Triglyceride Analysis by Gas Chromatography in Assessment of Authenticity of Goat Milk Fat

J. Fontecha[a,*], V. Díaz[a], M.J. Fraga[b], and M. Juárez[a]

[a]Department of Dairy Products, Instituto del Frío (CSIC), Ciudad Universitaria s/n, 28040 Madrid, Spain, and [b]Department of Animal Production, Universidad Politécnica, Madrid, Spain

ABSTRACT: The triglyceride (TG) composition of 35 samples of milk collected at different times of year from five herds of goats was analyzed using short capillary column-gas chromatography. The distribution of TG in goat milk fat was unimodal, peaking at C_{40} (12.6%); the sum of TG from C_{38} to C_{44} accounted for about 50%, whereas the three classes of TG from C_{48} to C_{52} did not exceed 6% each. These results were compared with the corresponding data for cow milk fat. Significant differences between herds were observed, mainly in long-chain TG. To detect foreign fats in goat milk fat, two multiple regression equations based on TG content of the goat milk fat were proposed. Analysis of known mixtures of tallow, palm oil, and cow milk fat with goat milk fat have experimentally confirmed the accuracy of the equations.
JAOCS 75, 1893–1896 (1998).

KEY WORDS: Adulteration, goat milk fat, short capillary column-GC, triglycerides.

The nutritional and commercial importance of cow milk fat has prompted extensive studies of its triglyceride (TG) composition, analyzed mainly by gas chromatography (GC) (1–3). Nevertheless the amount of information on TG composition of goat milk fat available to date is too small to enable determination of the ranges of variation of the content of each TG, owing to the low number of samples used (4–5).

Determination of classes of milk fat TG according to their carbon number (CN) has been reported to be a more effective criterion than fatty acid composition for determining their origin (6–7). TG determination has also been used to detect low levels of mixtures of foreign fats in milk fat (6–8) and has been proposed by the EC as an official method for controlling milk fat purity (9). According to that, the use of multiple regression equations based on TG composition of a large number of cow milk samples using GC with packed columns permits the detection and quantification of a low percentage (2–5%) of foreign fat in milk fat (10–13). On the other hand, it has been reported that short capillary analysis cuts down the analysis time and provides similar levels of accuracy (7,14,15).

The aim of this work was to determine the range of variation of TG composition of goat milk obtained by short capillary column-GC and to propose multiple regression equations based on the TG content of goat milk fat to detect foreign fats in goat milk.

*To whom correspondence should be addressed.
E-mail: Jfontecha@if.csic.es

EXPERIMENTAL PROCEDURES

Samples and standards. Thirty-five samples of raw goat milk were collected monthly (from November to May) from five herds belonging to five different breeders in the Murcia region (Spain). The refrigerated raw milk sample (250 mL) was tempered at 20°C for 20 min, then filtered and centrifuged in a Beckman (Fullerton, CA) J 2 MC at 6000 rpm for 30 min at 20°C prior to fat separation. The tubes containing the centrifuged milk were placed in ice until the milk fat had solidified, at which point it was removed and treated with anhydrous sodium sulfate. The mixture was washed four times with diethyl ether and the total organic fraction was filtered, exposed to a stream of N_2 and evaporation-dried under low pressure at 4°C. The fat residue extracted was stored frozen at –20°C until GC analysis. A butter oil, which had served as test fat in EC collaborative trials (6), was used to obtain the data on the TG composition of cow milk fat.

Twelve mixtures, prepared with different amounts of tallow, palm oil (of commercial origin), or the reference cow milk fat with goat milk fat (obtained from combining in equal volume all goat milk fat samples), were analyzed to test the accuracy of proposed equations.

For identification of TG, a mixture of synthetic TG (trilinolein, triolein, tristearin, tripalmitin, trimyristin, trilaurin, tricaprin, and tricaprylin) was first analyzed to determine both the best chromatographic conditions and the retention times of these components. To determine the response factors for quantitative studies of TG, the reference butter oil was used as described in a previous paper (15).

GC analysis. For analysis of TG, 0.2 μL dilutions of goat fat 5 mg/mL in hexane were injected into the gas chromatograph. Duplicate analyses were performed for each sample.

TG analyses were performed on a Perkin-Elmer gas chromatograph (Beaconsfield, United Kingdom) Model Autosystem, Gion 4072042, equipped with an automatic split/splitless injector (split ratio 1:20) and programmed temperature. A capillary column 2.5 m long (from a 30 m column, Rtx-65 TG [35% dimethyl, 65% diphenyl polysiloxane] $d_f = 0.10$ μm),

supplied by Restek (Bellefonte, PA), was used. Experimental chromatography conditions were as follows: the initial oven temperature of 210°C was raised to 355°C at a rate of 8°C/min and then held at this temperature for 10 min. The injector and detector temperatures were 355 and 370°C, respectively. The pressure at the top of the column was 10 psig, and helium was the carrier gas.

Statistical analysis. The results were analyzed according to the BMDP (16) software package. Sources of variation in variance analyses were herd and seasonal period. Multiple linear regression analyses were made to determine the most accurate relationships between TG as a means of discriminating between goat milk fat and other fats.

RESULTS AND DISCUSSION

TG composition. Figure 1 shows the chromatographic profiles of TG from goat and cow milk fat where there were 16 peaks quantified, corresponding to TG of 24 to 54 carbon atoms. In cow milk fat there are two clear maxima, located at C_{38} and C_{50}–C_{52} (12.8 and 12.0%, respective average values), whereas in goat milk the TG content increased with the number of carbon atoms, reaching maximum (about 13%) at C_{40} and C_{42}. Beyond this point the TG of goat milk decreased, but not uniformly, given that the values for C_{48} and C_{50} were close. The largest relative differences between goat and cow milk fat were found in TG C_{42}, C_{44}, C_{50}, C_{52}, and C_{54}. Previous studies of TG of goat milk fat have reported a similar gas chromatographic profile, with a maximum located near to TG C_{38}–C_{42}, although the values of TG C_{50} and C_{52} were slightly higher (4,5) than ours. The differences between authors' findings may be explained by both the small number of samples used in previous papers and the high coefficient of variation (CV) in some TG.

The CV found in this paper (Table 1) are comparable to those reported by Precht (12) for 775 cow milk samples, although slightly lower as one would expect from a narrower sampling range. While the lowest CV were found in the middle-chain TG (C_{38}–C_{44}), those of 52 and 54 carbon atoms were high in both species [17.7 and 30.3% in goat and 21.3 and 34.8% in cow (12), respectively]. This is attributed to the taking of samples from underfed herds (12).

The composition of TG in goat milk did not differ significantly with respect to the time of year of sampling (November to May), perhaps because of differences in milk yield, diet, and stage of lactation from one herd to another. For the same reason there were significant differences with respect to the herd effect (Table 2). Sauvant and Morant-Fehr (17) also reported that individual factors in goats significantly influenced milk fat composition, even between animals in the same herd. Only TG C_{26} and C_{34} were unaffected by herd factor. For the four quantitatively most important TG (C_{38}, C_{40}, C_{42}, and C_{44} totaled about 50% of the TG), the differences, although quantitatively low (less than 10% between the extreme values) were highly significant ($P < 0.001$); the highest values were found in herd 1. The differences ($P < 0.01$) for

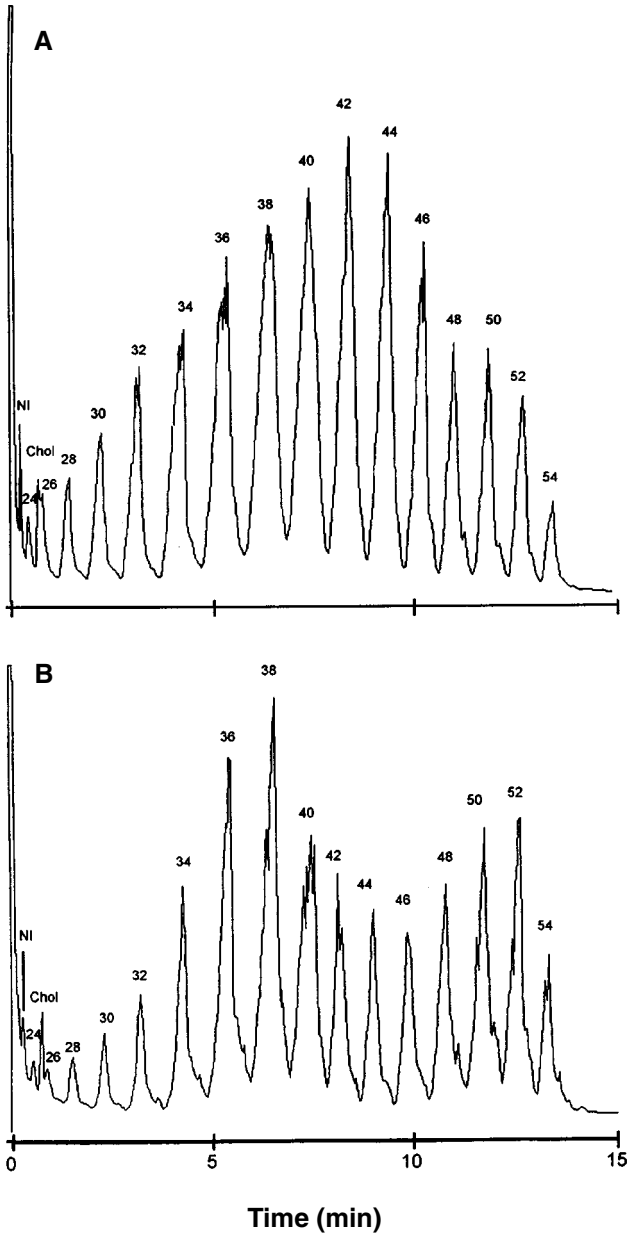

FIG. 1. Gas chromatographic profiles of triglycerides of a goat milk fat sample (A) and of the reference cow milk fat (B), using short capillary column.

long-chain TG (C_{48} to C_{54}) were quantitatively greater (up to 40% between extreme values); the lowest values were found in herd 1. This herd was included in a regional selection program to increase milk yield (maintaining fat and protein contents), and its management (including the nutritional aspects) was the most appropriate.

Detection of foreign fat in milk goat fat. Different methods for detecting foreign fats in cow milk fat using TG content as a variable have been proposed (10–13). The difference in TG composition between milk fat of cow and goat requires the calculation of specific equations to assess the purity of

TABLE 1
Triglyceride Composition of 35 Goat Milk Fat Samples (wt%)

Item	Mean value	CV (%)[a]	Range of variation Min	Max
C_{24}	0.15	33.3	0.06	0.27
C_{26}	0.49	18.3	0.29	0.93
C_{28}	1.23	16.2	0.57	1.83
C_{30}	2.47	8.9	2.09	3.02
C_{32}	4.06	7.1	3.48	4.73
C_{34}	6.20	6.7	5.44	7.08
C_{36}	9.40	6.7	8.17	10.80
C_{38}	12.08	4.2	11.15	13.59
C_{40}	12.62	4.3	11.79	14.14
C_{42}	12.51	4.6	11.56	14.46
C_{44}	11.57	3.3	10.81	12.62
C_{46}	8.10	5.4	7.01	9.21
C_{48}	5.84	8.0	4.39	6.57
C_{50}	5.85	12.6	3.50	7.30
C_{52}	4.92	17.7	2.15	6.83
C_{54}	2.01	30.3	0.69	3.24

[a]Coefficient of variation.

goat milk fat. The goat milk TG data from this study were fitted to a formula of the type:

$$\Sigma_i a_i C_i = M + e \quad [1]$$

where i is the carbon number, C_i is the percentage of TG with carbon number i, a_i is a coefficient to be estimated, M is a constant in which 100 is defined as pure goat milk, and e is the random error.

The variables first used were the percentages of TG C_{40}, C_{42}, and C_{44} as proposed by Timms (10) as being suitable for discriminating between cow milk fat and other fats (of animal or vegetable origin). The equation derived from our data was not selected because of its low R^2 value (0.42).

The next TG variables used were those utilized by Precht (13) in the formula established to detect mixtures of unknown foreign fats. This gave the following equation:

$$7.836C_{26} + 6.390C_{28} + 12.828C_{30} - 21.324C_{32} + 5.989C_{34} + 7.264C_{40} - 1.581C_{42} + 0.186C_{44} + 3.077C_{46} = 100 \pm 1.65 \text{ (SD)} \quad [2]$$

where $R = 0.898$ and 95% of the data are included in the interval 97.0–102.9.

Applying Equation 2 to the TG composition for several nonmilk fats (vegetable and animal fat) reported by Precht (12), the M values are very low (average 5.1 except for coconut oil). The equation is therefore considered useful for detection of adulteration of goat milk fat with foreign fats and is as sensitive as Precht's (13). In fact, mixtures of fats could be detected in 95% of cases studied using equations like Equation 2, always given concentrations of these fats in excess of 5.6–6.5% (11.8% in the case of coconut oil).

Nevertheless, application of the percentages of TG found in this study for the reference cow milk gave a value of M in Equation 2 close to 100 (87.90), which indicates the difficulty of detecting admixture with cow milk. Given that in practice adulteration of goat milk with cow milk may be quite frequent, another equation was calculated using as variables the TG in which the differences between cow and goat milk were relatively large (C_{40}, C_{42}, C_{44}, C_{50}, C_{52}, and C_{54}) according to Figure 1:

$$11.179C_{40} - 4.089C_{42} - 1.905C_{44} + 10.258C_{50} - 7.697C_{52} + 5.014C_{54} = 100 \pm 2.09 \text{ (SD)} \quad [3]$$

where $R = 0.932$ and 95% of the data are included in the interval 96.9–104.4.

TABLE 2
Effect of Herd on Triglyceride Composition of Goat Milk (wt%)

Item	Herd 1	2	3	4	5	MSE[d]	Significance[d]
n	7	7	7	7	7		
C_{24}	0.09^a	0.15^b	0.16^b	0.20^b	0.16^b	0.011	***
C_{26}	0.44	0.48	0.50	0.56	0.45	0.027	
C_{28}	0.98^a	1.19^b	1.25^b	1.45c	$1.26^{b,c}$	0.047	***
C_{30}	2.34^a	2.37^a	$2.42^{a,b}$	2.70^b	$2.48^{a,b}$	0.069	**
C_{32}	$4.20^{a,b}$	3.86^a	$4.02^{a,b}$	4.28^b	$3.97^{a,b}$	0.093	*
C_{34}	6.49	5.97	5.94	6.27	6.28	0.139	
C_{36}	9.86^a	$9.25^{a,b}$	8.81^b	$9.26^{a,b}$	9.78^a	0.194	**
C_{38}	12.75^a	11.65^b	$11.82^{a,b}$	$12.10^{b,c}$	12.17^c	0.118	***
C_{40}	13.55^a	12.12^b	12.59^c	$12.49^{b,c}$	$12.48^{b,c}$	0.092	***
C_{42}	13.35^a	12.53^b	12.13^b	11.97^b	12.64^b	0.122	***
C_{44}	11.94^a	11.69^a	$11.62^{a,b}$	11.20^b	$11.48^{a,b}$	0.111	***
C_{46}	$8.02^{a,b}$	$8.19^{a,b}$	8.47^a	$8.10^{a,b}$	7.77^b	0.148	*
C_{48}	5.27^a	6.23^b	$5.88^{b,c}$	$5.59^{a,c}$	$6.08^{b,c}$	0.118	***
C_{50}	4.73^a	6.47^b	$5.96^{b,c}$	5.71^c	$6.17^{b,c}$	0.159	***
C_{52}	3.94^a	5.23^b	5.43^b	5.19^b	$4.79^{a,b}$	0.253	**
C_{54}	1.64^a	$2.12^{a,b}$	2.51^b	$2.30^{a,b}$	1.48^a	0.176	**

[a,b,c]Means in the same row with different superscripts are significantly different ($P < 0.05$).
[d]MSE, mean standard error; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

If Equation 3 is applied for the reference cow milk data, it follows that although detection requires the presence of at least 15% cow milk fat in goat milk, this equation is more sensitive than the one calculated by other authors (11).

To test the actual potential of Equation 2 for detecting the addition of nonmilk fats to goat milk fat, the TG composition of eight mixtures of goat milk fat and tallow or palm oil was analyzed and the equation was applied to the results to obtain the respective *M* values. Figure 2 shows that the use of Equation 2 permits recognition of foreign fat additions in all the experimentally made mixtures, including those in which the concentrations of foreign fats (from 3 to 5%) are lower than the theoretically determined detection limit (5.6–6.5%). The *M* values obtained when applying Equation 3 to the results of the TG composition analysis for four mixtures of goat milk fat and cow milk fat indicated that the *M* values of the mixtures in which the concentrations of cow milk fat were lower than the detection limit (15%) are included in the range of variation obtained in Equation 3 (96.9–104.4) for the pure goat milk fat samples. Only the *M* value obtained in the mixture with 20% of added cow milk fat (105.6) was out of this range.

Not only could the application of these mathematical equations provide a fast and highly sensitive means of determining mixtures of nonmilk fats in goat milk (Eq. 2), but it would also be very useful for specific detection of mixtures of other milk fats with goat milk fat (Eq. 3). However, more data on TG composition of milk fat of goats of different feeding and genetic conditions seem necessary to increase the practical interest of this type of equation.

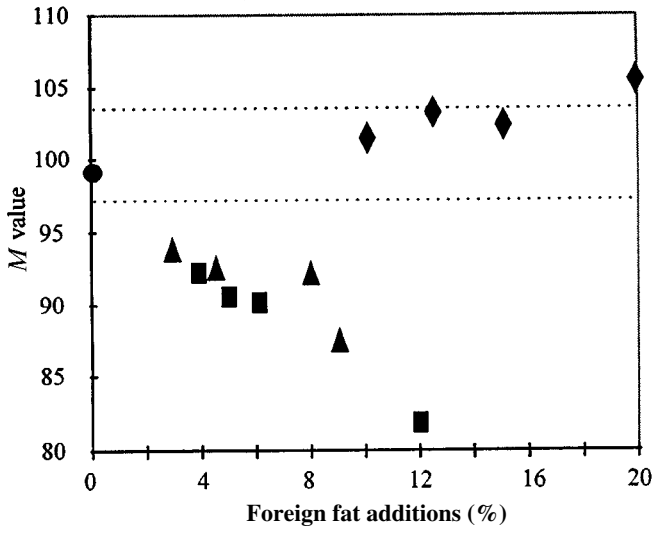

FIG. 2. *M* values obtained by applying the equations proposed in the text to the triglyceride composition of pure goat milk fat (●) and of mixtures with cow milk fat (◆), tallow (▲), and palm oil (■). The dotted lines are the lower and upper limits of intervals defined in Equations 2 and 3, respectively.

ACKNOWLEDGMENTS

The authors are grateful to A. Falagán for supplying the goat milk samples and to L. Barrios for her help in statistical analysis. This work was supported by a grant from the Comisión Interministerial de Ciencia y Tecnología (Project ALI-95 0046-C02-01).

REFERENCES

1. Precht, D., Qualitätseinstufung und Schnelle Jodzahlbestimmung von Butterfett anhan einer Triglyceridanalyse, *Z. Lebens. Unters. Forsch. 187*:457–462 (1988).
2. Alonso, L., Capillary Gas Chromatography of Some Triglycerides in Cheese Using Programmed-Temperature Injection, *Chromatographia 35*:649–652 (1993).
3. Kalo, P., and A. Kemppinen, Mass Spectrometric Identification of Triacylglycerols of Enzymatically Modified Butterfat Separated on a Polarizable Phenylmethylsilicone Column, *J. Am. Oil Chem. Soc. 70*:1209–1217 (1993).
4. Cerbulis, J., O.W. Parks, and H.M. Farrell, Composition and Distribution of Lipids of Goats' Milk, *J. Dairy Sci. 65*:2301–2307 (1982).
5. Luf, W., A. Stock, and E. Brandl, Detection of Foreign Fat in Milkfat by Means of Triglyceride Analysis. I. Triglyceride-Composition of Austrian Milkfat—Seasonal and Regional Fluctuation, *Oesterr. Milchwirtsch. 42*:23–28 (1987).
6. Precht, D., Control of Milk Fat Purity by Gas Chromatographic Triglyceride Analysis, *Kiel. Milchwirtsch. Forschungsber. 43*:219– 242 (1991).
7. Contarini, G., R. Leardi, C. Pezzi, and P.M. Toppino, Valutazione della Genuinità del Burro mediante Analisi di Trigliceridi ed Acidi Grassi, *Riv. Ital. Sostanze Grasse 70*:491–499 (1993).
8. Juárez, M., Test for Fraudulent Identification, in *Proceedings of the XXIII International Dairy Congress*, Int. Dairy Fed., Montréal, 1990, Vol. 2, pp. 1285–1300.
9. *Reference Method for the Detection of Foreign Fats in Milk Fat by Gas Chromatographic Analysis of Triglycerides*, Commission of the European Communities, Doc. VI/2648/94, 1994.
10. Timms, R.E., Detection and Quantification of Non-Milk Fat in Mixtures of Milk and Non-Milk Fats, *J. Dairy Res. 47*:295–303 (1980).
11. Luf, W., A. Stock, and E. Brandl, Detection of Foreign Fat in Milkfat by Means of Triglyceride Analysis. II. Detection of Milkfat Adulteration by Means of Triglyceride Combinations, *Oesterr. Milchwirtsch. 42*:29–35 (1987).
12. Precht, D., Detection of Foreign Fat in Milk Fat. I. Qualitative Detection by Triacylglycerol Formulae, *Ibid. 194*:1–8 (1992).
13. Precht, D., Detection of Foreign Fat in Milk Fat. II. Quantitative Evaluation of Foreign Fat Mixtures, *Ibid. 194*:107–114 (1992).
14. Lund, P., Analysis of Butterfat Triglycerides by Capillary Gas Chromatography, *Milchwissenschaft. 43*:159–161 (1988).
15. Lozada, L., M.A. de la Fuente, J. Fontecha, and M. Juarez, Considerations of the Quantitative Aspect of the Determination of Milkfat Triglycerides with Split PTV and On-Column Injection, *J. High Resolut. Chromatogr. 18*:771–775 (1995).
16. BMDP Statistical Software, Inc., Los Angeles, CA 90025 (1993).
17. Sauvant, D., and P. Morant-Fehr, Interactions Alimentation entre Individus en Production Laitière Caprine. 6ème Journée INRA-ITOVIC (1981).

[Received January 30, 1998; accepted July 29, 1998]