht (22), the results were in the range 98.0 to 101.6, that is, within the established range for cow's milk. Indeed, the equation proposed by Precht could not discriminate between different ewe's and cow's milk samples.

Given the utility of a specific equation that would make it possible to detect foreign fat, including cow's milk fat, in ewe's milk, the TAG data obtained in this study were used to calculate a series of multiple linear regressions of the type:

$$\sum a_i C_i = M + e$$

where i is the number of carbon atoms, C_i is the percentage of TAG with carbon number i, a_i is a coefficient to be estimated, M is a constant in which 100 represents pure ewe's milk, and e is the random error.

The variables used in the first regression (Eq. 1) were those that differed most from cow's milk, i.e., TAG of C_{42}, C_{50}, and C_{52}. Another regression was performed including more variables but limiting the number of TAG to 10 and selecting also those TAG with the greatest differences between cow's and ewe's milk, i.e., C_{42}, C_{50}, and C_{52} (Eq. 2). Finally, a third regression was tried using the selected variables in Equation 2 and taking into account the data concerning co-linearity. The selected variables were C_{42}, C_{44}, C_{48}, C_{50}, and C_{52} (Eq. 3). Table 3 shows the coefficients (a_i) and the statistical data for the three selected equations.

If the values reported by Precht (17) for vegetable fats are applied to Equations 1 to 3 as being characteristic of pure ewe's milk fat, the absolute range of variation around 100 is either not attained or is exceeded. The same would apply if the TAG composition for cow's and goat's milk reported by Precht (17) and Fontecha *et al.* (6) were fitted to Equations 1 to 3. With Equation 1, considering the confidence interval for the mean value at 99%, it is possible using a computer simulation to detect additions of more than 7% of vegetable or lard fats, more than 8% of goat's milk fat, or more than 24% of cow's milk fat. With Equations 2 and 3, it is possible to detect the presence of smaller quantities of some vegetable fats, but only higher levels of cow's and goat's milk fat. In consequence, Equation 1, which is the simplest, would be more useful for the detection of adulteration with fats of different origin.

TABLE 2
Effect of Herd on TAG Composition of Ewe's Milk[a] (wt%)

	Herd[b]						
CN	A	W	M	A × C	CH	MSE[c]	Significance[c]
26	0.84	0.73	0.75	0.75	0.57	0.024	
28	1.83	1.57	1.72	1.65	1.33	0.049	
30	2.88	2.45	2.73	2.63	2.05	0.073	
32	4.07	3.50	3.88	3.77	3.10	0.090	
34	6.46	5.70	6.15	6.13	5.99	0.099	
36	10.27	9.20	9.48	9.69	10.05	0.105	
38	13.52	12.55	12.99	12.71	12.66	0.079	
40	12.48[a]	12.15[a]	12.38[a]	11.80[a,b]	11.08[b]	0.102	*
42	9.69[a]	9.03[a]	9.52[a]	8.96[a]	8.00[b]	0.099	**
44	8.51[a,b]	7.98[b]	8.64[a]	7.97[b,c]	7.33[c]	0.080	**
46	6.57	6.81	6.89	6.61	6.81	0.071	
48	6.11[a]	6.56[a]	6.22[a]	6.60[a]	7.78[b]	0.134	*
50	6.64[a]	7.70[a,b]	6.85[a]	7.61[a,b]	9.13[b]	0.184	*
52	7.02	8.76	7.88	8.54	9.39	0.258	
54	3.12	5.32	3.93	4.59	4.73	0.186	

[a]Means in the same row with different roman superscripts are significantly different ($P < 0.05$).
[b]A, Assaf breed (480 head); W, Awassi breed (2,500 head); M, Manchega breed (2,200 head); A × C (cross between Assaf and Castellana breeds) (170 head); CH, Churra breed (130 head).
[c]MSE, mean standard error; *$P < 0.05$; **$P < 0.01$.

TABLE 3
TAG Equations for the Detection of Foreign Fats (coefficients a_i for the different TAG and statistical results)

	Equations		
Item	1	2	3
26	—	+3.753	—
28	—	−7.748	—
30	—	+0.0868	—
32	—	+4.239	—
40	—	−4.621	—
42	+7.733	+34.746	+20.794
44	—	−30.890	—
48	—	+19.076	+12.643
50	+2.428	−13.794	−7.174
52	+1.392	+7.575	+3.847
Scattering	94.87–110.32	91.66–109.60	94.42–106.31
Confidence interval (mean value 99%)	98.71–101.25	98.32–101.63	99.06–101.13
R^2 corrected	0.832	0.983	0.984
SD	3.181	4.182	2.607
Durbin–Watson statistics	1.642	1.391	1.406
Mean value	99.99	99.98	100.09

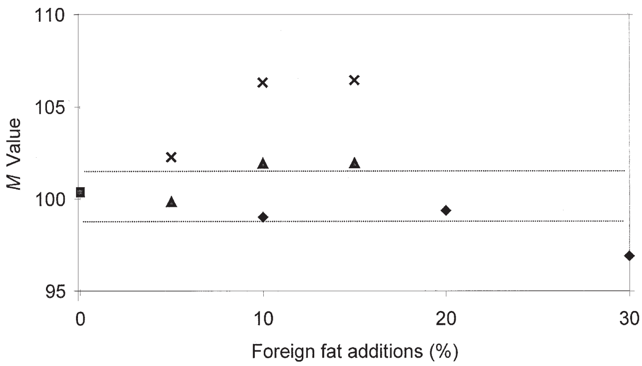

FIG. 2. *M* values (from regression equation of type $\sum a_i C_i = M + e$) obtained by applying Equation 1 as proposed in the text to the TAG composition of pure ewe's milk fat (■) and of mixtures with lard (▲), palm oil (×), and cow's milk fat (◆). The dotted lines are the lower and upper limits of confidence intervals for the mean value at 99%.

To test the potential of Equation 1 for detecting the addition of foreign fat such as palm oil or lard to ewe's milk fat, the TAG composition of the six mixtures of ewe's milk fat with lard or palm oil were analyzed and the equation was applied to the results to obtain the respective *M* values in the equation $\sum a_i C_i = M + e$. Figure 2 shows that with this equation it is possible to detect foreign fat additions as low as 5% of palm oil and 10% of lard. The detection limit does not improve with Equation 2. Equation 3 allows the detection of lard in concentrations as low as 5%.

When Equations 1 to 3 were applied to the results of the TAG composition for the three mixtures of ewe's milk fat and cow' milk fat, the *M* values of the mixtures in which the concentration of cow's milk fat was lower than 20% fell within the range of variation obtained in these equations for pure ewe's milk fat (98.71–101.25). Only the *M* value obtained with 30% added cow's milk fat (96.90) was outside this range.

The results suggest that these equations may be useful for detecting foreign fats in milk fat where present in concentrations comparable to those reported by Fontecha *et al.* (6) for goat's milk fat; however, the limit of detection for mixtures with cow's milk fat was higher.

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