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Seasonal changes in the technological and compositional quality of ewe's raw milks from commercial flocks under part-time grazing

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Rennet coagulation parameters, curd texture and gross compositional variables were studied in ewes' raw milk samples from nine commercial flocks using different concentrate : forage ratios and grazing times. From early lactation to March flocks were fed concentrate pellets and hay whereas from April to the end of lactation flocks were allowed to graze from 6 to 19 h/day receiving concentrate supplementation in the morning and evening. Milk from late-lactation flocks, when allowed to graze, showed higher content of fat, dry matter, protein, casein, soluble protein, total calcium, curd firmness and curd resistance to compression than the milk from early lactation flocks. Higher total calcium content and lower fat content were found when the early lactation flocks were fed high concentrate : forage ratio than when the flocks were fed low ratio. Curd firmness were lower for milk from flocks fed high concentrate : forage ratio, and the curd resistance to compression was greater, than for milk from flocks fed low ratio. At late lactation, when flocks grazed for a long time per day the total calcium content was higher than when the flocks grazed for a short time per day. Principal component analysis showed that protein and fat content were highly correlated with coagulum and curd firmness, whereas total calcium content was highly correlated with curd resistance to compression, and milk pH with rennet coagulation time.

Keywords: Seasonal changes, ewe's milk, milk composition, rennet coagulation, curd texture.

The production of ewe's milk is a traditional activity of most Mediterranean countries. The main use for sheep milk is for cheesemaking that is usually conducted at farm level or in small local dairies in most of these countries. Cheese quality depends closely on the composition and quality of milk, particularly for raw milk cheeses. Milk and cheese characteristics are mainly affected by breed, stage of lactation, health status and dietary factors. Studies on the effect of animal feeding on milk and cheese quality have mainly focused on the relationship between nutrient intake from the main feeding systems and the concentration of milk components (Coulon et al. 2004). Interesting reports have been recently published both on the physico-chemical characteristics of sheep milk, and on the influence of farming and feeding systems on composition and quality of sheep milk and sensory properties of cheese (Morand-Fehr et al. 2007; Park et al. 2007). It has been reported that increases in the content of protein and fat during lactation, or caused by the animal diet, produce changes in the rennet coagulation properties of milk, particularly increasing curd firmness (Malosini et al. 1996; Guinee et al. 1997). However, changes in rennet coagulation properties can be also affected by other upstream factors such as animal genetic characteristics, health status and physiological stage (Macheboeuf et al. 1993; Coulon et al. 2004).

In seasonal calving systems, such in those used for sheep flocks management, the effects of stage of lactation are confounded with those of season, i.e. the effects of variation in photoperiod, climate and weather, and in diet

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(Auldist et al. 1998; Walker et al. 2004). Feeding systems based on pasture may vary subject to the agro-climate conditions and different botanical species. Accordingly, depending on the available biomass and the nutritive value of the pasture, the use of feed supplements of various types has increased, particularly among local farmers resulting in increases in flock size (Jaeggi et al. 2005; Steinshamm et al. 2006). However, it has been reported that milk production in a semi-extensive feeding regime of part-time grazing and concentrate is comparable that in an intensive indoor regime (Min et al. 2005), and that supplementary feeding was useful for increasing the production and quality of milk from ewes under part-time grazing (D'Urso et al. 1993; García-Rodríguez & Oregui, 2004).

The objective of the present work was to report seasonal changes including stage of lactation and diet on compositional quality and technological aptitude of ewe's raw milk obtained from flocks under part-time grazing. The effect of concentrate : forage ratio was investigated in early lactation flocks whereas that of grazing time was studied in late-lactation flocks.

Materials and methods

Commercial flocks and milk samples

The experiment started in March in the Basque Country Region of northern Spain. Nine commercial flocks of *latxa* breed from farmhouses belonging to the Denomination of Origin of Idiazabal Cheese were selected, all the flocks having the same seasonal calving system. Flock size ranged between 200 and 400 ewes, with lambing periods extending approximately for 45 days and lactating lasting 4 to 5 months, form late winter to early summer. During the first part of the experiment (from the beginning of lactation to March), flocks were fed concentrate pellets and hay. Different commercial formulations of concentrate pellets were used in each farmhouse. Three farmhouses used a concentrate : forage ratio greater than 3 (Concentrate Fed-flocks), whereas for the other six farmhouses the ratio was lower than 1 (Forage Fed-flocks). During the second part of the experiment (from April to the end of lactation) the flocks were allowed to graze, receiving between 0·6 to 1 kg concentrate pellets per day and animal. In three farmhouses sheep grazed *ad libitum* for 16–19 h/day (Long Time Grazing-flocks) whereas in the other six farmhouses sheep grazed for 6–8 h/day (Short Time Grazing-flocks). Flocks were located at an altitude between 500 and 900 m. Flocks grazed both in intensively managed and fertilised grasslands dominated by ryegrass and in other community grasslands with a higher diversity of grass species. Samples of bulk milk from each flock (1·5 l) were taken in duplicate in early March when early lactation flocks were fed no pasture (Before Pasture-flocks) and at the end of May when late-lactation flocks were allowed to graze (After Pasture-flocks). Total number of milk samples was 36. Automatic milking machines were used in all farmhouses. Weather conditions during May were rather similar for all pasture locations, with average day temperature of 14·4 °C, relative humidity of 74·8% and precipitation of 2·9 l/m^2 per day.

Milk composition analysis

The amount (g/100 ml) of nitrogen fractions such as total nitrogen, total soluble nitrogen and non-protein nitrogen were directly determined by Kjeldahl procedure as described by Rowland (1938). Various protein fractions were converted into the corresponding amounts of protein (g/100 ml) by multiplying by 6·38. The amount (g/100 ml) of total fat was measured by the Gerber method according to IDF international standard 105 (1981). The amount of dry matter (g/100 g) was determined as described in IDF international standard 021B (1987). The pH of the milk samples was measured at 20 °C. The total calcium content (mg/l) was determined as described by De la Fuente et al. (1997) in an atomic absorption spectrometer (AAnalysit 200, Shelton, CN, USA) with a cathode lamp after wet mineralization of the milk samples assisted by microwave acid digestion in a laboratory microwave oven (MSP 1000, CEM, Matthews, NC, USA). Microwave digestion of milk samples was done in two steps. First step consisted of closed-vessel heating with full power under controlled pressure from 20 to 170 psig for 52 min, and the second step used full power under controlled pressure from 20 to 150 psig for 36 min. All analyses were made in duplicate.

Microbiological routine control of milks was done in the Dairy Institute of Lekunberri (Lekunberri, Spain). Total microbiological counts were lower than 50×10^3 cfu /ml, which indicated the high quality of the milks.

Rennet coagulation and curd texture

Commercial rennet powder (Naturen^{TM} Plus 1400 NB, CHR Hansen, Madrid, Spain) consisted of 80% (w/w) bovine chymosin and 20% (w/w) pepsin; the minimum rennet strength was 1300 IMCU/g of coagulant. Milk samples were coagulated at 32 °C using 3 mg rennet per 100 ml. The coagulation process was measured as described previously (Nájera et al. 2003) in a model NT Gelograph (Gel Instrumente AG, Thalwil, Switzerland) based on the near-infrared light absorption and scattering in the coagulating milk. Rennet coagulation time (min) was the time from rennet addition to the first appearance of an increase in viscosity of the coagulated milk. Coagulum and curd firmness were measured as the percentage of relative transmission (% RET) of the coagulated milk at the rennet coagulation time and at the cutting time (twice the rennet coagulation time), respectively. A higher % RET value equates to lower curd firmness. Gel firming rate was obtained by dividing the difference in firmness at cutting and rennet coagulation times by the time difference between

Table 1. Mean values and standard deviations of gross compositional variables, rennet coagulation parameters, and curd texture of ewe's raw milks from flocks according to season (diet and stage of lactation), concentrate : forage ratio and grazing time effects

	Season		Concentrate : forage ratio		Grazing time	
	Early lactation flocks	Late-lactation flocks	Early lactation flocks		Late-lactation flocks	
	Before Pasture-flocks	After Pasture-flocks	Concentrate Fed-flocks	Forage Fed-flocks	Long Time Grazing-flocks	Short Time Grazing-flocks
Total nitrogen (g/100 ml)	0·77 ± 0·05^a	0·84 ± 0·04^b	0·76 ± 0·06^a	0·78 ± 0·05^a	0·86 ± 0·05^a	0·83 ± 0·04^a
Total soluble nitrogen (g/100 ml)	0·15 ± 0·01^a	0·17 ± 0·02^b	0·15 ± 0·01^a	0·15 ± 0·01^a	0·17 ± 0·02^a	0·17 ± 0·02^a
Non-protein nitrogen (g/100 ml)	0·04 ± 0·00^a	0·04 ± 0·00^a	0·04 ± 0·00^a	0·04 ± 0·00^a	0·04 ± 0·00^a	0·04 ± 0·00^a
Protein (g/100 ml)	4·69 ± 0·35^a	5·14 ± 0·27^b	4·57 ± 0·40^a	4·75 ± 0·33^a	5·27 ± 0·30^a	5·08 ± 0·23^a
Casein (g/100 ml)	3·95 ± 0·35^a	4·27 ± 0·26^b	3·85 ± 0·42^a	4·00 ± 0·31^a	4·42 ± 0·27^a	4·20 ± 0·24^a
Total fat (g/100 ml)	6·10 ± 0·54^a	7·07 ± 0·55^b	5·75 ± 0·43^a	6·28 ± 0·51^b	7·10 ± 0·59^a	7·05 ± 0·56^a
Dry matter (g/100 g)	16·29 ± 0·63^a	17·75 ± 0·76^b	15·90 ± 0·67^a	16·48 ± 0·54^a	18·01 ± 0·86^a	17·62 ± 0·71^a
Total calcium (mg/l)	1514·76 ± 241·76^a	1664·72 ± 189·70^b	1719·00 ± 197·66^a	1412·65 ± 196·07^b	1796·92 ± 117·00^a	1598·63 ± 187·34^b
pH	6·68 ± 0·09^a	6·69 ± 0·07^a	6·68 ± 0·09^a	6·68 ± 0·09^a	6·72 ± 0·04^a	6·68 ± 0·08^a
Rennet coagulation time (min)	13·53 ± 1·92^a	12·85 ± 1·97^a	14·57 ± 1·07^a	13·01 ± 2·07^a	12·88 ± 1·64^a	12·84 ± 2·19^a
Coagulum firmness (%RET)	6·14 ± 0·38^a	5·09 ± 0·41^b	6·41 ± 0·37^a	6·00 ± 0·31^b	5·07 ± 0·56^a	5·10 ± 0·33^a
Curd firmness (%RET)	4·26 ± 0·28^a	3·59 ± 0·29^b	4·44 ± 0·26^a	4·17 ± 0·25^b	3·53 ± 0·33^a	3·63 ± 0·28^a
Gel firming rate (%RET/min)	0·15 ± 0·01^a	0·13 ± 0·00^b	0·14 ± 0·03^a	0·15 ± 0·02^a	0·12 ± 0·02^a	0·12 ± 0·02^a
Curd resistance to compression (g)	86·54 ± 9·30^a	97·11 ± 9·43^b	95·54 ± 3·46^a	82·05 ± 7·86^b	100·27 ± 11·65^a	95·53 ± 8·22^a

[a,b] Means followed by a different letter were significantly ($P \leqslant 0{\cdot}05$) different between flocks under each effect

these two points (% RET/min). Rennet coagulation parameters were determined in duplicate.

Curd texture was analysed by a compression test using a model TA.XT2i Texture Analyser (Stable Micro Systems, Surrey, UK) equipped with a local cell of 5 kg and P/25 probe. Milk samples were coagulated as described above, and once the coagulation process reached the cutting time the curd resistance to compression (g) was measured. Curd samples were compressed at room temperature to 50% of their original height using a cylindrical sample probe (contact area of 490·87 mm^2) and a surface sensitive force of 0·005 kg/m s^2. The force *vs.* time plots were recorded using the Texture Expert[TM] software with crosshead moved at a constant speed of 12 mm/min. Four measurements were performed for each milk sample.

Statistical analysis

SPSS statistical package, version 16.0 (SPSS Inc., Michigan, USA), was used for the statistical analysis. Analysis of variance (ANOVA) was used to determine the presence of significant ($P \leqslant 0{\cdot}05$) differences in the analytical variables between milks from Before Pasture-flocks and After Pasture-flocks. Milk pH was used as covariate when coagulation parameters were studied. Mixed linear model was used including 'season' as fixed effect nested within 'flock' as random effect when the interaction term 'season*flock' was not significant. F-test of the 'season' against the interaction term 'season*flock' was used when this interaction was significant ($P \leqslant 0{\cdot}05$). F-statistic was also used to determine the presence of significant differences ($P \leqslant 0{\cdot}05$) in the analytical variables either between milks from Concentrate Fed-flocks and Forage Fed-flocks or between milks from Long Time Grazing-flocks and Short Time Grazing-flocks. Principal component analysis (PCA) was performed on a matrix of the compositional variables, coagulation parameters and curd texture of the milk samples using the Kaiser criterion (eigenvalue >1) to select the principal components. Factors were rotated (Varimax method) for ease of interpretation.

Results and discussion

Table 1 shows the content of gross compositional variables of milk samples from Before Pasture-flocks (early lactation flocks) and from After Pasture-flocks (late-lactation flocks). Significant differences ($P \leqslant 0{\cdot}05$) were found for most of the gross compositional variables. As expected, as lactation period progressed the content of total nitrogen, total soluble nitrogen, protein, casein, total fat and dry matter was higher in milk from After Pasture-flocks than in milk from Before Pasture-flocks. The content of total nitrogen, protein and casein increased around 9% when flocks grazed on pastures, whereas the content of total soluble protein including whey proteins and other minor proteins increased above 13%. It has been reported that seasonal changes, mainly stage of lactation, diet and flock management, affect the composition of sheep milk (Perea et al. 2000; Barron et al. 2001; Pulina et al. 2006). The compositional parameter that increased most when the late-lactation flocks grazed on pastures was total fat (around 16%). It is well-known that as lactation progresses the content of fat and protein increase in sheep milk (Coulon et al. 1998). Some authors have reported higher casein and soluble protein content in milk from cows fed on pasture outdoors (Berry et al. 2001). Other authors have reported that fat and protein content in sheep milk decreased as the lactation period progressed because of hot weather and poorer quality pastures (Jaeggi et al. 2005). The content of non-protein nitrogen, which includes mainly urea, creatin and free amino acids (Park et al. 2007), and pH did not significantly ($P>0{\cdot}05$) vary between Before Pasture-flocks and After Pasture-flocks (Table 1). Other authors found significant increments ranging from 0·02 to 0·04 units in milk pH when cows were fed on pasture (Macheboeuf et al. 1993).

The content of total calcium was significantly ($P\leqslant0{\cdot}05$) higher in After Pasture-flocks than in Before Pasture-flocks showing an increment over 9% when late-lactation flocks grazed (Table 1). Khan et al. (2006) reported an increase from 551 to 990 mg/ml in total calcium content when comparing milk from late-lactation ewes feeding indoors and grazing for 2–5 h. Contradictory results have been reported for changes in total calcium content in sheep milk during the lactation period (Pellegrini et al. 1994; Coulon et al. 1998). The differences observed in the seasonal fluctuations in total calcium content of ewe's milk can be attributed to breed, diet, individual animal stage or status of udder health (Park et al. 2007).

Significant differences ($P\leqslant0{\cdot}05$) in most of the coagulation parameters were found between milks from Before Pasture-flocks and After Pasture-flocks (Table 1). Coagulum firmness (% RET) decreased around 17% when late-lactation flocks grazed and curd firmness (% RET) decreased over 15%. These values (% RET) indicated that the curd made with milk from After Pasture-flocks presented higher firmness than that made with milk from Before Pasture-flocks. Also, the curd resistance to compression increased over 12% in the milk from After Pasture-flocks. Rennet coagulation time did not significantly ($P>0{\cdot}05$) change when the flock feeding regime changed and the lactation period progressed, but the gel firming rate (% RET min^{-1}) decreased around 13% in milk samples taken when late-lactation sheep had been grazing for at least one month (After Pasture-flocks; Table 1). Several authors have reported that increments in the content of protein and fat as lactation progressed, or caused by diet, produce higher curd firmness and lower rennet coagulation time (Guinee et al. 1997; Auldist et al. 2002). In our work, the observed increase (around 5%) in rennet coagulation time in milk from After Pasture-flocks was not statistically significant ($P>0{\cdot}05$). Some authors reported higher curd firmness and lower rennet coagulation time in milks from cows with a part-time grazing regime than in milks from cows fed concentrate and forage, regardless the stage of lactation (Berry et al. 2001). However, these changes did not appear to result entirely from the parallel increase in milk protein content (Macheboeuf et al. 1993). Other authors have reported no significant differences in composition and rennet coagulation properties between milk samples from grazing dairy cows and milk samples from cows with different feeding regimes (Bovolenta et al. 2002). These divergent results imply that in addition to diet and stage of lactation, there must be other as yet unidentified factors that affect rennet coagulation time and curd firmness. No study on changes in rennet coagulation properties of ewe's milk due to changes in diet, particularly pasture feeding, has been found in the literature.

In early lactation flocks, significant differences ($P\leqslant 0{\cdot}05$) between milks from Concentrate Fed-flocks and from Forage Fed-flocks were only found for two of the compositional variables studied: total fat and total calcium (Table 1). Total fat increased over 9% whereas total calcium decreased nearly 18% when flocks were fed low concentrate : forage ratio (Forage Fed-flocks). It has been reported that changes in the diet of animals fed indoor due to different nutrient intake or nature of forage influence ewe's milk composition (Chilliard & Ferlay, 2004; Pulina et al. 2006; Sanz Sampelayo et al. 2007). Several authors have reported increases in the fat content of ewes' milk when supply of concentrates in diet increases (Morand-Fehr et al. 2007). Other authors did not find significant differences ($P>0{\cdot}05$) in the composition of milk from cows consuming different concentrate : forage ratios (Malossini et al. 1996). Coagulum and curd firmness were the rennet coagulation parameters that significantly varied between Concentrate Fed-flocks and Forage Fed-flocks (Table 1). Coagulum and curd firmness (% RET) decreased over 6% when flocks were given feed with a low concentrate : forage ratio. As described in Materials and Methods, the higher value of % RET, the lower firmness, and in consequence, the curds made with milks from Forage Fed-flocks were firmer than those made with milk from Concentrate Fed-flocks. However, the curd resistance to compression significantly ($P\leqslant0{\cdot}05$) decreased by over 14% in the milks from Forage Fed-flocks (Table 1). As several authors have pointed out, and as it will be pointed out in the next section, increments in fat content and calcium content of the milk can produce increments in curd firmness and in gel aggregation rate, respectively (Guinee et al. 1997).

In late-lactation flocks, significant differences ($P\leqslant0{\cdot}05$) were found only for total calcium content which was over 11% higher in Long Time Grazing-flocks than in Short Time Grazing-flocks (Table 1). It has been reported that a longer daily grazing time does not induce systematically an increase in level of intake, and in consequence increments in nutrient content of the milk (Morand-Fehr et al. 2007). Therefore, in addition to the longer grazing time, other factors such as individual animal stage or status of

Table 2. Rotated factor loadings for principal components (PC) 1, 2, 3 and 4 as applied to compositional variables, rennet coagulation parameters and curd texture of ewe's raw milks from Before Pasture-flocks (early lactation flocks) and After Pasture-flocks (late-lactation flocks). Factor loadings lower than |0·350| are set to 0

Variable	PC1[a]	PC2[b]	PC3[c]	PC4[d]
Protein	0·939			
Curd firmness	−0·937			
Coagulum firmness	−0·934			
Total nitrogen	0·932			
Dry matter	0·921			
Total fat	0·869			
Casein	0·861			
Gel firming rate	−0·586	−0·470	−0·499	
pH		0·817		
Rennet coagulation time		0·812		
Non-protein nitrogen			0·810	
Total soluble nitrogen	0·502	0·357	0·681	
Total calcium				0·858
Curd resistance to compression				0·778

[a] 47·3% variance; [b] 13·0% variance; [c] 11·5% variance; [d] 10·8% variance

udder health could increase the calcium content in the milk from Long Time Grazing-flocks (Celik & Ozdemir, 2003; Park et al. 2007). None of the rennet coagulation parameters showed significant differences ($P>0\cdot05$) between Long Time Grazing-flocks and Short Time Grazing-flocks (Table 1).

Principal component analysis

Principal component analysis (PCA) was applied to gross compositional variables, rennet coagulation parameters and curd texture. Four PCs accounting for 82·6% of the total variance described the variation in the compositional quality and technological aptitude of milks from flocks managed under part-time grazing (Table 2).

Compositional variables such as total nitrogen, protein, casein, total fat and dry matter content showed high positive loadings (>0·860) with PC1 whereas rennet coagulation parameters such as coagulum and curd firmness showed high negative loadings with this factor. Gel firming rate also showed negative loading (−0·586) with PC1. As above mentioned, the higher value (% RET) for coagulum or curd firmness, the lower firmness, and in consequence, both rennet coagulation parameters together with gel firming rate showed negative correlation with gross compositional variables in PC1 (Table 2). Significant ($P\leqslant 0\cdot05$) positive correlations have been found between the content of fat, protein, casein or total solids of cow's milk and curd firmness and curd firming rate (Auldist et al. 2004). Several authors have reported that the content and type of caseins affect the curd firmness (Auldist et al.

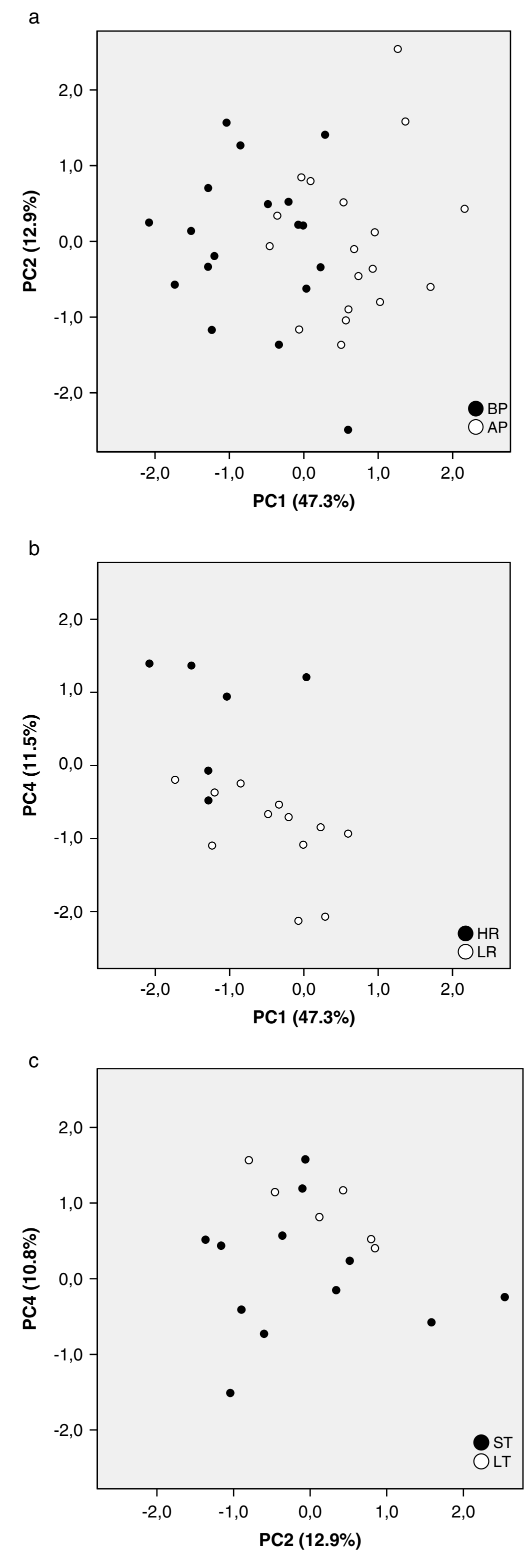

Fig. 1. Plots depicting milk sample distributions (factor score mean values) in the two-dimensional coordinate systems defined by (1a) PC1 and PC2, (1b) PC1 and PC4, and (1c) PC2 and PC4. AP: After Pasture-flocks; BP: Before Pasture-flocks; HR: Concentrate Fed-flocks; LR: Forage Fed-flocks; LT: Long Time Grazing-flocks; ST: Short Time Grazing-flocks.

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2002; Lucey et al. 2003) and that increasing the levels of milk fat the renneting properties are enhanced (Guinee et al. 1997). Accordingly, this factor was defined as 'gel firming factor'.

Rennet coagulation time and pH showed high positive loadings (>0·810) with PC2 (Table 2). Martin & Coulon (1995) found a strong correlation between bovine milk clotting time and the pH of the milk, regardless feeding practices during lactation; the influence of pH on rennet coagulation time is very strong because it affects chymosin activity for the hydrolysis of κ-casein (Hyslop, 2003). Accordingly, PC2 was defined as 'enzymic activity factor'.

Total calcium content and curd resistance to compression were highly correlated (>0·775) with PC4 (Table 2). It has been reported that calcium bridges are involved in the aggregation of casein micelles during the non-enzymatic phase of milk coagulation (Lucey, 2002). Accordingly, this factor was defined as 'gel aggregation factor'. Non-protein nitrogen and total soluble nitrogen content were positively correlated with PC3 which pointed out that these nitrogen fractions did not significantly affect the rennet coagulation process or curd textural properties. In rennet-induced gels, most of the serum which contains non-protein compounds and soluble proteins is lost as whey after the curd is cut (Lucey et al. 2003).

Figure 1 depicts milk sample distributions in two-dimensional coordinate systems defined by PC1, PC2 and PC4. Most milk samples from After Pasture-flocks (late-lactation flocks) or Before Pasture-flocks (early lactation flocks) could be distinguished by the 'gel firming factor' (PC1) in the coordinate system defined together with PC2. Therefore, the part-time grazing together with the stage of lactation of the flocks influenced the curd firming which strongly depends on milk composition. Most milk samples (early lactation flocks) from Concentrate Fed-flocks and Forage Fed-flocks could be distinguished by the 'gel aggregation factor' (PC4) in the coordinate system defined together with PC1. When flocks were managed indoors the content of total calcium in milk increased with the concentrate:forage ratio, and, as a result, curd resistance to compression also increased. Most milk samples (late-lactation flocks) from Long Time Grazing-flocks and Short Time Grazing-flocks could be distinguished by the 'gel aggregation factor' (PC4) in the coordinate system defined together with PC2. Then, when flocks were allowed to graze, the grazing time increased the total calcium content of the milk, but no increase in curd resistance to compression was observed.

In summary, in milks from early lactation flocks, the higher concentrate:forage ratio, the higher calcium content and the lower fat percentage. In milks from late-lactation flocks, the longer grazing time, the higher calcium content. When early lactation flocks fed indoors were compared with late-lactation flocks under part-time grazing, higher content of protein, fat and calcium were found. These changes were responsible for variations in rennet coagulation properties and curd texture, which will affect ultimately rheological and sensory properties of cheese.

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