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Adjusting the initial milk pH before freezing affected physico-chemical properties of thawed goat milk

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Abstract

This experiment was conducted to study the effects of initial goat milk pH on changes in some physico-chemical properties of milk during freeze-thaw cycles, and to investigate the possible causes by freeze-thawing for 5 cycles to magnify the changes. Raw goat milk was adjusted to 3 alternative pH levels, which were 1) original milk pH, 2) adjusted to original pH-0.2, and 3) adjusted to original pH+0.2. All the milk samples were packed in plastic bags and kept at -18±2°C for 5 days, then thawed at room temperature at 25°C (1 cycle) and this was repeated for 5 cycles. Milk samples from each treatment and each cycle were analyzed for pH, presence of sediment, viscosity, fat droplet size, particle size distribution, heat stability and changes in mineral content of milk serum. The result showed that the goat milk had an original pH of 6.69 ($T_{6.69}$) that was adjusted to 6.49 ($T_{6.49}$) and 6.89 ($T_{6.89}$). During freeze-thawing all the treatment groups exhibited decreasing pH and increasing sediment, milk viscosity and fat droplet size, with only slight differences between treatments. The important differences were in heat stability, as the $T_{6.49}$ treatment resulted in a heat stability decrease from 80 mins initially to 46 mins (42.50% decrease) at the 5th cycle, whereas $T_{6.89}$ treatment only had an 8.89% change in heat stability. Average particle diameter ($D_{3,2}$), volume distribution ($D_{4,3}$) and uniform distribution (span) increased with freeze-thaw cycles, which clearly showed in $T_{6.49}$ and $T_{6.69}$ cases but not distinctly in $T_{6.89}$. In conclusion, milk pH affected the quality of milk after freeze-thawing. Adjusting pH to 6.89 before freezing in this study reduced changes of pH, sediment formation, coalescence of protein, and heat stability of thawed goat milk; such adjustment is beneficial for further milk processing in the dairy industry.

1. Introduction

Goat milk is mainly produced in Asia, Europe, Africa and America, totalling approximately 18 million tons in 2014 (Rubio-Martin *et al*., 2017). Collecting milk after milking and then chilling or freezing is the common approach to preserving milk before further processed in a dairy plant. Preservation addresses problems from seasonal production (Kljajevic *et al*., 2016), milk transportation and small capacity factories. However, the freezing affects many properties of goat milk causing decreased pH (Kljajevic *et al*., 2016), partial coalescence of fat droplets (Nurliyani *et al*., 2015), protein destabilization (Wendorff, 2001), aggregation of casein micelles, imbalance of salt (Bienvenue *et al*., 2003), increase of soluble calcium concentration (Kljajevic *et al*., 2016) and changes in particle size distribution (Balde and Aider, 2016). These in turn impact heat stability, particularly in fluid milk processing. To prevent the instability of milk after pasteurizing or sterilizing, a practically applicable pretreatment would be useful. As the pH affects the physical properties of milk, the dairy industry adjusts pH before thermal processing to maintain heat stability and reduce coagulation (Pouliot and Boulet, 1991; McSweeney *et al*., 2004; Tsioulpas *et al*., 2010; Chen *et al*., 2015). However, when frozen goat milk is used, aggregation and precipitation of protein and minerals frequently occur. Therefore, adjusting the pH before freezing might be a solution to these problems, but there is no prior study on the physical properties of goat milk in this context. The objectives of the present research were to study the effects of milk pH and freeze-thawing on the physical properties of thawed goat milk, in particular on pH, sediment, milk viscosity, fat droplet

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size, particle size distribution, heat stability and minerals in milk serum. The causes of deterioration were investigated by performing freeze-thawing for 5 cycles to promote large changes.

2. Materials and methods

2.1 Milk sample preparation

Raw goat milk was collected from a dairy goat farm located in Yala province, Thailand. The goat milk was pooled into a composite sample that was analysed for milk composition in terms of total solids, protein (Kjeldahl method, Kjeldatherm, Gerhardt, Germany) and fat content (Gerber method, Gerber Soxhem, Gerhardt, Germany) by AOAC methods (Horwitz and Latimer, 2005). The lactose content in milk was analysed by iodometric titration following Hinton and Macara (1927). The analyses were performed in three replicates. To study the effects of pH and freeze-thawing, the goat milk sample was divided into 3 aliquots set at different pH levels, which were 1) original milk pH, 2) original milk pH-0.2 (adjusting with 3 M HCl), and 3) original milk pH+0.2 (adjusting with 3 M NaOH). All the milk samples were packed in 0.5 L polyethylene plastic bags and were kept in a freezer at -18±2°C for 5 days and were then thawed at 25°C for 2 hrs to a completely molten state (confirmed by checking the temperature profile before the study). The above counted as 1 freeze-thaw cycle. The experiments implemented 5 cycles, and samples from each cycle were analysed for certain physicochemical properties that relate to milk stability: pH, viscosity, sedimentation, fat droplet size, distribution of particle size of milk, heat stability and soluble mineral content in goat milk serum.

2.2 Changes in pH, viscosity and sediment

The pH of goat milk was measured by using a pH meter (pH Mettler, S20 SevenEasy, Toledo, U.S.A.) and the viscosity was analyzed at 25°C by a Brookfield Synchro-Lectricviscometer, Mode LV, Spindle S61 (Brookfield Synchro-Lectric viscometer, UK) with spindle speed set at 60 rpm (Fava *et al*., 2013). The sediment was analysed according to the method of Katsiari *et al*. (2002) by centrifuging at 1,540×*g* for 10 mins, after which the sediment was dried at 105°C for 3 hrs in a hot air oven. The sediment percentage was calculated as % sediment = (weight of sediment/weight of milk) × 100.

2.3 Fat globule size and particle size distribution

The fat globule size of milk was determined under a microscope (CH30; Olympus, Tokyo, Japan) with a magnification of 40×. Each microscope field was marked, and the area and radius were measured for 50–60 fat globules. The average area and radius were calculated using the Motic Images Plus 2.0 program choosing the Tri-circle mode (Thoh *et al*., 2017). The particle size distribution of milk was analyzed by laser particle size diffraction (Beckman coulter, USA.). Milk samples were centrifuged to separate fat and obtain skim milk (Wang *et al*., 2017) before analysis. The size distribution was determined in terms of average particle diameter ($D_{3,2}$), volume distribution ($D_{4,3}$) and uniform distribution (span). The span was calculated from span = $d_{0.9}$-$d_{0.1}$/$d_{0.5}$ where $d_{0.9}$, $d_{0.1}$ and $d_{0.5}$ are the diameters matching 90%, 10% and 50% of the cumulative distribution, respectively. Specific surface area (SSA) of milk was also calculated as the total area of the particles divided by total sample weight.

2.4 Heat stability

Heat stability of milk was represented by the duration at 100°C it takes for milk protein to be coagulated. To perform these analyses the sample was put in a glass tube, immersed in a thermostatically controlled oil bath (Thoh *et al*., 2017) and sampled every 2 mins to check for coagulation.

2.5Minerals in milk serum

The calcium (Ca), phosphorus (P), magnesium (Mg) and sodium (Na) contents in goat milk serum were analysed using an Inductively Couple Plasma-Optical Emission Spectrometer (ICP–OES). For sample preparation, the milk sample was dialyzed using a dialysis tube with 10 KDa molecular mass cut off (Amicon Ultra- Centrifugal, Germany, volume 15 mL, diameter 2.97 cm, and filtration area 7.6 cm^2) to retain the soluble mineral components in milk filtrate. Then the filtrate was centrifuged, and the clear solvent was collected and kept in a refrigerator at 4°C. The analysis was performed with ICP–OES (Optima 4300 DV, Perkin -Elmer, Norwalk, CT, U.S.A.) observing the following wavelengths: 317.933, 396.847 and 393.366 nm for Ca, 285.213, 280.271, and 279.553 nm for Mg, 213.617 nm for P and 766.490 nm for Na (Balde and Aider 2016).

2.6 Statistical analysis

The data are presented as mean ± SD and all data were subjected to analysis of variance (ANOVA) (Cochran and Cox, 1957). Significant differences between means were analyzed by Duncan's multiple range test (Steel and Torrie, 1980) at a 5% probability level (p<0.05).

3. Results and discussion

The raw goat milk in this study contained 13.77±0.07% total solids, 5.18±0.10% fat, 3.60±0.08%

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protein, and 5.38±0.35% lactose.

3.1 Changes in pH, viscosity and sediment

The pH of raw goat milk was 6.69 ($T_{6.69}$) thus, the treatments with adjustment had pH 6.49 ($T_{6.49}$) and 6.89 ($T_{6.89}$). The freeze-thaw cycles decreased the pH consistently with all pre-treatments (Table 1) with a significant decrease in the 1st cycle. The pH of $T_{6.89}$ decreased from 6.89 in the 1st cycle to 6.72 after the 5th cycle, whereas the pH of $T_{6.49}$ decreased from 6.49 to 6.46. The $T_{6.89}$ and $T_{6.69}$ pre-treatments tended to have a faster rate of decrease than the $T_{6.49}$, but the 5th cycle with $T_{6.49}$ showed the lowest pH. Similar findings were reported by Kljajevic *et al.* (2016). This change might be due to increasing ionic strength from losing phosphorylated β-casein (Salaun *et al.*, 2005) and precipitation of calcium phosphate from the dissociation of β-casein (Ribeiro and Ribeiro, 2010). This is related to sediment in the milk. Each freeze-thaw cycle induced sediment after centrifugation but without significant differences between the pre-treatments at the 1st and 2nd cycles. However, there was slightly more sediment with $T_{6.49}$ ($p<0.05$) than with $T_{6.89}$ and $T_{6.69}$ after the 5th cycle (Table 1). Even though the sediment results were similar when the samples were heated at 100°C to investigate heat stability the results clearly showed large differences between the pre-treatments.

The viscosity of the milk samples was initially 2.82 to 3.28 cP. The viscosities of $T_{6.49}$ and $T_{6.89}$ were slightly lower than of $T_{6.69}$, which might be a sheer effect of adjusting pH. During freeze-thaw cycles, all the treatments gave increasing viscosity. After the 5th cycle, the $T_{6.49}$ that had the lowest pH and the most sediment exhibited the highest apparent viscosity (5.99cP, see Table 1) but without significant difference from $T_{6.89}$ (5.76cP) and $T_{6.69}$ (5.62cP). The freeze-thaw cycles affected pH and might also impact the volume of casein micelles, particularly of β-casein that dissociated at low temperatures, resulting in increased viscosity (Fava *et al.*, 2013). Blade and Aider (2016) reported that the fragile (weak) inter-micellar links in frozen milk are confirmed by the non-Newtonian behaviour of cryoconcentrated skim milk after freezing for 5 weeks.

3.2 Fat droplet size and particle size distribution

Table 2 shows the fat droplet diameters determined by microscopic photography. Fat in the raw goat milk was in the form of spherical particles well dispersed in the milk serum. The distribution of fat diameters in raw goat milk was in the range of 0.53-3.43 μm (data not shown). The mean initial size of fat droplets in goat milk ranged from 1.43 to 1.54 μm and slightly increased during freeze-thaw cycles. After the 1st cycle, each treatment gave fat droplet size larger than initially ($p<0.05$, data not shown) and it increased with further freeze-thaw cycles. After the 3rd cycle, the fat globules became larger, and after the 4th cycle, fat clumps were floating on the surface of goat milk, due to partial coalescence (Degner *et al.*, 2014). After the 5th cycle, milk with $T_{6.49}$, $T_{6.69}$ and $T_{6.89}$ pre-treatments contained fat globules with diameters 3.13, 3.11 and 3.02 μm, respectively ($p>0.05$). The diameter of fat droplets in goat milk tended to increase, but not much, which can be attributed to the lack of agglutinin (lipoprotein) to agglutinate the fat globules (Nurliyani *et al.*, 2015).

To study the particle size distributions, the goat milk samples were skimmed by separating fat before analysis. The distribution of particle sizes in raw goat milk is here reported as the average particle diameter ($D_{3,2}$), the volume distribution ($D_{4,3}$), the specific surface area

Table 1. Observed pH, sediment and viscosity of goat milk after freeze-thaw cycles, with pre-treatments that adjusted the initial pH.

Treatment	Cycle 0	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
			pH			
$T_{6.49}$	$6.49±0.00^{Ca}$	$6.46±0.01^{Cc}$	$6.47±0.01^{Cb}$	$6.47±0.01^{Cbc}$	$6.47±0.00^{Cbc}$	$6.46±0.01^{Cc}$
$T_{6.69}$	$6.69±0.00^{Ba}$	$6.58±0.00^{Bb}$	$6.53±0.00^{Bd}$	$6.53±0.01^{Bd}$	$6.54±0.00^{Bc}$	$6.52±0.01^{Bc}$
$T_{6.89}$	$6.89±0.00^{Aa}$	$6.79±0.00^{Ab}$	$6.79±0.00^{Ab}$	$6.73±0.01^{Ad}$	$6.74±0.00^{Ac}$	$6.72±0.00^{Ae}$
			Sedimentation (%)			
$T_{6.49}$	$0.07±0.02^{Ae}$	$0.13±0.01^{Acd}$	$0.12±0.02^{Ad}$	$0.14±0.00^{Abc}$	$0.16±0.01^{Aab}$	$0.17±0.02^{Aa}$
$T_{6.69}$	$0.07±0.03^{Ac}$	$0.13±0.01^{Aab}$	$0.11±0.2^{Ab}$	$0.14±0.02^{ABa}$	$0.14±0.02^{Ba}$	$0.15±0.02^{ABa}$
$T_{6.89}$	$0.07±0.02^{Ac}$	$0.12±0.01^{Ab}$	$0.10±0.02^{Ab}$	$0.12±0.01^{Cb}$	$0.11±0.01^{Cb}$	$0.15±0.00^{Ba}$
			Viscosity (cP)			
$T_{6.49}$	$2.82±0.03^{Bd}$	$2.82±0.12^{Ad}$	$3.00±0.03^{Bd}$	$3.49±0.11^{Ac}$	$3.97±0.02^{Bb}$	$5.99±0.12^{Aa}$
$T_{6.69}$	$3.28±0.15^{ABd}$	$2.92±0.05^{Af}$	$3.10±0.08^{ABe}$	$3.50±0.03^{Ac}$	$4.08±0.03^{Ab}$	$5.62±0.05^{Aa}$
$T_{6.89}$	$3.08±0.17^{Ade}$	$2.88±0.10^{Aef}$	$3.18±0.03^{Ad}$	$3.48±0.06^{Ac}$	$3.68±0.03^{Cb}$	$5.76±0.29^{Aa}$

Values are presented as mean±SD. Values with different lowercase superscripts within the row while values with different uppercase superscripts within the column are significantly different ($p<0.05$).

Table 2. Fat droplet diameters (μm) in goat milk after freeze-thaw cycles, with the pre-treatments that adjusted initial pH.

Treatment	Cycle 0	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
$T_{6.49}$	1.54±0.09^{Ad}	2.21±0.35^{Ac}	2.09±0.28^{Ac}	2.57±0.40^{Ab}	3.04±0.06^{Aa}	3.13±0.43^{Aa}
$T_{6.69}$	1.43±0.01^{Ac}	1.87±0.01^{Ab}	2.04±0.22^{Ab}	2.11±0.08^{Ab}	3.10±0.03^{Aa}	3.11±0.18^{Aa}
$T_{6.89}$	1.54±0.20^{Ad}	2.02±0.09^{Ac}	2.53±0.41^{Ab}	2.72±0.34^{Aab}	2.70±0.03^{Bab}	3.02±0.52^{Aa}

Values are presented as mean±SD. Values with different lowercase superscripts within the row while values with different uppercase superscripts within the column are significantly different (p<0.05).

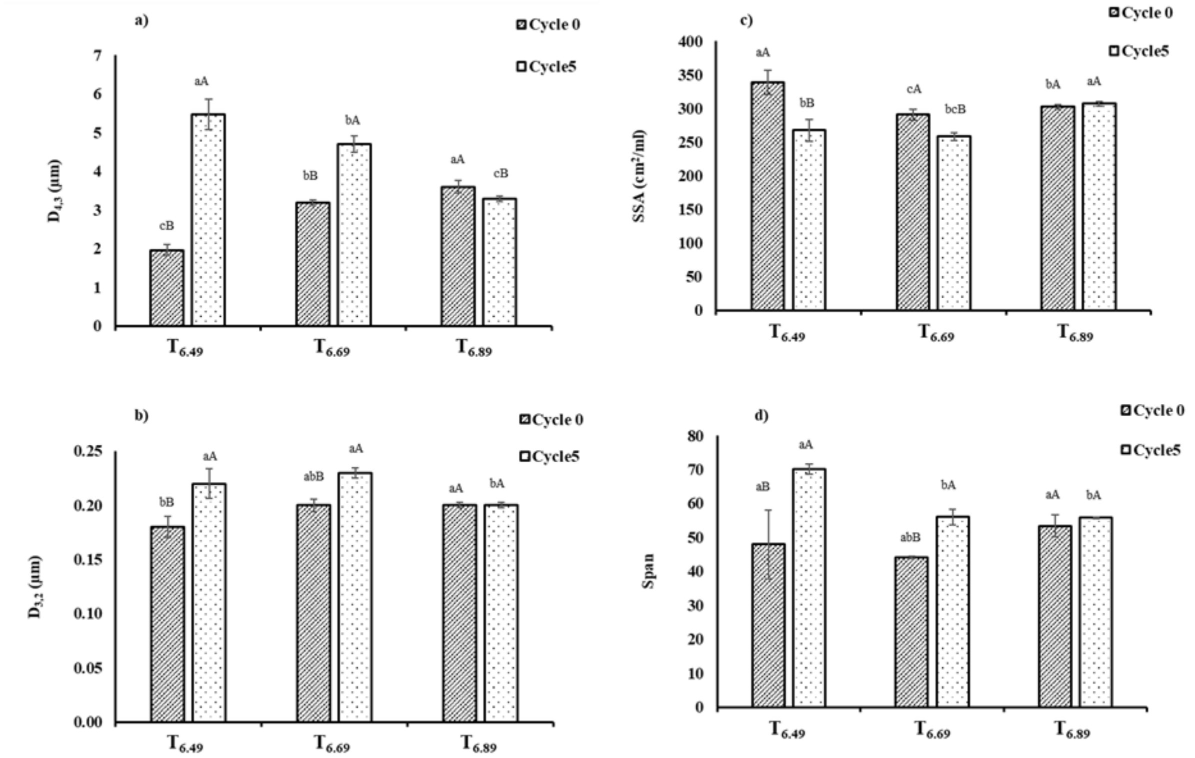

Figure 1. Effects of initial goat milk pH on volume distribution ($D_{4,3}$), average particle diameter ($D_{3,2}$), uniformity of particle size distribution (span), and specific surface area (SSA) in control (0th) and after the 5th cycle of freeze-thawing. Error bars indicate standard deviations. Different uppercase superscripts compare between freeze-thaw cycles, and different lowercase small superscripts compare between pH adjustment pre-treatments, for significant differences (p<0.05).

(SSA), and the uniform distribution (span). These are compared between the pre-treatments in Figure 1. The freeze-thaw cycles increased $D_{3,2}$, $D_{4,3}$ and span in goat milk with pre-treatments $T_{6.49}$ and $T_{6.69}$ but not with T6.89. The freeze-thaw and milk pH both affected the coalescence of protein. For example, particle diameter in $T_{6.49}$ changed from 0.18 μm to 0.22 μm, volume increased from 1.96 μm to 5.47 μm and specific surface area decreased from 338 cm^2/mL to 267 cm^2/mL. On the other hand, the $T_{6.89}$ cases were not much affected by the cycles. The span in Figure 1 represents the homogeneity of the sample: if most particles in a sample have nearly similar sizes, this value will be low. The pre-treatments with lower pH showed larger changes from the initial to after the 5th cycle, while interestingly adjusting milk pH to 6.89 before freeze-thaw cycles made these measured parameters stable from the initial state to the 5th cycle.

3.3 Heat treatment of goat milk

The heat stability of goat milk is expressed in minutes required for a milk sample at 100°C to become precipitated. The previous study found that the heat stability of fresh goat milk was 78-108 mins when milk pH ranged from 6.4-7.0 (Thoh *et al*., 2017). In the current study, the stability of all treatments decreased with freeze-thaw cycles. The $T_{6.49}$ had a heat stability decrease from 80 mins to 74 mins after the 1st cycle, whereas the $T_{6.69}$ and $T_{6.89}$ had a heat stability decrease from 90 mins to 82 and 86 mins, respectively. With $T_{6.49}$ stability rapidly declined from 80 mins to 46 mins (42.50%) from the initial to the 5th cycle (Table 3), while with $T_{6.69}$ and $T_{6.89}$ the decreases were 17.78% and 8.89 %, respectively. Temperature and pH are two main factors that influence the stability of goat and cow milk. Li *et al*. (2014) reported that below pH 6.40, there was a complexation of κ-casein and whey protein to casein micelles. These precipitated more easily than with higher pH. Adjusting milk pH to 6.89 before freezing in this current study reduced heat stability changes, and this could find practical application in the dairy industry.

3.4 Changes in some minerals in goat milk serum

The instability of milk in some cases is due to a mineral imbalance. The salt constitutes only a small part

Table 3. Heat stability expressed in minutes for the goat milk samples after freeze-thaw cycles.

Treatment	Cycle 0	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
$T_{6.49}$	80.0 ± 0.00^{Ba}	74.0 ± 0.00^{Cb}	64.0 ± 0.00^{Cc}	58.0 ± 0.00^{Cd}	50.0 ± 0.00^{Ce}	46.0 ± 0.00^{Cf}
$T_{6.69}$	90.0 ± 0.00^{Aa}	82.0 ± 0.00^{Bb}	78.0 ± 0.00^{Bc}	76.0 ± 0.00^{Bd}	74.0 ± 0.00^{Be}	74.0 ± 0.00^{Be}
$T_{6.89}$	90.0 ± 0.00^{Aa}	86.0 ± 0.00^{Ab}	84.0 ± 0.00^{Ab}	82.0 ± 0.00^{Ac}	82.0 ± 0.00^{Ac}	82.0 ± 0.00^{Ac}

Values are presented as mean±SD. Values with different lowercase superscripts within the row while values with different uppercase superscripts within the column are significantly different ($p<0.05$).

of milk (8–9 g/L) and this fraction contains calcium, magnesium, sodium and potassium as the main cations and inorganic phosphate, citrate and chloride as the main anions (Gaucheron, 2005). To determine the minerals, milk samples were filtered with an Amicon tube with a 10 kDa molecular cut-off. The mineral contents in serum of $T_{6.69}$ milk were initially: phosphorus, magnesium, calcium and sodium at 503.90, 256.50, 462.30 and 92.80 mg/mL (Figure 2 a-d), respectively. Adjusting pH to 6.49 decreased Ca, P and Mg in the milk serum, while the opposite effect was found in $T_{6.89}$. After 5 freeze-thaw cycles, the Ca and P in milk serum were clearly increased with all pre-treatments. Gaucheron (2005) reported that in natural cow milk approximately 31% of Ca was soluble and 69% was partially associated with casein molecules to form large colloidal casein micelles. When milk protein was denatured, the Ca dissociated from casein and was dispersed into the aqueous phase (Fuente *et al.*, 1997). Most of the Na was soluble in milk. After freeze-thaw, the Na decreased ($p<0.05$). This might be due to it associating with phosphoseryl residues of casein molecules (Gaucheron, 2005). A consequence of these changes is increased hydration of casein micelles (Gaucheron, 2005) and a possible increase in their particle size.

4. Conclusion

The milk pH and freeze-thaw cycles caused a mineral imbalance increasing the size of casein micelles, which accumulated with repeated cycles. Goat milk had improved heat stability when the initial pH was increased to 6.89. This pre-treatment gave less sediment, smaller fat droplet size, stable particle size, and importantly significantly improved heat stability over untreated milk, or milk with reduced pH. Therefore, increasing the milk pH before freezing could help preserve milk by freezing as this prevents changes and improves heat stability, which is important in further processing, particularly sterilization.

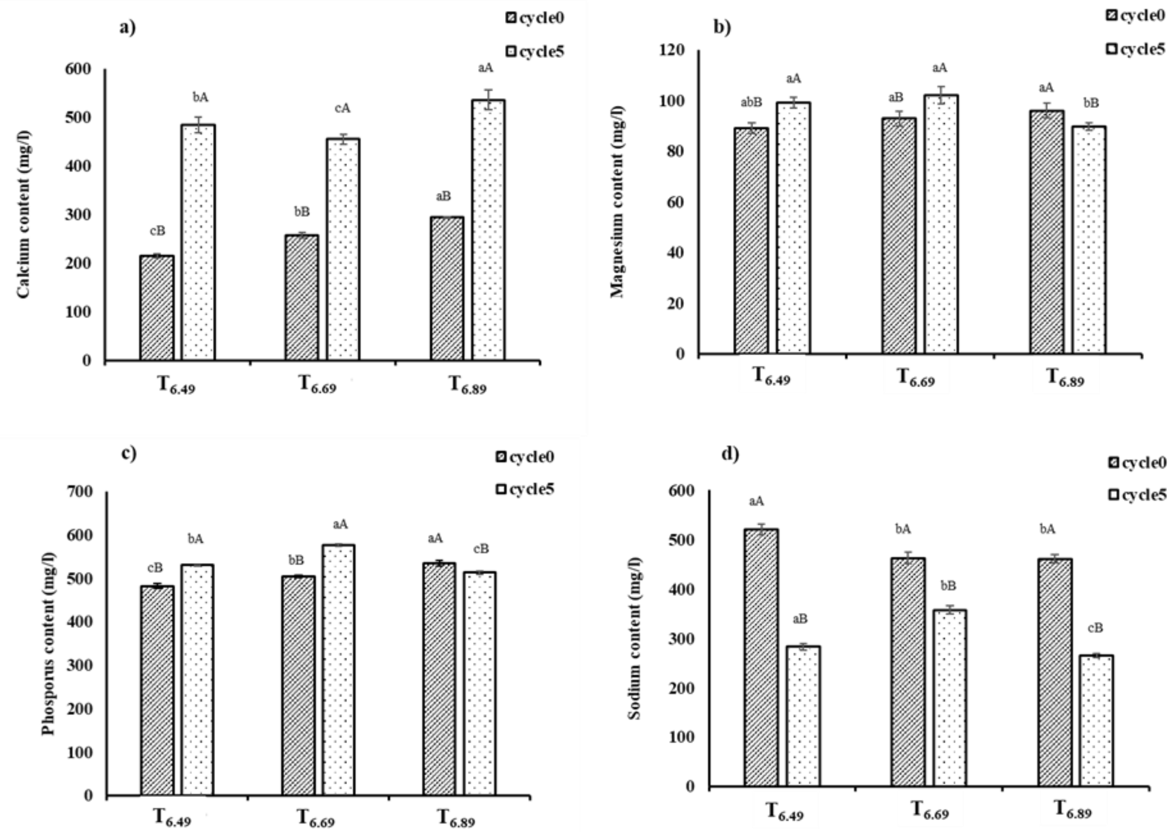

Figure 2. Calcium, phosphorus, magnesium and sodium contents in goat milk serum initially and after the 5th freeze-thaw cycle, with the alternative pre-treatments (mean±SD). Different uppercase superscripts compare between freeze-thaw cycles, and different lowercase small superscripts compare between pH adjustment pre-treatments, for significant differences ($p<0.05$).

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Conflict of interest

The authors declare no conflict of interest.

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