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in

Baumont R. (ed.), Carrère P. (ed.), Jouven M. (ed.), Lombardi G. (ed.), López-Francos A. (ed.), Martin B. (ed.), Peeters A. (ed.), Porqueddu C. (ed.).
Forage resources and ecosystem services provided by Mountain and Mediterranean grasslands and rangelands

Zaragoza : CIHEAM / INRA / FAO / VetAgro Sup Clermont-Ferrand / Montpellier SupAgro
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 109

2014
pages 275-278

Applicability of the genuineness official parameters to the milk fat from cows consuming mountain pasture: the case of triglyceride composition

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Abstract. It is well known that mountain pasture affects the fatty acid (FA) composition of milk fat. The Official EU method for the evaluation of cow milk fat purity is based on the triglyceride (TAG) analysis. The percentage of each TAG is inserted into five specific formulae (S). The resulting five S parameters, for a genuine milk fat, have to fall inside the corresponding limits; if only one of them is outside the limits, the milk fat is judged as adulterated. In this research, milk samples deriving from cows feeding mountain pasture, in absence or with low amounts of concentrates, were analyzed for their FA and TAG composition and the purity was calculated by using the S parameters. All the samples showed the characteristic FA composition of milk fat from mountain pasture-fed cows: high concentration of linolenic acid (1.1 ± 0.32), vaccenic acid (3.8 ± 0.57), *cis*-9,*trans*-11 CLA (1.7 ± 0.25), as well as low concentration of saturated fatty acids (62.9 ± 1.50). However, all the samples provided false positive results for at least one of the five S parameters. In order to avoid improper charges of adulteration, this behaviour should be taken into account by the competent authorities.

Keywords. Mountain pasture – Milk fat – Genuineness – Triglycerides – Fatty acids.

Applicabilité des paramètres officiels d'authenticité à la matière grasse du lait de vache à l'alpage : le cas de la composition en triglycérides

Résumé. *Il est bien connu que les pâturages de montagne modifient la composition en acides gras (AG) de la matière grasse du lait. La méthode officielle de l'UE pour l'évaluation de la pureté de la matière grasse du lait de vache est basée sur l'analyse des triglycérides (TG). Le pourcentage de chaque TG est introduit dans cinq formules spécifiques. Les cinq paramètres S résultants, doivent être compris dans des limites correspondant à une matière grasse du lait pure; même si un seul des paramètres est en dehors des limites, la matière grasse du lait est jugée non conforme. Dans cette étude, des échantillons de lait provenant de vaches pâturant des alpages, en l'absence ou avec de faibles quantités de concentrés, ont été analysés pour leur composition en AG et TG et la pureté a été calculée en utilisant les paramètres S. Tous les échantillons ont montré une composition en AG, caractéristique de la matière grasse du lait de montagne : une concentration élevée en acide linolénique (1,1 ± 0,32), acide vaccénique (3,8 ± 0,57), CLA (1,7 ± 0,25), ainsi qu'une concentration réduite en acides gras saturés (62,9 ± 1,50). Cependant, tous les échantillons ont donné des résultats faux positifs pour au moins l'un des cinq paramètres S. Afin d'éviter des déclarations de falsification incorrectes, ce résultat doit être examiné par les autorités compétentes.*

Mots-clés. *Alpage – Matière Grasse du lait – Authenticité –Triglycérides – Acides gras.*

I – Introduction

The Official EU method for the evaluation of cow milk fat purity is based on the triglyceride (TAG) analysis (Reg. CE 273, 2008) and on the values obtained inserting TAG results into five specific equations. If only one of the results of these five equations falls outside the limits that are characteristic for pure milk fat, the sample is judged as adulterated.

Since TAG are molecules in which three FA are esterified to the glycerol backbone, their composition is strictly dependent on the linked fatty acids and, consequently, they are affected by the same factors of variation, first of all, the feeding system. The high diversity in the botanical composition of grass, the environmental conditions, the low density of net energy of alpine herbage that increases the body fat mobilization, are some of the factors responsible for the particular characteristics of the fatty acid composition of milk fat from cows consuming mountain pasture (Lourenço *et al.*, 2008; Povolo *et al.*, 2013).The saturated FA content, particularly palmitic acid, was lower in mountain milk than that found in milk derived from the intensive breeding systems; at the same time, except for linoleic acid, monounsaturated and polyunsaturated FA, particularly linolenic acid, were higher (Leiber *et al.*, 2005). Finally, the mountain milk fat was characterized by the high content of isomer *cis*-9, *trans*-11 of conjugated linoleic acids and their precursor, vaccenic acid (C18:1 *trans*-11).

In this research, milk samples deriving from cows feeding mountain pasture, in absence or with low amount of concentrates, were analyzed for their FA and TAG composition and the purity was calculated by using the S parameters.

II – Materials and methods

Bulk milk samples were collected in two mountain areas, at altitude varying from 1800 to 2100 m, (East and West Italian Alpine region) during the summer period from cows grazing on pasture in absence (20 samples) or with 1.5 (14 samples) or 3 (13 samples) kg /(cow x day) of concentrate. The pastures of the East and West area were characterized by *Festuca rubra* and *Nardus stricta*, respectively. The concentrate supplemented to the cows grazing in the East zone of the Alpes had a higher protein and a lower fat content than that supplemented to the cows grazing in the West zone.

Milk samples were stored at -20°C until the analyses, which were done in duplicate. The fat fraction was extracted according to ISO 14156 (2001). The FA composition was carried out on methyl esters obtained according to ISO 15884 (2002) and applying the GC conditions described by Povolo *et al.* (2013). The analysis of TAG composition was performed following the Official EU method (Reg. CE 273, 2008).

Data obtained were evaluated by both univariate (ANOVA) and multivariate (PCA) statistical analyses.

III – Results and discussion

The first evaluation of the results was made by applying PCA analysis to the whole data set (47 objects and 43 variables) subdivided into the east (E) and west (W) Alpine area both labelled 0, 1.5, 3, according to the amount of concentrate in the diet (Fig. 1). A well defined separation between the two Alpine zones occurred on the first-component axis. On the contrary, no clear distinction was observed among the different contents of concentrate in the diet, even though a slight tendency to a distribution according to the concentrate content was observed along the second component axis. This result demonstrated that the FA composition of milk was largely influenced by the type of pasture growing in the different Alpine areas and this difference was not affected by the presence of supplementation. This result was confirmed by the ANOVA applied to the FA grouped on the basis of the presence of double bonds (SFA = saturated, MUFA = monounsaturated, PUFA = polyunsaturated), on the position of double bonds (omega-3) and on the geometrical isomerization (Trans). As it is reported in Table 1, all the FA classes showed high significant differences between the types of pastures, independently of the amount of supplementation.

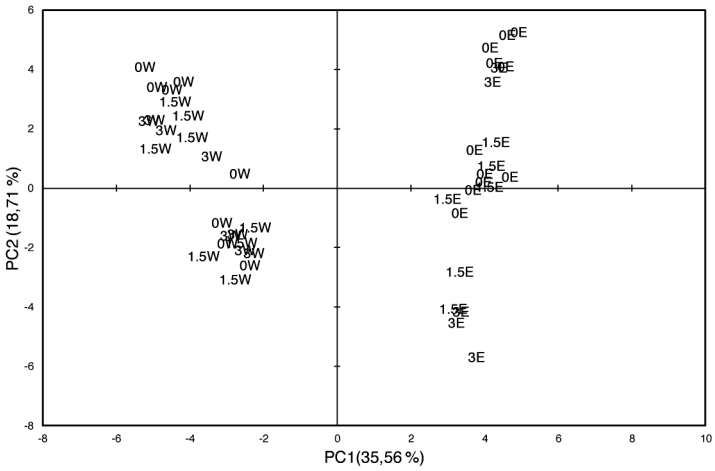

Fig. 1. Plot of the scores of the 47 milk samples on the first and second principal components.

Table 1. Mean and standard deviation of fatty acids, expressed as% on the total fatty acids

	Only pasture			+ 1.5 kg conc.			+ 3 kg conc.		
	East	West		East	West		East	West	
SFA	62.1±1.01	64.2±0.96	**	62.0±1.08	63.9±1.23	*	61.2±1.23	63.3±1.40	*
MUFA	33.1±0.80	29.2±0.74	**	33.1±0.88	29.5±1.04	**	33.6±0.80	29.9±1.00	**
PUFA	4.8±0.35	6.6±0.31	**	4.9±0.43	6.7±0.35	**	5.1±0.57	6.8±0.49	**
Omega-3	0.9±0.11	1.5±0.18	**	0.9±0.08	1.5±0.09	**	0.9±0.13	1.6±0.25	**
Trans	5.5±0.28	7.5±0.40	**	5.2±0.20	7.5±0.43	**	5.4±0.14	7.4±0.29	**
C18:1 *t11*	3.4±0.13	4.3±0.39	**	3.1±0.12	4.3±0.38	**	3.3±0.15	4.2±0.18	**
CLA	1.5±0.12	1.9±0.11	**	1.4±0.09	1.9±0.16	**	1.4±0.13	1.9±0.10	**
C18:2 *t*11,*c*15	0.3±0.08	0.7±0.04	**	0.3±0.03	0.7±0.05		0.3±0.03	0.7±0.08	**

** P<0.01; * P<0.05.

Moreover all the samples showed a FA composition typical of mountain milk fat: low SFA content, high PUFA, CLA and *trans* fatty acids (Collomb *et al.*, 2002). Among the latter category, it is worth noting the high values of both vaccenic acid (C18:1 *t*11) and C18:2 *t*11,*c*15, the highest non-conjugated linoleic acid isomer found in mountain milk samples, accordingly to Collomb *et al.* (2008).

Figure 2A shows an example of the TAG profile obtained applying the EU official method. Sixteen peaks identified on the basis of the sum of the number of carbon atoms of FA esterified into the TAG, and quantified as percentage on the total TAG content, were obtained.

The values of the five S parameters (S1 to S5) calculated to evaluate the genuineness of milk fat provided the following results: all the samples fell out the limits for S1, 20% of samples for S2, 67% of samples for S3, and 52% of samples for S4 and S5. An example is given in Fig. 2B, where the results of S1 formula are reported, together with the range that is characteristic of the pure milk fat. All the mountain milk fat samples, independently of the possible presence of concentrate in the diet, did not meet the law requirements to be considered as genuine milk fat.

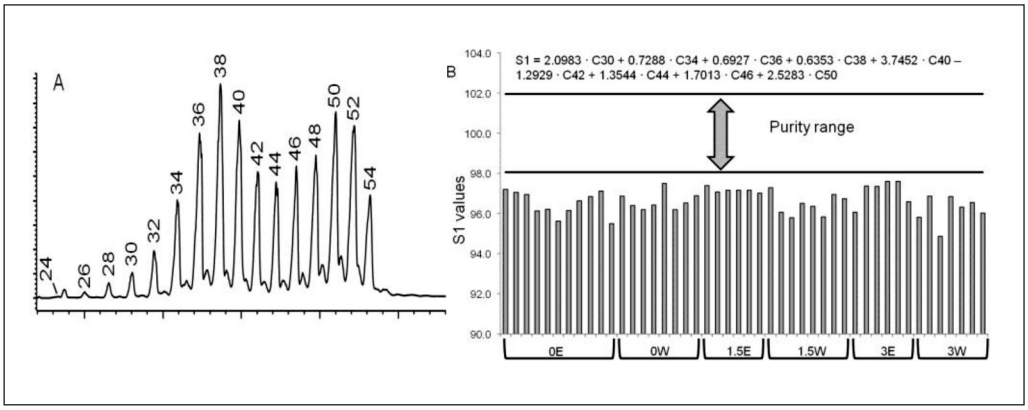

Fig. 2. GC profile of TAG of a mountain milk fat sample (A) and values obtained by the application of S1 purity parameter to all the mountain milk fat samples (B).

IV – Conclusions

Independently of the factors responsible for the particular characteristics of the fatty acid composition of milk fat deriving from cows consuming mountain pasture, this feeding system also affects the TAG composition on which the EU Regulation defines the parameters of the milk fat genuineness. It seems very important to avoid improper charges of adulteration related to milk and dairy products that very often are characterized by high quality composition and unique value. Thus, this evidence has to be pointed out to the competent authorities in order to guarantee the protection of these productions. A first action on this direction has been already undertaken by the Italian delegation of the ISO Committee, to mention this problem within the scope of the ISO 17678 (2010) standard that reports the reference method for milk fat TAG determination.

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