

Variations in the Contents of Vitamins A and E during the Ripening of Cheeses with Different Compositions

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Abstract

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We investigated the composition in vitamins A and E of cheeses made from the milks of ewes, goats and cows. A total of 84 cheeses of known composition were prepared and controlled to determine the influence of different factors, e.g. the variable proportions of cow's, ewe's, and goat's milks, seasonality (winter/summer), and evolution during the course of ripening. The variable proportions of milk from the different species did not vary in either the amount of vitamin A or that of vitamin E in the cheeses. Seasonality and ripening were seen to have a significant effect on the concentration of vitamin A.

Keywords: vitamins; cow; ewe; goat; season

Fat content is the most quantitatively and qualitatively variable component of milk, depending on the stage of lactation, season, breed, genotype, and feeding (RAYNAL-LJUTOVAC *et al.* 2008). A significant nutritional aspect of milk lipids is that vitamins A, D, E, and K are dissolved in the fat phase. Milk fat represents a good dietary source of vitamin A, vitamin E, and β -carotene (PANFILI *et al.* 1994), but is a poor source of vitamins D and K (MCBEAN & SPECKMANN 1988).

Vitamin A is a fat-soluble vitamin involved in critical biological functions. It occurs in three primary forms: retinol, retinal, and retinoic acid. The retinol content in milk is influenced by the level of β -carotene in forage (SHARMA *et al.* 1983; GOYAL *et al.* 1984). Dietary β -carotene is converted to retinal

in the intestinal epithelium and in the liver by the enzyme β -carotene-15-15'-dioxygenase. In contrast to that of cows, the milks of goats and ewes contain only retinol and generally no β -carotene, which is entirely converted into retinol (RAYNAL-LJUTOVAC *et al.* 2008), thus accounting for the observed differences in colour between bovine and smaller ruminant dairy products (PARK *et al.* 2007).

Vitamin E is considered to act primarily as a lipid-soluble antioxidant, protecting polyunsaturated fatty acids and related substances from peroxidation and hence from rancidity (BATES & PRENTICE 1994). The chemistry of vitamin E is rather complex since there are eight compounds (four tocopherols and four tocotrienols) that exhibit vitamin E activity. In milk, α -tocopherol accounts for nearly all of vitamin E. In

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bovine milk, α -tocopherol is the main compound with vitamin E activity (LINDMARK-MANSSON & AKES-SON 2000), while very small amounts of β -tocopherol and γ -tocopherol are present. Goat milk is poor in vitamin E (RAYNAL-LJUTOVAC *et al.* 2008) while sheep milk generally has higher contents of it than cow and goat milks (PARK *et al.* 2007).

The variability in the cheese composition as regards fat-soluble constituents (fatty acids, carotenoids, retinol, α -tocopherol) mainly depends on the conditions in which the milk is produced (LUCAS *et al.* 2008a). Thus, the species of animal from which it is obtained is also likely to influence the nutritional characteristics of a cheese to a significant extent (CHILLIARD & LAMBERET 2001; LUCAS *et al.* 2006a). Indeed, the animal diet is another factor that strongly influences the milk composition. Regarding this, the predominant effect of the nature of the basic fodder ration on fat-soluble vitamin contents in milk has been broadened to those present in cheese (LUCAS *et al.* 2008b). The results show that when cows are fed a preserved forage-based ration, the retinol content in cheese fat is increased in a dose-dependent way due to the supplementation with vitamin A. Similarly, the α -tocopherol content in cheese fat is positively influenced by the vitamin E supplementation of preserved forage-based rations, but only at the highest level of supplementation. However, vitamin supplementation of pasture-based rations has no significant effect on the retinol and α -tocopherol contents in cheeses (LUCAS *et al.* 2008b). Moreover, lower retinol concentrations and higher levels of α -tocopherol have been found in organic rather than in conventional mozzarella buffalo milk samples and cow milk products. (BERGAMO *et al.* 2003).

Vitamin A is partially influenced by both the original milk composition and the cheese-making process (LUCAS *et al.* 2006c). Given that carotenoids and retinol are fat-soluble, they mainly behave like milk fat. However, a small proportion of the retinol and carotenoids is associated with whey proteins (PUYOL *et al.* 1991) and/or concentrated in the milk fat globule membrane (ZAHAR *et al.* 1995). As a result, a certain amount of these micronutrients could be lost to the whey during cheese-making. Finally, a study on cow milk cheeses and goat milk cheese has revealed that vitamin E does not depend on the technology used but rather on the composition of the milk (e.g. breeding systems: hay vs. pasture) for both species (LUCAS *et al.* 2006a,b).

Taking into account the scarce data available concerning fat-soluble vitamin contents in cheeses and

the influence of several factors on their levels, the aim of the present study was to investigate the effects of the origin of milk (ewe, goat, and cow), of the season and of the ripening time on the vitamin composition of different types of cheese.

MATERIAL AND METHODS

To perform the present study, a total of 84 cheeses of known composition were prepared and controlled. They were made of milk collected directly from farms, bovine, ovine, and caprine raw tank milks obtained directly from the producers in Zamora (Spain). Cheeses were prepared in the laboratory according to GONZÁLEZ MARTÍN *et al.* (2007): raw milk (40 l), not standardised, was incubated with direct-vat-set starters (MA400; Arroyo Laboratories, Santander, Spain) at 30°C. After 10 min at 32°C, calf rennet (90% chymosin, 10% trypsin, 1 : 150 000 strength) was added to each vat. Coagulation was allowed to take place over 20–70 minutes. When the curds had developed the desired firmness, which was evaluated subjectively, they were cut with a cheese harp until pieces similar in size to a grain of rice were obtained. The curd was then stirred for 30 min and heated for 10–20 min at 37°C until it had reached the desired consistency to improve its drainage with sieves. The curd was packed in round hoops (1 kg) and pressed for 6 h at 1.5 kg/cm² at 20°C. After pressing, the cheeses were salted by soaking them in sodium chloride brine

Table 1. Composition of the reference cheeses elaborated (winter and summer milk)

Milk cow (%)	Milk ewe (%)	Milk goat (%)
100	0	0
0	100	0
75	25	0
50	50	0
25	75	0
0	0	100
25	0	75
50	0	50
75	0	25
0	25	75
0	50	50
0	75	25
33	33	33
10	45	45
45	10	45
45	45	10

(18%) at 18°C for 6 hours. The cheeses were then transformed to a drying chamber, where temperature (15°C) and relative humidity (80%) were controlled. Cheeses with 16 different compositions were made, prepared with varying amounts of milk from cows, ewes, and goats, with percentages ranging between 0 and 100%, as shown in Table 1.

Determination of vitamins in cheese samples. The concentrations of vitamin A (all *trans* retinol) and vitamin E in the cheeses were measured simultaneously by means of normal phase HPLC using a UV-VIS photodiode-array detector after saponification and hexane extraction, adapted from LUCAS *et al.* (2006b). The results were expressed as µg vitamin/100 g fat.

Statistical analyses. To study the different factors – month of ripening, seasonality, and the percentages of cow's, ewe's, and goat's milks, the SPSS package (Statistical Package for the Social Sciences) for Windows 15.0 was employed for all the samples analysed. The mean values of the quantitative variables were compared by analysis of variance (ANOVA) considering the differences as significant when *p* was lower than 0.05. With a view to studying which means were different, a Tukey's multiple comparison test was implemented.

RESULTS AND DISCUSSION

Table 2 shows the minimum, maximum, and mean concentrations together with the standard deviations (SD) of vitamins A and E contents in the samples of the cheese analysed. Of the 84 cheese analysed, three lacked detectable amounts (< 1 µg/100 g fat) of vitamins: two samples in the case of vitamin A and one sample of vitamin E. The variation in the range obtained for the total number of samples had lower values for vitamin A than those reported for the processed cheese from cows, buffalos and goats (BALESTRIERI *et al.* 2002, LUCAS *et al.* 2008b).

Figure 1 shows the mean values in the cheese samples whose composition included mainly cow's, ewe's, or goat's milks (this situation was considered when the cheese contained amounts equal to or greater than 75% of milk from each of these species). The mean concentrations found for vitamin A were 272.66 ± 52.75 , 156.48 ± 76.45 , and 189.21 ± 21.50 µg vita-

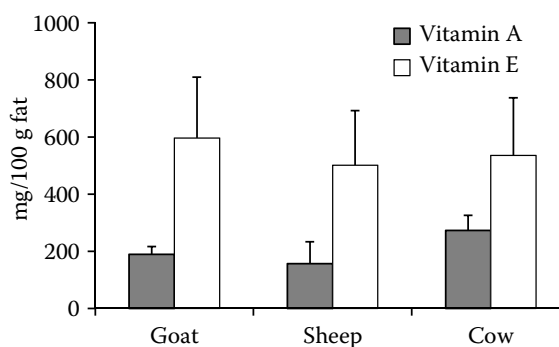


Figure 1. Mean concentration of vitamin A and E with respect to the type of milks (cows, ewes, goats) present in the highest amounts in the cheese

min/100 g fat depending respectively on the contents of cow's, ewe's, and goat's milks. For vitamin E, higher values were found, i.e. 535.91 ± 201.37 , 501.23 ± 191.40 , and 596.49 ± 213.59 µg vitamin/100 g fat. These results are in agreement with the findings of LUCAS *et al.* (2008b) who found higher mean values for vitamin E than for vitamin A in cow and goat milk cheeses. Similar results were obtained when the vitamin contents of sheep (PACCARD & LAGRIFFOUL 2006b) and cow milks (PACCARD & LAGRIFFOUL 2006a) were analysed, the vitamin E contents being much higher than those of vitamin A.

Since the cheese-making technology was exactly the same for all the cheeses studied, the vitamin losses during the process should be similar regardless of the milk type.

Indeed, previous works have revealed that the variability in the cheese composition with respect to fat soluble components (fatty acid, carotenoids, vitamins A and E) mainly depends on the conditions of the milk production (LUCAS *et al.* 2006b). Therefore, the cheese-making technology did not seem to be the reason for the differences between the vitamins A and E concentrations because previous works had reported a decrease in the initial total vitamin A content in milk fat up to 70% during the manufacture of cheeses while the transfer of vitamin E from milk to cheese did not exceed 30% (LUCAS *et al.* 2006b).

Owing to the higher average fat content in the sheep's milk an even higher proportion of lipophilic vitamins in the sheep's cheese compared to the cow's

Table 2. Chemical reference data

	<i>n</i>	Min	Max	Mean	SD
Vitamin A (µg/100 g fat)	82	16.36	366.83	207.20	78.75
Vitamin E (µg/100 g fat)	83	70.37	907.07	514.70	201.94

and goat's cheeses was expected. However, the results did not reveal statistically significant differences for either of these vitamins analysed, depending on the type of milk. Previous results from several authors do not allow any clear influence of species on lipolytic vitamins to be confirmed. Some authors have reported that, generally, ewe's milk contains higher amounts of vitamins (PARK *et al.* 2007), in particular vitamins A and E (RAYNAL-LJUTOVAC *et al.* 2008), than goat and cow milks. However, KONDYLI *et al.* (2012) found higher contents of these vitamins in cow's and sheep's milks than in goat's milk while other authors (PANDYA & GHODKE 2007; LUCAS *et al.* 2008b) have reported the opposite. Finally according to JANDAL (1996) sheep's and goat's milks show the highest values of vitamins A and E. Previous results suggest a stronger influence of diet than of species on vitamins A and E contents. The amounts of retinol and α -tocopherol secreted to ruminant milk fat depend directly upon their levels in the ration (SHARMA *et al.* 1983). All previous studies focused on the analysis of vitamin concentrations in milk and there are few references in the literature to the contents of these vitamins in cheeses.

The second factor studied here was cheese ripening. The cheeses were processed from the same batch of raw milk and ripened under strictly controlled conditions. Statistical analysis revealed that ripening had an effect ($P = 0.05$) on the concentration of vitamin A, as concerns both the month of ripening and the type of milk. In the case of ewe's and goat's cheeses, Figure 2 shows an increase in the vitamin A concentration up to the second month of ripening (8 weeks), and this was statistically significant from month 0 to month 2. Cow's milk cheeses showed a progressive decrease in the vitamin A content, which was statistically significant for month 0 of ripening,

after which a marked decrease in the concentration was observed.

Previous studies of Edammer cheese revealed a retention of retinol in 12-day-old Edammer cheese of 81% but further ripening led to a retention of about 40% (HULSHOF *et al.* 2006). However, in Gouda cheese it seems that the main losses of retinol occur during the first 12 days while the differences in the retinol content between young (8 week ripening) and mature Gouda cheeses (26 weeks ripening) were small, indicating that ripening does not result in a loss of retinol (HULSHOF *et al.* 2006).

In the case of vitamin E, the global analysis of the mean values did not show any significant effect ($P = 0.05$) of either the month of ripening or the type of milk employed. The analysis of the data for the different types of milk revealed that only cow's milk showed significant differences between month 0 and the other months of ripening for this vitamin.

The third factor studied, seasonality, was seen to have a significant effect on the concentration of vitamin A (200.70 $\mu\text{g}/100\text{ g}$ fat during winter and 212.79 $\mu\text{g}/100\text{ g}$ fat during summer) but not on the concentration of vitamin E. Seasonal differences in animal feeding practices may be the main cause for these differences in the nutrients content. The influence of the feeding treatment observed on milk retinol concentration could be due to a difference in retinol precursor concentration in the animal diet and/or a difference in bioavailability of the precursors in the animal body. The grazing herbage positively influences the *trans* retinol level in milk and seems to be the most critical factor vs. the higher the herbage intake, the higher the retinol concentration in milk in summer and winter (FEDELE *et al.* 2004). Thus, the contents of retinol are higher in the milk from goats reared in the pasture system than

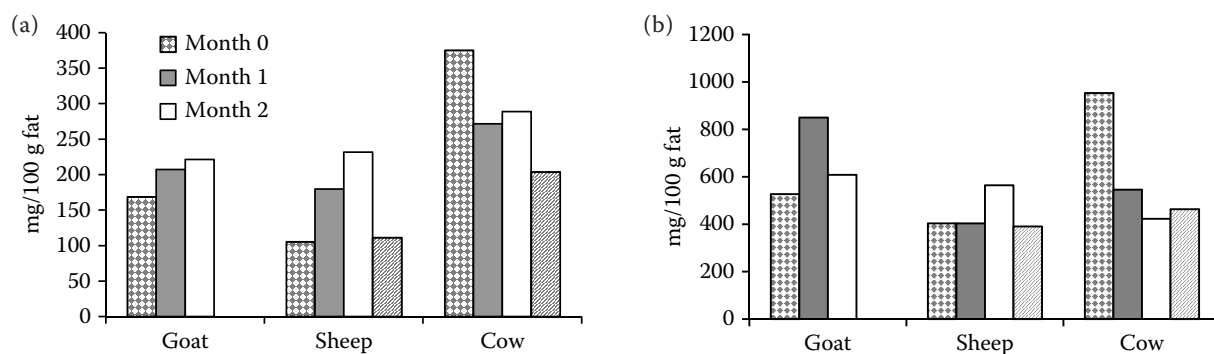


Figure 2. Evolution of the mean concentration of (a) vitamin A and (b) vitamin E during ripening with respect to the type or milk present at the highest amount in the cheese

indoors (MORAND-FEHR *et al.* 2007) which has also been observed for cows (COULON *et al.* 2005), while supplying concentrates to grazing goats reduced the retinol content in milk (FEDELE *et al.* 2004).

Previous results regarding the season effect showed that in general the highest values of retinol equivalents were found in the summer and early autumn (HULSHOF 2006). These results are in agreement with those of AGABRIEL *et al.* (2004) who found significantly higher contents of vitamins A and E in cow milk during the grazing period (May–September) than were those measured during winter (February–March). This was because in these works the animals mainly remained in the pasture during summer and autumn or, when not, were fed freshly cut grass, in addition to ensilage and pellets containing grains. During winter and spring remained in stables and were mainly fed with ensilage.

CONCLUSION

The results obtained in the present study reveal higher levels of vitamin E in all the cheeses analysed, regardless of the type of milk from which they were prepared. The cheeses made of cow's milks had higher vitamin A levels than those made with ewe's or goat's milk. However, the goat milk cheeses had the highest vitamin E contents. Vitamin A proved to be more influenced by the factors analysed in this study, with the observation of during summer and autumn significance of seasonality, and higher values found in the cheeses made in summer. In the cheeses made from ewe's and goat's milks, only vitamin A was seen to be affected by the length of ripening whereas in the case of cheeses made of cow's milk a decrease in the levels of both vitamins was observed over the ripening period.

References

- AGABRIEL C., FERLAY A., JOURNAL C., SIBRA C., TEISSIER D., GROlier, P., BONNEFOY J.C., ROCK E., CHILLIARD Y., MARTIN B. (2004): Composés d'intérêt nutritionnel de laits de grand mélange: teneurs en acides gras et vitamines selon l'altitude et la saison. *Rencontre Recherche Ruminants*, **11**: 51–54.
- BALESTRIERI M., BALESTRIERI M., STEFANIA SPAGNUOLOA M., CIGLIANO L., STORTIB G., FERRARAB L., ABRESCIAA P., FEDELE E. (2002): Evaluation of oxidative damage in mozzarella cheese produced from bovine or water buffalo milk. *Food Chemistry*, **77**: 293–299.
- BATES C.J., PRENTICE A. (1994): Breast milk as a source of vitamin essential minerals and trace elements. *Pharmacology & Therapeutics*, **62**: 193–220.
- BERGAMO P., FEDELE E., IANNIBELLI L., MARZILLO G. (2003): Fat-soluble vitamin contents and fatty acid composition in organic and conventional Italian dairy products. *Food Chemistry*, **82**: 625–631.
- CHILLIARD Y., LAMBERET G. (2001): Biochemical characteristics of goat milk lipids and lipolytic systems. A comparison with cow and human milk. Effects of lipid supplementation. In: FREUND G. (ed.): *Recent Advances on Goat Milk Quality, Raw Material for Cheesemaking*. ITPLC Editions, Surgères: 71–114.
- COULON J.B., DELACROIX-BUCHET A., MARTIN B., PIRISI A. (2005): Facteurs de production et qualité sensorielle des fromages. *INRA Productions Animales*, **18**: 49–62.
- FEDELE V., CLAPS S., RUBINO R., MANZI P., MARCONI S., PIZZOFERRATO L. (2004): Seasonal variation in retinol concentration of goat milk associated with grazing compared to indoor feeding. *South African Journal of Animal Science*, **34** (Suppl. 1): 148–150.
- GONZÁLEZ MARTÍN I., HERNÁNDEZ HIERRO J.M., MORÓN SANCHO R., SALVADOR ESTEBAN J., VIVAR QUINTANA A.M., REVILLA I. (2007): Determination of the percentage of milk (cow's, ewe's and goat's) in cheese with different ripening times using near infrared spectroscopy technology and a remote reflectance fibre-optic probe. *Analytica Chimica Acta*, **604**: 191–196.
- GOYAL K.C., SHARMA Y.K., MISTRA U.K. (1984): Enrichment of milk with vitamin A using provitamin A carotenoids in the diet of goats. *Nutrition Report International*, **29**: 397–404.
- HULSHOF P.J.M., ROEKEL-JANSEN T., BOVENKAMPY P., WESTZ C.E. (2006): Variation in retinol and carotenoid content of milk and milk products in The Netherlands. *Journal of Food Composition and Analysis*, **19**: 67–75.
- JANDAL J.M. (1996): Comparative aspects of goat and sheep milk. *Small Ruminant Research*, **22**: 177–185.
- KONDYLI E., SVARNAS C., SAMELIS J., KATSIARI M.C. (2012): Chemical composition and microbiological quality of ewe and goat milk of native Greek breeds. *Small Ruminant Research*, **103**: 194–199.
- LINDMARK-MANSSON H., ÅKESSON B. (2000): Antioxidative factors in milk. *British Journal of Nutrition*, **84** (Suppl. 1): 103–110.
- LUCAS A., HULIN S., MICHEL V., GABRIEL C., CHAMBA J.F., ROCK E., COULON J.B. (2006a): Relations entre les conditions de production du lait et les teneurs en composés d'intérêt nutritionnel dans le fromage: étude en conditions réelles de production. *INRA Productions Animales*, **19**: 15–28.
- LUCAS A., ROCK E., CHAMBA J.F., VERDIER-METZ I., BRACHET P., COULON J.B. (2006b): Respective effects of milk composition and cheese-making process on the cheese

- composition variability in components of nutritional interest. *Lait*, **86**: 21–41.
- LUCAS A., AGABRIEL C., MARTIN B., FERLAY A., VERDIER-METZ I., COULON J.B., ROCK E. (2006c): Relationships between the conditions of cow's milk production and the contents of components of nutritional interest in raw milk farmhouse cheese. *Lait*, **86**: 177–202.
- LUCAS A., ROCK E., AGABRIEL C., CHILLIARD Y., COULON J.B. (2008a): Relationships between animal species (cow versus goat) and some nutritional constituents in raw milk farmhouse cheese. *Small Ruminant Research*, **75**: 243–248.
- LUCAS A., COULON J.B., AGABRIEL C., CHILLIARD Y., ROCK E. (2008b): Relationships between the conditions of goat's milk production and the contents of some components of nutritional interest in Rocamadour cheese. *Small Ruminant Research*, **74**: 91–106.
- MCBEAN L.D., SPECKMAN, E.W. (1988): Nutritive value of dairy foods. In: WRONG N.P. (ed.): *Fundamentals of Dairy Chemistry*. Van Nostrand Reinhold Company, New York: 343–407.
- MORAND-FEHR P., FEDELE V., DECANDIA M., LEGRILEUX Y. (2007): Influence of farming and feeding systems on composition and quality of goat and sheep milk. *Small Ruminant Research*, **68**: 20–34.
- PACCARD P., LAGRIFFOUL G. (2006a): Synthèse bibliographique sur la composition du lait de brebis en composés d'intérêt nutritionnel. [Personal communication.]
- PACCARD P., LAGRIFFOUL G. (2006b): Synthèse bibliographique sur la composition des fromages de brebis en composés d'intérêt nutritionnel. [Personal communication.]
- PANDYA A.J., GHODKE K.M. (2007): Goat and sheep milk products other than cheeses and yoghurt. *Small Ruminant Research*, **68**: 193–206.
- PANFILI G., MANZI P., PIZZOFERRATO L. (1994): High-performance liquid chromatography method for the simultaneous determination of tocopherols, carotenes, and retinol and its geometric isomers in Italian cheese. *Analyst*, **119**: 1161–1165.
- PARK Y.W., JUÁREZ M., RAMOS M., HAENLEIN G.F.W. (2007): Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research*, **68**: 88–113.
- PUYOL P., PEREZ M.D., ENA J.M., CALVO M. (1991): Interaction of β -lactoglobulin and other bovine and human whey proteins with retinol and fatty acids. *Agricultural and Biological Chemistry*, **55**: 2515–2520.
- RAYNAL-LJUTOVAC K., LAGRIFFOUL G., PACCARD P., GUILLET I., CHILLIARD Y. (2008): Composition of goat and sheep milk products: An update. *Small Ruminant Research*, **79**: 57–72.
- SHARMA Y.K., GOYAL K.C., MISTRA U.K. (1983): Vitamin A and RBP contents of milk of goats given a single massive dose of vitamin A orally. *Nutrition Report International*, **28**: 593–596.
- ZAHAR M., SMITH D.E., MARTIN F. (1995): Vitamin A distribution among fat globule core, fat globule membrane, and serum fraction in milk. *Journal of Dairy Science*, **78**: 498–505.

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