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Changes in Native Whey Protein Content, Gel Formation, and Endogenous Enzyme Activities Induced by Flow-Through Heat Treatments of Goat and Sheep Milk

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Abstract: The aim of the present study was to assess the effects of different flow-through heat treatments—68, 73, 78, 85, 100 °C for 16 s—applied to in-line homogenized goat and sheep milk. Alkaline phosphatase (ALP) activity in raw goat milk was 324.5 ± 47.3 μg phenol/mL, and that of lactoperoxidase (LPO) was 199.3 ± 6.7 U/L. The respective activities in raw sheep milk were 7615 ± 141 μg phenol/mL and 319 ± 38.6 U/L. LPO activity was not detected in both milk kinds treated at 85 °C for 16 s. Residual enzyme activities at 73 °C for 16 s with respect to the initial levels in raw milk were higher in goat than in sheep milk. The whey protein fraction of sheep milk was more heat sensitive compared to goat counterpart. Sheep milk rennet clotting time (RCT) was not affected by the treatments, while curd firmness decreased significantly ($p < 0.05$) at 100 °C for 16 s. Treatments more intense than 73 °C for 16 s increased the RCT of goat milk significantly but inconsistently and decreased curd firmness significantly, while yoghurt-type gels made from 73 °C or 78 °C for 16 s treated goat milk exhibited the highest water-holding capacity.

Keywords: goat milk; sheep milk; heat treatment; alkaline phosphatase; lactoperoxidase; whey protein denaturation; rennet clotting behavior; goat milk yoghurt-type gels

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1. Introduction

Goat and sheep milk are excellent bases for the manufacture of various types of dairy products due to their qualitative and quantitative compositional characteristics [1–6]. The heat treatment of milk is a necessary step in the manufacture of several milk products. Apart from addressing sanitary or shelf-life issues, heating can be a means for the development of specific textural properties of dairy foods. The effect of various combinations of time, temperatures, and heating methods on the characteristics and technological behavior of cow milk is constantly studied, and emphasis has been given to heat stability under various conditions [7,8].

Publications in relation to the heat treatment of small ruminants' milk are considerably fewer compared to those of their cow counterpart. Indicatively, the following topics appear in the literature, the most part of which are about goat milk. The effect of heat load on casein micelles of small ruminants' milk has been studied [9–15]. The heat stability and heat-induced changes in the mineral balance of goat or sheep milk have been reported [9,13–17]. The rennet clotting behavior of small ruminants' milk under various heating conditions has been assessed [13,18–20]. There are also publications for the effect of heat treatments on the activity of endogenous enzymes that are indices for heat load such as alkaline phosphatase [21–25] and lactoperoxidase [23,26,27].

Most of the above-mentioned research has been performed in non-homogenized milk using batch heating. Exceptions are the inactivation enzyme studies of Klotz et al. [21]

and Lorenzen et al. [23], who applied flow-through heating, and the heat-stability study of Heilig et al. [9], who heated homogenized goat milk in a pilot plant heating facility. The aim of the present study was to investigate heat-induced changes in goat milk in parallel to its sheep counterpart. For this purpose, whey protein denaturation, enzyme activities and gel-forming behaviour were studied.. A novel element of the present study is the simulation of conditions utilized in the dairy practice, which involves continuous flow-through heat treatments combined with in-line homogenization.

2. Materials and Methods

2.1. Heat Treatments

In homogenization, continuous heat treatments of raw goat and sheep milk by means of tubular heat exchanger and subsequent cooling were carried out in the laboratory heating system HT220 HTST/UHT (OMVE Lab and Pilot Equipment, 3454 MZ, De Meern, The Netherlands) equipped with a two-stage in-line homogenizer (Twin Panda, Gea Niro Soavi, Type NS2002H, Parma, Italy). On each experimental day, raw sheep or goat milk was homogenized—25/5 MPa, 55 °C—heated at 68 °C (1), 73 °C (2), 78 °C (3), 85 °C (4), and 100 °C (5) for 16 s, and then cooled down to 30 °C. In addition, a portion of milk was heated under batch conditions in an open vat at 90 °C for 5 min (6), then cooled down immediately in a water-ice bath. Heat treatment experiments for both milk kinds were carried out in triplicate.

2.2. Milk Analyses

Milk analyses were performed after cooling at 20 °C and allowing it to stand for approx. 30 min. The titratable acidity of 10 mL milk was determined by the Dornic method using N/9 NaOH and phenolphthalein. For the assessment of the level of denaturation of whey proteins, two types of analyses were performed. Firstly, the fraction of milk soluble at pH 4.6 (acid whey) was prepared after the isoelectric precipitation of casein. The nitrogen contents of milk (total N, TN) and of the acid whey (soluble nitrogen, SN) were determined by means of the Kjeldahl method. The ratio of SN to TN expressed as a percentage was used as an index for the level of heat-induced denaturation of whey proteins. In addition, the RP-HPLC method applied by Sakkas et al. [28] for the assessment of the heat treatment of milk was used for the analysis of SN fractions. In brief, a Vydac C4 214 TP 5415 column (Columbia, MD, USA) was used, the elution was performed by a water-acetonitrile gradient in the presence of trifluoroacetic acid at 1 mL/min, and the eluent was monitored at 214 nm. The estimation of the heat-induced denaturation of α-lactalbumin and β-lactoglobulin was based on the residual chromatographic area of the respective peaks, with the raw milk counterparts as controls.

The activity of alkaline phosphatase (ALP) was determined by two methods. Firstly, The photometric method was carried out; it is based on the liberation of phenol from the disodium phenylphosphate, subsequently added to the sample solution in buffer at pH 10.6, and incubated at 37 °C for 60 min [29]. The second method was the continuous fluorimetric direct kinetic assay, which utilized a non-fluorescent aromatic monophosphoric ester substrate that is hydrolyzed in the presence of ALP, producing a fluorescent product [30]. The determination of lactoperoxidase (LPO) was carried out by the assay based on the oxidation of ABTS by the LPO of the sample at pH 6.0 and 25 °C, which resulted in $ABTS^{++}$ radical estimated at 420 nm [31].

2.3. Rennet Clotting Behavior of Heat-Treated Goat and Sheep Milk

Rennet clotting behavior was assessed by means of a Formagraph apparatus (Lattodinamographo, Foss, Padova, Italia), as described by Bakopanos et al. [32]. Ten mL milk inoculated with 200 μL of a 0.3% (*w/v*) calf rennet in 10 mM acetate buffer pH 5.5 were analyzed in triplicate, at 35 °C. The rennet clotting time in min (RCT), and the width of the recorder span 30 min after the addition of rennet, in mm (A30), corresponding to curd firmness, were estimated.

2.4. Yoghurt-Type Gels from Heat-Treated Goat Milk

Heat-treated goat milk was inoculated with a commercial yoghurt starter and incubated at 43 °C until pH 4.6. Reconstituted skim (cow) milk powder (SMP) treated at 90 °C for 5 min (6) was inoculated and incubated in parallel, to serve as control. Then, yoghurt-type gels were stored at 4 °C for the next three weeks. Titratable acidity—as described above—and pH were used for the assessment of the acidification course during incubation. The stability of yoghurt-type gels during cold storage was determined by changes of acidity, pH, and water holding capacity (WHC) according to Moschopoulou et al. [33]. In brief, WHC was expressed as the percentage of the remaining fraction after the expulsion of the serum induced by the centrifugation of the yoghurt-type gels.

2.5. Statistical Analysis

Statgraphics Centurion XVI (Manugistics, Inc., Rockville, MA, USA) was utilized for statistical analysis of the results. The effect of heat treatment on the studied parameters was tested by analysis of variance (ANOVA) and the significant differences by the least significance difference method (LSD) at $p < 0.05$.

3. Results and Discussion

3.1. pH and Acidity

The pH and acidity of goat and sheep milk heated under different conditions are shown in Table 1. The pH of heated goat milk changed significantly ($p < 0.05$) at 85 °C and remained steady thereafter, while the reduction of sheep milk pH was not statistically significant. The acidity of both kinds of milk did not change. During heating, the pH of the milk decreases, while the titratable acidity increases due to organic acids coming from the degradation of lactose and to the hydrogen ions resulting from the transfer of calcium and phosphate from the soluble phase to the casein micelles. This change is small below 60 °C, and the effect of temperature above 80 °C is partially reversed at room temperature [7]. The significant reduction of the pH of goat milk heated at temperature above 78 °C (Table 1) can be assigned to the formation of additional colloidal phosphate since the conditions of our experiments do not coincide with lactose degradation phenomena.

Table 1. pH and titratable acidity (% lactic acid) of heat-treated homogenized goat and sheep milk; means ± standard deviation. Different letters indicate significant differences (LSD, $p < 0.05$) between the means of different heat treatments.

Treatment	Goat Milk		Sheep Milk	
	pH	Acidity (%)	pH	Acidity (%)
raw	6.65 ± 0.105 [b]	0.15 ± 0.005	6.59 ± 0.070	0.18 ± 0.009
68 °C/16 s [1]	6.55 ± 0.025 [a,b]	0.15 ± 0.010	6.55 ± 0.069	0.19 ± 0.010
73 °C/16 s [1]	6.56 ± 0.065 [a,b]	0.14 ± 0.006	6.56 ± 0.071	0.18 ± 0.019
78 °C/16 s [1]	6.52 ± 0.106 [a,b]	0.15 ± 0.006	6.55 ± 0.090	0.19 ± 0.017
85 °C/16 s [1]	6.49 ± 0.064 [a]	0.15 ± 0.006	6.53 ± 0.115	0.18 ± 0.016
100 °C/16 s [1]	6.46 ± 0.086 [a]	0.14 ± 0.015	6.53 ± 0.104	0.18 ± 0.014
90 °C/5 min [2]	6.43 ± 0.105	0.14 ± 0.007	6.49 ± 0.025	0.18 ± 0.013

[1] two-stage homogenization and heating by means of tubular heat exchanger; [2] batch heating of homogenized milk using an open container placed in a water bath.

In the experiments of Raynal and Remeuf [13], the pH of goat and sheep milk heated from 75 to 90 °C, for 0.5–10 min did not vary by more than 0.05 unit before and after heating. No significant pH changes were observed by Zhao et al. [15] after the heating of goat milk at 65, 85, 95, and 110 °C for 7 s, although they noticed a significant decrease of calcium and phosphorus in the supernatant and an increase in the sediments. In the experiments of De la Fuente et al. [16], the treatment of skimmed goat milk at 85 °C for 30 min decreased the pH, the ionic calcium, and the soluble calcium, phosphorus, and magnesium contents of goat milk, similarly to cow milk heated under the same conditions; however, soluble

mineral contents did not change when goat milk was treated at 85 °C for 20 s. The same group [17] reported that the significant decrease of diffusible calcium observed in skimmed sheep milk after heating at 85 °C for 20 s was almost completely reversible 30 min after cooling down to room temperature; this coincides with the time margin under which pH and acidity determinations were performed in the present study.

3.2. Denaturation of Whey Proteins

The reduction in the native whey protein of goat and sheep milk due to the applied heat treatments is presented in Table 2. Statistical analysis did not include batch heating at 90 °C in order to avoid an additional effect from the method of heating. Treatment under these conditions is usually applied for the manufacture of traditional yoghurt from small ruminants' milk; in this respect, it can be considered as a high-heat control.

Table 2. Soluble nitrogen and residual native whey proteins (expressed as peak areas of the RP-HPLC profiles) of heat-treated goat and sheep milk as compared to the respective raw milk; means ± standard deviation. SN/TN, pH 4.6 soluble N/total N by means of the Kjeldahl method; α-la, α-lactalbumin and β-lg, β-lactoglobulin. Different letters indicate significant differences (LSD, $p < 0.05$) between the means of different heat treatments.

Treatment	Goat Milk			Sheep Milk		
	% SN/TN	α-la	β-lg	% SN/TN	α-la	β-lg
Raw	24.0 ± 1.28 [a]	6.26 ± 0.305 [a]	10.68 ± 1.327 [a]	21.0 ± 1.05 [a]	6.19 ± 0.439 [a,b]	24.44 ± 1.605 [a]
68 °C/16 s [1]	23.3 ± 1.48 [a]	6.42 ± 0.364 [a]	10.61 ± 1.341 [a]	20.1 ± 0.27 [a]	6.16 ± 0.733 [a,b]	24.76 ± 3.097 [a]
73 °C/16 s [1]	20.8 ± 0.91 [b]	6.34 ± 0.366 [a]	10.19 ± 1.257 [a]	18.1 ± 1.42 [b]	6.33 ± 0.384 [a]	24.23 ± 1.872 [a]
78 °C/16 s [1]	19.1 ± 1.08 [b]	6.62 ± 0.197 [a]	9.87 ± 1.746 [a]	14.8 ± 0.56 [c]	5.96 ± 0.628 [a,b]	19.30 ± 1.838 [b]
85 °C/16 s [1]	15.4 ± 0.63 [c]	5.69 ± 0.248 [b]	4.82 ± 1.53 [b]	10.9 ± 0.90 [d]	5.32 ± 0.285 [b]	8.91 ± 1.112 [c]
100 °C/16 s [1]	13.7 ± 1.09 [c]	5.57 ± 0.161 [b]	0.88 ± 158 [c]	8.1 ± 0.44 [e]	4.38 ± 0.454 [c]	1.74 ± 0.247 [d]
90 °C/5 min [2]	8.7 ± 0.91	0.33 ± 0.283	0.044 ± 0.036	5.5 ± 0.89	0.32 ± 0.034	0.056 ± 0.015

[1] two-stage homogenization and heating by means of tubular heat exchanger; [2] batch heating of homogenized milk using an open container placed in a water bath.

The nitrogen content of the SN fraction of milk decreases when the denaturation of whey proteins takes place due to their inclusion in the insoluble fraction at pH 4.6. In fact, the most important cause of the decrease of SN is the denaturation of β-lactoglobulin (β-lg) and α-lactalbumin (α-la), especially that of β-lg because it is the most abundant whey protein. The ratios of β-lg to α-la are differentiated in sheep and goat milk as compared to cow milk, being considerably higher in the former and lower in the latter [34]. Information on the behavior of whey proteins of small ruminants' milk under variable heating conditions is limited, while the heat denaturation of cow whey proteins has been extensively reviewed. In brief [35], the first stage of the denaturation of cow milk β-lg, e.g., by heating at 60–75 °C for 12 min, is the dissociation of dimers into monomers and corresponds to 90% of the native protein. In the second stage, i.e., heating at 75–93 °C for 12 min, S-S dimers and S-S polymers are formed and the native protein level is up to 20% of the initial level. On the other hand, extensive heating at 100 °C for at least 10 min is needed for the formation of S-S bonded dimers or trimers of α-la. The denaturation of minor whey proteins begins at 65 °C—that is, of bovine serum albumin (BSA), immunoglobulins, and lactoferrin—and becomes significant at 72 °C for 15 s.

In both goat and sheep milk, typical (low) pasteurization at 72 °C for 16 s caused a statistically significant decrease ($p < 0.05$) in the ratio of pH 4.6 soluble nitrogen (SN) to total nitrogen (TN), although the quantity of the major native whey proteins α-lactoglobulin (α-la) and β-lactoglobulin (β-lg) did not change significantly (Table 2). This reduction is due to the above-mentioned denaturation of minor whey proteins that occurred under low pasteurization conditions. A significant decrease ($p < 0.05$) was observed at 85 °C and above, in accordance with the significant decrease of β-lg and α-la that occurred in goat milk. The whey fraction of sheep milk was more heat-sensitive and significant reduction was observed at 78 °C. The high amount and contribution of heat-sensitive β-lg in the

whey proteins of sheep milk can be related to the different behavior of the two milk kinds, which is depicted in the changes of the β-lg/α-la and SN/TN ratios in Figure 1. The whey proteins of goat milk also exhibited higher heat tolerance as compared to sheep counterparts at 100 °C. In particular, after treatment at 100 °C for 16 s, with 57%, 89%, and 8% of the initial native SN, α-la and β-lg remained soluble in goat milk; the respective residual percentages in sheep milk were 39, 71, and 7%.

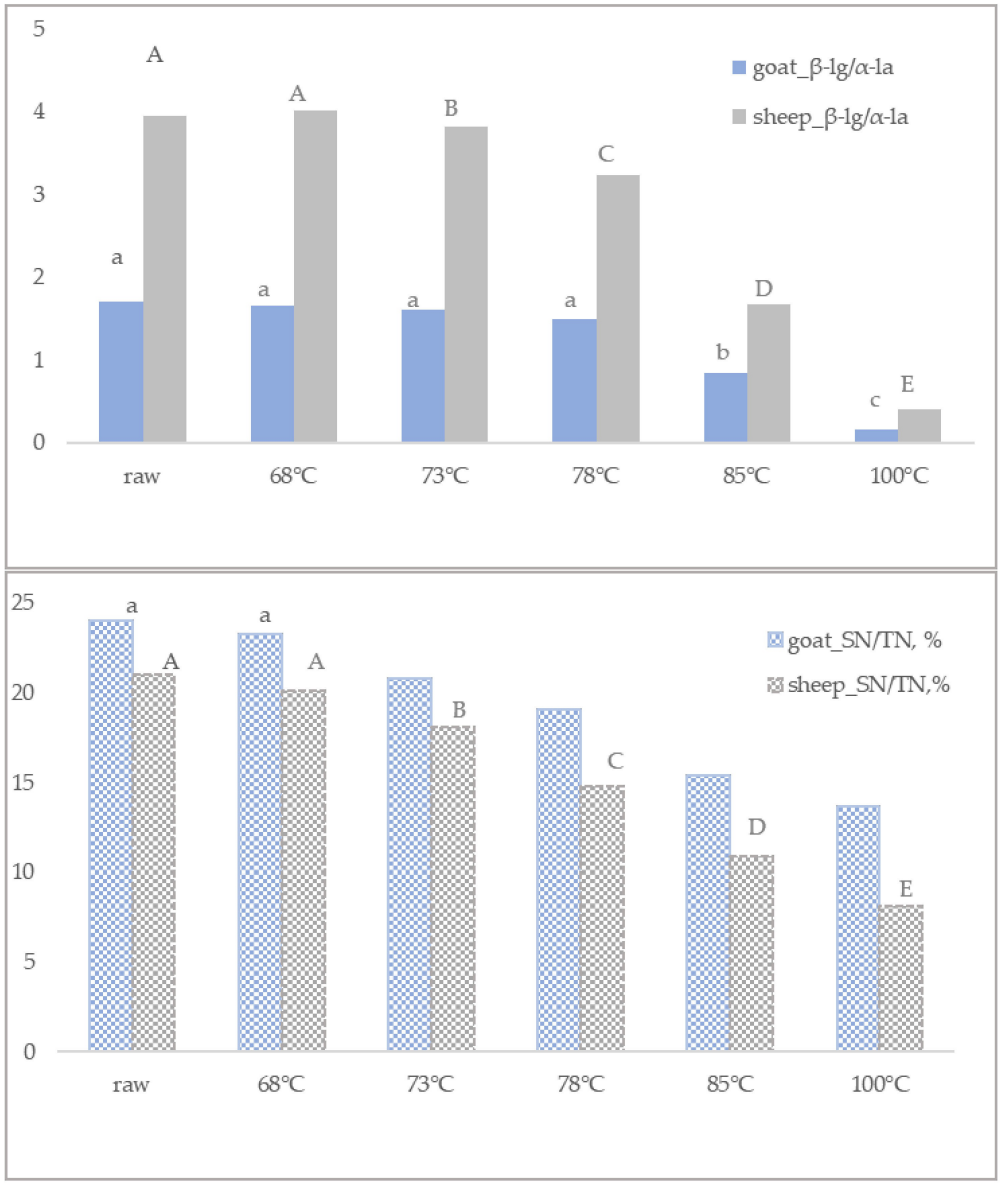

Figure 1. Effect of continuous flow-through treatments at different temperatures for 16 s on the β-lg/α-la and SN/TN ratios of goat and sheep milk. Different letters indicate statistically significant differences ($p < 0.05$) between the treatments of each milk kind.

An opposite trend was reported by Raynal and Remeuf [13], who found that the maximum degree of denaturation at 85 °C was after 1 min heating for goat milk, 1–3 min for sheep milk, and 10 min for cow milk. A few qualitative studies that could contribute to the understanding of the heat-induced denaturation of goat milk whey proteins have been performed. Pesic et al. [12] report different distribution patterns of denatured whey protein/κ-casein complexes of goat milk as compared to cow milk. No heat-induced complexes were formed in the serum fraction of goat milk, in contrast to the 30% found in the serum of heated cow milk. Zhao et al. [15] studied the changes in the secondary

structures of the whey proteins of goat milk at 65, 85, 95, and 110 °C for 7 s. They observed a significant decrease of β-sheets and β-turns related to protein stretching at 65 and 85 °C, with a respective increase in α-helix related to protein globularization. Interestingly, the increase in temperature, from 85 to 95 °C, did not cause significant changes; a further significant increase in α-helix took place at 110 °C.

3.3. Rennet Clotting Behaviour

The rennet clotting time (RCT) and the firmness (A30) of the resulting gel of heat-treated milk is presented in Table 3. Heat-treated sheep milk exhibited shorter RCT than goat milk, similarly to the findings of Pazzola et al. [36] and Allogio et al. [18]. Likewise, a high curd consistency (A30) of sheep milk rennet curds was evident.

Table 3. Rennet clotting behavior of heat-treated goat and sheep milk; means ± standard deviation. RCT, rennet clotting time; A30, curd firmness. Different letters indicate significant differences (LSD, $p < 0.05$) between the means of different heat treatments.

Treatment	Goat Milk		Sheep Milk	
	RCT (min)	A30 (mm)	RCT (min)	A30 (mm)
Raw	12.3 ± 0.21 ^{a}	34.2 ± 3.44 ^{e}	13.3 ± 1.0	43.0 ± 6.05 ^{b}
68 °C/16 s ^{1}	14.2 ± 0.87 ^{b}	30.5 ± 2.75 ^{d,e}	12.6 ± 1.44	43.8 ± 2.04 ^{b}
73 °C/16 s ^{1}	15.0 ± 0.79 ^{b,c}	26.7 ± 0.48 ^{c,d}	12.6 ± 1.40	43.5 ± 1.62 ^{b}
78 °C/16 s ^{1}	16.0 ± 0.96 ^{c,d}	21.1 ± 2.99 ^{a,b}	13.0 ± 1.05	39.3 ± 2.00 ^{b}
85 °C/16 s ^{1}	15.5 ± 1.00 ^{b,c}	22.5 ± 3.71 ^{b,c}	13.6 ± 1.04	39.4 ± 1.89 ^{b}
100 °C/16 s ^{1}	17.4 ± 1.05 ^{d}	17.3 ± 3.21 ^{a}	14.4 ± 0.34	33.9 ± 1.69 ^{a}
90 °C/5 min ^{2}	15.0 ± 0.51	23.7 ± 8.28	13.0 ± 0.69	36.6 ± 1.06

^{1} two-stage homogenization and heating by means of tubular heat exchanger; ^{2} batch heating of homogenized milk using an open container placed in a water bath.

The heat treatment of cow milk under conditions that induce the considerable denaturation of whey proteins has adverse effects on the rennet clotting behavior and the rheological properties of the rennet gel that have been assigned to the coating of casein micelles with denatured whey proteins. The modification of the casein surface does not affect the start of flocculation, but impairs the flocculation rate, e.g., by a factor of 2 when there is 60% denaturation [37]; no effect on rennet clotting has been observed below a whey protein denaturation rate of 15% [38]. Apart from the complexes of casein micelles with denatured whey proteins, it is suggested that the serum whey protein/κ-casein complexes [39] and the distribution pattern of denatured whey proteins between soluble aggregates and complexes with caseins [38] play an important role.

The findings presented in Table 3—in particular, those for sheep milk—are not in accordance with the known behavior of heat-treated cow milk. The rennet clotting time (RCT) of sheep milk was not affected significantly by the heat treatment even at the substantial level of denaturation of β-lg observed at 85 or 100 °C. For a significant decrease ($p < 0.05$) in gel firmness (A30), treatment at 100 °C with less than 10% of residual native β-lg content in milk was necessary (Table 2). The RCT of heat-treated goat milk was affected by the heat treatments, as shown in Table 3. Heating at 100 °C for 16 s increased the RCT by 40% and 20% as compared to raw and pasteurized milk, respectively; consequently, gel firmness decreased substantially. Interestingly, the rennet clotting parameters of control goat milk heated at 90 °C for 5 min were similar to those of pasteurized milk. A similar finding was described by Raynal and Remeuf [13], who reported that the RCT and gel firmness of rennet gels from goat milk heated at 90 °C for 1–10 min were close to its pasteurized counterpart.

Our findings are consistent with reports of various studies about the differences in the rennet clotting behavior of heat-treated milk of small ruminants as compared to the typical behavior of cow milk. Balcones et al. [40], estimated a slight decrease in the rennet clotting time of sheep milk treated at 85 °C with no native β-lg content. Montilla et al. [20], report

that in contrast to cow milk, the rennet clotting time of goat milk treated at 85 °C for 30 min and the gel firmness were not affected, although a more intense whey protein denaturation took place. Treatment at 70 °C for 30 min, which doubled the RCT of cow milk, did not affect the RCT of its goat and sheep counterparts [41]. The heating of goat milk at 70, 80, 95 °C for 1.3 and 10 min decreased the RCT, contrary to the intense increase observed in cow milk [18]. Raynal and Remeuf [13] did not notice any correlation between the RCT and the level of whey protein denaturation in the heat-treated milk of small ruminants, in contrast to cow milk. The maximum increase in the RCT of goat and sheep, i.e., by 1.3 to 1.5 times, was estimated after heating at 80 °C for 1 min without being significantly affected by a further increase in holding time, up to 10 min; treatments at 75 °C for 1 to 10 min that induced a 40% whey protein denaturation had no effect. Moreover, they report that heated goat milk gel exhibited a cross-linking capacity similar to that of untreated milk, in contrast to sheep milk gel. Recently, Miloradovic et al. [19] found that the coagulation time of goat milk was not affected by treatments at 65 °C for 30 min, at 80 °C for 5 min, or at 90 °C for 5 min, whereas the aggregation rate and firmness were substantially decreased. The size of the casein micelle, which is an important factor for rennet clotting phenomena, is not similarly affected by heating in various milk kinds. An increase of 40% and 25% in the size of goat milk micelles was observed after heating at 85 °C for 5 min by Hovjecki et al. [11] and Raynal and Remeuf [13], respectively. The latter group observed no increase for the size cow milk micelles and a substantial increase of 75% for sheep milk micelles under the same conditions.

3.4. Goat Milk Yoghurt-Type Gels

The development of acidity and the evolution of pH during the thermophilic lactic acid fermentation of the heat-treated goat milk are presented in Table 4 with respect to the two experimental factors. The statistically significant differences ($p < 0.05$) are indicated by different letters. As expected, the % lactic acid content and pH values were strongly and linearly correlated ($r = -0.967$). Approx. 5 h or 300 min of incubation resulted in pH 4.6 for all types of gels. The acidification course of reconstituted cow skim milk powder (RSMP) treated at 90 °C for 5 min—which was used as control—was significantly ($p < 0.05$) slower compared to its goat milk counterparts. The course of acidity development during the incubation of heat-treated goat milk (Table 4) was not affected by the heating conditions. In contrast to our results, Hovjecki et al. [11] reported that an increase in the heat treatment of goat milk, from 72 °C for 30 s to 85 °C for 5 min, substantially decreased the fermentation and gelation time of the resulting acid gels.

Post-acidification of yoghurt-type goat milk gels is shown in Figure 2. According to the results of the statistical analysis, a significant ($p < 0.05$) increase in acidity took place on day 7 for treatments at 73 °C/16 s, on day 14 for heating at 78 °C/16 s or 85 °C/16 s, and on day 20 for heating at 90 °C/5 min. The acidity of gels from goat or cow skim milk powder heated at 90 °C/5 min did not change significantly during storage. A significant ($p < 0.05$) pH decrease was observed on day 14 for G2, G3, G4, and G5 gels, on day 7 for G6 and on day 4 for the control C7 gel. Overall, the acidity of the control gel from reconstituted skim milk powder (C7) was significantly lower ($p < 0.05$) than that of goat milk gels at all storage stages, while no significant differences were observed for the pH values.

As shown in Table 5, the water holding capacity (WHC) of yoghurt-type gels was significantly affected by the conditions of heat treatment, while those treated under continuous heating conditions were not affected by the storage time. The WHC was significantly lower ($p < 0.05$) in the gels from goat milk treated at 85 and 100 °C for 16 s and at 90 °C for 5 min than those heated at 73 °C and 78 °C for 16 s, despite the high level of whey protein denaturation of the former group. Nevertheless, the WHC of goat milk gels was high, in accordance with Moschopoulou et al. [33], who estimated approx. 60% and 50% WHC for goat and cow milk yoghurt, respectively.

Table 4. Acidification of heat-treated homogenized goat milk, after inoculation with yoghurt starter and incubation at 43 °C; means ± standard deviation. Reconstituted cow skim milk powder (RSMP) treated at 90 °C for 5 min was used as control.

Min	Goat Milk					Cow RSMP
	73 °C/16 s (G2)	78 °C/16 s (G3)	85 °C/16 s (G4)	100 °C/16 s (G5)	90 °C/5 min (G6)	90 °C/5 min (C7)
			Acidity (% Lactic Acid)			
30	0.15 ± 0.012 ^{a,A}	0.15 ± 0.013 ^{a,A}	0.15 ± 0.009 ^{a,A}	0.15 ± 0.005 ^{a,A}	0.15 ± 0.008 ^{a,A}	0.13 ± 0.087 ^{a,B}
60	0.16 ± 0.016 ^{a,A}	0.18 ± 0.039 ^{a,A}	0.16 ± 0.018 ^{a,A}	0.18 ± 0.025 ^{a,b,A}	0.17 ± 0.005 ^{a,A}	0.14 ± 0.013 ^{a,B}
90	0.16 ± 0.016 ^{a}	0.18 ± 0.039 ^{a}	0.18 ± 0.018 ^{a,b}	0.18 ± 0.025 ^{a,b}	0.17 ± 0.005 ^{a,b}	0.16 ± 0.032 ^{a,b}
120	0.19 ± 0.010 ^{a}	0.25 ± 0.069 ^{a,b}	0.26 ± 0.103 ^{b}	0.20 ± 0.017 ^{b}	0.21 ± 0.022 ^{b,c}	0.18 ± 0.020 ^{a,b}
150	0.28 ± 0.068 ^{b}	0.32 ± 0.117 ^{b}	0.37 ± 0.133 ^{c}	0.34 ± 0.060 ^{c}	0.25 ± 0.013 ^{c}	0.24 ± 0.071 ^{b}
180	0.43 ± 0.108 ^{c}	0.44 ± 0.126 ^{c}	0.47 ± 0.031 ^{d}	0.43 ± 0.020 ^{d}	0.39 ± 0.061 ^{d}	0.35 ± 0.082 ^{c}
210	0.56 ± 0.067 ^{d,A}	0.52 ± 0.055 ^{c,d,A}	0.55 ± 0.025 ^{d,e,A}	0.52 ± 0.005 ^{e,A}	0.51 ± 0.006 ^{e,A}	0.42 ± 0.041 ^{c,d,B}
240	0.59 ± 0.014 ^{d,e,A}	0.61 ± 0.035 ^{d,e,A}	0.60 ± 0.029 ^{e,A}	0.53 ± 0.044 ^{e,A,B}	0.53 ± 0.068 ^{e,A,B}	0.49 ± 0.053 ^{d,e,B}
270	0.64 ± 0.016 ^{d,e,A}	0.64 ± 0.041 ^{e,A}	0.63 ± 0.014 ^{e,A,B}	0.60 ± 0.015 ^{f,A,B}	0.60 ± 0.008 ^{f,A,B}	0.56 ± 0.822 ^{e,B}
300	0.65 ± 0.023 ^{e,A}	0.67 ± 0.023 ^{e,A}	0.65 ± 0.01 ^{e,A,B}	0.64 ± 0.042 ^{f,A,B}	0.60 ± 0.007 ^{f,A,B}	0.56 ± 0.080 ^{e,B}
			pH			
30	6.49 ± 0.314 ^{d}	6.59 ± 0.177 ^{f}	6.58 ± 0.111 ^{f}	6.52 ± 0.121 ^{f}	6.50 ± 0.092 ^{f}	6.69 ± 0.121 ^{f}
60	6.52 ± 0.179 ^{d}	6.44 ± 0.176 ^{f}	6.41 ± 0.131 ^{e,f}	6.47 ± 0.144 ^{e,f}	6.51 ± 0.021 ^{f}	6.58 ± 0.226 ^{f}
90	6.41 ± 0.270 ^{c,d}	6.30 ± 0.121 ^{e,f}	6.17 ± 0.225 ^{d,e}	6.32 ± 0.125 ^{e,f}	6.35 ± 0.22 ^{e,f}	6.35 ± 0.194 ^{e,f}
120	6.38 ± 0.517 ^{c,d}	6.01 ± 0.229 ^{d,e}	6.01 ± 0.450 ^{d}	6.21 ± 0.213 ^{e}	6.11 ± 0.193 ^{d,e}	6.15 ± 0.122 ^{e}
150	5.96 ± 0.45 ^{c}	5.81 ± 0.375 ^{d}	5.57 ± 0.298 ^{c}	5.63 ± 0.248 ^{d}	5.90 ± 0.187 ^{d}	5.73 ± 0.366 ^{d}
180	5.28 ± 0.329 ^{b}	5.38 ± 0.198 ^{c}	5.21 ± 0.135 ^{b,c}	5.34 ± 0.031 ^{c}	5.39 ± 0.270 ^{c}	5.40 ± 0.226 ^{c,d}
210	4.94 ± 0.091 ^{a,b,A}	4.99 ± 0.157 ^{b,A}	4.96 ± 0.038 ^{a,b,A,B}	5.04 ± 0.045 ^{b,A,B}	5.02 ± 0.078 ^{b,A,B}	5.15 ± 0.131 ^{b,c,B}
240	4.97 ± 0.129 ^{a,b}	4.85 ± 0.104 ^{a,b}	4.91 ± 0.251 ^{a,b}	4.99 ± 0.222 ^{b}	4.87 ± 0.173 ^{a,b}	4.93 ± 0.056 ^{a,b}
270	4.72 ± 0.108 ^{a}	4.64 ± 0.060 ^{a}	4.64 ± 0.078 ^{a}	4.67 ± 0.106 ^{a}	4.72 ± 0.114 ^{a}	4.74 ± 0.127 ^{a}
300	4.61 ± 0.085 ^{a}	4.58 ± 0.067 ^{a}	4.53 ± 0.078 ^{a}	4.63 ± 0.106 ^{a}	4.69 ± 0.155 ^{a}	4.62 ± 0.106 ^{a}

Different lowercase letters indicate significant differences (LSD, $p < 0.05$) between the means of the different incubation stages (within columns). Different capital letters indicate significant differences (LSD, $p < 0.05$) between the means of heat treatments (within rows).

Table 5. Water holding capacity (WHC, %) during the storage of yoghurt-type gels made from heat-treated homogenized goat milk at 43 °C; means ± standard deviation. Reconstituted cow skim milk powder (RSMP) treated at 90 °C for 5 min was used as control.

Yoghurt-Type Gels	Water Holding Capacity (%)		
	7 Days	14 Days	20 Days
From goat milk			
73 °C/16 s (G2)	67.4 ± 9.22 ^{b,c}	70.0 ± 8.16 ^{b,c}	70.4 ± 5.33 ^{a,b}
78 °C/16 s (G3)	74.2 ± 9.80 ^{c}	73.0 ± 9.72 ^{c}	76.2 ± 8.73 ^{b}
85 °C/16 s (G4)	59.6 ± 4.48 ^{a,b}	62.0 ± 3.32 ^{a}	64.5 ± 6.31 ^{a}
100 °C/16 s (G5)	58.4 ± 5.40 ^{a}	62.4 ± 3.28 ^{a}	64.3 ± 5.93 ^{a}
90 °C/5 min (G6)	59.3 ± 4.64 ^{a,b,A}	62.6 ± 1.65 ^{a,A,B}	64.8 ± 4.36 ^{a,B}
From cow RSMP			
90 °C/5 min (C7)	55.4 ± 3.43 ^{a,A}	62.7 ± 6.84 ^{a,b,A,B}	66.0 ± 4.55 ^{a,B}

Different lowercase letters indicate significant differences (LSD, $p < 0.05$) between the means of the different incubation stages (within columns). Different capital letters indicate significant differences (LSD, $p < 0.05$) between the means of heat treatments (within rows).

3.5. Alkaline Phosphatase and Lactoperoxidase

The effect of heat treatments on alkaline phosphatase (ALP) and lactoperoxidase (LPO) activities, which are used as indices for heat treatments of milk, is presented in Table 6. Mean ALP activity in raw goat milk was much lower than that in raw sheep milk. Up to 20 times higher ALP was reported in sheep milk as compared to that in goat milk, ranging on average from 8000 to 17,000 and from 120 to 1300 μg phenol/mL, respectively [1,13,24,42]. As illustrated in Table 4, after (low) pasteurization at 73 °C for 16 s, the residual ALP activity of goat milk, expressed in μg/mL, was 0.8% of the initial level; that is, it is much higher than the respective residual activity of 0.07% observed in

pasteurized sheep milk. A similar trend has been found for pasteurized whole goat and sheep milk by other research groups [21–23]. With a residual ALP activity of less than 350 mU/L, pasteurized (73 °C for 16 s) goat milk fulfilled this requirement by means of the fluorometric method [43]; the same was marginally true for sheep milk in the present study.

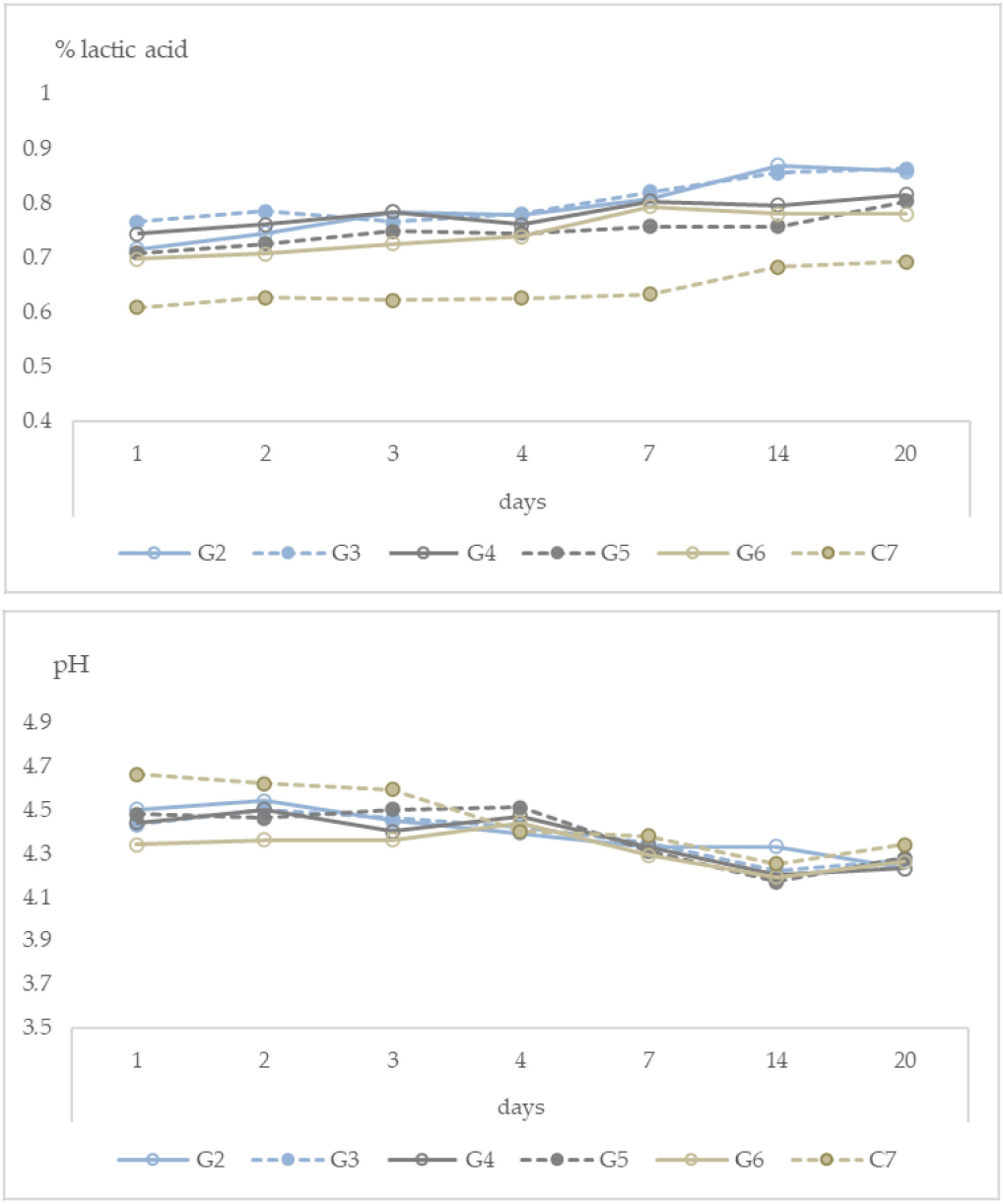

Figure 2. Post-acidification expressed as % lactic acid and pH changes of yoghurt-type gels made from heat-treated homogenized goat milk, after inoculation with yoghurt starter and incubation at 43 °C. G2, 73 °C/16 s; G3, 78 °C/16 s; G4, 85 °C/16 s; G5, 100 °C/16 s; G6, 90 °C/5 min. Control C7, reconstituted cow skim milk powder, 90 °C/5 min.

Highly variable LPO activity has been reported for different milk kinds, which, according to several publications, is higher in goat than in cow and sheep milk (1,42). However, the order of LPO activity in raw milk reported by Dumitraşcu et al. [26] was sheep > cow > goat, which coincides with our findings (Table 4). Zou et al. [27] reported higher LPO activity in cow milk as compared to that in goat. After typical (low) pasteurization at 73 °C for 16 s, the residual LPO in goat and sheep milk was 62.5% and 28% of the initial levels, respectively. The activity in both kinds of milk treated at 78 °C for 16 s was lower than the detection limit of 50 U/L in the International Standard [31].

Table 6. Alkaline phosphatase (ALP) activity determined by the photometric (μg/mL) and by the fluorometric (mU/L) methods, and lactoperoxidase (LPO) activity (U/L) in heat-treated goat and sheep milk; means ± standard deviation. G, goat milk; S, sheep milk. Different letters indicate significant differences (LSD, $p < 0.05$) between the means of different heat treatments. n.r., not recorded; n.d., not detected.

Treatment	Goat Milk			Sheep Milk		
	ALP, μg/mL	ALP, mU/L	LPO, U/L	ALP, μg/mL	ALP, mU/L	LPO, U/L
Raw	324.5 ± 47.28 [a]	11,810 ± 3738 [a]	199.3 ± 6.71 [a]	7615 ± 141 [a]	n.r.	319.1 ± 38.59 [a]
68 °C/16 s [1]	19.7 ± 5.14 [b]	4682 ± 1935 [b]	164.9 ± 1.68 [a,b]	147 ± 37.21 [b]	n.r.	167.7 ± 5.97 [b]
73 °C/16 s [1]	2.6 ± 1.86 [b]	305.7 ± 78.7 [c]	124.6 ± 31.88 [b]	5.7 ± 1.61 [c]	418 ± 98.8 [a]	89.4 ± 7.25 [c]
78 °C/16 s [1]	n.r.	186.2 ± 23.68 [c]	19 ± 0 [c]	2.4 ± 0.19 [c]	165.5 ± 18.24 [b]	2.4 ± 3.36 [d]
85 °C/16 s [1]	0.8 ± 0.39 [b]	196.5 ± 68.59 [c]	n.d.	0.5 ± 0.26 [c]	143 ± 22.77 [b]	n.d.
100 °C/16 s [1]	0.4 ± 0 [b]	139.5 ± 39.32 [c]	n.d.	n.d.	120.5 ± 9.1 [b]	n.d.
90 °C/5 min [2]	n.d.	15.6 ± 2.37 [c]	n.d.	0.6 ± 0.06 [c]	22.5 ± 1.27 [c]	n.d.

[1] two-stage homogenization and heating by means of tubular heat exchanger; [2] batch heating of homogenized milk using an open container placed in a water bath.

4. Conclusions

The present findings from the parallel experiments performed with goat and sheep milk suggest that similar heating conditions affect the technological properties of homogenized goat and sheep milk in a different manner. The whey fraction of sheep milk was more heat-sensitive than that of goat milk. Interestingly and contrary to goat milk, the rennet clotting time and rennet-curd firmness of the sheep counterpart were not significantly affected by the heat treatment even when a considerable denaturation of β-lg occurred. Treatments more intense than typical (low) pasteurization substantially increased the rennet clotting time of goat milk and decreased the firmness of rennet curd, while high water holding capacity was observed in yoghurt-type gels made from pasteurized goat milk. Residual ALP and LPO activities expressed as fractions of the initial levels in raw milk were higher in pasteurized goat milk than those in its sheep counterpart. The results of the present study can be exploited in the quality control of heat-treated goat and sheep milk, and in the development of various types of dairy products made from these types of milk. Moreover, they can be used as a base for further kinetic experiments that combine the effects on various endogenous enzymes and microorganisms.

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