



The impact of heat treatment conditions on the stability of selected biochemical parameters of small ruminant's milk in Armenia

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ABSTRACT

Background: There is a growing need to precisely identify key biological markers to assess the pasteurization of milk from small ruminants accurately. In the case of milk from cattle, such indicators have been specified, whereas for small ruminants, they have not; therefore, this is an area that requires further research.

Objective: The research aimed to assess the stability of the biochemical parameters of goat and sheep milk pasteurized under two different sets of conditions. The overall goal was to precisely determine the key biochemical markers for accurate pasteurization of milk from small ruminants to produce safe, functional food for consumption.

Materials and Methods: Alkaline phosphatase (ALP), lactoperoxidase (LP), and furosine (FRS) were measured

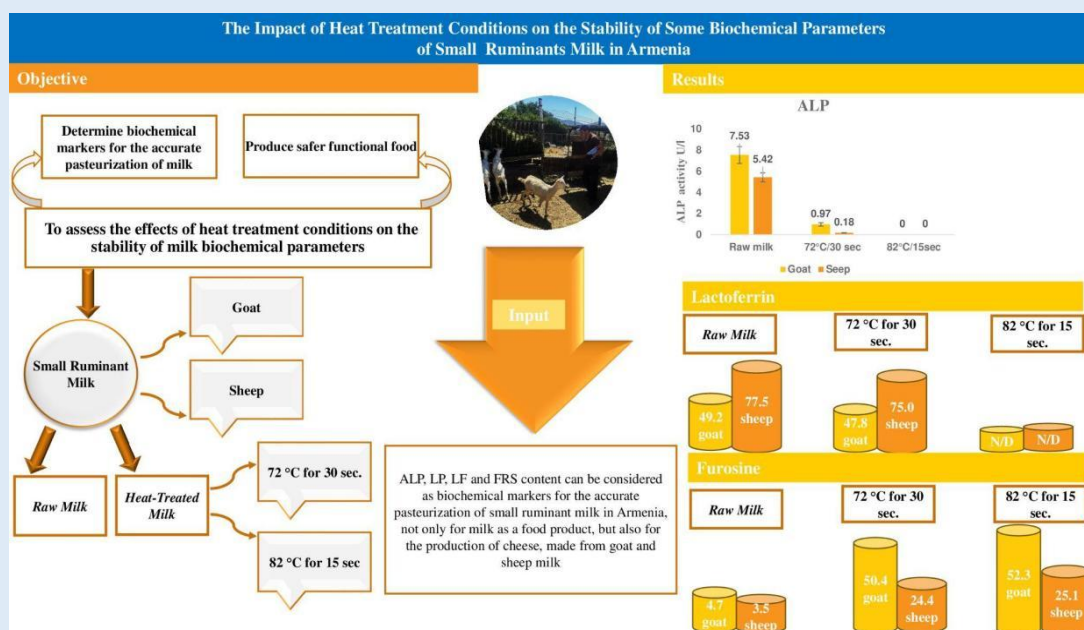
photometrically on a CDR FoodLab Analyzer. γ -glutamyl transferase (GGT), lysozyme, and lactoferrin (LF) levels were determined spectrophotometrically, while casein, lactose, and fat levels were measured using a milk analyzer. The milk heat treatment conditions were 72 °C for 30 seconds and 82 °C for 15 seconds.

Results: Heat treatment decreased ALP, LP, and GGT levels in goat and sheep milk at 72 °C/30 sec by 87.2%, 61.3%, and 83.1%, respectively, and by 96.6%, 61.4%, and 64.9%, respectively. In contrast, levels of lysozyme and LF were unaffected by heat treatment. ALP and LF were not detected in goat and sheep milk heat-treated at 82 °C/15 sec, and their absence in both types of milk provides a basis for proposing them as biochemical markers for precise pasteurization. Furosine was reliably generated in goat and sheep milk as the temperature increased. The total casein, fat, and lactose in raw sheep milk exceeded goat milk by 1.7, 1.3, and 1.2 times, respectively, and a similar trend was observed during heat treatment.

Novelty: We propose a species-specific biomarker panel for goat and sheep milk pasteurization—ALP, LP and GGT suppression with FRS increase, plus LF loss at 82 °C/15 s—validated under 72 °C/30 s and 82 °C/15 s. This fills the gap beyond cattle-based indicators and enables precise pasteurization for safe, functional dairy and cheese production.

Conclusion: The practical implication of the research is that ALP, LP, LF, and FRS can be considered as biochemical markers for the accurate pasteurization of goat and sheep milk in RA. This proposal provides a basis for clarifying indicators for the production of fermented dairy products under UHT conditions.

Keywords: furosine, alkaline phosphatase, small ruminant, proper pasteurization marker



Graphical Abstract: The impact of heat treatment conditions on the stability of selected biochemical parameters of small ruminant's milk in Armenia

INTRODUCTION

Milk and dairy products comprise a wide range of foodstuffs marketed and consumed worldwide [1]. As a result, there is a growing need to precisely determine key biochemical parameters to accurately assess pasteurization of milk, especially from small ruminants such as goats and sheep. Although the heat-treatment processes are similar across all types of animals, there are type-specific peculiarities that can influence the stability of milk's biochemical parameters. Accurate pasteurization indicators have been specified for cattle milk, whereas for small ruminants, this core issue requires further research [2-3]. Therefore, based on the European Food Safety Agency's (EFSA's) recommendation, there is a need to identify key biological markers in the pasteurization of milk from small ruminants [4], which is the objective of this study. This work comprises a logical continuation of our previous research [5], which focused on identifying biochemical markers of pasteurization in raw and heat-treated goat milk and dairy products. In that study, pasteurization was carried out using the low-temperature, long-time method at 63 °C for 30 min. In the work described in this paper, we continue exploring potential biochemical markers of precise pasteurization, this time testing different heat treatment conditions: high-temperature, short-time treatments at 72°C for 30 seconds and 82°C for 15 seconds. To gather more complete data on milk from small ruminants, we expanded the study to include not only goats but also sheep.

During pasteurization, milk is subject to a specific heat treatment for a particular period of time, and, as a result, the milk produced can be affected by the combination of time and temperature used. Heat treatment itself is a necessary process that affects the quality of milk produced. In the case of small ruminants, it is known that goat and sheep milk is less stable in

relation to heat treatment than that from cattle. Heat treatment inactivates milk enzymes, which can serve as a barometer of accurate pasteurization and as a means of meeting legal requirements for milk processing [6-8]. Accurate pasteurization of milk is crucial for the continued development of functional food production in Armenia as well. According to the Functional Food Center, functional foods are defined as those containing active ingredients that, at appropriate doses, have proven benefits in preventing chronic diseases, and bioactive compounds are key components in the development of functional foods that offer health benefits beyond core nutrition [9-12].

In the work described here, we were guided by the EFSA questionnaire on Alkaline Phosphatase (ALP), which emphasizes the importance of ALP testing in milk, colostrum, dairy, and colostrum-based products from non-bovine species [13]. We were also guided by our knowledge of increasing industry demands and by the fact that many countries have now accepted the ALP test as the standard assay for the rapid validation of milk pasteurization [14]. As a result, the first biochemical parameter chosen in our study was ALP activity measurement in goat and sheep milk in Armenia, to determine whether this activity changed before and after different heat treatment conditions.

It is known that ALP is more resistant to thermal inactivation than the most heat-resistant bacterial pathogens present in milk (i.e., *Coxiella burnetii* and *Mycobacterium tuberculosis*). Thus, if ALP activity is highly reduced, it can be concluded that the legal thermal requirements for pasteurization have been met and that bacterial pathogens have been similarly reduced [15].

In addition to ALP, other enzyme systems were also evaluated in this study. These enzymes included γ -Glutamyl Transferase (GGT) and Lactoperoxidase (LP). It has been found that γ -Glutamyl Transferase (GGT) is more heat-resistant than ALP but less so than

Lactoperoxidase (LP). Most research on GGT detection has focused on bovine milk, and only a few studies have targeted non-bovine milk. GGT catalyzes the breakdown of glutathione and therefore plays a vital role in the biosynthesis of milk proteins, making its study essential [16-17].

Lactoperoxidase has antimicrobial activity and plays a vital role in protecting against bacterial infections. The LP system acts as an antioxidant by protecting cells against reactive oxygen species (ROS). It is known that LP has high thermal stability in milk, though thermal inactivation varies across species. Most studies on the activity and thermal denaturation of LP have focused on bovine milk, and little research has been conducted on non-bovine dairy [18].

Thermal treatments are used to improve milk microbial safety, enhance the biological activity of some milk components, extend shelf life, and inactivate certain enzymes. That said, thermal treatments can also reduce milk's nutritional quality by altering the molecular structure of proteins, such as lysozyme, an important milk component due to its antimicrobial activity against gram-positive bacteria. For example, the research literature shows that sheep lysozyme displayed high enzymatic activity at 40-70°C but was inactivated at temperatures above 80°C [19-24].

Another beneficial protein found in milk is Lactoferrin (LF). This protein belongs to the transferrin family and is an iron-binding protein in milk [23]. It has antimicrobial, antioxidant, antibacterial, and antifungal properties and is widely used in probiotic foods for human consumption [26]. Shabeeb *et al.* (2024) reported that LF levels vary across species, with the average percentage content in goat milk higher than in cow milk but lower than in sheep milk [27]. Liu *et al.* (2020) studied the thermal denaturation of LF in raw bovine milk over a temperature range of 65 to 121°C for 2 to 300 sec and concluded that a model for the 65 to 95°C range can be

applicable for evaluating existing processes in food manufacturing [28].

To evaluate milk heat damage during heating, several indicators have been proposed. For example, Furosine (FRS) is the stable product of the first stages of the Maillard reaction produced by the hydrolysis of lactulose-lysine, which accumulates in heat-treated milk [29-30]. The research literature suggests that FRS can be used to monitor the heat treatment of milk, and that higher concentrations of Maillard reaction compounds may result from excessive or repeated heat treatment [31-32]. With this in mind, and within the context of our research, FRS levels in treated and untreated milk from goats and sheep were studied to determine whether this marker could serve as one indicator of proper milk pasteurization in Armenia. In addition, levels of total casein, fat, and lactose in milk were studied.

The objective of the study was to assess the effects of treatment and untreated conditions on the stability of biochemical parameters in goat and sheep milk pasteurized under various conditions, to determine the key biochemical markers for accurate pasteurization.

MATERIAL AND METHODS

Milk sample collection and preparation: Goat milk samples were collected from Shirak, and sheep milk samples were collected from Kotayk, RA, and transferred to the university laboratories in accordance with the requirements of Technical Regulation of the Customs Union TP TS 033/2013 [33]. Milk sampling for both ruminant species was carried out in the second month of lactation in 2024 and included 1.5-6-year-old 22 Saanen breed female goats and 2-6-years-old 15 semi fine-wool local female sheep. Milk heat treatment related time-temperature conditions were fulfilled at the HTST conditions, specifically, at 72°C/30 sec. and 82°C/15 sec., according to Technical Regulations [33].

The analysis was conducted in the Food Quality

Control Laboratory of the Armenian National Agrarian University (ANAU). For spectrophotometric

measurements, an AQ7100 spectrometer (Thermo Fisher Scientific, UK) was used.



Pic. 1. Goat breeding farm, milk sampling, pasteurization.

Analysis of biochemical and chemical parameters:

Alkaline Phosphatase (ALP) and Lactoperoxidase (LP) activities, and Furosine (FRS) concentration were measured photometrically on a CDR FoodLab Analyzer (CDR, Italy). The ALP activity was determined by measuring the absorbance at 420 nm after hydrolysis of p-nitrophenyl phosphate. The LP activity was determined by measuring the formation of a red compound proportional to peroxidase concentration in milk at 505 nm. FRS concentration was determined by the reaction between a tetrazol salt and ϵ -fructosyl-lysine, with the intensity of the resulting purple compound measured at 545 nm. γ -Glutamyl Transferase (GGT) activity was measured spectrophotometrically by quantifying p-nitroaniline, following the method described by Zehetner

et al. [34], with absorbance measured at 410 nm. Lysozyme activity was determined according to the method described by Selested and Martinez [35]. A suspension of *Micrococcus lysodeikticus* (35 mg%) in 0.05 M potassium phosphate buffer, pH 7.4, was used as a substrate, and absorbance was measured at 450 nm. Iron in lactoferrin was measured by using o-phenanthroline, with absorbance of the formed compound measured at 520 nm [36]. pH was measured using a digital pH meter (Jenway 3540, UK). The total casein, lactose, and fat were measured using a Milk Analyzer "Expert Standard" (Laboratorika, RF). The biochemical and chemical parameters in raw and heat-treated milk were measured four times.



Pic 2. Experiment implementation, Food Quality Control Laboratory, ANAU.

Animal Feeding: The daily feed per sheep during the first period of suckling (March-April), apart from grass, comprised a 0.4-0.5 kg concentrate, which included wheat, oat, bran, and mineral mixture. The outdoor stall scheme was used for goats; therefore, in addition to grass, the feeding diet structure per dairy goat included hay and a combined forage consisting of sunflower, soybeans, maize, and barley.

Statistical Analysis: The data were analyzed using Microsoft Excel 2018 [37] and are presented as the mean $\pm$ standard error of the mean (SEM). Variation between experimental groups was evaluated using a one-way ANOVA, and any statistical differences are indicated where $P < 0.05$.

RESULTS AND DISCUSSION

The biochemical parameters in raw and heat-treated milk under different conditions: The findings of our study indicate that species-specific characteristics were observed. The activity of ALP in raw goat milk was significantly higher ($p < 0.05$) than in sheep milk, measuring 7.53 ± 0.79 U/l against 5.42 ± 0.43 U/l respectively (Fig.1). However, it is interesting to note that when subjected to heat treatment at $72^\circ\text{C}/30$ sec., a sharp and statistically significant drop ($p < 0.05$) in ALP

activity was recorded in both goat and sheep milk. In goat milk, ALP activity decreased from 7.53 ± 0.79 U/l to 0.97 ± 0.16 U/l (a 87.2% reduction), while in sheep milk, it dropped from 5.42 ± 0.43 U/l to 0.18 ± 0.02 U/l (a 96.6% reduction). Therefore, it is worth noting that ALP in sheep milk appears to be sensitive to heat processing than goat milk, since under the same pasteurization conditions, ALP activity in goat milk decreased approximately 7.7-fold. In contrast, in sheep milk, it dropped by about 30.1-fold. Comparing these findings to our previous study, where goat milk was heat-treated at $63^\circ\text{C}/30$ min., the ALP activity decreased around 3.3 times – from 7.05 U/l to 2.1 U/l [5]. Remarkably, when the milk samples were exposed to a higher temperature treatment – $82^\circ\text{C}/15$ seconds – no ALP activity was detected in either goat or sheep milk. Summarizing the results of our experiments, we can conclude that ALP activity can serve as a reliable biochemical marker for the precise pasteurization of small ruminant milk. This assumption will be further studied in the following stages of our research, particularly under UHT conditions and through complementary microbiological analysis – a decisive factor not only for cheese, but also for fermented dairy product production.

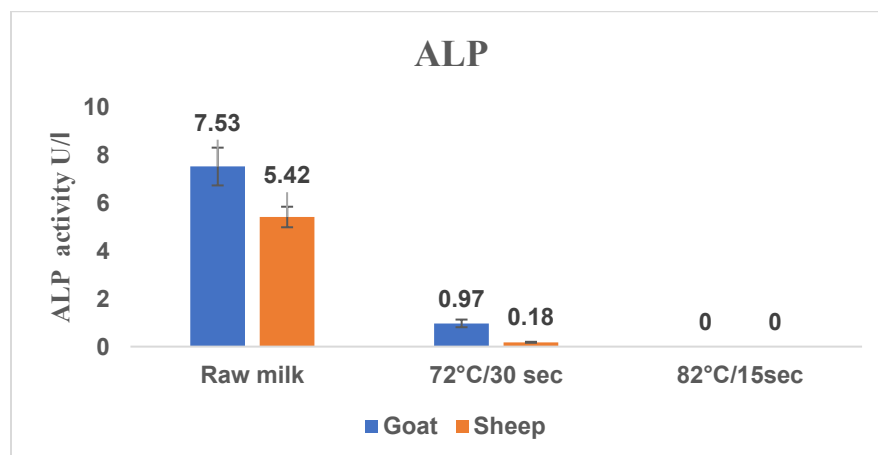


Figure 1. ALP activity trend of small ruminant raw and heat-treated milk

The literature indicates that differences in thermal resistance mean some milk enzymes can serve as indicators of heat treatment for milk and dairy products. Even though ALP has been used as a marker in evaluating the accuracy of cow milk pasteurization, there is still a need to clarify whether this marker is applicable to non-cow milk and dairy products [38-40]. Lorenzen *et al.* (2010) concluded that ALP average activity in raw cow, sheep, and goat milk was 774, 1413, and 67 U/l, respectively, and after pasteurization at 62°C/32 min. The enzyme activity in the three mammals' milk was below 0.6 U/l, whereas at HTST pasteurization conditions (75°C/28 seconds), ALP activity was lower than 0.1 U/l [41]. That said, Tsiamita *et al.* (2022) report that ALP commercial kits should not be used as pasteurization indicators for donkey and camel milk and that other markers should be considered [42].

Figure 2 illustrates the dynamics of GGT activity in raw and heat-treated milk from goat and sheep under different pasteurization conditions. The research presented indicates that, like ALP, GGT activity also demonstrates species-specific characteristics. However, while ALP activity was higher in goat milk compared to sheep milk, the opposite trend was observed for GGT. In raw milk, GGT activity in sheep significantly exceeded that in goat milk by approximately 2.1 times ($p < 0.05$), measuring 1172.0 ± 127.3 U/l in sheep milk and 558.0 ± 34.3 U/l in goat milk, respectively. A similar species-specific pattern was reported in the study by Lorenzen *et al.* (2010), in which the authors found the following order of GGT activity in raw milk: cow (4143 U/l), sheep (1878 U/l), and goat (603 U/l) [41]. Our results also show that GGT in both goat and sheep milk is more heat-resistant than ALP, with this effect being more pronounced in sheep milk. For instance, when milk was treated at 82°C for 15 seconds, ALP activity was inactivated entirely in both types of milk. However, residual GGT activity remained – 1.49 ± 0.52 U/l in goat

milk (a 99.7% decrease compared to raw milk), and 70.4 ± 4.8 U/l in sheep milk (a 94% decrease). It is worth noting that our findings regarding the relative thermal stability of GGT and ALP enzymes are consistent with existing literature [13, 16, 39]. To summarize the impact of different heat treatment conditions on GGT activity in goat and sheep milk, we can conclude that increasing the temperature results in a statistically significant ($p < 0.05$) decline in enzyme activity. In goat milk, raw GGT activity started at 558.0 U/l and decreased by 83.1% at 72°C (down to 94.6 U/l), and by 98.5% at 82°C (down to 1.49 U/l). In sheep milk, initial GGT activity was 1172 U/l, which dropped by 64.9% (to 411.9 U/l) at 72°C, and by 94% (to 70.4 U/l) at 82°C. These results were therefore similar to those reported by Dumitraşcu *et al.* (2013), who found that thermal treatment at 65 °C for 5min reduced GGT enzyme activity in sheep, goat, and cow milk by 96.07%, 85.9%, and 70.7%, respectively [18].

With respect to LP, our research demonstrated significant ($p < 0.05$) inactivation following heat treatment in both goat and sheep milk (Table 1); however, the enzyme activity differences between goat and sheep milk are not clearly expressed, as observed for ALP and GGT. The results indicate that the LP activity in goat raw milk was 3.2 ± 0.38 U/ml after heat treatment at 72 °C for 30 sec. condition decreased to 1.24 ± 0.18 U/ml (61.3%) and at 82°C/15 sec. the residual activity constituted 0.30 ± 0.19 U/ml (90.6%). Regarding sheep milk, the data showed that in raw milk, the LP enzyme activity was 4.35 ± 0.5 U/ml, which decreased in parallel with increasing temperature to 1.68 ± 0.12 U/ml (61.4%) and 0.44 ± 0.07 U/ml (89.9%). Thus, LP heat treatment inactivation in goats' milk at 82°C/15 sec reached 90.6%, and in sheep milk, 89.9%. It should be noted that LTLT heating (63°C/30 min) performed in our previous research resulted in 69.7% LP inactivation in goat milk compared with raw milk [5]. Sharma *et al.* (2014) studied LP and GGT activity in cow milk and concluded that in milk

heated at 65 °C, 70 °C, and 75 °C, substantial enzyme activity remained, whereas at 80 °C both enzymes were completely inactivated [44]. Lorenzen *et al.* (2010) demonstrated that the LP activity of cow, ewe, and goat

milk at 75 °C/28 sec. heat treatment condition reduced from 2267 U/l (raw milk) to 887 U/l; from 1850 to 980 U/l and from 4277 to 1820 U/l respectively [41].

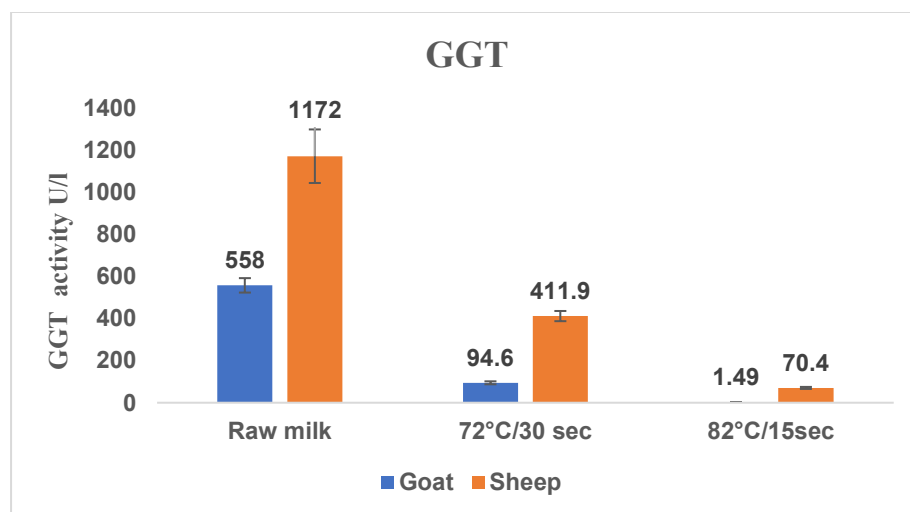


Figure 2. GGT activity trend of small ruminant raw and heat-treated milk.

Table 1 presents the results of our study on the levels of FRS, lysozyme, and LF in raw goat and sheep milk, as well as under different heat treatment conditions. The data show that raw goat milk contained slightly higher levels of FRS when compared to sheep milk – 4.7 ± 0.69 mg/100g protein and 3.5 ± 0.67 mg/100g protein, respectively. Following heat treatment, the generation of FRS in goat milk was twice as intense as in sheep milk. After pasteurization at 72°C for 30 seconds, FRS content in goat milk increased by 90.7%, reaching 50.4 ± 3.7 mg/100g protein, while in sheep milk it rose by 85.7%, reaching 24.4 ± 5.2 mg/100g protein. It is worth noting that FRS generation slowed when milk was heated to 82°C for 15 seconds. In goat milk, FRS content increased by only 3.64% compared to the 72°C treatment, amounting to 52.3 ± 5.6 mg/100g protein, while in sheep milk it increased by just 2.8%, reaching 25.1 ± 5.0 mg/100g protein. When we incorporate these results with findings from our previous study, the accumulation of FRS in goat milk under different heat treatment conditions followed this trend: 47.8 mg/100g

protein at 63°C for 30 minutes [5], 50.4 mg/100g protein at 72°C for 30 seconds, and 52.3 mg/100g protein at 82°C for 15 seconds. FRS can accumulate in milk after heat treatment and its formation is undoubtedly related to the heat treatment conditions, therefore it has been shown to be an indicator of heat damage during sterilization [45–46]. Zhao *et al.* (2023) followed the FRS content in camel raw milk and under different heating condition and reported that with the increase in heat treatment temperature and time, the FRS concentration increased significantly and reached a maximum value at 135 °C heating. In addition, compared with that of cow's milk, it was higher than that of camel milk at the same heat treatment conditions [47]. Sakkas *et al.* (2014) assessed the effects of different heat treatments (80–140°C/4 sec..) on the FRS content in various types of milk. They reported that the average FRS concentration was 1.9 and 126.5 mg/L in raw and 140 °C-treated milk, respectively [48].

According to our study, lysozyme content in raw goat milk was 2.3 times higher than that in sheep milk

and ranged from 0.73 ± 0.07 to 0.31 ± 0.09 mg/ml, indicating the higher bactericidal properties of goat milk. It should be emphasized that heat-treatment at 72°C for 30 sec. The condition resulted in a moderate decrease in lysozyme activity in both types of milk. It constituted 0.70 mg/ml in goat and 0.27 mg/ml in sheep milk. In contrast, heat-treatment at 82°C for 15 sec. led to complete inactivation of enzyme activity in sheep milk, with a residual concentration of 0.15 mg/ml observed in goat milk. Priyadarshini and Kansal (2002) studied the effect of heat processing on the lysozyme content of buffalo and cow milk. They concluded that the enzyme activity was higher in buffalo milk than in cow milk. In addition, lysozyme in buffalo milk was fully stable compared with cow milk [49].

The LF levels found in our research were approximately 1.6 times higher in raw sheep milk than in raw goat milk, at 77.5 ± 8.7 mg/l and 49.2 ± 6.7 mg/l, respectively. In this context, our research is consistent with the literature, which indicates that the LF average concentration in sheep's milk is higher than that in goats' milk [27]. Our research shows that pasteurization at 72°C for 30 seconds resulted in a statistically significant ($p < 0.05$) reduction in LF levels in both types of milk compared to raw milk. Considering that, under these

conditions, goat milk contained 47.8 mg/l of LF and sheep milk contained 75.0 mg/l, and given that milk used for cheese production is typically pasteurized using HTST methods – at a minimum of 72°C for 15 seconds or 63°C for 30 minutes [50], we regard this finding as positive from a practical standpoint. It supports the potential for preserving the antimicrobial and antioxidant properties of the final product, contributing to both the development of high-quality milk products and the expansion of goat and sheep milk cheese production in Armenia. At the same time, the complete absence of LF in both goat and sheep milk following heat treatment at 82°C for 15 seconds provides a strong rationale for proposing LF as an additional biochemical marker for precise pasteurization. The literature indicates that heat treatment at 72°C for 20 seconds has no statistically significant effect on lactoferrin content between raw and pasteurized goat milk, and that lactoferrin concentration increases during the lactation period [51-52]. Another study reported that LF in donkey milk completely disappeared at heat treatment temperatures above 65°C [53]. Litwińczuk *et al.* (2011) showed that LF concentration in cow milk varied by breed and season, ranging from 66 to 119 mg/L [54].

Table 1. Impact of heat treatment conditions on small ruminant milk biochemical parameters. *Data are represented as the Mean $\pm$ SEM.

Parameters	Raw milk	Heat-treated heterogeneous milk	
		72°C/30 sec.	82°C/15 sec.
Goat (n=22)			
Furosine (mg/100 g protein)	4.7±0.69	50.4±3.72	52.3±5.66
Lactoferrin (mg/l)	49.2±6.69	47.8±6.07	N/D
Lysozyme (mg/ml)	0.73±0.07	0.70±0.06	0.15±0.06
LP (U/ml)	3.20±0.38	1.24±0.18	0.30±0.19
Sheep (n=15)			
Furosine (mg/100 g protein)	3.5±0.67	24.4±5.21	25.1±5.03
Lactoferrin (mg/l)	77.5±8.7	75.0±5.8	N/D
Lysozyme (mg/ml)	0.31±0.09	0.27±0.08	N/D
LP (U/ml)	4.35±0.50	1.68±0.12	0.44±0.07

Impact of heat treatment conditions on the chemical characteristics of milk: The chemical composition of basic nutrients in goat and sheep raw milk and their trend under different pasteurization conditions is presented in Table 2. It is noteworthy that, in terms of the key nutritional components studied – total casein, fat, and lactose – raw sheep milk exceeded goat milk by 1.7, 1.3, and 1.2 times, respectively. In this context, it should be noted that the casein concentration in raw milk positively correlated with GGT activity, which plays a crucial role in

milk protein synthesis [17]. It should also be emphasized that the levels of these nutrients in both goat and sheep milk fall within the normative limits set by the Technical Regulations of the Customs Union [33], as well as the Technical Conditions for "*Pure Goat Milk*", accepted by the Armstandard of Armenia [55]. A similar trend was observed during heat treatment. The levels of the primary nutrients must remain within the normative range at 72 °C for 30 seconds, given their nutritional value in dairy functional foods.

Table 2. Effects of heat treatment conditions on the chemical composition of small ruminant milk.

Parameters	Raw milk	Heat-treated heterogeneous milk	
		72°C/30 sec.	82°C/15 sec.
Goat (n=22)			
Total casein (%)	2.5±0.59	2.1±0.47	1.7±0.49
Fat (%)	4.7±0.51	4.7±0.51	4.67±0.52
Lactose (%)	4.1±0.57	3.9±0.63	2.98±0.45
pH	6.7	7.0	7.1
Sheep (n=15)			
Total casein (%)	4.3±0.63	3.9±0.47	3.0±0.47
Fat (%)	6.1±0.75	6.0±0.72	5.8±0.65
Lactose (%)	4.9 ±0.60	4.8±0.53	3.7±0.39
pH	6.55	6.7	6.8

As seen in Table 2, as the pasteurization temperature increases, the milk pH increases, which is beneficial for shelf-life. However, it is also noted that nutrient content declined, most significantly at 82 °C for 15 seconds. Despite this, our experiments showed that fat remained the most stable component in both goat and sheep milk during heat treatment. The pH of raw goat milk was around 6.7, and the total casein content was 2.5%, which decreased significantly ($p<0.05$) to 1.7% after pasteurization at 82 °C for 15 sec. The same pattern was observed for lactose and fat, decreasing from 4.1% to 2.9% and from 4.7% to 4.67%, respectively. With sheep milk, our results indicate that the initial pH was 6.55,

which increased progressively with temperature to 6.7, and then to 6.8—after pasteurization at 82 °C for 15 sec, total casein and lactose contents significantly decreased ($p<0.05$) compared to raw milk, reaching 4.3% and 3.0%, down from 4.9% and 3.7%, respectively. In contrast, fat content showed no significant decline, changing only slightly from 6.1% to 5.8%. Regarding the chemical composition of milk from different species, our findings align with those of Park et al. (2007), who reported that sheep milk contains higher levels of fat, lactose, and casein than goat milk [56]. According to Dauda *et al.* (2025), the highest total protein level was found in sheep milk (6.02%), followed by goat milk (4.38%) and then

cattle milk (3.54%) [57]. To summarize the results, they support the following: (1) the data suggest biochemical standards for the accurate pasteurization of goat and sheep milk; (2) milk from goats and sheep that has been pasteurized at 72°C/30 second keeps the pH and essential nutrients at required levels; (3) the presence of lactoferrin, lactoperoxidase, and GGT, and even the residual activity of lysozyme can contribute to antimicrobial and antioxidant properties of goat and sheep milk, and so will increase the nutritional value of final dairy products. In short, the data will assist in developing and expanding field-related legal standards and in increasing safer, functional goat and sheep milk food production in Armenia.

CONCLUSION

Heat treatment significantly decreased ALP, LP, and GGT activity in goat and sheep milk at 72 °C/30 sec. compared with the raw milk by 87.2%, 61.3%, 83.1% and 96.6%, 61.4%, 64.9% accordingly. The most unstable compounds were ALP and LF, which were not detected in either sheep or goat milk at 82°C/15 sec., while GGT activity was significantly reduced in both cases. The data show that heat treatment at 72°C/30 seconds did not affect lysozyme activity or LF levels, whereas at 82°C/15 seconds, Lysozyme was hardly detected in goat milk. LF and Lysozyme detection in both types of milk following treatment at 72°C/30 sec. is positive from a practical point of view, in the context of the potential antimicrobial properties of the final product and the expansion of cheese production in Armenia using goat and sheep milk. The absence of LF in both goat and sheep milk following heat treatment at 82°C for 15 seconds provides a basis for proposing it as an additional biochemical marker of precise pasteurization. The results of the study confirm that FRS reliably accumulated in goat and sheep milk as temperature increased; moreover, its

accumulation in goat milk was approximately twice that in sheep milk. The breed-specific characteristic is that raw sheep milk exceeded goat milk by GGT, LF, and basic nutrients concentrations -total casein, fat, lactose, and lactoferrin concentrations. It could be concluded that sheep milk is preferentially nutritional as a functional food. The practical implication of the study is that ALP, GGT activity, LF, and FRS can be considered biochemical markers for accurate pasteurization of goat and sheep milk and for cheese production in Armenia. This proposal will be further substantiated in the next phase of the research by evaluating the indicators' responses under UHT heat-treatment conditions and by conducting microbiological studies. These steps are essential not only for milk and cheese production but also for the production of fermented dairy products in RA.

Novelty: Given that there are no standards for the accurate pasteurization of milk and dairy products derived from small ruminant milk, this research proposes the use of biochemical markers, which are crucial for safer functional food production in Armenia.

List of Abbreviations: HTST: High-Temperature Short-Time, ALP: Alkaline Phosphatase, LP: Lactoperoxidase, LF: Lactoferrin, FRS: Furosine.

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Authors' contributions: GM contributed to conceptualization, data curation, writing – original draft, and writing – review and editing; HG contributed to experimental work, methodology development, data interpretation, and software analysis. DN contributed to investigation, methodology, and

formal analysis; GHa contributed to writing, editing, validation, and preparation of the final draft. AK contributed to the interpretation of results, writing, editing, and validation; TS contributed to data collection, experimental work, and software analysis; RQ contributed to writing, editing, and validation; GHx contributed to experimental work, data collection, and visualization; YM contributed to project administration, conceptualization, supervision, writing, editing, validation, and funding acquisition.

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