Salt Balance in Ewe's and Goat's Milk during Storage at Chilling and Freezing Temperatures

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This article examines the change in salt balance in the soluble fraction of ewe's and goat's raw milk over storage periods from 1 to 7 days, at 3 and 7 °C. It also examines the balance of salts in identical samples frozen and stored at −18 °C for 3 months. Renneting properties were determined in all treatments. Phosphorus and divalent cations contents of the soluble phase increased over chilled storage, particularly in goat's milk. In this milk, after storage for 2 days at 3 °C, the levels of serum Ca, Mg, and P had increased by 10.3, 3.1, and 9.4%, respectively. As pH dropped, after 4 days, there was a considerable increase in the presence of these elements in the soluble phase. In milk samples of both species kept in frozen storage, colloidal phosphorus, calcium, and magnesium contents increased slightly. Renneting properties were less affected in frozen than in chilled samples.

Keywords: *ewe's and goat's milk; mineral balance; refrigeration; freezing.*

INTRODUCTION

Ewe's and goat's milk are currently gaining considerably in economic importance, particularly in Mediterranean countries, as a result of growing acceptance of products made from them—chiefly cheeses. Collection at regular intervals and cold storage on the farm to improve the bacteriological quality of the raw material is now normal practice.

The balance of soluble and colloidal salts plays a fundamental role, in that it affects to the coagulation properties of the milk with the rennet and the physical characteristics of the curds (Qvist, 1979; Shalabi and Fox, 1982). It is well-known that cold storage gives rise to changes in the colloidal and soluble phases and likewise in aptitude to coagulate (Reimerdes and Klostermeyer, 1976; Qvist, 1979; Ali et al., 1980). Although it has been reported that the changes in the pH play also a important role in the milk salts equilibrium (Shalabi and Fox, 1982; Van Hooydonk et al., 1986; Dalgleish and Law, 1989; Le Graet and Brulé, 1993), its influence can be limited in milk with high microbiological quality (Guinot-Thomas et al., 1995). The distribution of salts and alteration of their balance through lower storage temperatures have been investigated and reviewed in cow's (Qvist, 1979; Ali et al., 1980; Brulé and Fauquant, 1982; Holt, 1985; Dalgleish and Law, 1989) and buffalo's milk (Sindhu and Roy, 1982). The effects of refrigeration after heating on the milk salt and their interaction with casein has also been studied and reviewed (Pouliot et al., 1989; Holt, 1995). There is however relatively little information on this distribution in milk from ewes and goats and practically none at all on alterations during low-temperature storage.

The milk output of goat and sheep is seasonal; therefore, to maintain cheese production throughout the year, a number of researchers (Addeo et al., 1992; Voutsinas et al., 1995) have proposed frozen storage of milk during periods of peak output, for thawing when output drops. Despite these references dealing with the quality characteristics of cheeses made with frozen milk, there is no literature on salt balance in nonbovine species as a consequence of this process.

This article examines the minerals in the soluble fraction of ewe's and goat's raw milk during cold storage at two different temperatures (3 and 7 °C) and their evolution over 7 days considering the natural acidification. It also examines the effects of the freezing and frozen storage process on salt contents in the soluble fraction of milk from either species. It further examines the way that these treatments affect the aptitude of the milk to coagulate.

MATERIALS AND METHODS

Samples. Raw pooled ewe's and goat's milk used for this study was from flocks in the central region of Spain. Freshly drawn milk was shipped to the laboratory in less than 1 h in isothermal containers. For each of the treatments assayed, four aliquots were taken from either species.

Treatment of Samples. Untreated aliquots of each sample were chilled at 3 ± 1 and 7 ± 1 °C in cold chambers. Over the storage period, controls were carried out (1, 2, 4, and 7 days) on the different elements. Samples were skimmed following heating (37 °C) and centrifugation (1540*g*; 10 min).

In addition, 250-mL volumes of ewe's and goat's milk, skimmed and nonskimmed, were vacuum-packed in plastic bags and frozen, by following two different procedures: (a) with liquid nitrogen at −80 °C (fast freezing) and (b) in a multiple-plate freezer at −35 °C (slow freezing). Following freezing, all of these samples were stored in freezers at −17 ± 1 °C for 90 days. Samples were thawed over 24 h at 5 °C and left at 22 ± 3 °C for a further 45 min.

In addressing the study of low-temperature storage, the question arose of whether to chill the milk whole or skimmed, given that analytical determinations were to be carried out on samples without fat. Since the fat separation process entails prior warming of the milk at between 30 and 37 °C and this could alter the salt balance, preliminary assays were performed to verify possible alterations to this balance as a consequence of warming (Figure 1). Aliquots of a single sample each of ewe's and goat's milk, both whole and skimmed (following warming at 37 °C), were kept chilled for 4 days at 4 °C (paths A and B, respectively, in Figure 1). At the end of this period, the soluble phase was separated by ultracentrifugation and calcium and magnesium contents were determined (the whole milk was skimmed before ultracentrifugation). At the same time, a fraction of the skimmed milk stored for 4 days was warmed at 37 °C immediately prior to ultracentrifugation and a soluble fraction obtained in order to ascertain the extent to which this second warming affected distribution of calcium and magnesium (path C in Figure 1).

Analytical Methods. Psychrotrophic bacteria counts were performed using standardized procedures (International Dairy Federation, 1981).

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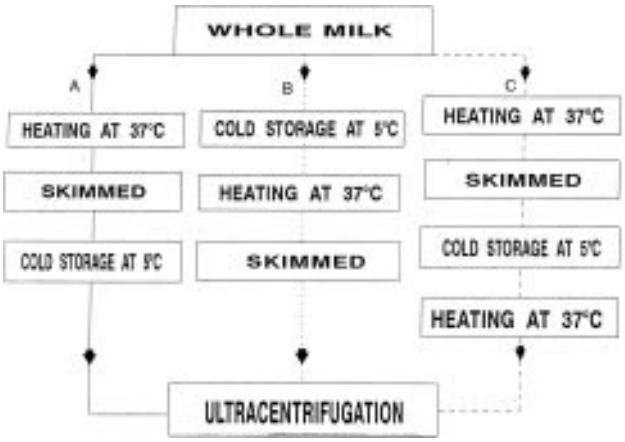

Figure 1. Scheme of the preliminary refrigeration assays.

The soluble phase was separated by high-speed centrifugation in a Sorvall Combi Plus ultracentrifuge (Wilmington, Delaware): 30 mL of milk was centrifuged (100000*g*) at 20 °C for 1 h using a 50-RT-1250 rotor. The supernatant was carefully removed and vacuum-filtered through Whatman no. 40 paper.

P, Ca, Mg, Na, K, ionic calcium, and pH were determined in skimmed samples. P was determined colorimetrically by the molybdenum blue method (de la Fuente and Juárez, 1995a), Ca, Mg, Na, and K by flame atomic spectrometry (de la Fuente and Juárez, 1995b), and ionic calcium by selective electrode in skim milk as described Geerts et al. (1983). The analytical methodology is more widely described in a previous paper (de la Fuente et al., 1996).

Determination of Renneting Properties. Evolution of coagulation time and coagulum characteristics were analyzed using a model D thromboelastograph (Hellige GmbH, Freiburg, Germany). This instrument draws a *firmness versus time* diagram as clotting occurs. Rennet clotting time represented by *r* indicates time from addition of enzyme until two lines diverge. The time in minutes from *r* until two lines are 20 mm apart represents the rate of curd formation (k_{20}). The curd at this time is firm enough for cutting. A_m corresponds to the measurement of the greatest width in millimeters between both lines. It represents the curd firmness.

To determine in goat's milk the rate of curd formation the distance applied was 10 mm in place of 20 mm due to the characteristics of its coagulum.

The whole milk sample for the determinations was tempered at 32 ± 1 °C, and 2% $CaCl_2$ was added (4% v/v). After 10 min at this temperature, animal rennet (0.4% v/v) (Chr. Hansen, Denmark, strength = 1:50 000) prepared in solution at 2.5% was added. The mixture was stirred, then immediately 0.8 mL was taken with a syringe and added to each thromboelastograph cuvette. The apparatus was then started up, the analysis being completed in 40 min.

Statistical Analysis. Results were analyzed by means of a multiple-range analysis, using the LSD (least significance difference) test with a 95% confidence interval for the comparison of the test means.

RESULTS AND DISCUSSION

Effect of Heating Prior to Skimming and the Addition of Preservative. Table 1 shows the soluble calcium and magnesium contents at 0 and 4 days for the different treatments. Release of calcium and magnesium into the serum was greater in milk that was warmed to 37 °C, skimmed then chilled (path A in Figure 1), than in milk that was stored whole then warmed to 37 °C before skimming (path B in Figure 1). This effect was more pronounced in goat's than in ewe's milk. By storing milk whole instead of skimmed, almost 3.1% more of total calcium in goat's milk and over 0.7% more in ewe's milk was retained in the colloidal phase (Table 1). Where milk was warmed both before and after chilling (path C in the Figure 1), part of the calcium solubilized during chilling was recovered in the colloidal phase. This was the more appreciable in goat's milk, where chilling had caused more displacement of Ca and Mg. During storage, 4.8% of the total Ca and 7% of the total Mg moved to the soluble fraction, whereas after the final warming, 3.4 and 5.2% of Ca and Mg, respectively, remained in the soluble fraction. These changes were less pronounced in ewe's milk, where even magnesium content was not altered by chilling of previously skimmed sample. These findings give an idea of the importance of the effect that temperature alterations have on the salt balance of milk, however small these alterations may be in extent and duration.

In view of the fact that acidification has a major influence on salt balance and the effects of cold storage can be confused, a further prior experiment was performed. Aliquots of a single whole milk sample containing psychotrophic bacteria levels of 10^4 cfu/mL considered of acceptable bacteriological quality were stored for 4 days at 4 °C, with and without added preserving agent. The agent used was chloroform (0.25% v/v), as recommended by Davies and White (1960), which can be kept with no detectable change in milk composition. The results are given in Table 2. As the table shows, the samples did not differ significantly in statistical terms of soluble Ca and Mg content, as reported in cow's milk by Guinot-Thomas et al. (1995), although there was a slight fall of pH in samples with no added preservative. On the other hand, Ca and Mg values in the soluble phase did increase with respect to the control (0 days) after 4 days chilled storage at 4 °C (with and without preservative). This increase should therefore be attributed to cold storage.

The milk kept in cold storage by the dairy industry is whole milk, and therefore, the rest of the chilling assays were carried out using whole milk (see path B, Figure 1). On the other hand, the milk was stored without preservative in order to ascertain the effect of natural souring in the last stage of storage.

Change in Salt Balance over Chilling Period. Table 3 shows the change in salt balance at 3 and 7 °C in ewe's and goat's milk over 1 week of storage. During the first day of storage, there was no appreciable increase in any of these elements in the soluble fraction of ewe's milk or of Mg in goat's milk. Ca and P in the soluble fraction of goat's milk stored at 3 °C increased by 3.8 and 1.5%, respectively. After 2 days, at that temperature, the changes were more appreciable, particularly in goat's milk (Ca, Mg, and P in the soluble phase increased by 10.3, 3.1, and 9.4%, respectively). In ewe's milk soluble Ca and P increased 4.8 and 6.1%, respectively. Since, at 3 °C, the changes in the pH were negligible during the first days of storage (Figure 2) especially in ewe's milk, the increases in the soluble salt in this period should be attributed to the temperature.

In the goat's milk stored 2 days at 7 °C, the increase in soluble Ca was smaller (4.8%) than in sample stored at 3 °C. The increases in P and Mg were comparable (2.1 and 9.1% in 7 °C sample). In ewe's milk, the changes in Ca and Mg in sample stored at 7 °C were similar to those in sample stored at 3 °C. However the increase in P was smaller (1.5%).

Table 1. Mean Values[a] and Standard Deviation (mg L^{-1}) of Total and Soluble Calcium and Magnesium Content (mg L^{-1}) of Different Treatments[b] of Ewe's and Goat's Milk Chilled at 4 °C for 4 Days[c]

			soluble fraction			
species	element	total content	control	treatment A	treatment B	treatment C
ewe	calcium	2161 ± 23	406 ± 4[a]	437 ± 3[b]	421 ± 2[c]	427 ± 5[d]
	magnesium	212 ± 3	120 ± 1[a]	121 ± 1[a]	120 ± 1[a]	120 ± 1[a]
goat	calcium	1343 ± 11	417 ± 4[a]	481 ± 9[b]	439 ± 5[c]	462 ± 4[d]
	magnesium	115 ± 2	83 ± 1[a]	91 ± 1[b]	86 ± 1[c]	90 ± 1[b]

[a] Each datum is the mean value of three determinations. [b] Treatments A, B, and C correspond to the same paths of the Figure 2. [c] Different letters in the same row indicate significant different ($p \leq 0.05$).

Table 2. Mean Values and Standard Deviation[a] (in Percent) of Soluble Calcium and Magnesium in Goat's and Ewe's Raw Milks (Control) and Stored at 4 °C over a Period of 4 Days with (Treatment 1) and without (Treatment 2) Addition of Chloroform 0.25% (v/v) as Preservative[b]

	ewe's milk			goat's milk		
	Ca	Mg	pH	Ca	Mg	pH
control	23.4 ± 0.4[a]	59.7 ± 0.7[a]	6.65	33.1 ± 0.6[a]	72.3 ± 0.6[a]	6.63
treatment 1	24.8 ± 0.5[b]	64.0 ± 0.7[b]	6.60	34.6 ± 0.6[b]	74.8 ± 0.6[b]	6.61
treatment 2	24.8 ± 0.4[b]	63.8 ± 0.4[b]	6.57	35.7 ± 0.7[b]	75.2 ± 0.8[b]	6.53

[a] Each datum is the mean value of three determinations. [b] Different letters in the same column indicate significant different ($p \leq 0.05$).

Table 3. Mean Values and Standard Deviation[a] (mg/L) of Total and Soluble Calcium, Magnesium, and Phosphorus during the Cold Storage at 3 and 7 °C over a Period of 7 Days in Goat's and Ewe's Milk

	ewe			goat		
	Ca	Mg	P	Ca	Mg	P
total content	2351 ± 5	202 ± 1	1582 ± 35	1468 ± 6	133 ± 1	862 ± 13
soluble	477 ± 12	115 ± 1	611 ± 5	497 ± 5	97 ± 1	351 ± 6
3 °C						
1 day	473 ± 11	113 ± 1	620 ± 3	516 ± 13	98 ± 1	355 ± 16
2 days	500 ± 4	115 ± 1	648 ± 3	548 ± 2	100 ± 1	384 ± 6
4 days	517 ± 3	115 ± 1	665 ± 10	550 ± 5	100 ± 1	410 ± 11
7 days	637 ± 8	129 ± 1	716 ± 19	598 ± 8	102 ± 1	464 ± 2
7 °C						
1 day	463 ± 14	115 ± 1	614 ± 10	512 ± 4	97 ± 1	357 ± 6
2 days	499 ± 6	115 ± 1	620 ± 10	521 ± 5	99 ± 1	383 ± 7
4 days	644 ± 8	127 ± 1	702 ± 9	771 ± 3	112 ± 1	462 ± 20
7 days	1592 ± 9	178 ± 1	1413 ± 38	1315 ± 28	132 ± 2	668 ± 14

[a] Each result is the average of three determinations.

Such solubilization of Ca and P during cold storage has previously been reported by different authors and have been reviewed by Holt (1985) in cow's milk, but there are no references for ewe's or goat's milk. Davies and White (1960), working with skimmed milk refrigerated at 3 °C for 1 and 2 days, found that the concentrations of phosphorus and calcium in the soluble phase increased by 4 and 7%, respectively. Greater changes still were reported by Brulé and Fauquant (1982) when partitioning calcium in bovine milk concentrated by ultrafiltration during storage periods of several hours at 2 °C. Ali et al. (1980) also reported increases of Ca and P in the soluble phase which were greater than those found in the present study for ewe's and goat's milk over the first 2 days of storage at 3 and 7 °C. Davies and White (1960) and Ali et al. (1980) added preservatives to the milk to prevent bacterial growth and, in this way, avoided the effects of the natural acidification. Although, more recently, Dalgleish and Law (1989) reported that the composition of the micellar calcium phosphate and the manner of its dissociation appeared to be largely unaffected by the temperature of the milk, they also reported that calcium bound to casein can be reduced at 4 °C.

The change in pH and ionic calcium during storage is shown in Figure 2. Over the 1 week in storage, pH declined, resulting in a progressive increase of ionic calcium. In ewe's milk, pH dropped from 6.58 to 5.89 (3 °C) and 5.20 (7 °C). In goat's milk, pH dropped from 6.62 to 6.18 (3 °C) and 5.21 (7 °C). It should be noted, however, that the drop in pH (Figure 2) was in fact negligible during the first 2 days or so, especially at 3 °C. Ionic calcium levels reflected alterations in the balance of calcium between the two phases, and concentration increased as pH declined. Acidification of milk, whether the result of direct addition with CO_2 or an acid or of bacterial activity, is always accompanied by progressive solubilization of colloidal calcium phosphate and of the cations linked to the casein complex (Van Hooydonk et al., 1986; Dalgleish and Law, 1989; Le Graet and Brulé, 1993; Ould Eleya et al., 1995). Through natural acidification of milk with release of lactic acid until pH reaches 4.6, almost all the colloidal calcium and all the inorganic phosphorus can be solubilized before isoelectric precipitation of the caseins, which remain virtually free of salts (Le Graet and Brulé, 1993).

In the present study, the milks with the lowest pH (those stored at 7 °C for 4 days or more) exhibited the highest levels of soluble elements at the end of storage. Most studies in cow (Van Hooydonk et al., 1986; Dalgleish and Law, 1989; Gastaldi et al., 1996) and nonbovine milks (Ould Eleya et al., 1995) agree that the cations linked to the casein are released almost entirely at pH levels close to 5, peak release occurring at pH between 5 and 6. At lower pH levels, the casein was practically free of minerals (Le Graet and Brulé, 1993; Gastaldi et al., 1996).

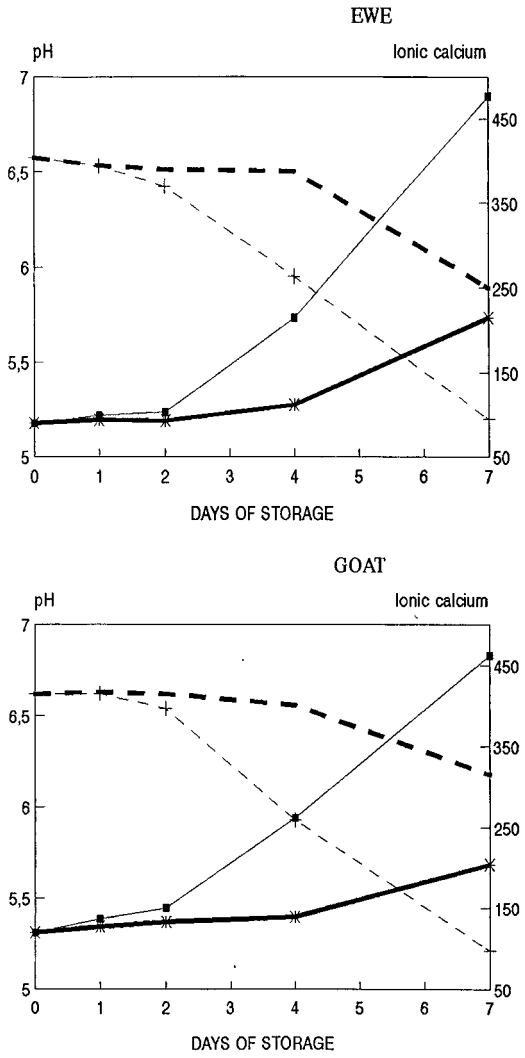

Figure 2. Change in the pH (broken lines) and the content of ionic calcium in mg L^{-1} (solid lines) in goat's and ewe's milk refrigerated at 3 (wide lines) and 7 °C (narrow lines) during 7 days.

The residual cations not solubilized at these pH levels could have been due to the presence of caseins which had been only partially decalcified and to the presence of occluded colloidal calcium phosphate, which is difficult to solubilize at this stage (Le Graet and Brulé, 1993).

About Na and K, although most of these elements occur in soluble form, it must be stressed that a percentage, ranging from 5% to 10%, is bound to the colloidal casein complex. This point had been noted earlier by Davies and White (1960) and Le Graet and Brulé (1993) in cow's milk, where amounts of these elements in excess of 5% were associated with the colloidal phase. Although the chilling process caused fluctuations in sodium and potassium values for either species, no clear progressive trend was observed.

Effect of Freezing. Table 4 shows soluble calcium, magnesium, and phosphorus contents in ewe's and goat's milk stored (either whole or skimmed) after slow and fast freezing in the conditions described in Materials and Methods.

In goat's milk, the decrease of soluble calcium and magnesium was less than 7%, while colloidal phosphorus concentrations remained stable over the different treatments. The composition of the soluble phase in ewe's milk also altered after 3 months of storage (Table 4); there was also decline in percentages of soluble phosphorus, calcium, and magnesium. The salt content in milk is greater than can be maintained in solution, and processes such as freezing can disturb the balance of salts (Chen and Yamauchi, 1969). Insolubilization of salts during freezing can occur in various stages, which need not necessarily coincide over time or be confined to simple precipitation of calcium phosphate. In studies on frozen storage of cow's milk over a period of 6 months, Chen and Yamauchi (1969) and Yamauchi and Yoneda (1977) found that the calcium became less soluble during the early stages of storage, whereas phosphorus became less soluble at a later stage.

The similar soluble phosphorus levels found in goat's milk and the moderate drop in levels found in ewe's milk indicate that freezing, as carried out in this study, would not cause serious deterioration of the casein micelles. Incorporation of phosphorus and calcium to the micellar phase over longer frozen storage periods may be connected with the formation of a casein precipitate (Chen and Yamauchi, 1969) or protein flocculated (Muir, 1984) that is not readily dispersed by stirring after thawing. Chen and Yamauchi (1969) suggested that the phosphorus might penetrate the casein micelles and cause more drastic alteration to their structure, producing a complex that is more resistant to dissociation than that originally occurring in the milk and hence destabilizing the micelles. Muir (1984) indicated that Ca is also a key factor in the destabilization process since it would favor physical aggregation of the casein micelles.

The fact that soluble phosphorus in the ewe's and goat's milk assayed did not decrease as sharply during freezing as reported by Chen and Yamauchi (1969) could be due to more than one reason: first, as noted above, our samples were kept in storage for a shorter time (they were analyzed after 3 months), which could result in less precipitation of phosphorus on the micelles, and above all due to the storage temperature. Milk was kept at −17 °C, while Chen and Yamauchi (1969) in their experiments employed higher temperatures (−7 °C). It is well-known that frozen milk stored at −20 °C will remain stable for long periods of time. It has been demonstrated that samples stored at −4 to −12 °C exhibited poorer stability than samples stored at higher or lower temperatures (Koschak et al., 1981).

Note the influence of the freeze−thaw procedures followed in the experiment. In this study, the product was frozen in a matter of seconds (fast freezing with liquid nitrogen) or in a few minutes (slow freezing in a multiple plate freezer), whereas freezing was much slower in previous experiments (Chen and Yamauchi, 1969). Again unlike the authors cited, thawing in the present case was very slow, 24 h at chilling temperatures before moving up to room temperature (22 ± 3 °C). The consequence of this could be give time to solubilize colloidal elements as in refrigeration experiments.

In either species, ionic calcium retained levels very similar to those found in unfrozen milk (Table 4). This is consistent with the scant variation found in the technological suitability of the milk consequent upon freezing, as explained further below.

Finally, it should be noted that only slight differences were found between the two types of freezing (fast and slow) and Na and K levels in the soluble phases of the frozen milk samples were comparable to those of milk not subjected to these treatments.

Table 4. Total and Soluble Calcium, Magnesium and Phosphorus Content[a] (mg/L) in Ewe's and Goat's Milk Frozen and Stored at −17 °C for 3 Months[b]

			soluble content				
				freezing skim milk		freezing whole milk	
species	element	total content	control	fast	slow	fast	slow
ewe	Ca	2351 ± 5	477 ± 12	460 ± 12	462 ± 8	434 ± 19	467 ± 13
	Mg	202 ± 1	115 ± 1	110 ± 1	111 ± 2	107 ± 1	109 ± 1
	P	1582 ± 35	613 ± 5	586 ± 10	566 ± 13	575 ± 34	583 ± 10
	Ca^{2+}	90 ± 3		93 ± 3[c]	95 ± 3[c]		
goat	Ca	1422 ± 7	478 ± 6	452 ± 14	445 ± 9	461 ± 6	448 ± 8
	Mg	135 ± 1	97 ± 1	93 ± 1	92 ± 1	93 ± 1	93 ± 1
	P	862 ± 13	351 ± 6	354 ± 13	351 ± 9	360 ± 6	342 ± 8
	Ca^{2+}	120 ± 4		118 ± 4[c]	118 ± 4[c]		

[a] Each result is the average of three determinations. [b] Whole and skimmed milk samples were frozen with liquid nitrogen (fast method) and in a multiple-plate freezer (slow method). [c] Concentration in total milk.

Curd Characteristics. Figure 3 shows the evolution of parameters r, K_{20}, and A_m in ewe's and goat's milks samples according to cold storage treatment. Ewe's milk exhibited greater renneting properties than did goat's milk, as reflected by shorter coagulation times, higher rates of curd formation, and greater elasticity. In fact it is well-known (Storry et al., 1983; Remeuf and Lenoir, 1986) that gels produced by the action of rennet in goat's milk tend to be fragile and not very firm, and hence give a poorer yield in cheesemaking.

The increase in coagulation time in the first 2 days of chilled storage was especially pronounced in milk stored at 3 °C. In the first 2 days of chilling at 7 °C, there was a smaller increase in coagulation time (ewe's milk) or even a decrease (goat's milk) with respect to samples stored at 3 °C.

No noticeable increases in ionic calcium were found in the first 2 days of storage (Figure 2), and therefore, changes in thrombodynamometric parameters must be attributed to other causes. The transition of part of the caseins from the micelles to the soluble fraction, accompanied by reduction in colloidal phosphate particularly after the second day of storage, was possibly the decisive factor in the increase of coagulation time, given that other key parameters such as pH remained stable (Figure 2). Research into the balance of the protein fraction in cow's milk (Reimerdes et al., 1977; Ali et al., 1980; Davies and Law, 1983) has shown that, after cooling, hydrophobic proteins from the micelles pass into the soluble phase. Ali et al. (1980) reported that milk stored at 3 and 7 °C having increased soluble casein shows slower clotting, weaker curds, and lower curd yield than that stored at higher temperature. This shift is strongly temperature-dependent: in milk stored at 4 °C the micelles partly dissociates even if the pH is not changed (Reimerdes and Klostermeyer, 1976; Davies and Law, 1983; Dalgleish and Law, 1988). Banks et al. (1988) reported that the increase in soluble caseins during storage was about 6% higher at 2 than at 6 °C. Although Van Hooydonk et al. (1986) and Ali et al. (1980) support that β-casein is the protein which is predominantly dissociated from the micelles at low temperatures, Dalgleish and Law (1988) showed that also appreciable amounts of α_{s1}-caseins were present in the serum, especially at pH lower than 6. Coagulation time is related to α_s- and β-casein content in the micelles (Storry et al., 1983; Grandison et al., 1985), and the passage from the micelles into the soluble fraction, where they may be broken down by natural enzymes of the milk (plasmine) or microbial proteases, could affect the micellar structure as the curd is formed. Thermization (Ali et al., 1980), heating at 37 °C (Okigbo et al., 1985) or longer storage at 20 °C (Davies and White, 1960; Davies and Law, 1983), has been found to restore micellar casein levels and reduce coagulation times. In the present study, there was no subsequent heating to offset the storage chilled. Tempering at 32 °C prior to measurement of rheological properties of curds was insufficient to re-establish the original equilibrium, and coagulation time increased with respect to the controls.

Although the enzymatic action of rennet in coagulation of milk is virtually independent of salt concentrations, the micellar aggregation phase is largely dependent on the composition and balance of salts in the milk. This second stage is particularly sensitive to colloidal calcium phosphate levels and ionic calcium concentration. Acidification of milk and neutralization resulted in improved renneting properties and a reduction in rennet coagulation time due to an elevated ionic calcium activity (Lucey et al., 1996). Acidification solubilizes calcium phosphate and concomitantly increases ionic calcium levels. Nevertheless if this solubilization is too large the increase of the calcium ion concentration is less importance for the coagulation time than the intact state of the micellar structure and colloidal calcium. Coagulability of para-κ-casein is determined by its concentration. In cow's milk, the rate of gel formation and curd consistency have been found to be closely linked to colloidal calcium phosphate content (Qvist, 1979; Storry et al., 1983; McMahon et al., 1984; Grandison et al., 1985); other elements may exert some influence, but to a lesser degree. For example, magnesium, as a divalent cation, positively affects coagulation, although to a lesser extent than calcium because its amounts were much smaller (Dalgleish and Law, 1989).

The determinations carried out at the end of the storage period revealed a reduction in coagulation time, and in goat's milk, in addition, a loss of curd consistency. This reflects the deterioration of the product which was particularly apparent in milk stored at 7 °C, as pH fell below 6, causing release of colloidal salts and consequent destruction of the micellar structure. Holt and Horne (1996) reported in a recent review that acidification removed the calcium phosphate cement bonding the micellar casein and also neutralized the negative charge of the protein, reducing the electrostatic repulsive forces which would promote dissociation. High levels of ionic calcium (Figure 2) were insufficient to offset dissolution of micellar calcium phosphate.

The results regarding the influence of freezing on renneting properties are not conclusive. Previously, in an earlier study on buffalo's milk, Addeo et al. (1992) found that the coagulation characteristics of samples in frozen storage at −20 °C showed no marked differences between fresh and rapidly frozen milk, regardless of the thawing technique or storage time. In the present

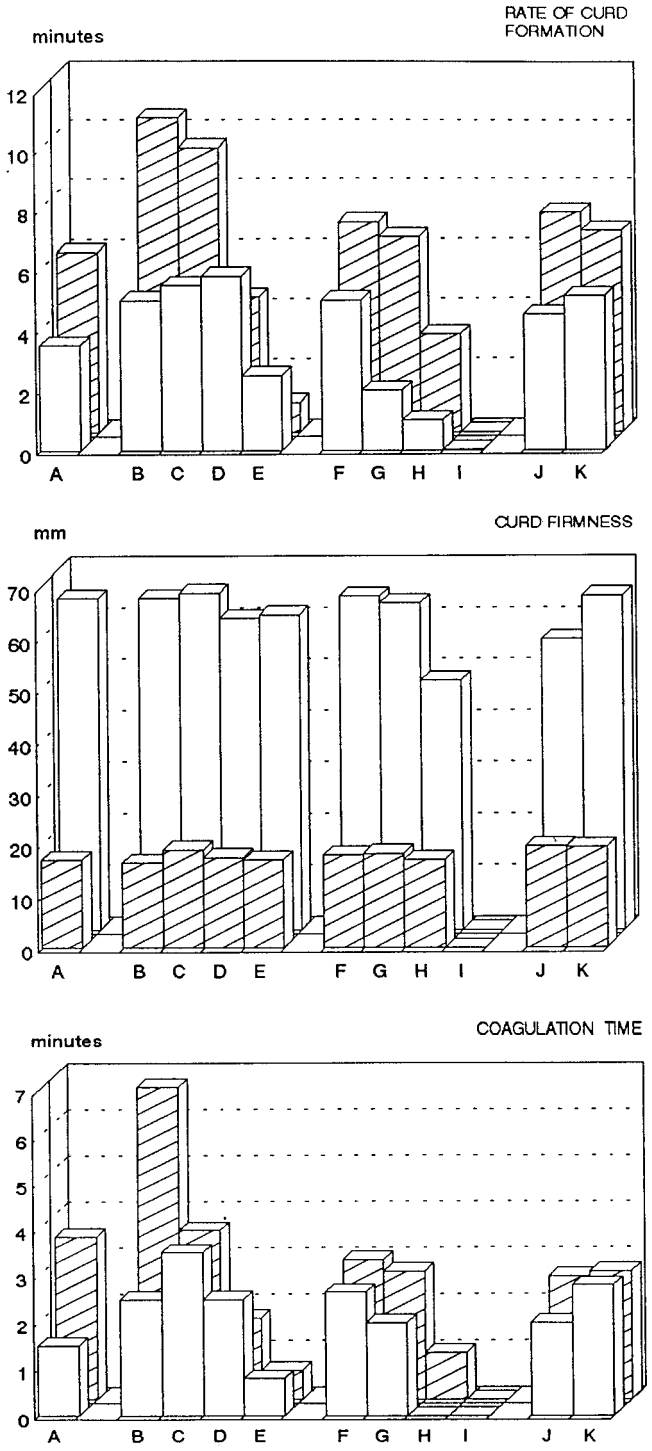

Figure 3. Coagulation time (min), rate of curd formation (min), and curd firmness (mm) in ewe's (white bars) and goat's milk (stripped bars) refrigerated during 1, 2, 4, and 7 days at 3 (B, C, D, and E) and 7 °C (F, G, H, and I) and frozen in a multiple-plate freezer (J) and with liquid nitrogen (K) and freezing storage during 3 months. "A" corresponds to the fresh milk.

case, however, effects were detected, although less pronounced than those found in chilling, especially at 3 °C. Even in goat's milk, curd consistency was augmented slightly and coagulation time diminished. These effects can be caused by the relative rapidity of the freezing process, which made for less abrupt alterations and a smaller impact on coagulation parameters. It should be taken into account that the influence of pH in frozen samples was very limited, unlike chilled samples. In ewe's milks samples, pH remained constant throughout frozen storage, and in goat's milk, it rose by no more than 0.05 units.

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