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COMPOSITION OF FREE FATTY ACIDS AND LIPASE ACTIVITY IN EWES' MILK THROUGHOUT THE LACTATION PERIOD

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SUMMARY

The amount of free fatty acids (FFAs) produced by lipolysis is in part responsable for some characteristics of ewe cheese. The ones exerting the major effects are those of the chain C_4 - C_{12}, which may cause alterations of the organoleptic features of the sheep milk and the dairy products derived from it. The effect of variation in FFAs amount and composition on cheese quality is even more important when ewes are managed in separated groups and the lambing is synchronized.

The main goal of this work was to analyse the variation in the FFAs amount and compositions and lipase activity in raw milk of Manchega ewes during the lactation period.

Milk samples were collected from the morning milking of 44 sheep, every two weeks starting 15 days after lambing and for 90 days. Samples were analysed by gas chromatography.

Variations in the FFAs amount and composition were found in milk samples at different stages of lactation, especially as regards of short- and medium-chain FFAs. Differences were mainly observed in milk samples from the middle lactation, which can affect profoundly the quality of cheese obtained with milk produced in this period.

Key words: ewe's milk, free fatty acids, lipase activity.

RESUMEN

Composición de ácidos grasos libres y actividad de la lipasa en leche de oveja a través del período de lactación.

La cantidad de ácidos grasos libres (AGLs) producidos por la lipólisis es responsable en gran medida de algunas propiedades del queso de oveja. Los únicos que ejercen mayores efectos son aquellos ácidos grasos de cadena comprendidas entre C4 y C12, ya que pueden causar alteraciones de las características organolépticas de la leche de oveja y los productos lácteos que derivan de ella.

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El efecto de la variación de la cantidad y composición de los AGLs sobre la calidad de queso resulta más importante cuando las ovejas se manejan en grupos separados y se llevan a cabo sincronización de partos.

El objetivo principal de este trabajo fue analizar la variación en la cantidad y composición de AGLs y la actividad de la lipasa en leche cruda de ovejas de raza Manchega durante un periodo de lactación. Las muestras de leche procedentes de 44 ovejas fueron recolectadas durante el ordeño de la mañana, con una frecuencia de dos semanas, comenzando 15 días después del parto y durante un periodo de 90 días. Estas muestras fueron analizadas por cromatografía en fase gaseosa.

Se encontraron variaciones en la cantidad y composición de AGLs de las muestras de leche en los diferentes momentos de la lactancia, especialmente en los AGLs de cadenas cortas y medias. Se observaron diferencias principalmente en las muestras de leche que procedían de la mitad de la lactación que podrían afectar marcadamente la calidad de los quesos elaborados con la leche de este período.

Palabras claves: leche de oveja, ácidos grasos libres, actividad de la lipasa.

INTRODUCTION

The amount of free fatty acids (FFAs) in raw milk and the by-products originating from the lipolysis of milk fat varies, depending on the fat composition and the activity of the enzymes. Many factors favour the lipolysis of milk fat, the most important being the lactation stage, the level of milk production and age and the diet of the animals, amongst others which cause ‘spontaneous lipolysis’. Others factors relating to milking, refrigeration and transportation may cause the so called ‘induced lipolysis’ (Fleming, 1979; Meffe, 1994). Finally, contamination deriving from ineffective cleaning and desinfecting of milking and refrigeration equipment which cause ‘microbial lipolysis’ (Heuchel & Chilliard, 1988).

Additionally, FFAs composition affects also the characteristics of ewe cheese, especially C_4 to C_{12} FFAs (Gonzalez-Llano & Ramos, 1988), which, in some cases, can cause defects and organoleptic alterations of cheese.

High concentration of certain FFAs in raw milk can inhibit the growth of some microorganisms important in cheese processing. They can also interfere negatively in the screening methods used to detect inhibitors in raw milk (Barbosa, 1997).

The variation in milk composition due to lactation stage can affect the characteristics of dairy products when ewes are managed in groups and lambing is synchronised.

The aim of this study was to analyse the evolution of FFAs concentrations and lipase activity in Manchega ewes’ milk throughout the lactation period.

MATERIAL AND METHODS

Forty-four Manchega ewes, permanently housed at the Animal Science Department of the Polithecnical University of Valencia, were used for this study. Milk samples were taken from individual animals at morning milking, and they were collected in disposable plastic tubes at twice a month intervals, starting after 15 days from lambing to the 90^{th} day of lactation.

Fat was extracted from milk samples to analyse the FFAs, according to the method proposed by Needs *et al.* (1983). Following methylation, using the method described by Gandemberg *et al.* (1991), samples were analysed and quantified by gaseous phase chromatography.

Free fatty acid methyl esters were separated and quantitated using a Fisson GC-8160 gas chromatograph with an FID-80 flame ionization detector and a slitless injection system. Capillary column (25 m x 0.25 mm i.d) was with a mobile nitroterflalic acid phase modified with polyethylene glycol. (DB-FFAP Fisson). After sample injection column temperature was held for 2 min at 50ºC and then increased at a rate of 7,86ºC/min to a temperature to 160ºC and then increased a rate of 10ºC to a final temperature to 220ºC (held for 50 min). Nitrogen carrier gas pressure was 80 kPa. The temperature of the detector was 250ºC. Free fatty acid methyl esters were identified by comparison of their retention times with those of high purity FFA methyl ester standards. The recovery factor of each extraction was calculated by adding the standard of the C_5 (87,3%) C_9 (92,1 %) and C_{13} (96,7 %) acids.

Lipase activity was determined photometrically according to Humbert *et al.* (1997) with a Shimadzu UV-1601.

Statistical analysis was carried out using the RANDOM option from the GLM (General Linear Models) procedure for repeated samples (SAS, 1998). The following model was used:

$$Y_{ijk} = m + L_i + S_j + (L*S)_{jj} + e_{jjk}$$

Where: Y_{ij}= FFAs concentration or lipase activity, m= general mean, L_i= lactation stage effect (days), S_j= sheep effect, $(L*S)_{jj}$ = lactation stage*sheep interaction, and e_{jjk}= residual error.

RESULTS AND DISCUSSION

Table 1 reports the mean values, standard deviations (SD), coefficients of variance (CV), the maximum and minimum concentrations of FFAs and lipase activities found in the milk samples

Table 1: Statistical parameters for FFAs concentrations (mg/l) and lipase activity (µmol p-nitrophenol/ml min) in Manchega ewes' milk.

FFA	Average	SD	CV	Minimum	Maximum
C_4	3.74	1.44	0.39	0.35	14.91
C_6	4.36	2.47	0.57	0.62	15.33
C_8	1.75	1.22	0.70	0.11	8.15
C_{10}	3.79	2.62	0.69	0.43	14.38
C_{12}	2.65	1.81	0.68	0.18	10.05
C_{14}	4.45	2.75	0.62	0.43	16.11
C_{15}	0.54	0.31	0.57	0.01	1.78
C_{16}	10.78	6.14	0.57	0.10	35.66
$C_{16:1}$	0.47	0.31	0.66	0.02	1.82
C_{17}	0.49	0.80	1.63	0.05	4.28
C_{18}	3.77	1.75	0.46	n.d.	10.66
$C_{18:1}$	7.35	3.93	0.53	n.d.	23.08
$C_{18:2}$	1.57	0.89	0.57	n.d.	5.91
$C_{18:3}$	0.45	0.61	1.35	n.d.	4.88
C_{20}	0.22	0.23	1.05	n.d.	0.92
Lipase	0.11	0.04	0.36	0.01	0.25

n.d.: not detected.

collected from 44 ewes throughout lactation.

The concentration of C4, C6, C10, C12, C14, C16, C18, C18:1 fatty acids is higher than C15, C16:1, C17, C18:3 and C20 fatty acids, as already found for FFAs of Latxa ewe milk (Chavarri, 1998).

As far as the FFAs variability, our results show that the values of the coefficient of variance vary from a minimum of 0.39 (C4) and a maximum of 1.63 (C17).

From Table 2, which shows the evolution of FFA concentrations, it can be seen that the concentrations of C18:1, C18:2, C18:3 and C20 decrease significantly to 60 days of lactation. The short chain FFAs (C4 and C8) increase to this period. The quantities of short and medium chain change FFAs are more elevated in the second month of lactation.

The values of lipase activity are reported in Table 2. A significant increase in the activity of this enzyme was detected in milk samples collected between days 45 and 60 of the lactation period. This increase paralleled with an increase in the concentration of short-chain FFAs and a decrease in the concentration in long-chain FFAs, suggesting a high lipolytic activity during this period.

CONCLUSIONS

Differences in the concentrations of short and medium-chain FFAs in sheep milk at different lactation stages could cause variations in the quality of cheese obtained with milk produced from the middle of the lactation period compared with to cheese manufactured with milk from other lactation stages.

FFAs are a possible cause of anomalous flavours. Problems due to high concentrations of certains FFAs are more likely to occur in home made cheese, produced with milk from a single flock, because of a greater proportion of the FFAs in a similar stage of lactation. However, such problem are less common for industrial cheese producers, that make use of large volumes of milk from different flocks and because the milk

Table 2: Mean concentration values for FFA (mg/l) and lipase activity (µmol p-nitrofenol/ml min) throughout the lactation period

FFA	Lactation stage (days)					
	15	30	45	60	75	90
C_4	3.35_a	3.80_a	3.60_a	5.27_b	3.26_a	3.33_a
C_6	5.10	4.80	3.70	4.38	4.25	3.98
C_8	1.62_a	1.77_a	1.10_{ac}	2.67_b	1.83_a	1.64_{ab}
C_{10}	4.01_a	3.78_a	2.59_b	4.52_a	4.34_a	3.60_{ab}
C_{12}	2.86_a	2.88_a	1.81_b	2.87_a	3.08_a	2.51_{ab}
C_{14}	4.29	4.78	3.24	4.66	5.20	4.68
C_{15}	0.49_a	0.61_b	0.43_a	0.67_{bc}	0.55_{ab}	0.51_{ab}
C_{16}	10.55_a	11.98_a	7.48_b	11.74_a	11.98_a	11.06_a
$C_{16:1}$	0.49_{ac}	0.50_{ac}	0.34_b	0.41_{ab}	0.53_a	0.59_c
C_{17}	0.28_a	0.15_b	0.29_a	0.32_a	0.26_a	0.42_a
C_{18}	4.04_a	4.23_a	2.98_b	3.80_a	4.02_a	3.63_{ab}
$C_{18:1}$	8.23_a	8.77_a	5.30_b	5.97_{bc}	7.75_a	8.12_{ac}
$C_{18:2}$	1.81_a	1.85_a	1.32_{bc}	1.38_{bd}	1.59_{ac}	1.49_{acd}
$C_{18:3}$	0.46_{ac}	0.69_a	0.44_{ac}	0.18_b	0.30_{bc}	0.59_a
C_{20}	0.20_a	0.11_b	0.10_b	0.03_c	0.36_d	0.53_e
Lipase	0.09_a	0.09_a	0.13_b	0.15_b	0.11_c	0.09_a

Lipase

a. b. c. d. e: different letters in the same row indicate significant differences ($P < 0.05$).

used comes from animals over a range of lactation stages.

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