

Original article

Cheese-making properties of ovine and caprine milks submitted to high pressures

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Abstract — The possibility to improve the yield and rennetability of ovine and caprine milks by the application of high pressures (100–400 MPa) has been investigated. The application of pressures over 200 MPa induced denaturation of β -lactoglobulin, enhancing protein and moisture retention in the curd. The effect of pressure on the coagulation properties depended on the magnitude and duration of the process and on the milk species, but in general terms, pressurization did not improve the renneting properties of ewe and goats' milk. However, treatments at 400 MPa, which provided the highest cheese yields, did not considerably lengthen the rennet coagulation times, and in the case of goat's milk, pressurization improved the consistency of the curd. Therefore, pressurization of ovine and caprine milks seems to be a promising way of improving their technological properties for cheese-making. © Inra/Elsevier, Paris.

high pressure / ewe milk / goat milk / cheese yield / coagulation

Résumé — Comportement fromager des laits de brebis et de chèvre soumis à de hautes pressions. La possibilité d'accroître le rendement et l'aptitude à la coagulation par la présure des laits de brebis et de chèvre par application de hautes pressions (100–400 MPa) a été étudiée. L'application de pressions supérieures à 200 MPa induit la dénaturation de la β -lactoglobuline, augmentant la rétention de protéines et d'humidité dans le lait caillé. L'effet de la pression sur les propriétés de coagulation dépend de la force et de la durée du processus et de l'origine du lait, mais de façon plus générale, la pressurisation n'a pas amélioré les propriétés de coagulation par la présure du lait de brebis ou de chèvre. Cependant, les traitements à 400 MPa, qui ont fourni les rendements en fromage les plus élevés, n'ont pas allongé considérablement les temps de coagulation par la présure, et dans le cas du lait de chèvre, la pressurisation a augmenté la consistance du gel. Ainsi, la pressurisation des laits de brebis et de chèvre semble être une voie prometteuse pour améliorer l'aptitude fromagère de ces laits. © Inra/Elsevier, Paris.

haute pression / lait de brebis / lait de chèvre / rendement en fromage / coagulation

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1. INTRODUCTION

Production of ovine and caprine milks is of great importance in Mediterranean countries, where they are mainly used in the cheese industry. Owing to their peculiar organoleptic properties, cheeses made from milks of these two species or their mixtures are increasingly gaining worldwide acceptance, and during the period 1987–1994, the world production of cheeses from ewes' and goats' milk increased by 4 and 10% respectively [11].

The study of the genetic variants of milk proteins has received considerable interest in relation to the technological quality of milk, because genetic polymorphism is responsible for variations in the cheese yield and coagulation properties [8]. However, in addition to animal selection, the application of technological treatments such as calcium addition [16], controlled acidification [27], ultrafiltration [25] or heat treatment [17] can be attractive approaches to improve the cheese-making characteristics of milk.

High pressure treatment of bovine milk has been found to improve its microbiological quality and functionality [5, 9, 10, 20, 30]. It was observed that pressurization of milk under certain conditions increased protein and moisture retention in the curd, and decreased the rennet coagulation time and the curd firming time [13], but very few studies have been carried out on the effects of pressurization on milks other than bovine milk [3, 6, 7]. In the present work, the possibility to improve the yield and rennetability of ovine and caprine milks by the application of high pressures (100–400 MPa, 5–30 min) was investigated.

2. MATERIALS AND METHODS

2.1. Milk samples

Raw ovine and caprine milks were obtained from a local dairy farm and were kept refrigerated

a maximum of 3 h before use. Samples of 300 mL were poured into polyethylene bottles, avoiding any head space, and vacuum-sealed in polyethylene bags before being pressurized.

2.2. High pressure treatments

Milk samples were pressurized using a 900 HP apparatus (Eurotherm Automation, Lyon, France). The pressure was raised to the desired value (100 to 400 MPa) at a rate of 2.5 MPa/s, then maintained for 5 to 60 min, and finally released at the same rate. Before the pressure treatments, the temperature of the hydrostatic fluid medium was controlled at 25 ± 1 °C by circulating water through a jacket surrounding the pressure vessel, and the temperature increase, as a result of the pressure treatment, was 2 °C/100 MPa. High pressure treatments were performed in quadruplicate and replicates were conducted on different days with milk from different batches. Samples were kept refrigerated at 5 °C and analyzed within 20 h after pressurization.

2.3. Whey protein analyses

Whey proteins were determined by reverse-phase HPLC [26] both in the fractions soluble at pH 4.6 and in the rennet wheys. The fractions soluble at pH 4.6 were separated from raw and pressurized milk samples after the pH was adjusted with 2 mol/L HCl, and denaturation of β -lactoglobulin (β -Lg) following the pressure treatments was expressed as percentage of the β -Lg content of the corresponding raw milk.

2.4. Cheese yield

Cheese yields were estimated by centrifugation. Milk (30 mL) prewarmed to 30 °C was treated with 600 μ L of standard rennet (85% chymosin and 15% bovine pepsin, strength 1:10 000, Chr Hansen's Lab Copenhagen, Denmark) solution (0.3 g/100 mL) in acetate buffer (0.2 mol/L, pH 5.4), and stirred for 1 min. After 40 min at 30 °C, the curd was cut and 10 min later centrifuged at 15 000 g for 15 min at 5 °C. The curd and whey were then separated and weighed.

2.5. Coagulation properties of milk

A Formagraph (N. Foss and Co., Hellerup, Denmark) was used to determine the rennet coagulation properties: coagulation time (r), curd firming time (k_{20}), and curd firmness (a_{30}) [18]. 200 μ L of rennet solution (see above) was added to 10 mL of milk preequilibrated to 36 °C for 30 min, and the rennet clotting properties were measured.

2.6. Statistical analysis

One-way ANOVA of the results was carried out by using the Statgraphics Statistical System [28]. The results employed corresponded to four independent experiments with three analytical determinations made for each set.

3. RESULTS AND DISCUSSION

3.1. Effects of high pressure on cheese yield

Figure 1 shows the effect of pressure, applied for 30 min, on β -lactoglobulin (β -Lg) denaturation and cheese yield of ovine and caprine milks. In agreement with previous results on bovine milk [13], α -lactalbumin (α -La) did not undergo pressure induced denaturation under 400 MPa at the temperatures assayed. Denaturation of ovine and caprine β -Lg (*figure 1a*) increased with pressure at a faster rate than that of bovine β -Lg, in the order ovine > caprine > bovine. This is in accordance with the rates of heat induced denaturation of β -Lg from the three species at temperatures over 80 °C found by other authors [12, 22]. The denaturation behavior of caprine whey proteins on pressurization agrees with the results published by Felipe et al. [6].

There is evidence that, on heating, denatured whey proteins became associated with the casein micelles, initially through hydrophobic interaction, and then by disulphide linkage mainly with κ -casein [4]. Aggregation of bovine β -Lg through

the formation of disulphide bonds, and its precipitation at pH 4.6 with the casein fraction were found as a result of milk pressurization, but it was not possible to determine whether β -Lg formed homopolymers or was disulphide linked to κ -casein [14]. The formation of disulphide linked polymers was also observed as a result of pressurization of goats' milk [6]. The observation that ovine and caprine β -Lg denature at a faster rate than bovine β -Lg can be attributed to differences in the concentration of κ -caseins and in their content in -SH groups ovine and caprine caseins having double concentration of -SH capable of forming disulphide bonds with β -Lg than bovine milk. In addition, the higher concentration of β -casein in ovine casein with respect to bovine and caprine casein may promote the initial hydrophobic binding of β -Lg to caseins [12].

It was previously observed that denaturation of β -Lg enhanced protein retention in the curds giving a substantial increase in yield, provided that pressurization is carried out above 200 MPa [13]. Caprine milk behaved very similarly to bovine milk, and treatments at 300 and 400 MPa, applied for 30 min, significantly ($P < 0.05$) improved cheese yield (*figure 1b*). Cheese yield of raw ovine milk was almost double than that of raw bovine and caprine milks owing to its higher protein and fat contents [1]. Unlike bovine and caprine milks, the cheese yield of ovine milk pressurized at 200 MPa was higher ($P < 0.05$) than that of the raw milk (*figure 1b*). This is in accordance with a higher level of β -Lg denaturation than that of β -Lg of milks of the other two species at 200 MPa, together with the fact that ewes' milk contains more β -Lg than cows' and goats' milks.

After treatments at 400 MPa for 30 min, the curd weights increased by 15.6 and 16.7% in ovine and caprine milks respectively. The analyses of the levels of α -La and β -Lg by RP-HPLC showed substantial decreases in β -Lg loss in the rennet wheys

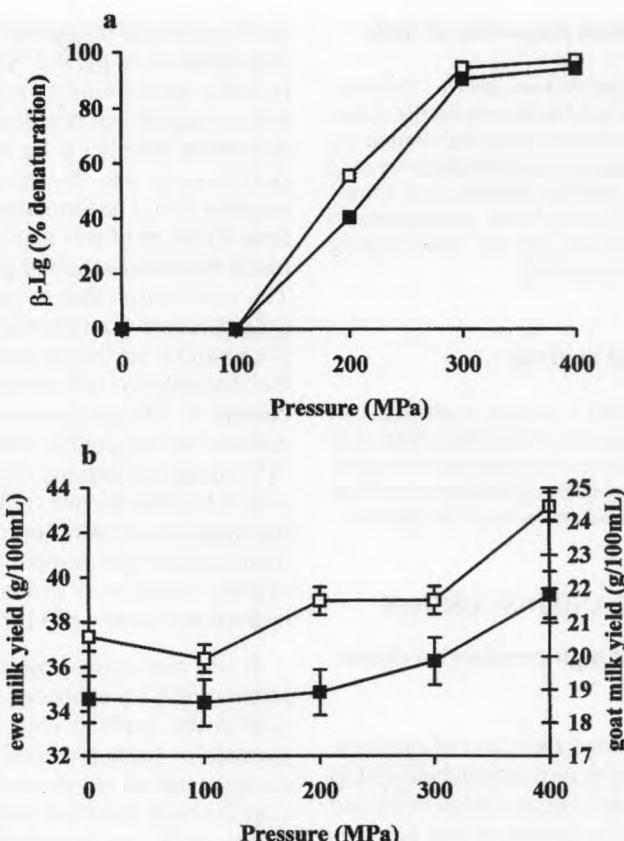


Figure 1. Effect of pressurization of ovine (□) and caprine (■) milks for 30 min on the percentage of denaturation of β -lactoglobulin (a), and the cheese yield as estimated by centrifugation (b). The means of four independent experiments and the 95% error bars are shown, except for a, where the error bars are included in the symbols.

Figure 1. Effet de la pressurisation des laits de brebis (□) et de chèvre (■) pendant 30 minutes, sur le pourcentage de dénaturation de la β -lactoglobuline (a), et le rendement fromager estimé par centrifugation (b). La moyenne de quatre expériences indépendantes et la barre d'erreur à 95 % sont indiquées (sur la figure 1a, elles sont incluses dans les symboles).

with increasing pressure, that were equivalent to the levels of denaturation as measured in the fractions soluble at pH 4.6 (*figure 1a*). This indicated that denatured β -Lg was retained in the curd, although, as it was the case of bovine milk [13], the increases in yield were mainly due to an enhanced retention of moisture, since the

total volume of whey decreased by 4.2 and 11.4% in ovine and caprine milks treated for 30 min at 400 MPa. It should also be noted that, although treatments at 400 MPa did not increase the percentage of β -Lg denaturation over treatments at 300 MPa, they significantly improved cheese yield.

To assess the influence of the treatment duration, ovine and caprine milks were submitted to 300 MPa for different periods (from 5 to 30 min) (*figure 2*). β -Lg from both species underwent a very rapid denaturation during the first 5 min of treatment. The main changes in the estimated curd weights were also produced on pressurization for 5–10 min. The fact that at this pressure (300 MPa) most of the increase is achieved by 10 min of treatment can be favorable since high pressure treatment of foods is most often a batch

process and short exposure times allow maximum throughput of product. However, the effect of the treatment time can vary with different combinations of pressure and temperature.

3.2. Effects of pressure on rennet coagulation properties of milk

Figure 3 shows the coagulation properties of ovine and caprine milks subjected to pressures of 100 to 400 MPa for

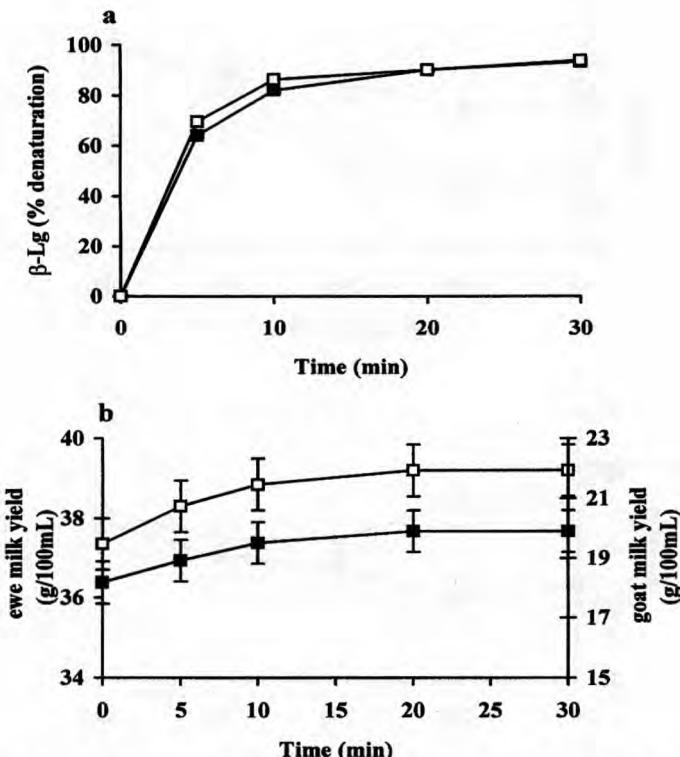


Figure 2. Effect of pressurization of ovine (□) and caprine (■) milks at 300 MPa for different times on the percentage of denaturation of β -lactoglobulin (a), and the cheese yield, as estimated by centrifugation (b). The means of four independent experiments and the 95% error bars are shown, except for a, where the error bars are included in the symbols.

Figure 2. Effet de la pressurisation des laits de brebis (□) et de chèvre (■) à 300 MPa pour différentes durées, sur le pourcentage de dénaturation de la β -lactoglobuline (a), et le rendement fromager estimé par centrifugation (b). La moyenne de quatre expériences indépendantes et la barre d'erreur à 95 % sont indiquées (sur la figure 2a, elles sont incluses dans les symboles).

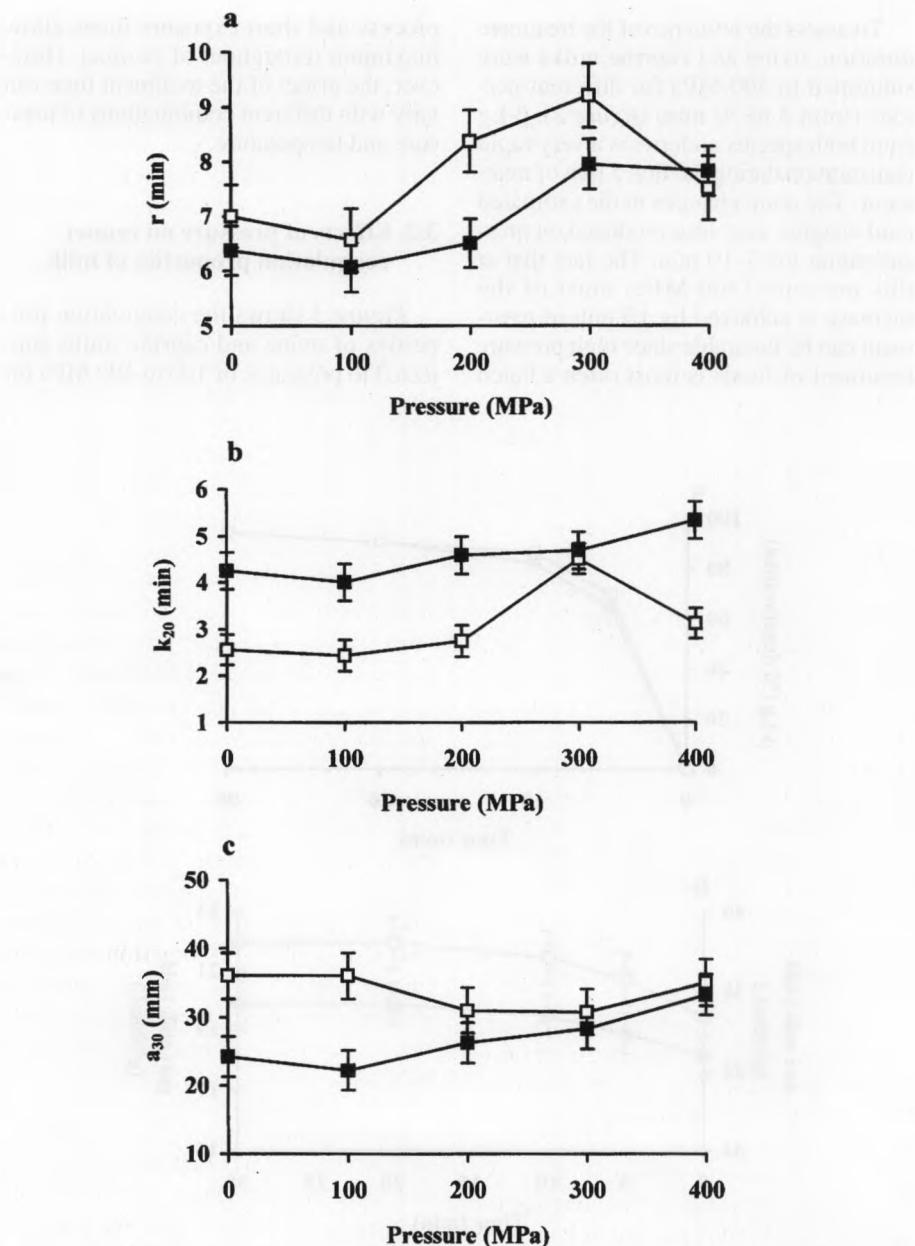


Figure 3. Effect of pressurization of ovine (□) and caprine (■) milks for 30 min on the rennet clotting properties: coagulation time (*r*) (a), curd firming time (*k*₂₀) (b), and curd firmness (*a*₃₀) (c). The means of four independent experiments and the 95% error bars are shown.

Figure 3. Effet de la pressurisation des laits de brebis (□) et de chèvre (■) pendant 30 minutes, sur les propriétés de coagulation par la préasure : temps de coagulation (*r*) (a), vitesse de raff du gel (*k*₂₀) (b), et fermeté du gel (*a*₃₀) (c). La moyenne de quatre expériences indépendantes et la barre d'erreur à 95 % sont indiquées.

30 min as determined by the Formagraph. In accordance with the literature data, the rennet coagulation times and curd firming rates of raw milks from these two species were shorter than those of cows' milk [29]. Raw ewes' milk exhibited a great coagulum strength [21], while raw goats' milk gave more fragile gels [24] (*figure 3c*).

In the case of ovine milk, r decreased slightly with pressurization at 100 MPa and then increased significantly ($P < 0.05$) at 200 and 300 MPa, decreasing again at 400 MPa (*figure 3a*). In caprine milk, r did not change significantly with pressures up to 200 MPa, but treatments at 300 and 400 MPa increased the coagulation time (*figure 3a*). Because k_{20} is strongly related to r , it followed a similar trend in both species (*figure 3b*). The a_{30} value of ewes' milk was not considerably affected by the pressure treatments applied, whereas pressurization at 300 and 400 MPa improved ($P < 0.05$) curd firmness of goats' milk (*figure 3c*). The firmness of the curd is considered very important in the cheese-making process. Goats' milk curds are known to present low cohesion that can lead to losses of fine particles into the whey and lower yields, owing to a high average diameter of the micelles, together with a high degree of size dispersion [23].

Pressurized ewes' and goats' milks followed a pattern different from that of pressurized cows' milk [13], since, in general terms, their coagulation parameters were less affected by pressure. It has been reported that heat treatments up to 85 °C for 30 min did not affect the rennet coagulation time of goats' milk [19] and only caused slight changes in ewes' milk [2], despite the observation that the degrees of thermal denaturation of β -Lg were very high in both cases. However, in cows' milk, heat-induced denaturation and complex formation between β -Lg and κ -casein is known to render milk less susceptible to

coagulation, lengthening the rennet clotting time [31].

The higher proportion of β -Lg denatured at 200 and 300 MPa in ovine and caprine milks, with respect to bovine milk, could be partially responsible for the comparative lengthening observed in the rennet coagulation times of milks submitted to these pressures. However, treatments at 100 MPa that did not induce β -Lg denaturation in any of the three species, significantly shortened the rennet coagulation time of bovine milk but did not significantly affect the rennet coagulation times of ovine or caprine milks. It is, therefore, likely that the differences found in the rennet clotting properties of pressurized milks from the three species could be in part attributed to differences in the individual casein composition and size distribution of the micelles, or to changes in the salt equilibrium during pressurization. Indeed, previous research has shown that pressurization increased the levels of non-sedimentable casein in bovine, ovine and caprine milks, as well as the levels of Ca, P and Mg in the serum, but the extent of the effects observed varied with the milk species. In the case of bovine and caprine milks, maximum dissociation from the micelle was observed in milks treated at 300 MPa, while in ewes' milk dissociation increased with pressure up to 400 MPa [15].

Figure 4 illustrates the effect of the holding time at 300 MPa on the coagulation properties. As it was the case of the cheese yield and β -Lg denaturation, treatment of ovine and caprine milks at 300 MPa from 5 to 30 min showed that the most important changes took place during the first 10 min of treatment. However, the positive effect of prolonged treatments at 300 MPa on the curd firmness of goat's milk should be noticed.

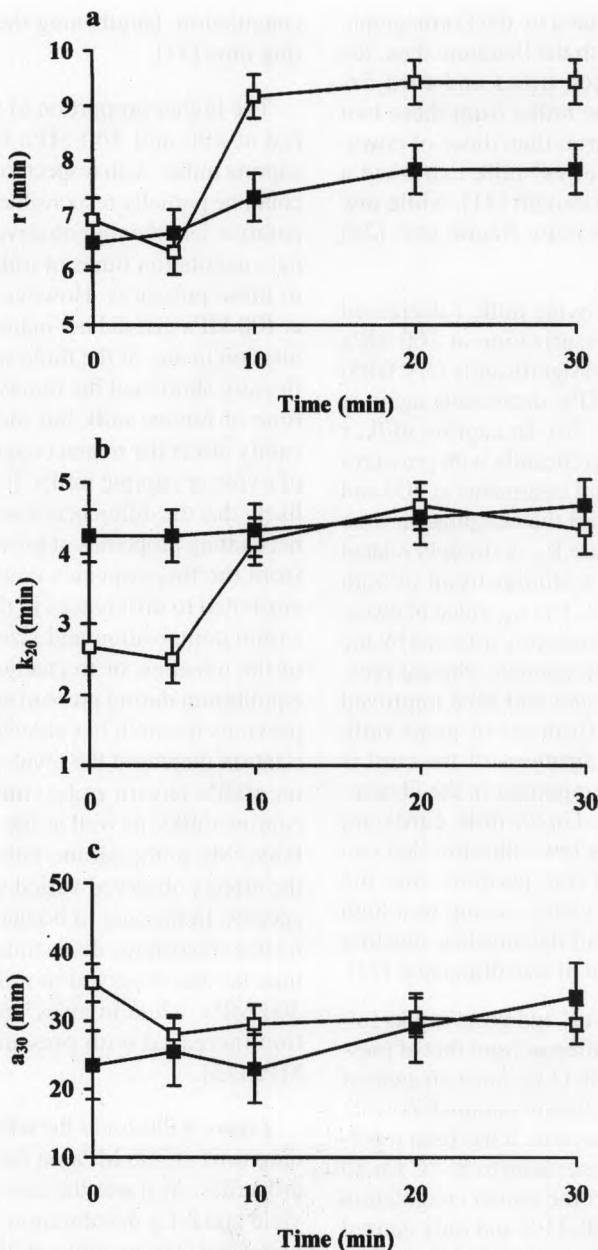


Figure 4. Effect of pressurization of ovine (□) and caprine (■) milks at 300 MPa for different times on the rennet clotting properties: coagulation time (r) (a), curd firming time (k_{20}) (b), and curd firmness (a_{30}) (c). The means of four independent experiments and the 95% error bars are shown.

Figure 4. Effet de la pressurisation des laits de brebis (□) et de chèvre (■) à 300 MPa pour différentes durées, sur les propriétés de coagulation par la présure : temps de coagulation (r) (a), vitesse de raff du gel (k_{20}) (b), et fermeté du gel (a_{30}) (c). La moyenne de quatre expériences indépendantes et la barre d'erreur à 95 % sont indiquées.

4. CONCLUSION

The present results show that pressurization of ovine and caprine milks is a promising way of improving their technological properties for cheese-making, since pressures over around 200 MPa enhanced protein and moisture retention in the curd. The effect of pressure on the coagulation properties depended on the magnitude and duration of the process and on the milk species. For instance, at 300 MPa, most of the observed effects took place on treatment for 5–10 min and fewer changes on prolonged pressure treatment. Unlike the case of cows' milk, pressurization did not improve the renneting properties of ewes' and goats' milk, although treatments at 400 MPa, which provided the highest cheese yields, did not considerably lengthen the rennet coagulation times, and in the case of goats' milk, pressurization improved the consistency of the curd. Further research on the pressure-induced modifications in milk proteins and salt equilibrium might help to understand the effect of high pressures on milk and, thus, know the full potential of these processes and increase their efficiency.

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