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Rheological Properties of Goat Milk Coagulation as Affected by Rennet Concentration, pH and Temperature

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Abstract: Various factors affect rennet coagulation and consequently cheese yield, but the subject of research has been mainly the cow milk. For the purpose of goat cheese production optimization, this paper investigated the influence of enzyme concentration (0.01–0.054 g/L), pH (6.5–6.1) and temperature (27–35 °C) on rennet coagulation of goat milk. Coagulation time (RCT), aggregation rate (AR), and gel firmness (G'_{60} and GF), were measured by oscillatory rheometry. The decrease in rennet concentration extended RCT. At lower rennet concentrations, a lower AR was recorded, which ranged from 0.02 Pa/s to 0.05 Pa/s. The decrease in pH from 6.5 to 6.1 caused a two times shorter RCT, and a two times faster AR. There was no effect of pH on the firmness of the rennet gel. The increase in coagulation temperature from 27 °C to 35 °C reduced the RCT of pasteurized milk from 12.6 min to 8.6 min, and caused a linear increase in the AR, but did not significantly affect the firmness of the gel. The present study revealed that the optimization of the rennet coagulation process could be directed towards pH lowering, or temperature increase, since they accelerate the process, but do not alter the examined gel firmness parameters.

Keywords: goat milk; rheology; rennet coagulation; coagulation time; aggregation rate; curd firmness

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1. Introduction

The basics of the cheese structure in cheesemaking are traditionally established through the rennet coagulation of milk and therefore the examination of the parameters that define this phase in the production of cheeses is of great importance. The firmness of the gel is the most important coagulation property because it affects the quality of cheese, the yield and therefore, the cost-effectiveness of production. Based on the research on cow milk it was established that a firmer curd enables better retention of milk components and thus increases the yield of cheese. In addition, achieving a shorter coagulation time and a higher coagulation rate would shorten the time required to make the cheese, so the optimization of these parameters is of great importance to producers [1].

The optimal cutting time of rennet-coagulated gel in cheesemaking is determined empirically, based on a subjective assessment of the textural and visual properties of the gel, while the evolution of its rheological properties and cheese yield is strongly influenced by the coagulation process itself. Some of the most important factors determining the course of rennet coagulation are: rennet concentration, coagulation temperature, milk pH, protein content, previous heat treatment of milk (raw material history), and ionic calcium content Ca^{2+} [2,3].

Investigations on cow milk have shown that increasing the concentration of the coagulating enzyme shortens the coagulation time, by enabling a higher level of κ-casein initial cleavage. A linear relationship between enzyme concentration and reciprocal value of coagulation time was established, and an increase in gel firming rate was also associated with higher enzyme concentration [2,4]. On the other hand, some literature data on cow milk implies a reduction in gel firmness with increasing rennet concentration in the range 0.010–0.040 g/L [2].

Milk coagulation shows a high dependence on temperature [5]. It is known that temperature affects both the enzymatic and non-enzymatic phase of aggregation during milk coagulation, and it was found that this effect is far greater on the secondary phase, i.e., on the aggregation reaction itself [6].

According to some authors, the rate of gel formation shows a linear increase in the range from 20 °C to 40–42 °C for cow milk, however, at higher temperatures the process slows down. The temperature of the milk affects protein aggregation to a large extent and increasing temperature increases the rate of gel firming (aggregation rate) [2]. Panthi et al. [7] found that lowering the coagulation temperature results in a finer protein network, and that a higher temperature increases the strength of the gel and the gel firming rate [7]. However, goat cheeses usually fall into the group of fresh or white unripened cheeses and soft cheeses, because of their fragile rennet gel, and the coagulation temperature in their production is lower than for the hard type of cheeses [8].

The effect of high temperatures on the components of goat milk, especially on proteins, is different compared to the effect they have on cow milk, so high heat treatments of milk can be applied in the production of goat cheese. The application of high temperatures causes the denaturation of whey proteins and the formation of coaggregates, whereby these components are incorporated into the cheese mass, which contributes to the increase of goat cheese yield and improves its biological value [9].

The influence of milk pH on coagulation time is very pronounced. Lowering the pH from 7.0 to 5.2 shortens the RCT, with the optimal pH for hydrolysis of κ-casein in the range of 5.1–5.3 [2,4]. The most important effects of lowering the pH of milk are the dissolving of micellar calcium phosphate, reduction in the net charge of the casein as well as dissociation of caseins from micelles [3,10]. Additionally, it has been reported that limited pH lowering leads to an increase in the gel firming rate [11].

The influence of factors affecting rennet coagulation has been extensively studied on cow milk [2,7,12], while to a much lesser extent on goat milk [6]. Goat milk has certain specificities that influence its different technological properties compared to cow milk, such as lower isoelectric point of casein [12], higher calcium content, and different casein micelle structure [13]. Often low protein content, especially αs1-casein in goat milk, contributes to lower firmness of rennet coagulated gel compared to cow milk, and also to lower production yield due to higher losses during curd processing [8]. Furthermore, high heat treatment of goat milk has a different effect on milk components, primarily proteins [14] and the coagulation process [15]. Despite the foregoing, goat milk products are usually produced based on technological procedures which apply to cow milk [16].

Therefore, as a subject of this study, the examination of various factors on the rennet coagulation of goat milk and the properties of the resulting gel was imposed. It was of interest to investigate how the lowering of milk pH, or different rennet concentrations and coagulation temperature would affect rheological parameters of goat milk gel and the course of coagulation. New information would serve as the basis for optimization of technological parameters in goat cheese production.

2. Materials and Methods

2.1. Materials

The raw milk used in these experiments was taken from a commercial flock of *Alpine* goats during the autumn period.

Immediately after milk collection, basic composition was determined by the following methods: total solids—by standard drying method at 102 ± 2 °C [17]; fat content—according to the Gerber method [18]; protein content—by the Kjeldahl method [19]. The pH was measured using a calibrated digital pH-meter (Consort, Turnhout, Belgium). Three replicates of these analytical determinations were carried out.

Veal rennet powder was used for rennet coagulation (Caglificio Clerici, 96% chymosin, 4% bovine pepsin, 765 to 1620 IMCU/g, optimal action temperature 35 °C, Clerici-Sacco Group, Cadorago, Italy).

2.2. Preparation of Milk Samples

Factors examined in this study were: (i) heat treatment of goat milk (raw or pasteurized milk at 65 °C/30 min) and (ii) the other factor varied depending on the experiment and comprised the concentration of rennet, pH and coagulation temperature.

A fixed conditions of milk sample preparation: pasteurization regime (65 °C/30 min), coagulation temperature (31 °C), milk pH (6.5), rennet (0.054 g/L) and calcium chloride concentration (0.2 g/L added to pasteurized milk at coagulation temperature), were employed as reference conditions for milk coagulation [20,21].

2.2.1. Examination of Rennet Concentration Influence

Prior to coagulation, all described fixed conditions were applied, except the concentration of rennet. Coagulation was initiated by adding the four different levels of rennet concentrations to milk (0.054 g/L, 0.020 g/L, 0.015 g/L and 0.010 g/L), which was preheated at coagulation temperature.

2.2.2. Examination of pH Influence

The pH value of goat milk was adjusted to 6.5, 6.3 and 6.1 at the coagulation temperature (31 °C), by adding the appropriate amount of 10% (v/v) lactic acid to raw milk samples. The pH of pasteurized milk was adjusted after adding calcium chloride (0.2 g/L).

2.2.3. Examination of Coagulation Temperature Influence

The milk was divided in two equal parts. The first part was not subjected to heat treatment, and the second part was pasteurized by a low pasteurization regime (65 °C/30 min). The raw and pasteurized samples were heated to the appropriate coagulation temperature (27 °C, 31 °C and 35 °C).

For each set of experiments, milk was taken for three consecutive weeks so that the experiment was repeated three times.

2.3. Rheological Measurements

The rennet coagulation was monitored by the oscillatory rheometry with small amplitude on the Kinexus Pro+ Rheometer (Malvern, Worcestershire, UK) with the four blade vane as a tool. The measurement started 4 min after the rennet addition by inserting the sample into the lower part of the geometry which had previously been tempered to the coagulation temperature. Gel formation was monitored at an oscillation frequency of 1 Hz and a deformation of 0.01% for 60 min by measuring the modulus of elasticity (G′). To characterize the final curd firmness, a subsequent frequency sweep test was performed in the range of 0.01–20 Hz at deformation of 0.01% at 31 °C.

Based on the obtained graphs, the aggregation rate (AR) was calculated according to Steffl et al. [22]. Gel firmness was determined according to the method of Wang et al. [23] by measuring the modulus of elasticity after 60 min (G'_{60}) since the start of measurement. Another parameter describing final gel firmness (GF) was obtained as a value of G′ modulus recorded at 1.5 Hz from the frequency sweep test, based on the method by Dimassi et al. [24].

Coagulation time (RCT) was calculated as the moment when the value of the modulus of elasticity ($G' \geq 1$ Pa) [25].

These parameters were calculated using OriginPro 8.0 (OriginLab Corporation, Northampton, MA, USA). All experiments were done in triplicate and measurements at least twice.

2.4. Statistical Analysis

Two-way analysis of variance (ANOVA) was performed to determine the significant effect of factors as well as their interactions on examined parameters—coagulation time, aggregation rate and modulus of elasticity after 60 min, as an indicator of gel firmness. In this regard, one factor was the heat treatment of goat milk (raw or pasteurized) and the other factor: the concentration of rennet, pH or coagulation temperature, depending on the experiment. Comparison of means within groups was performed with post hoc Tukey HSD test or LSD test, using Statistica 10.0 (Stat Soft. Inc., Tulsa, OK, USA) software.

3. Results and Discussion

3.1. Influence of Rennet Concentration

The parameters of the physicochemical composition of raw milk from this part of the experiment were as follows: 3.60 ± 0.17% protein, 4.57 ± 1.46% milk fat, 13.27 ± 2.07% dry matter and pH 6.54 ± 0.02.

Based on the results shown on Figure 1, it can be observed that the influence of rennet concentration on coagulation time (RCT) was significant in both raw and pasteurized milk. The decrease in rennet concentration significantly prolonged the coagulation time when comparing the average RCT values between all four levels of this factor. Also, a significant difference in coagulation time was found between raw and pasteurized milk at lower rennet concentrations (0.010–0.020 g/L). Thus, the assumption that increasing the concentration of rennet would shorten the coagulation time was also confirmed in goat milk, which is in accordance with the results published by Bencini [26] for cow and sheep milk. This conclusion is not surprising given that the primary phase of coagulation is a first-order enzymatic reaction whose rate depends on the enzyme concentration.

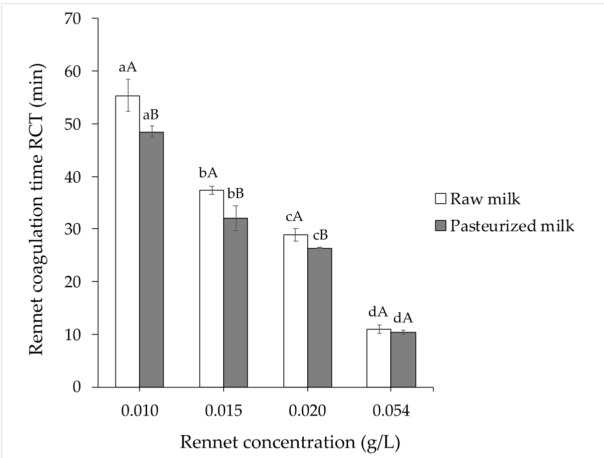
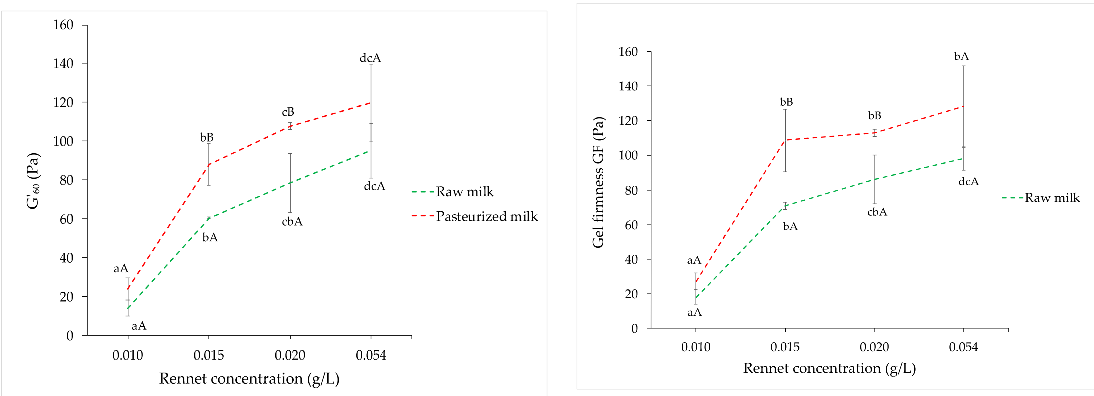


Figure 1. Influence of rennet concentration and heat treatment of milk on rennet coagulation time RCT (min). Values with different lowercase letters (a, b, c, d) represent differences between levels of rennet concentrations, different uppercase letters (A, B) represent differences between milk heat treatments (raw/pasteurized). Mean comparisons were performed with the Tukey HSD test ($p < 0.05$).

Nájera et al. [2] investigated the coagulation properties of cow milk and they have observed that the concentration of rennet has a very pronounced effect on shortening the coagulation time when it increases in the range of 14–56 IMCU/L (equivalent to 0.01–0.04 g/L), after which further increase has no effect and RCT tends to constant value. As can be seen from Figure 1, considering goat milk, higher enzyme concentrations continue to shorten RCT.

Regarding gel firmness, Najera et al. [2] reported that an increase in the concentration of enzyme produced a slight decrease in curd firmness, particularly when the enzyme concentration was risen from 0.010 g/L to 0.040 g/L, as opposed to our results, where gel firmness parameters (G'_{60} and GF) increased in the concentration interval 0.010–0.054 g/L.

Furthermore, it is evident that more than a double increase in the concentration of rennet (from 0.020 to 0.054 g/L) did not significantly influenced gel firmness parameters (G'_{60} and GF), but neither did cause a decrease as the aforementioned authors reported (Figure 2), for the both raw and pasteurized milk. The reduction of the rennet concentration to 0.015 g/L, caused significantly lower G'_{60} of the pasteurized milk gel, while this difference was not detected with a frequency sweep test. Both gel firmness parameters were significantly reduced by a further concentration drop up to 0.010 g/L, considering raw as well as pasteurized milk. The results on Figure 2 illustrate that the increase in gel firmness with increasing rennet concentration was sharper for the pasteurized gel compared to that of the raw milk. Additionally, there is a significant effect of heat treatment of milk on both G'_{60} and GF parameters at two concentration levels (0.015 and 0.020 g/L), indicating higher values of modulus of elasticity when milk was pasteurized.The results obtained by this experiment indicate that increasing the concentration of rennet to a certain extent significantly affects the firmness of rennet gel from goat milk and that the pasteurization of milk leads to higher values of gel firmness expressed as GF and as G'_{60} when the rennet concentrations are 0.015 and 0.020 g/L.



(**a**) (**b**)

Figure 2. Influence of rennet concentration and heat treatment of milk on: (**a**) gel firmness G'60 (Pa) and (**b**) GF (Pa). Values with different lowercase letters (a, b, c, d) represent differences between levels of rennet concentrations, different uppercase letters (A, B) represent differences between milk heat treatments (raw/pasteurized). Mean comparisons were performed with the Tukey HSD test ($p < 0.05$).

Since the role of rennet is to hydrolyze κ-casein and release the macropeptide, its concentration should affect the firmness of rennet gel only if rennet is a limiting factor, i.e., if there is not enough enzyme to perform hydrolysis. The rennet present in a limiting amount restricts the formation of the gel because a smaller number of calcium bridges can be established between casein micelles and consequently, a much softer and weaker rennet gel is formed [26].

The concentration of rennet significantly affected the aggregation rate (AR), so that lower aggregation rates were recorded at lower rennet concentrations (Figure 3). A significant effect of heat treatment of milk was recorded ($p < 0.05$), with a lower rate of aggregation observed in the coagulation of raw milk. There was found significant difference in AR between concentration levels 0.010 and 0.020 g/L, whereas 0.054 g/L resulted in the highest AR values.

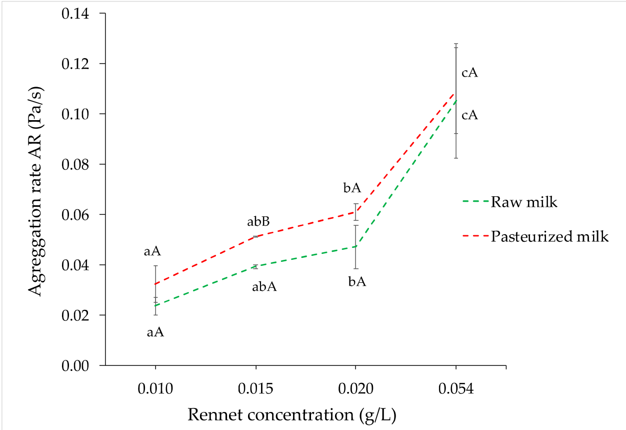


Figure 3. Influence of rennet concentration and heat treatment of milk on aggregation rate AR (Pa/s). Values with different lowercase letters (a, b, c) represent differences between levels of rennet concentrations, different uppercase letters (A, B) represent differences between milk heat treatments (raw/pasteurized). Mean comparisons were performed with the Tukey HSD test ($p < 0.05$).

The rate of aggregation of casein micelles depends on the concentration of micelles in milk [27] and this factor should be taken into account when comparing the results of the aggregation rate for goat milk with other types of milk. A higher aggregation rate indicates faster solidification (maturation) of the milk gel. Bencini [26] found that the concentration of rennet did not have a significant effect on the rate of hardening of the gel from sheep milk, which is characterized by a significantly higher content of casein micelles, while this effect was observed in cow milk. However, when drawing conclusions about the influence of certain factors on rennet coagulation of different types of milk, it should be borne in mind that the high casein content in sheep milk is the dominant factor prevailing over other parameters, and that a different experimental design (casein content adjustment) would probably gave different results. According to other authors, a progressive increase in the rate of hardening of rennet gel from goat [6] and cow milk [2] was observed with increasing enzyme concentration.

3.2. Influence of Milk pH Value

The basic parameters of the physico-chemical composition of raw milk used for this part of the study were as follows: 3.21 ± 0.15% protein, 3.64 ± 0.32% milk fat, 12.03 ± 0.41% dry matter and pH 6.54 ± 0.04.

The lowering of the milk pH caused a significant shortening of the coagulation time (Figure 4). Also, a significant difference in RCT was found between raw and pasteurized milk at pH 6.5, with a shorter time being recorded for pasteurized milk. Observed effect of pH on RCT is consistent with published results for cow and sheep milk, where coagulation times were also shorter if milk pH was lowered [26]. Lowering the pH of cow milk is associated with achieving shorter coagulation times and better rennet gel consistency [28].

The current study did not show a significant effect of pH on rennet gel firmness (G'_{60} and GF) as presented on the Figure 5. There was no statistical difference between raw and pasteurized milk for the values of this parameter. Similarly, Nájera et al. [2] also found no significant effect of pH on the firmness of rennet gel from cow milk.

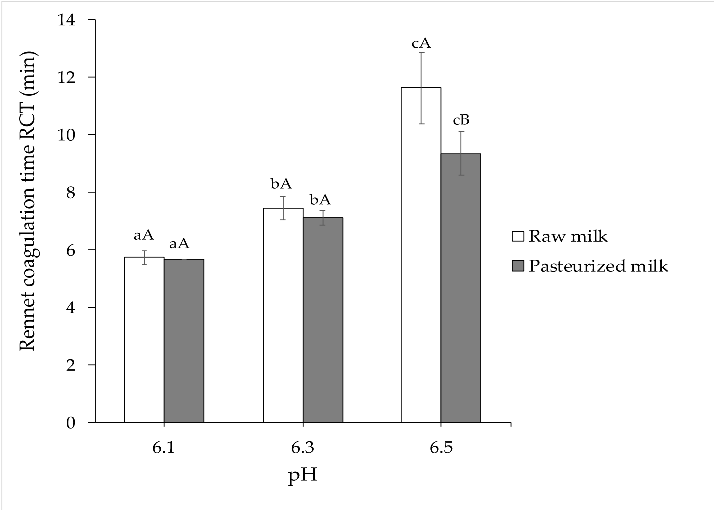


Figure 4. Influence of milk pH and heat treatment of milk on rennet coagulation time RCT (min). Values with different lowercase letters (a, b, c) represent differences between levels of milk pH, different uppercase letters (A, B) represent differences between milk heat treatments (raw/pasteurized). Comparison of means was performed by LSD test ($p < 0.05$).

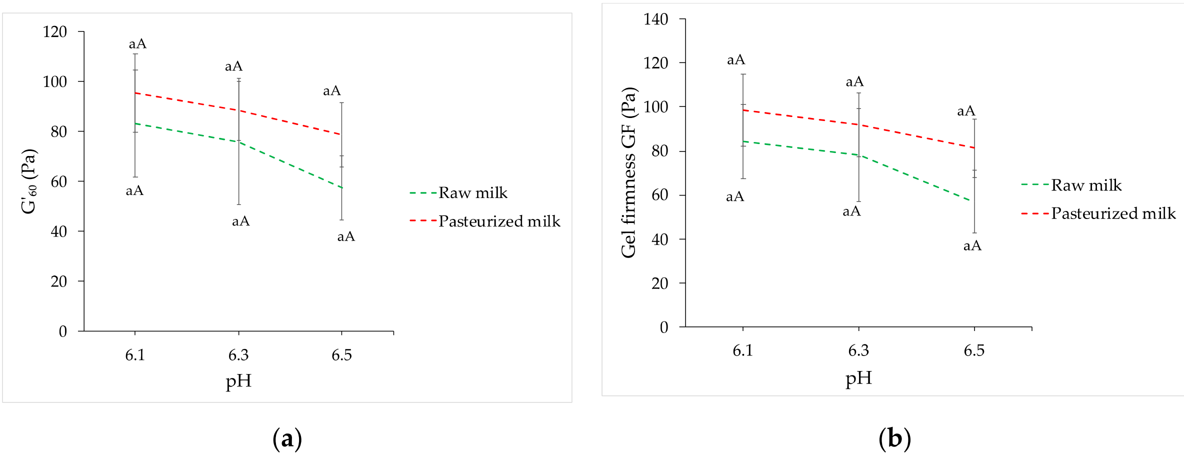


Figure 5. Influence of milk pH and heat treatment of milk on: (**a**) gel firmness G'_{60} (Pa) and (**b**) GF (Pa). Values with lowercase letters indicate no differences between levels of milk pH, and uppercase letters indicate no differences between milk heat treatments (raw/pasteurized). Comparison of means was performed by LSD test ($p < 0.05$).

A lower pH increases the affinity between micelles by reducing their surface charge and thus promotes the aggregation process during the secondary coagulation phase [11]. The aggregation rate increased significantly at the point where pH decreased from 6.5 to 6.3 of both raw and pasteurized milk, but with no further increase (Figure 6). There was a statistically significant difference in aggregation rate between raw and pasteurized milk only at pH 6.5. On the other hand, Bencini [26] found a very strong effect of lowering the pH on the increase of the gel firming rate in cow milk, while in the case of sheep milk, the pH had no effect on the examined parameter.

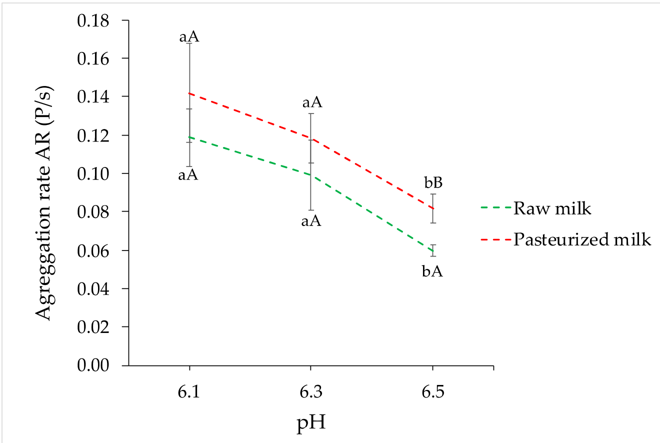


Figure 6. Influence of pH and heat treatment of milk on aggregation rate AR (Pa/s). Values with different lowercase letters (a, b) represent differences between the pH levels of the same milk, different uppercase letters (A, B) represent differences between milk heat treatments (raw/pasteurized). Comparison of means was performed by LSD test ($p < 0.05$).

Nájera et al. [2] examined the influence of a number of factors on the properties of rennet coagulation of cow milk and multivariate analysis established that of all the examined factors, pH had the most pronounced influence on the coagulation time and the gel firming rate. Similar to the results of our research Balcones et al. [29] also reported that lowering the pH of sheep milk shortens the coagulation time and increases the firming rate of the rennet gel.

3.3. Influence of Coagulation Temperature

The basic parameters of the physico-chemical composition of raw milk used in this part of the experiment were as follows: 3.60 ± 0.17% protein, 4.57 ± 1.46% milk fat, 13.27 ± 2.07% dry matter and pH 6.54 ± 0.02.

The coagulation temperature had a significant effect on the coagulation time (RCT) of both raw and pasteurized milk, i.e., with increasing temperature, the RCT was shorter (Figure 7). No significant differences were found in RCT among raw and pasteurized milk when observed at the same coagulation temperature.

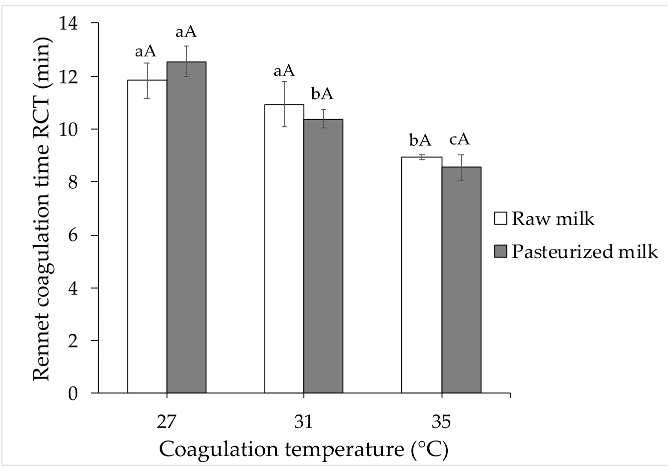
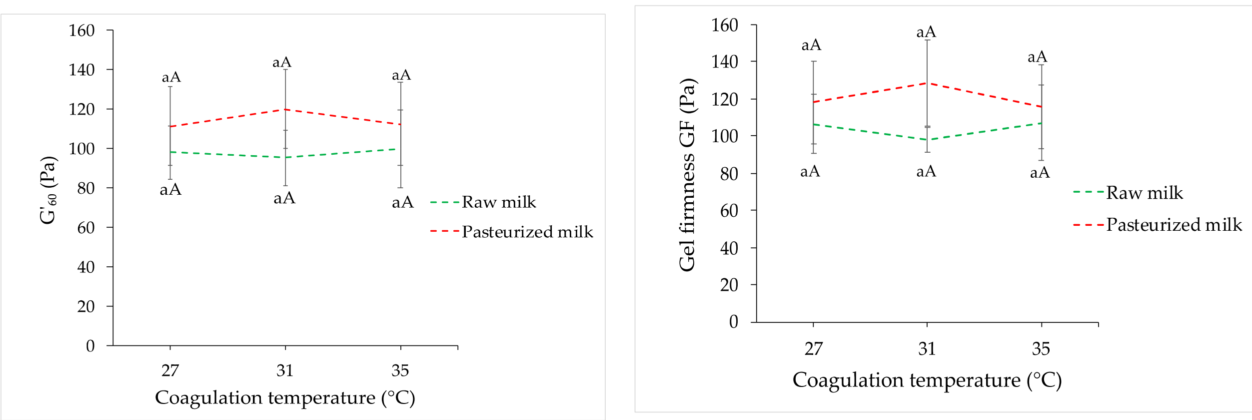


Figure 7. Influence of coagulation temperature and heat treatment of milk on rennet coagulation time RCT (min). Values with different lowercase letters (a, b, c) represent differences between the coagulation temperature levels of the same milk, uppercase letters indicate no differences between milk heat treatments (raw/pasteurized). Comparison of means was performed by Tukey HSD test ($p < 0.05$).

Similar to these results, Bencini [26] reported that an increase in coagulation temperature from 30 to 38 °C caused a shortening of the coagulation time in both cow and sheep milk. Also, Castillo et al. [6] examined the factors influencing the coagulation of goat milk and found the same trend in terms of RCT, when increasing coagulation temperature from 28 to 36 °C.

Based on the results on the Figure 8, it can be concluded that in this study neither the coagulation temperature nor the milk heat treatment significantly affected the rennet gel firmness parameters (G'_{60} and GF). The literature data on the effects of coagulation temperature on this parameters varies and depend on the type of milk on which the test was performed, as well as on the moment at which the gel firmness was measured. Thus, Bencini [26] pointed out that the effect of coagulation temperature on the consistency of rennet gel was significant only in cow milk, where the increase in temperature caused higher consistency values, as opposed to sheep milk where it was found that temperature had no effect on this parameter. Nájera et al. [2] by multivariate analysis of coagulation factors of cow milk demonstrated that the coagulation temperature was of the greatest importance for predicting the parameters for coagulum as well as for curd. These authors suggested that with the increase of the coagulation temperature, also a progressive increase in the curd firmness was recorded. On the other hand, Storry and Ford [30] noted a decrease in rennet gel firmness with increasing coagulation temperature.



(**a**) (**b**)

Figure 8. Influence of coagulation temperature and heat treatment of milk on: (**a**) gel firmness G'_{60} (Pa) and (**b**) GF (Pa). Values with lowercase letters indicate no differences between levels of milk pH, and uppercase letters indicate no differences between milk heat treatments (raw/pasteurized). Comparison of means was performed by Tukey HSD test ($p < 0.05$).

The coagulation temperature had a significant effect on the aggregation rate (Figure 9), where the increase in temperature caused a linear increase of this parameter. On the other hand, the heat treatment of milk did not significantly affect AR.

The secondary coagulation phase accelerates 1.3–1.5 times with each higher degree as the coagulation temperature increases, so that the effect of increasing the coagulation temperature is much more significant for the aggregation phase. It was found that in cow milk, higher coagulation temperatures shorten the coagulation time and increase the rate of rennet gel formation [2], as they are close to the optimal temperature of coagulant activity. The literature data on this temperature range varies, so Mistry [31] suggests 40–45 °C, and Miocinovic [32] reports that the optimal temperatures for coagulant activity are in the 38–40 °C interval. Furthermore, this author states that lowering the coagulation temperature to 32 °C only slightly reduces the reaction rate, while at temperatures below 25 °C the coagulation time is significantly extended. Kumar et al. [33] published that the optimum temperature varies from 30 to 50°C. Nevertheless, the optimal temperature for coagulant activity is not suitable for cheese making, and the rennet coagulation generally occurs at 30–35 °C [31].

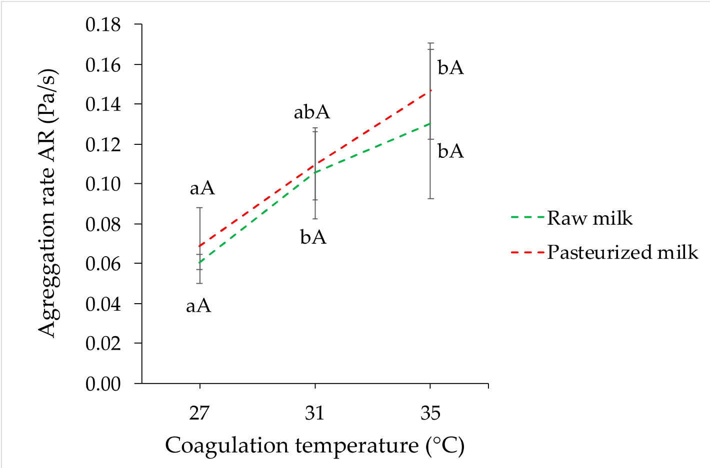


Figure 9. Influence of coagulation temperature and heat treatment of milk on aggregation rate AR (Pa/s). Values with different lowercase letters (a, b) represent differences between the coagulation temperature levels of the same milk, uppercase letters indicate no differences between milk heat treatments (raw/pasteurized). Comparison of means was performed by Tukey HSD test ($p < 0.05$).

Bencini [26] found that the increase in coagulation temperature (from 30 to 34 and 38 °C) slightly increased the firming rate of cow milk gel, while there was practically no effect on the rate of firming of sheep milk gel. The rate of firming had a linear increase with increasing coagulation temperature of cow milk gel according to other authors also [2].

Table 1 shows the significance of the examined factors' influence (rennet concentration, pH, coagulation temperature, milk heat treatment) on all evaluated parameters. Factors were observed individually and the significance is presented as *p*-value in the table.

Table 1. Influence of individual factors on measured parameters for each set of experiments.

	RCT	AR	G'_{60}	GF
MHT	<0.01	<0.05	<0.01	<0.01
Rennet concentration	<0.01	<0.01	<0.01	<0.01
MHT	<0.05	<0.05	ns	ns
pH	<0.01	<0.01	ns	ns
MHT	ns	ns	ns	ns
CT	<0.01	<0.01	ns	ns

Influence of examined factors at the level of significance $p < 0.05$ and $p < 0.01$; ns—not significant; MHT—milk heat treatment; CT—coagulation temperature.

4. Conclusions

The decrease in rennet concentration extended the RCT and reduced the AR. Contrary to some literature data, a significant increase in gel firmness parameters has been recorded with the rise in concentration up to a certain level or remained unchanged in the examined range. This would be interesting to examine in more detail in further research, concerning the differences between cow and goat milk.

The decrease in pH from 6.5 to 6.1 caused two times shorter RCT and two times higher AR. There was no significant effect of pH on the firmness parameters of the rennet gel.

The increase of coagulation temperature significantly reduced the RCT, as well as increased the AR, of both raw and pasteurized milk, without affecting gel firmness.

Bearing in mind that goat milk cheese is mainly produced by craft cheesemakers, it is of importance that there is a wider range of pH and temperature that would not significantly affect gel firmness, which would allow achieving a similar yield of cheese.

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References

1. Clark, S.; Sherbon, J.W. Alphas1-casein, milk composition and coagulation properties of goat milk. *Small Rumin. Res.* **2000**, *38*, 123–134. [CrossRef]
2. Nájera, A.I.; de Renobales, M.; Barron, L.J.R. Effects of pH, temperature, CaCl2 and enzyme concentrations on the rennet-clotting properties of milk: A multifactorial study. *Food Chem.* **2003**, *80*, 345–352. [CrossRef]
3. Horne, D.S. Casein micelle structure and stability. In *Milk Proteins*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 213–250. [CrossRef]
4. López, M.; Lomholt, S.; Qvist, K. Rheological Properties and Cutting Time of Rennet Gels. Effect of pH and Enzyme Concentration. *Int. Dairy J.* **1998**, *8*, 289–293. [CrossRef]
5. Fox, P.F.; Guinee, T.P.; Cogan, T.M.; McSweeney, P.L.H. Enzymatic coagulation of milk. In *Fundamentals of Cheese Science*; Springer: Berlin/Haidelberg, Germany, 2017; pp. 185–229.
6. Castillo, M.; Payne, F.; Hicks, C.; Lopez, M. Predicting cutting and clotting time of coagulating goat's milk using diffuse reflectance: Effect of pH, temperature and enzyme concentration. *Int. Dairy J.* **2000**, *10*, 551–562. [CrossRef]
7. Panthi, R.R.; Kelly, A.L.; Sheehan, J.J.; Bulbul, K.; Vollmer, A.H.; McMahon, D.J. Influence of protein concentration and coagulation temperature on rennet-induced gelation characteristics and curd microstructure. *J. Dairy Sci.* **2019**, *102*, 177–189. [CrossRef] [PubMed]
8. Nicolau i Villellas, N. *Influence of High Pressure Treatments on Goat's Milk Cheese for the Improvement of Its Sensory and Commercial Characteristics*; Universitat Autònoma de Barcelona: Barcelona, Spain, 2015.
9. Miloradovic, Z.; Kljajevic, N.; Miocinovic, J.; Tomic, N.; Smiljanic, J.; Macej, O. High heat treatment of goat cheese milk. The effect on yield, composition, proteolysis, texture and sensory quality of cheese during ripening. *Int. Dairy J.* **2017**, *68*, 1–8. [CrossRef]
10. Schiffer, S.; Scheidler, E.; Kiefer, T.; Kulozik, U. Effect of temperature, added calcium and pH on the equilibrium of caseins between micellar state and milk serum. *Foods* **2021**, *10*, 822. [CrossRef]
11. Daviau, C.; Famelart, M.H.; Pierre, A.; Goudédranche, H.; Maubois, J.L. Rennet coagulation of skim milk and curd drainage: Effect of pH, casein concentration, ionic strength and heat treatment. *Lait* **2000**, *80*, 397–415. [CrossRef]
12. Zhang, Y.; Chen, R.; Ma, H.; Chen, S. Isolation and identification of dipeptidyl peptidase IV-inhibitory peptides from trypsin/chymotrypsin-treated goat milk casein hydrolysates by 2D-TLC and LC–MS/MS. *J. Agric. Food Chem.* **2015**, *63*, 8819–8828. [CrossRef]
13. Park, Y.W.; Juárez, M.; Ramos, M.; Haenlein, G.F.W. Physico-chemical characteristics of goat and sheep milk. *Small Rumin. Res.* **2007**, *68*, 88–113. [CrossRef]
14. Pesic, M.B.; Barac, M.B.; Stanojevic, S.P.; Ristic, N.M.; Macej, O.D.; Vrvic, M.M. Heat induced casein–whey protein interactions at natural pH of milk: A comparison between caprine and bovine milk. *Small Rumin. Res.* **2012**, *108*, 77–86. Available online: http://www.sciencedirect.com/science/article/pii/S0921448812002556 (accessed on 27 September 2020). [CrossRef]
15. Miloradovic, Z.; Kljajevic, N.; Miocinovic, J.; Levic, S.; Pavlovic, V.B.; Blažić, M.; Pudja, P. Rheology and Microstructures of Rennet Gels from Differently Heated Goat Milk. *Foods* **2020**, *9*, 283. [CrossRef]

16. Hovjecki, M.; Miloradovic, Z.; Rac, V.; Pudja, P.; Miocinovic, J. Influence of heat treatment of goat milk on casein micelle size, rheological and textural properties of acid gels and set type yoghurts. *J. Texture Stud.* **2020**, *51*, 680–687. [CrossRef] [PubMed]
17. IDF. *Milk, Cream And Evaporated Milk–Total solids, Standard 21 B*; International Dairy Federation: Brussels, Belgium, 1987.
18. IDF. *Milk. Determination of Fat Content. Gerber Butyrometers*; International Dairy Federation: Brussels, Belgium, 1981.
19. AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Washington, DC, USA, 1990; pp. 807–809, 841–842.
20. Silanikove, N.; Merin, U.; Shapiro, F.; Leitner, G. Milk metabolites as indicators of mammary gland functions and milk quality. *J. Dairy Res.* **2014**, *81*, 358–363. [CrossRef]
21. Fox, P.F.; Guinee, T.P.; Cogan, T.M.; McSweeney, P.L.H. *Fundamentals of Cheese Science*; Springer: Berlin/Heidelberg, Germany, 2017; Available online: https://link.springer.com/book/10.1007%2F978-1-4899-7681-9 (accessed on 12 October 2020).
22. Steffl, A.; Schreiber, R.; Hafenmair, M.; Kessler, H.G. Effect of denatured whey proteins on the rennet-induced aggregation of casein micelles. *Int. Dairy J.* **1999**, *9*, 401–402. [CrossRef]
23. Wang, Q.; Bulca, S.; Kulozik, U. A comparison of low-intensity ultrasound and oscillating rheology to assess the renneting properties of casein solutions after UHT heat pre-treatment. *Int. Dairy J.* **2007**, *17*, 50–58. [CrossRef]
24. Dimassi, O.; Neidhart, S.; Carle, R.; Mertz, L.; Migliore, G.; Mané-Bielfeldt, A.; Zárate, A.V. Cheese production potential of milk of Dahlem Cashmere goats from a rheological point of view. *Small Rumin. Res.* **2005**, *57*, 31–36. [CrossRef]
25. Lucey, J.A.; Teo, C.T.E.T.; Munro, P.A.; Singh, H. Rheological properties at small (dynamic) and large (yield) deformations of acid gels made from heated milk. *J. Dairy Res.* **1997**, *64*, 591–600. [CrossRef]
26. Bencini, R. Factors affecting the clotting properties of sheep milk. *J. Sci. Food Agric.* **2002**, *82*, 705–719. [CrossRef]
27. Payens, T.A.J. On enzymatic clotting processes. *Biophys. Chem.* **1977**, *6*, 263–270. [CrossRef]
28. McMahon, D.J.; Richardson, G.H.; Brown, R.J. Enzymic Milk Coagulation: Role of Equations Involving Coagulation Time and Curd Firmness in Describing Coagulation. *J. Dairy Sci.* **1984**, *67*, 1185–1193. [CrossRef]
29. Balcones, E.; Olano, A.; Calvo, M.M. Factors Affecting the Rennet Clotting Properties of Ewe's Milk. *J. Agric. Food Chem.* **1996**, *44*, 1993–1996. [CrossRef]
30. Storry, J.E.; Ford, G.D. Some factors affecting the post clotting development of coagulum strength in renneted milk. *J. Dairy Res.* **1982**, *49*, 469–477. [CrossRef]
31. Mistry, V.V. Chymosin in cheese making. *Food Biochem. Food Processing* **2012**, *52*, 1170–1176.
32. Miočinović, J. *Tehnologija mleka II Posebno Sirarstvo*; Univerzitet u Beogradu, Poljoprivredni Fakultet: Belgrade, Serbia, 2020.
33. Kumar, A.; Grover, S.; Sharma, J.; Batish, V.K. Chymosin and other milk coagulants: Sources and biotechnological interventions. *Crit. Rev. Biotechnol.* **2010**, *30*, 243–258. [CrossRef] [PubMed]