Effect of Heat Treatment and Ultrafiltration Process of Cow's, Ewe's, or Goat's Milk on Its Coagulation Properties

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The influence of ultrafiltration (UF) and the intensity of the heat treatment applied to milk prior to the UF process on the rennet clotting properties of cow's, ewe's, and goat's milk was analyzed. Significant differences in the rennet clotting time, and the curd firming of the ×0 (skim milk), ×1, ×1.5, and ×2 retentates between species were detected. A slight decrease of rennet clotting time with increasing concentration was found when cow's milk was heated prior to UF; however, a significant increase was detected with increasing concentration when ewe's or goat's milk was submitted to heating. The curd firming rate of the retentates increased when milk samples were heated prior to UF.

Keywords: *Ultrafiltration; rennet clotting properties; cow's, ewe's, goat's milk*

INTRODUCTION

The use of milk ultrafiltration (UF) to separate and to concentrate milk constituents is widely recognized and can be applied to manufacture various cultured dairy products, such as some cheese varieties. Changes in chemical composition of milk could be considered (Renner and El-Salam, 1991; St-Gelais et al., 1992), since changes in the relative composition, as well as physical changes such as casein aggregation, can occur during the UF process.

The physicochemical changes in the composition of milk during UF could affect the rennet clotting properties (RCP) of milk. Sharma et al. (1990) found that the coagulation time of UF milk, used in Cheddar manufacture, is too short and curd firmness is high, which makes cutting of the curd in conventional equipment impossible. Dalgleish (1981) and Fernández-García et al. (1993) showed that the rennet clotting time (RCT) is relatively little affected by the concentration of milk by UF, although the structure of the curd may be affected.

Other cheese manufacture properties, such as a slight reduction of curd hardness and a decrease in whey separation rate, were described by Casiraghi et al. (1989a); these changes could be due to the mechanical and thermal stresses associated with UF treatment. These authors also found that UF modifies the viscoelastic behavior of curd, resulting in a marked increase in the viscous component at increasing protein concentration.

Effects of other milk heat treatments, pasteurization and UHT, prior to UF on RCP of retentates with different protein concentration were also investigated (Sharma et al., 1990, 1994; Ferron-Baumy et al., 1991; McMahon et al., 1993; Guinee et al., 1996; Smith and McMahon, 1996). It is known that the heat treatments increase the RCT and the time at which the curd is considered to be firm enough for cutting (k_{20}). However, when milk is submitted to high heat treatments (100 °C for 2 min) prior to UF, the RCT is only slightly affected (Guinee et al., 1996). These results disagree with those found by McMahon et al. (1993). These authors heated milk to 140 °C and found that the concentration by UF of the heated milk shortened coagulation time and increased gel firmness.

Most of these investigations on the effect of UF and heat treatment on the RCP of milk have been conducted on cow's milk, with some on buffalo's milk (Fakhar and Dien, 1994); however, given the differences in milk composition among species, the results obtained should not be extrapolated to other ruminant species.

Some cheeses are manufactured from ewe's or goat's milk; the application of UF to the manufacture of cheese from these species could be interesting. Since renneting behavior is a major factor in cheese making, information dealing with the RCP of UF or heated and UF ewe's and goat's milk, compared to cow's milk, would allow the improvement of cheese-making processes from these types of milk.

MATERIALS AND METHODS

Samples. Raw bulk cow's, ewe's, and goat's milks from different herds in the central region of Spain were used.

Milk samples were skimmed by centrifugation before analysis. Whole milk was incubated in a water bath at 30 °C for 20 min before centrifugation at 3000*g* for 30 min at 5 °C; the milks were then placed in a water−ice bath, and after 30 min, the solidified fat was removed using a spatula. The skimmed milk was filtered through glass fiber pads to remove any residual fat.

Milk samples were adjusted to the initial pH after heat treatment with 1 M NaOH, since the final lower final pH after heat treatment following by UF was 6.54. The lower final pH of raw samples submitted to the UF process was 6.84; samples were adjusted to the initial pH with 1 M HCl.

All experiments were performed with four different cow's, ewe's, or goat's milks collected on different days from the same source.

Ultrafiltration. Milk, twice concentrated (×2), and the corresponding ultrafiltrate were obtained by passing milk samples, kept at 50 °C, in a water bath through a Pellicon (Millipore, Madrid, Spain) stirred ultrafiltration cell fitted with a 10000 M_r exclusion membrane. The ultrafiltrate and ×2

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Table 1. Composition (Grams per 100 g) of Skim Milk and ×1, ×1.5, and ×2 Retentates Obtained from UF of Cow's, Ewe's and Goat's Milks[a]

		composition of concentrates			
milk component	milk sample	control	×1	×1.5	×2
total solids	cow's	11.6 (0.06)^{a,A}	9.8 (0.06)^{b,A}	14.7 (0.01)^{c,A}	19.6 (0.06)^{d,A}
	ewe's	17.2 (0.02)^{a,B}	16.7 (0.19)^{a,B}	23.5 (0.02)^{b,B}	31.9 (0.01)^{c,B}
	goat's	12.5 (0.34)^{a,A}	11.3 (0.03)^{a,C}	16.9 (0.04)^{b,C}	22.5 (0.03)^{c,C}
ash	cow's	0.61 (0.02)^{a,A}	0.58 (0.01)^{b,A}	0.88 (0.01)^{c,A}	1.17 (0.02)^{d,A}
	ewe's	0.83 (0.00)^{a,B}	0.80 (0.00)^{b,B}	1.20 (0.01)^{c,B}	1.59 (0.01)^{d,B}
	goat's	0.74 (0.02)^{a,C}	0.70 (0.02)^{a,C}	1.01 (0.01)^{b,C}	1.36 (0.06)^{c,C}
proteins	cow's	3.1 (0.08)^{a,A}	3.0 (0.09)^{a,A}	4.5 (0.08)^{b,A}	6.2 (0.05)^{c,A}
	ewe's	4.7 (0.01)^{a,B}	4.7 (0.01)^{a,B}	6.8 (0.04)^{b,B}	9.3 (0.05)^{c,B}
	goat's	3.5 (0.17)^{a,A}	3.3 (0.06)^{b,A}	4.8 (0.04)^{b,B}	6.4 (0.03)^{c,A}
caseins	cow's	2.3 (0.01)^{a,A}	2.1 (0.00)^{b,A}	3.3 (0.00)^{c,A}	4.5 (0.01)^{d,A}
	ewe's	4.5 (0.01)^{a,B}	4.1 (0.01)^{b,B}	5.8 (0.06)^{c,B}	7.8 (0.00)^{d,B}
	goat's	3.1 (0.00)^{a,C}	2.9 (0.00)^{b,C}	3.2 (0.00)^{c,A}	4.4 (0.00)^{d,A}

[a] Mean (standard deviation), $n = 4$. Means in the same row without a common lower case supercript differ ($P < 0.05$). Means in the same column for the same milk component without a common capital supercript differ ($P < 0.05$).

retentate were stored at 7 °C for 24 h. The ×1 and ×1.5 retentates were obtained by mixing the corresponding amount of the ultrafiltrate with the ×2 retentate.

Heat Treatments. Portions (10 mL) of milk or the concentrates were heated at 75, 80, or 85 °C in a water bath for 30 min in tightly sealed Pyrex glass tubes (16 × 162 mm). Heated samples were immediately cooled in an ice−water bath and kept under refrigeration until analysis.

All experiments were replicated four times using milk samples collected on different days.

Renneting Properties. Rennet solution was obtained by diluting 300 mg of rennet powder containing 85% of chymosin and 15% of bovine pepsine (Hansen, Copenhagen, Denmark; rennet strength 1:100000) to 100 mL with 0.2 M sodium citrate buffer at pH 5.2.

RCP (RCT, K_{20}, a_{30}) were determined by use of the Formagraph apparatus (Electric Foss, Barcelona, Spain) [McMahon and Brown, 1982, as was described in detail in a previous paper by Montilla et al. (1995)].

Composition of Milk. The total solids were determined after drying to constant weight at 115 °C. The total proteins were determined by N content of milk; the non-casein N was determined by measuring the supernadant obtained after centrifugation of acidified milk at pH 4.6, and the casein N was determined by difference between the total nitrogen less the nom-casein nitrogen. The N content was measured by using the Kjeldahl technique, and the protein concentration was calculated as N concentration × 6.38. Ash was calculated by weight after burning at 550 °C for 16 h.

Statistical Analysis. Means, standard deviations (SD), and variance among batches were calculated by a BMDPpv2 program (Brussels, Belgium) with a CDC Ciber 180/855 computer. A one-way analysis of variance was calculated to test the influence of the concentration by UF and the intensity of the heat treatment on RCP of cow's, ewe's, and goat's milks.

RESULTS AND DISCUSSION

Composition of the Retentates. The compositions of four cow's, ewe's, and goat's milk samples and their ×1, ×1.5, and ×2 retentates were analyzed (Table 1).

The results for skim milk of the three species were similar to those reported by O'Connor and Fox (1977), Anifantakis (1986), and De Rafael and Calvo (1996). Protein and total solids concentrations were highest in ewe's milk, and cow's milk had the lowest ash concentration.

When the increase in the concentration of the different components with increasing UF concentration was analyzed, some differences were found between species. Considering that the concentration of the analyzed compounds in the skim milk samples was 100%, the concentrations of the proteins in the ×2 concentrates were 200, 193, and 182% for cow's, ewe's, and goat's milks, respectively. The ×2 concentrate from goat's milk showed the lowest increase, which could be due to a higher retention in the filtration membranes of the casein micelles, pephaps due to the micelles size. Richardson et al. (1974) reported that the micelles size of goat's milk is higher than those of cow's and ewe's milks.

Table 2. RCT of Raw Skim Milk and ×1, ×1.5, and ×2 Retentates from Cow's, Ewe's, and Goat's Milks[a]

	rennet clotting time (min) of		
rate of concentration	cow's milk	ewe's milk	goat's milk
not concentrated	15.5 (0.08)^{a}	5.4 (0.21)^{a}	7.5 (0.30)^{a}
×1.0 retentate	12.6 (0.13)^{b}	5.7 (0.2)^{b}	8.0 (0.21)^{b}
×1.5 retentate	13.6 (0.80)^{b}	6.9 (0.18)^{c}	8.8 (0.26)^{c}
×2.0 retentate	15.6 (0.52)^{a}	7.0 (0.23)^{c}	10.6 (0.27)^{d}

[a] Mean (standard deviation), $n = 4$. Means in the same column without a common superscript differ ($P < 0.05$).

The percentages of the ash in the ×2 concentrates, considering the 100% percentage of milk samples, were 192, 193, and 182% for cow's, ewe's, and goat's milks, respectively. The lower percentage of goat's concentrates could be due also to the major retention of the casein micelles in the ultrafiltration membranes, taking into account that Aoki et al. (1993) found higher levels of caseins cross-linked by micellar calcium phosphate in goat's milk.

The obtained results show that UF does not have the same influence in the final composition on the retentates from milk of different species.

RCT. As has been indicated above, the influence of the milk concentration, the heat treatment before milk concentration, and the rate of milk concentration on the RCT was studied.

The influence of the UF process on RCT can be observed in Table 2. RCT of milk and retentates from cow's showed slight differences. These results are in agreement with those found by Dalgleish (1981) and Fernández-García et al. (1993). RCT is significantly affected by the UF process of ewe's and goat's milks (see Table 2). RCT increases significantly ($P < 0.05$) with increasing milk concentration in goat's milk, whereas a significant ($P < 0.05$) increase was found for ewe's milk concentrated up to ×1.5, but no significant differences were found between ×1.5 and ×2 retentates. Although the effect of UF on goat's and ewe's milks RCT is significant, there is not a great variation in the RCT for all of the assayed retentates. The ×2 retentates

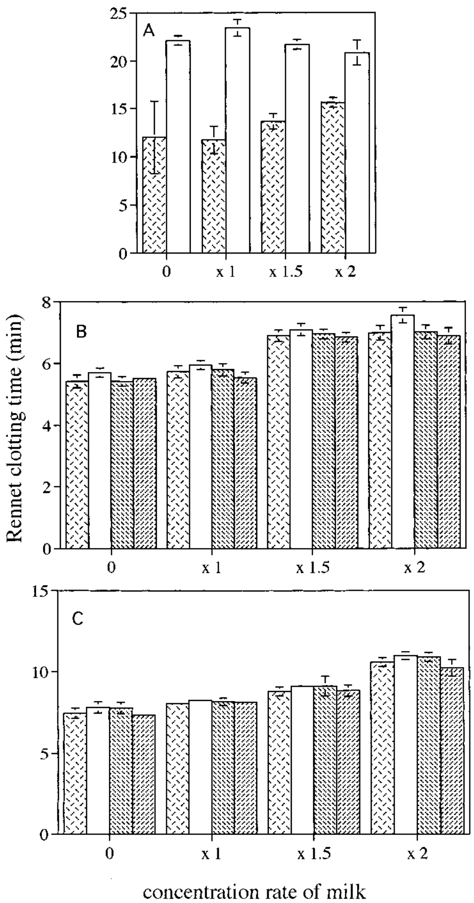

Figure 1. Influence of UF concentration, ×0 (no UF samples), ×1, ×1.5, and ×2 retentates, and heat treatment prior to UF on the RCT (in minutes) of cow's (A), ewe's (B), and goat's (C) milk samples. Bars represent, from left to right in each grouping, raw milk, milk heated at 75 °C for 30 min, milk heated at 80 °C for 30 min, and milk heated at 85 °C for 30 min. Error bars have been included.

obtained by UF could be used in cheese manufacture, due to the low increase in the RCT.

The differences found in the influence of the UF in the RCT of the milk of the three species could be due to various factors. As has been indicated above, the composition of the retentates shows differences between species. It is known that the composition and structure of the micelles of the three species are different as is the salt composition; these factors could influence the RCT.

Influence of heat treatment before the UF process was also analyzed. Effects of various heat treatments from 75 to 85 °C for 30 min prior to UF of milk and up to ×2 retentates were investigated (see Figure 1). As can be observed, the RCT of cow's milk increases with increasing intensity of the heat treatment. The milk heated at 80 °C for 30 min did not coagulate; however, the assayed heat treatments have no significant influence on the RCT of ewe's and goat's milks. These results are in agreement with those described by Montilla et al. (1995) and Balcones et al. (1996).

A significant ($P < 0.05$) decrease in RCT of cow's milk retentates heated at 75 °c was observed. RCT decrease significantly with increasing rate of concentration at the assayed heat treatment. These results are in agreement with those found by Ferron-Baumy et al. (1991), McMahon et al. (1994), and Smith and McMahon (1996).

Table 3. k_{20} Values (Time from RCT until the Two Lines Are 20 mm Apart, Representing the Rate of Curd Firming) of Raw Skim Milk and ×1, ×1.5, and ×2 Retentates from Cow's, Ewe's, and Goat's Milks[a]

	k_{20} (min) of		
rate of concentration	cow's milk	ewe's milk	goat's milk
not concentrated	10.7 (0.29)[a]	1.7 (0.06)[a]	2.0 (0.08)[a]
×1.0 retentate	10.0 (0.19)[b]	1.4 (0.04)[b]	2.1 (0.11)[b]
×1.5 retentate	5.0 (0.00)[c]	1.1 (0.10)[c]	1.6 (0.12)[c]
×2.0 retentate	2.9 (0.10)[d]	0.8 (0.26)[d]	1.0 (0.08)[d]

[a] Mean (standard deviation), $n = 4$. Means in the same column without a common superscript differ ($P < 0.05$).

RCT of heated goat's and ewe's milks and retentates showed a behavior similar to those described previously for the RCT of the raw milk and retentates; in general, the RCT increased with increasing milk concentration. No significant ($P < 0.05$) influence of the intensity of the heat treatment was detected.

No previous data in the literature were found about the influence of the heat treatment previous to the UF in the RCT of ewe's and goat's milk. The obtained results for ewe's and goat's samples are in disagreement with those described and also found by us for cow's milk. The different behavior of heated samples of these species could be influenced by differences in casein aggregation, such as a possible different rate of micelle aggregation, during the UF process. Green et al. (1983) reported that the flow rates, the high temperature (50 °C), and the shear stresses associated with recirculation of milk retentates through UF equipment may affect casein micelle aggregation. We observed that the ×1 retentates from nonheated ewe's and goat's milks showed a significantly higher RCT than those of the skim milk, whereas RCT fron the ×1 retentates from cow's milk was significantly lower than the control.

Differences in the composition of samples could also influence the RCT. As has been observed previously, the denaturation of whey proteins has no influence on the RCT of ewe's or goat's milk (Montilla et al., 1995; Balcones et al., 1996). On the other hand, we have observed that the intesity of the heat treatment has no influence in glycomacropeptide formation (unpublished data). The differences found in the behavior of the concentrates from the milk of the three studied species could be due to the different casein composition and size as well as the ionic calcium concentration.

Curd Firmness (k_{20}) and a_{30} Values. These RC properties were also analyzed following the same scheme used in the RCT analysis.

The k_{20} value of raw cow's milk was significantly higher than those of raw goat's or ewe's milk; these results had been observed in our laboratory (Montilla et al., 1995; Balcones et al., 1996).

A significant decrease in k_{20} was observed with increasing concentration of the UF retentates (see Table 3), indicating an increase in the curd firmness with UF. Chapman et al. (1979) and Dalgleish (1981) described the production of some kinds of cheeses from milks that have been concentrated and showed that the curd has a coarse texture; Sharma et al. (1990) found that the curd from UF milk has a high curd firmness.

The a_{30} value also increased with increasing rate of concentration of raw milk samples of the three studied species (see Table 4). Although different influences of concentration were found on RCT of the three species, all analyzed samples showed an increase of firmness with increasing concentration.

Table 4. a_{30} Values (Width of Graph Obtained in the Formagraph 30 min after Enzyme Is Added) of Raw Skim Milk and ×1, ×1.5, and ×2 Retentates from Cow's, Ewe's, and Goat's Milks[a]

	a_{30} (mm) of		
rate of concentration	cow's milk	ewe's milk	goat's milk
not concentrated	28.5 (0.37)[a]	54.9 (0.62)[a]	47.7 (0.68)[a]
×1.0 retentate	27.9 (0.14)[b]	56.6 (0.31)[b]	47.5 (0.76)[a]
×1.5 retentate	40.5 (0.42)[c]	59.4 (0.70)[c]	51.6 (0.48)[b]
×2.0 retentate	48.0 (0.20)[d]	66.5 (0.34)[d]	60.5 (0.89)[c]

[a] Mean (standard deviation), $n = 4$. Means in the same column without a common superscript differ ($P < 0.05$).

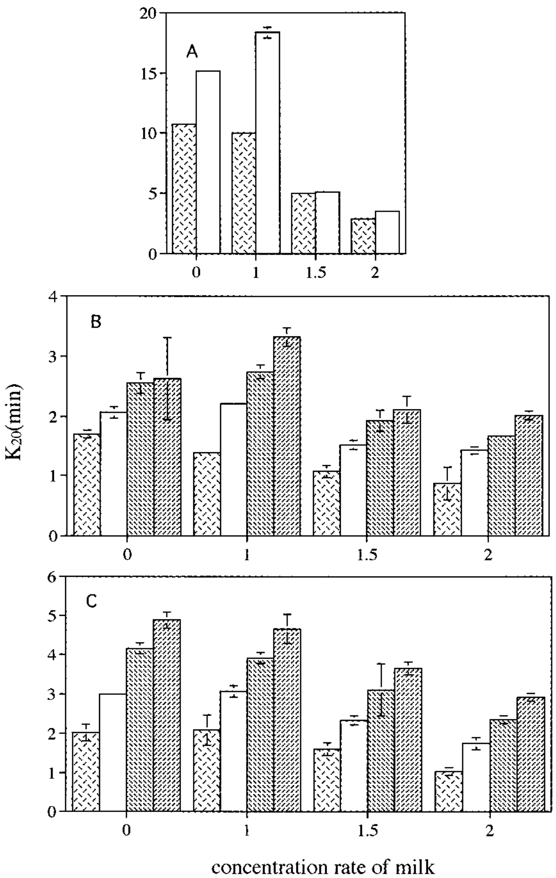

Figure 2. Influence of UF concentration, ×0 (no UF samples), ×1, ×1.5, and ×2 retentates, and heat treatment prior to UF on the rate of curd firming k_{20} (in minutes) of cow's (A), ewe's (B), and goat's (C) milk samples. Bars represent, from left to right within each grouping, raw milk, milk heated at 75 °C for 30 min, milk heated at 80 °C for 30 min, and milk heated at 85 °C for 30 min. Error bars have been included.

These results could indicate that during UF there are different changes in the milk of the three analyzed species that influenced the primary phase of the coagulation; the influence of the UF in the secondary phase is not as clear as in the primary one, since all samples showed an increase in the firmness of the curd due to the UF treatment. However, the increases of firmness with increasing concentration were different between species; the ×2 retentates from cow's milk showed an increase in the a_{30} value of 168% with respect of the nonconcentrated samples, an increase of 121% of the ×2 retentates from ewe's milk, and an increase of 126% of that from goat's milk. On the other hand, the decreases of the k_{20} value of the ×2 retentates with respect to the nonconcentrated samples were 27, 51, and 51% for cow's, ewe's, and goat's milk samples, respectively.

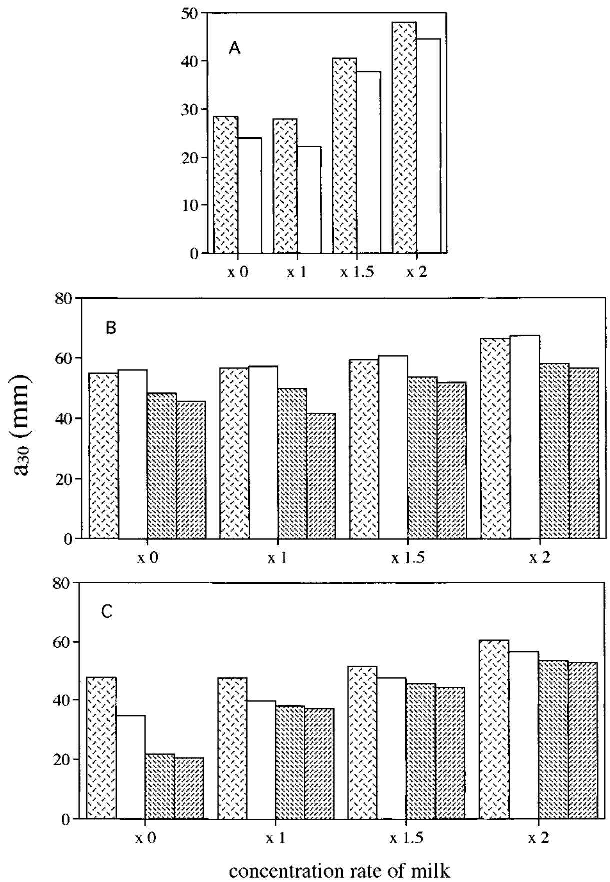

Figure 3. Influence of UF concentration, ×0 (no UF samples), ×1, ×1.5, and ×2 retentates, and heat treatment prior to UF on the a_{30} value (in millimeters) of cow's (A), ewe's (B), and goat's (C) milk samples. Bars represent, from left to right in each grouping, raw milk, milk heated at 75 °C for 30 min, milk heated at 80 °C for 30 min, and milk heated at 85 °C for 30 min. Error bars have been included.

As has been indicated above, cow's milk and the concentrate samples heated at 80 or 85 °C for 30 min did not coagulate. The results of the influence of the intensity of the heat treatment on the k_{20} and a_{30} values of UF samples are shown in Figures 2 and 3. More samples showed an increase in the k_{20} value and a decrease in the a_{30} with increasing heating intensity.

As can be observed in Figure 2, the k_{20} values of cow's ×1.5 and ×2 retentates were similar in raw samples and samples heated at 75 °C for 30 min. A significant ($P < 0.05$) increase was found in ×1.5 or ×2 retentates from heated ewe's or goat's samples, increasing the k_{20} value with increasing heating intensity. However, although the heat treatment prior to the UF affected significantly the k_{20} values, the variations between the analyzed samples were of 2 or 3 min.

The a_{30} value from cow's milk samples was significant higher in raw than in heated samples in all of the studied retentates. Ewe's milk was not significantly ($P < 0.05$) affected by the treatment at 75 °C for 30 min; higher heat treatments decreased the a_{30} value of all the studied retentates. The a_{30} value decreased when goat's milk samples were heated; no significant differences were found between samples heated at 80 or 85 °C.

The obtained results indicate that the curd firmness is affected by the intensity of the heat treatment prior to the UF process. This influence had been described for cow's milk; however, different results were obtained for the other species. The different behaviors could be due to the different compositions of the retentates, such as the casein and ionic calcium concentration, as well as the changes in casein aggregation during the UF process.

The effect of UF and the heat treatment of milk prior to the UF in the RCP of ewe's and goat's milks should be taken into account in the manufacture of cheese from UF milk. Although the high heat treatments applied and the UF process significantly influenced the RCP of goat's and ewe's milks, the changes in these properties are low and could not influence cheese elaboration from heated and UF milk from milk of these species.

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