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Research Article

The effect of heat treatment on the nutritional and antioxidant content of different milk types

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ABSTRACT

Heat treatments may cause some chemical and physicochemical changes in milk, although milk is a heat-stable system. Heat treatments can cause different changes in different types of milk. This study aimed to compare the effects of pasteurization and boiling on goat and cow milk's macro-molecular contents, glutathione levels, and superoxide dismutase activities. The protein level of both types of milk decreased with the pasteurization process, and boiling also reduced the protein level of goat milk. Both heat treatments reduced superoxide dismutase activity and glutathione levels in both types of milk. While the boiling process did not change the cow's milk lactose level, it increased the goat milk lactose level. It was determined that pasteurization reduced the lactose level in both types of milk. Pasteurization did not change the fat level in cow milk but decreased the fat level in goat milk. In conclusion, cow milk was less affected by these heat treatments, which can be attributed to having large fat globules, high lactose concentration, and high heat resistance protein content compared to goat milk.

Keywords: Milk, Boiling, Pasteurization, Antioxidants, The Nutritional Content of Milk

Introduction

Milk and dairy products are the major sources of protein, fat, carbohydrate, mineral, and vitamin in the human diet (Kliem et al. 2013). The macronutrients of milk are protein, fat, and carbohydrate and micronutrients are minerals and vitamins. The milk macronutrient content varies depending on the animal's diet, the season the milk is collected, and the type of animal. When the nutrient content of goat and cow milk is compared, there is a structural difference between them. Goat milk is richer in protein and fat; on the other hand, cow milk is rich in lactose content. Goat and cow milk have different biochemical properties because of these differences in nutritional content. Goat milk is less allergenic compared to cow milk, due to the low α-s1 casein and β-lactalbumin protein levels in goat milk. The goat milk fat globules are small in size (<3.5 µm), have a homogeneous structure and are easier to digest compared to other animal-based milk. Goat milk also has a higher oligosaccharide (25-30 mg/100 mL) concentration when compared to cow milk oligosaccharide concentration (2-3 mg/100 mL). These oligosaccharides stimulate the release of interleukin-2, increase the growth of bifidobacteria and help the nervous system to develop by elevating the ability to make long-chain polyunsaturated fatty acids (Altun and Sarici 2017).

Milk has antioxidant effects in addition to nutritional benefits. The antioxidant properties of milk are due to its high-quality protein content (Korycka-Dahl, Richardson, and Hicks 1979; Pocius, Clark, and Baumrucker 1981). It has been suggested that glutathione (GSH) and superoxide dismutase (SOD) are the antioxidant parameters for the oxidative stability of milk (Granelli, Björck, and Appelqvist 1995; Talukder et al. 2015). Glutathione is the non-protein sulfhydryl compound in mammalian cells and is generally considered a good indicator of the scavenging of reactive oxygen species (Talukder et al. 2015). Dismutation of superoxide anion by SOD may be of importance in preventing lipid peroxidation (Granelli et al. 1995). It has been observed that the GSH and SOD are excreted into the milk from mammary secretory cells. SOD is only found in skim milk fractions of cow milk, with concentrations ranging from 0.15 mg to 2.4 mg/L. (Khan et al. 2019). Öner et al. reported that goat milk contains higher free radical scavengers than cow milk. They also stated that dry matter, protein, and fat levels did not correspond to the antioxidant capacity (Öner, Sanlıdere-Aloglu, and Dedebaş 2011).

Heat treatment of raw milk reduces the microbial population, inactivates enzymes, and minimizes chemical reactions and physical changes during storage. Pasteurization, sterilization, ultra-high temperature, and high-temperature processing are accepted methods for extending the shelf life of dairy products in the dairy industry (Stojanovska et al. 2017). Pasteurization is one of the most commonly used techniques for the processing of fluid milk. During pasteurization, milk is exposed to a certain heat treatment for a specific period (Khan et al. 2017). It has been demonstrated that industrial pasteurization may cause remarkable modifications in milk structure, like enzyme inactivation, protein denaturation, modification, masking or unmasking cross-linking between proteins and other food components, such as lactosylation, lipid oxidation products, and the generation of Maillard reaction products (Lamberti et al. 2017). During pasteurization, approximately 5 - 15% of milk whey protein is denatured by the pasteurization process. Pasteurization does not cause dephosphorylation and reduction in pH and ionic calcium and causes a negligible effect on the heat-sensitive water-soluble vitamins (Deeth and Lewis 2017). In addition, SOD activity and GSH level are also affected by the heat treatments (Hicks, Bucy, and Stofer 1979; Li et al. 2018; Martysiak-Żurowska, Puta, and Kiełbratowska 2019). Much is known about the industrial thermal processing of food whereas there is a lack of information about the impact of domestic heat treatments (boiling) on the biochemical quality of milk (Lamberti et al. 2017). Boiling is the mildest heat treatment given to milk. Boiling aims to reduce the growth of psychrotrophic bacteria that may release heat-resistant proteases and lipases into the milk if allowed to reach high levels (Deeth and Lewis 2017). Boiling does not denature the milk whey proteins, does not affect the heat stability of milk as measured by the heat coagulation time at 130°C (Coghill, Mutzelburg, and Birch 1982), and reduces lipase activity by about 50% (Humbert et al. 1985).

Although heat treatments are effective in removing germs from milk, it is known that they change the milk's biochemical composition. However, no research has been conducted to determine which types of milk are more influenced by these processes. Therefore, this study aimed to see how different heat treatments affected the nutritional content of cow and goat milk, as well as glutathione levels and superoxide dismutase activity for antioxidant characteristics.

Materials and Methods

Milk Samples

Raw and pasteurized milk were obtained from the same local brand (Berk Süt, Kocaeli, Turkey). Each milk sample was purchased as one liter and used as a pool. The study was carried out by taking 8 different samples from cow and goat milk. Raw milk was boiled at 100ºC for 1 minute, and pasteurized milk of the same brand was purchased. Milk samples were divided into six groups designated as follows; RC: raw cow milk, BC: boiled cow milk, PC: pasteurized cow milk, RG: raw goat milk, BG: boiled goat milk, and PG: pasteurized goat milk.

Total Protein Determination

Cow and goat milk total protein levels were determined according to the method of Bradford (Bradford 1976). The fat (cream) layer was removed before the milk protein determination. Subsequently, the skim-milk samples were incubated with Coomassie Brillant Blue dye solution. The absorbance of the blue colour formed at the end of the incubation was recorded at 595 nm.

Lactose Determination

The milk lactose level was detected by the colorimetric picric acid method (Khramov, Kolomeĭtseva, and Papichev 2008). The milk samples were mixed with an aqueous and saturated solution of picric acid (Sigma-Aldrich, 197378) and then incubated in a boiling water bath. At the end of incubation, the color of the solution changes from yellow to red. The quantity of lactose in milk is directly proportional to the reddish color intensity in the solution.

Fat Determination (Lucas et al. 1978)

The milk samples were drawn into a capillary tube (ISOLAB, length of 75 mm, wall thickness of 0.2 mm) and sealed with a lighter flame for fat content analysis. Each milk sample performed a double determination. Sealed tubes were centrifuged by a hematocrit centrifuge for 40 minutes. The percentage of the fat (cream) layer was calculated by measuring the fat (cream) layer and the full milk length with a ruler. The calculated percentage of the fat (cream) layer is linearly related to the fat content in goat and cow milk.

Determination of GSH Level

GSH level was determined by the method of Beutler (Beutler 1975). The colored product resulting from the reaction of the sulfhydryl groups with Ellmann's reagent, 5-5 'dithiobis 1-2 nitrobenzoic acid (Merck, D8130) was evaluated spectrophotometrically. Results were presented as % mg glutathione using an extinction coefficient of 13600 $M^{-1}cm^{-1}$.

Determination of SOD Activity

SOD activity was measured by the method of Mylorie et al. (Mylroie et al. 1986). This method measures the ability of SOD to increase the effect of riboflavin (Sigma-Aldrich, R9504) sensitized photo-oxidation of o-dianisidine (Sigma-Aldrich, D3252). The activity of SOD is generated by illuminating the reaction mixture that contains o-dianisidine dihydrochloride and riboflavin with light from a fluorescent lamp. The oxidation of o-dianisidine, which is sensitized by riboflavin, is enhanced by superoxide dismutase, and the increase in the absorbance is linearly dependent on superoxide dismutase concentration. The absorbance of the resulting colored product is evaluated spectrophotometrically at 460 nm. Results were presented as kU/mL.

pH Measurement and Milk Energy Level

Cow and goat milk pH levels were determined by a benchtop pH meter (Mettler Toledo FE20-Basic Five Easy). The milk's macro components are multiplied by the suitable conversion factor to determine the total level of energy contained in the milk samples. Conversion factors are 4 kcal/dL for protein values, 9 kcal/dl for fat values, and 4 kcal/dl for lactose values, respectively. The total energy content was calculated as follows:

Total energy (kcal/dl)= (Fat x 9)+(Protein x 4)+(Lactose x4) (García-Lara et al. 2012).

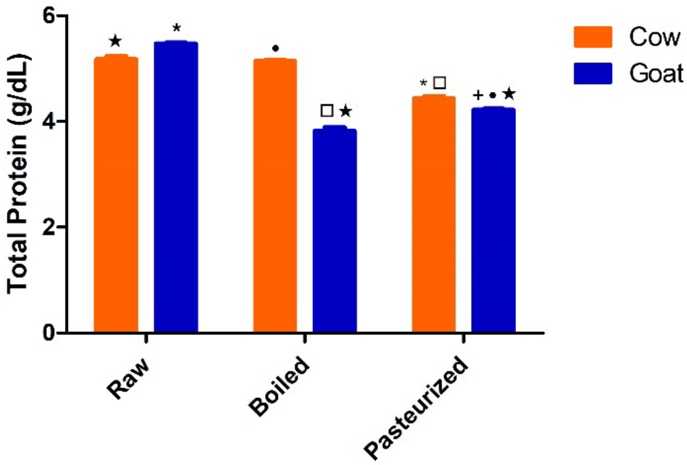
Statistical Analysis

Graph Pad Prism 5.0 (Graph Pad Software, San Diego, CA, USA) statistical package program was used for the statistical analysis. Groups of data were analyzed by using ANOVA followed by Tukey's multiple comparison tests. Values of $p<0.05$ were regarded as significant. All data were given as mean and standard deviation.

Results and Discussion

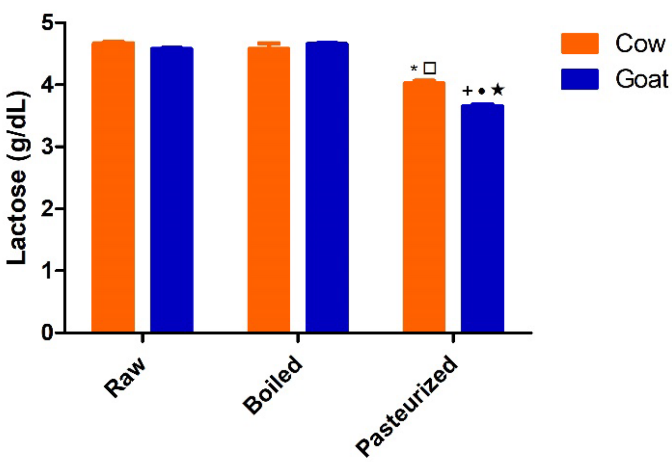
The effect of heat treatments on milk macronutrients and antioxidant capacity has been the subject of many researchers over the years (Hicks et al. 1979; Coghill et al. 1982; Villamiel and de Jong 2000; García-Lara et al. 2012; Deeth and Lewis 2017; Khan et al. 2017). Milk heat treatments are used to eliminate microorganisms that might spoil the milk and to maintain its safety for daily consumption. Heat treatments, on the other hand, may change the nutritional characteristics of milk. In this study, the nutritional composition, GSH level, and SOD activity changes of cow and goat milk were investigated to determine the most affected milk type.

The total protein level of raw goat milk was significantly higher than raw cow milk ($p<0.05$, Figure 1). Raw cow milk's total protein level was not significantly changed with boiling, while pasteurization reduced the total protein level by 14%. However, after boiling and pasteurization, the total protein level of raw goat milk significantly decreased ($p<0.05$, Figure 1). Raw cow and goat milk lactose levels were not significantly changed by boiling (Figure2). Pasteurization significantly reduced the lactose level of both cow and goat milk ($p<0.05$). For goat milk, the decline was 20%, while for cow milk, it was 13%. Heat treatment had no significant effect on the fat level of raw cow milk. While the fat level of goat milk did not change significantly after boiling, it decreased significantly after pasteurization ($p<0.05$, Figure 3).



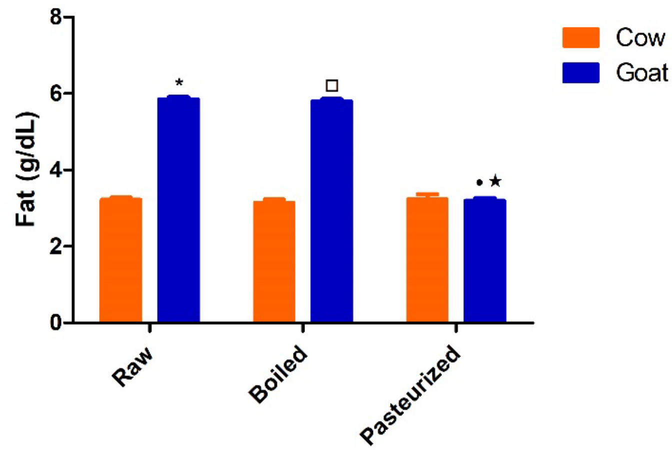
*: p<0.05 compared to Raw Cow Milk, □: p<0.05 compared to Boiled Cow Milk, +: p<0.05 compared to Pasteurized Cow Milk, ★: p<0.05 compared to Raw Goat Milk, •: p<0.05 compared to Boiled Goat Milk, n= 8

Figure 1. Total protein levels of cow and goat milk after the thermal processes



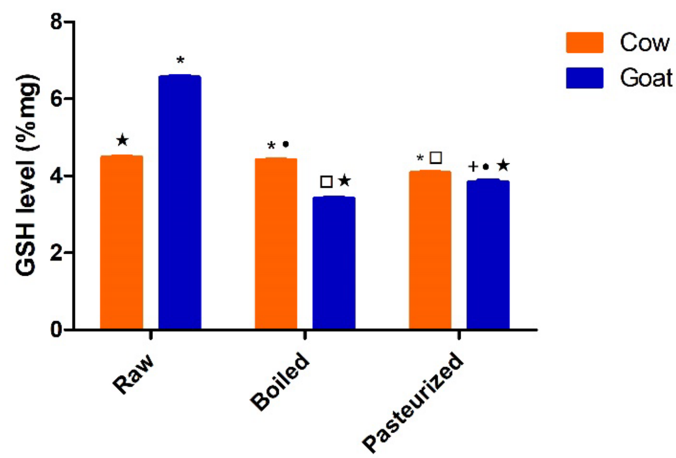
*: p<0.05 compared to Raw Cow Milk, □: p<0.05 compared to Boiled Cow Milk, +: p<0.05 compared to Pasteurized Cow Milk, ★: p<0.05 compared to Raw Goat Milk, •: p<0.05 compared to Boiled Goat Milk, n= 8

Figure 2. Lactose levels of cow and goat milk after the thermal processes



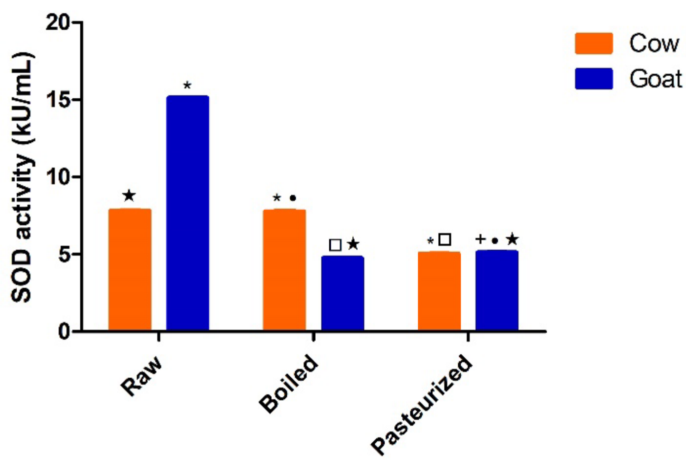
*: p<0.05 compared to Raw Cow Milk, □: p<0.05 compared to Boiled Cow Milk, ★: p<0.05 compared to Raw Goat Milk, •: p<0.05 compared to Boiled Goat Milk, n= 8

Figure 3. Fat levels of cow and goat milk after the thermal processes



*: p<0.05 compared to Raw Cow Milk, □: p<0.05 compared to Boiled Cow Milk, +: p<0.05 compared to Pasteurized Cow Milk, ★: p<0.05 compared to Raw Goat Milk, •: p<0.05 compared to Boiled Goat Milk, n= 8

Figure 4. GSH levels of cow and goat milk after the thermal processes.



*: p<0.05 compared to Raw Cow Milk, □: p<0.05 compared to Boiled Cow Milk, +: p<0.05 compared to Pasteurized Cow Milk, ★: p<0.05 compared to Raw Goat Milk, •: p<0.05 compared to Boiled Goat Milk, n= 8

Figure 5. SOD activities of cow and goat milk after the thermal processes

Raw cow and raw goat milk consists of basic parts (fat, protein and lactose) but goat milk has higher protein and fat level compared to cow milk (Posati & Orr, 1976; Jenness, 1980; Park & Haenlein, 2016). In the study by Khan et al., it was found that protein levels of cow's milk increased after 30 minutes of boiling, while the total protein level decreased with pasteurization. The reason for the increased total protein level was attributed to the decrease in water loss during boiling (Khan et al. 2017). Farrell et al. suggested that boiling milk induces structural changes in protein structure by disrupting intramolecular or intermolecular interactions and disulfide bonds, causing the loss of secondary structure and protein aggregation (Farrell et al. 2004).

Boiling raw milk for more than 5-10 minutes at home causes the milk to lose its structural and content properties. Laszlo et al (2017) advised shorter milk boiling times as the antibiotic residues that can be found in milk of animal origin may be more harmful than microorganisms (László, Lányi, and Laczay 2017). In the literature, there are also many milk-boiling procedures according to the purposes of the study. Condas et al (2012) boiled milk for 1 and two minutes at 100 ºC (Condas et al. 2012). Kilango et al. boiled milk for 5 minute at 95ºC (Kilango et al. 2012). Khan et al. boiled milk for 1 minute at 100ºC (Khan et al. 2017). Joishy et al. boiled milk for 10 minutes at 100ºC (Joishy, Dehingia, and Khan 2019). In this study, raw cow and goat milk samples were boiled for 1 minute at 100ºC. There was no significant change in the total protein level of cow milk at the end of controlled boiling, a decrease was found in the total protein level of goat milk. The heat stability of lactoferrin is such that typical pasteurization processes have little effect on structure (Farrell et al. 2004). The fact that cow's milk contains more lactoferrin than goat's milk causes cow's milk to be less affected by the pasteurization process (Rachman, Maheswari, and Bachroem 2015). The five major proteins of goat milk, α-lactalbumin, β-lactoglobulin, κ-casein, β-casein, and αs2-casein are very similar to their homologs in cow's milk. Goat milk lacks bovine αs1-casein, the most abundant protein in cow's milk. Caseinate micelles of goat milk contain more calcium and inorganic phosphorus, are less soluble and less heat resistant, and lose β-casein more easily than bovine micelles. For this reason, goat's milk proteins are less heat resistant (Jenness, 1980; Montilla & Calvo, 1997; Farrell et al., 2004) .

Khan et al. found that the lactose level of cow's milk increased with boiling and decreased with pasteurization (Khan et al. 2017). In this study, the lactose level was not affected by boiling in raw cow and goat milk, and it was decreased with pasteurization. As heat treatment causes several chemical modifications in milk nutrients, the lactose level of milk can change depending on the severity of heating. Heat treatment causes the degradation of lactose to acids (with a concomitant decrease in pH), isomerization of lactose (e.g., to lactulose), production of compounds such as furfural, and interactions with amino groups of proteins (by Maillard reaction) (Lamberti et al. 2017). Lactose is a reducing sugar that reacts with the amino groups of proteins. Since the proteins in goat milk are less heat resistant, lactose reacts more easily with the proteins in goat milk. With the pasteurization of goat milk, the lactose level decreases more than in cow's milk.

Heat treatments have been found to reduce the diameters of fat globules of cow milk (Villamiel and De Jong 2000). The decrease in the diameter of fat globules does not indicate a change in fat levels. The core of all milk fat globules contains triacylglycerols, polyunsaturated fatty acids, and monounsaturated fatty acids. The small fat globules, which are not heat resistant, are more affected by the boiling and pasteurization process (Jenness 1980). In light of this information, it can be concluded that the small-sized fat globules of goat milk were disrupted as a result of the pasteurization process.

Figures 4 and 5 show the effects of heat treatments on GSH levels and SOD activities in cow and goat milk, respectively. Raw goat milk's GSH level and SOD activity were significantly higher than raw cow's milk ($p<0.05$). The raw cow milk GSH level decreased after boiling and pasteurization. On the other hand, the GSH level decrease of raw goat milk was higher than the cow milk after boiling and pasteurization. Both heat treatments were significantly reduced the SOD activity of cow milk ($p<0.05$). On the other hand, the SOD activity of raw goat milk decreased by 68% and 66% by boiling and pasteurization processes, respectively.

One of the most often used methods for processing fluid milk is pasteurization, but another widely used method is boiling the milk at home for use in the home. In this study, the effect of pasteurization and boiling on antioxidant characteristics of cow and goat milk in terms of GSH level and SOD activity were investigated for improved consumption patterns. GSH is an antioxidant that protects cells from the toxic effects of reactive oxygen species such as free radicals, peroxides, and heavy metals, in a tripeptide structure consisting of the amino acids glutamate, cysteine, and glycine. When the amino acid profiles of cow and goat milk were examined, it was found that the amount of glutamate, cysteine, and glycine amino acids in goat milk was higher than the cow milk (Kamal et al., 2007; Barlowska et al., 2011; Medhammar et al., 2012). In this study, the GSH level was higher in raw goat milk compared to raw cow milk. Pasteurization and boiling significantly decreased the high raw goat milk GSH level ($p<0.05$).

SOD is the only antioxidant enzyme that scavenges the superoxide anion by converting this free radical to oxygen and hydrogen peroxide (Wang and Zhang 2015). SOD enzyme is rich in amino acids alanine, glycine, leucine, arginine, serine, and valine amino acids (Folz and Crapo 1994). In the amino acid profiles of cow and goat milk, the amount of alanine, arginine, serine, and valine amino acids were found to be higher in cow milk (Kamal et al., 2007; Barlowska et al., 2011; Medhammar et al., 2012). This situation can be thought of as if the SOD enzyme activity in cow's milk should be more than in goat's milk, but in this study, SOD enzyme activity was found to be higher in goat milk compared to cow milk. This finding is compatible with the studies that show the high SOD activity of goat milk compared to cow milk (Granelli et al. 1995; Li et al. 2018). The antioxidant content of the milk also changes as the macro components of milk decrease as a result of heat treatment. In the study of Li et al., it was determined that heat treatments higher than 75ºC inactivated more than 20% of SOD activity in goat milk. SOD activity is easily affected by temperature, pH, rotation speed, calcium ion concentration, and fermentation time. SOD activity gradually decreased as the storage time increased, the heating temperature and the Ca^{2+} concentration decreased (Li et al. 2018). In the study by Hicks et al. was found that cow milk SOD activity decreased depending on the increasing heat intensity (Hicks et al. 1979). Another study with breast milk revealed that convective heating at temperatures above 66ºC caused significant changes in the activity of antioxidant enzymes in human milk when applied for more than 20 minutes (Martysiak-Żurowska et al. 2019). In this study, heat treatments decreased both raw cow and raw goat milk SOD activity.

Both boiling and pasteurization did not significantly change the pH of cow and goat milk but decreased the cow milk energy level. This energy decrease was higher in the pasteurization process compared to the boiling process (Table 1). In goat milk, pasteurization significantly decreased the energy level ($p<0.05$, Table 2). When the energy levels of the cow and goat milk were compared, the energy level of goat milk was found to be higher than cow milk in raw and boiled samples ($p<0.05$). This difference can be associated with the high fat content of goat milk.

Table 1. Cow milk pH□ and energy level changes (means) after the heat treatments

	Raw Cow Milk	Boiled Cow Milk	Pasteurized Cow Milk
pH	6.76 ±0.16	6.71 ±0.18	6.75 ±0.13
Energy (kcal/mol)	87.64 ±2.7	80.69 ±2.8*	75.98 ±1.6□ Δ.

* $p<0.05$ compared to raw cow milk, □: $p<0.05$ compared to boiled cow milk, n= 8

Table 2. Goat milk pH• and energy level changes (means) after the heat treatments

	Raw Goat Milk	Boiled Goat Milk	Pasteurized Goat Milk
pH	6.75 ±0.17	6.75 ±0.17	6.70 ±0.13
Energy (kcal/mol)	111.8 ±5.6	114.1 ±5.8	101.7 ±1.5• Δ.

• $p<0.05$ Compared to raw goat milk, Δ: $p<0.05$ Compared to boiled goat milk, n=8

Conclusion

Goat milk was more affected by boiling and pasteurization than cow's milk. The reason that cow milk was less affected by these heat treatments can be attributed to having large fat globules, high lactose concentration, and high heat resistance protein content compared to goat milk.

Compliance with Ethical Standard

Conflict of interests: The author declares that for this article they have no actual, potential, or perceived conflict of interests.

Ethics committee approval: The author declares that this study does not include any experiments with human or animal subjects; therefore, no ethics committee approval is needed.

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Disclosure: -

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