

Probiotic Fermented Goat's and Sheep's Milk with Collagen

Summary

Supplementation is becoming an increasingly popular practice across various age groups, aimed at complementing daily diets with concentrated vitamins and/or minerals in the form of medications or dietary supplements exhibiting nutritional or physiological effects. Increasing attention is given to collagen supplementation due to its decline with age. The body's ability to produce collagen starts to decrease between the ages of 18 and 29, with an average decline of 1 % per year after the age of 40 and a 75 % reduction in collagen production by the age of 80. Therefore, collagen supplementation is beneficial starting as early as 25 or 30 years old. The nutritional and functional properties of collagen and its hydrolysate are utilized in the dairy sector, among others. Adding bioactive ingredients to dairy products, particularly fermented milk, results in products with higher nutritional value and bioavailability of components. To increase the content of well-absorbed protein in milk, collagen or its hydrolysate can be applied, yielding milk with potential therapeutic properties and tailored texture parameters. Dairy products are currently at the forefront of probiotic food development. The trend of increasing consumption of fermented products stems not only from the wide range of products available on the market but also from their high nutritional and dietary values and their therapeutic and preventive properties. Due to their specific properties, fermented dairy products also serve as an excellent matrix for incorporating nutrients that impart functional properties to the final product. Considering the growing interest in health-promoting foods and the occurrence of allergies to the α_1 casein fraction in cow's milk, there is an increasing demand for milk and dairy products derived from other mammalian species. Aside from cow's milk, sheep's and goat's milk are also used in processing in Poland and globally. These are becoming more popular as alternatives to cow's milk products due to their nutritional values and potential as substitutes for cow's milk.

This study aimed to determine the impact of adding different types of collagens (hydrolysate and bovine collagen) at two concentrations (1.5 % and 3.0 %), storage duration (1 day and 21 days), and probiotic strains (*Lacticaseibacillus casei*, *Lactobacillus acidophilus*, *Lacticaseibacillus paracasei*, and *Lacticaseibacillus rhamnosus*) on the quality of fermented sheep and goat milk, as well as the survival of these strains in the digestive system.

In the initial phase of the research, the technological suitability of raw sheep and goat milk was assessed. The quality evaluation of sheep and goat milk indicated the high quality of the raw milk and appropriate processing properties, enabling its use in the production of probiotic fermented milk. Upon analyzing the total microbial count, it was determined that both raw sheep and goat milk intended for the production of fermented milk require thermal treatment (pasteurization) to ensure the resulting milk's high quality and microbiological safety.

In the subsequent phase of the study, the feasibility of adding collagen into the production of probiotic fermented sheep and goat milk was evaluated by assessing the resulting milk's physicochemical, organoleptic, and microbiological properties.

In all groups of sheep and goat milk, it was found that fermented milk with added collagen exhibited a higher pH value after the first day of storage compared to the control milk without collagen. It was demonstrated that the type of collagen participates in differentiating pH values, as sheep and goat milk samples with hydrolysate had a higher pH on the first day of storage than those with bovine collagen, although these differences were not always statistically significant. Additionally, there was a tendency for increasing the collagen concentration from 1.5 % to 3.0 % to result in higher pH values, regardless of whether hydrolysate or bovine collagen was added.

Depending on the type and dose, collagen addition also influenced the lactic acid content in the analyzed samples. Fermented sheep milk with 1.5 % hydrolysate had a lower lactic acid content than the control counterparts. Conversely, in fermented goat milk, higher lactic acid content was observed in milk with bovine collagen compared to hydrolysate. An exception was the KLP1.5H sample, which had the same amount of lactic acid as KLP. Increasing the collagen dose from 1.5 % to 3.0 % increased lactic acid content in fermented goat milk. On analyzing the results on the 21st day of storage, it was found that a 1.5 % addition of bovine collagen favorably impacted the lactic acid content in fermented goat milk, with all milk groups showing significantly higher lactic acid content than their control counterparts. Similarly, increasing the dose from 1.5 % to 3.0 % in milk with bovine collagen significantly increased the lactic acid content.

The addition of hydrolysate most effectively limited the syneresis of sheep milk, both on the 1st and 21st day of storage. Increasing the hydrolysate dose from 1.5 % to 3.0 % significantly reduced syneresis compared to the control sheep milk counterparts and sheep milk with a 1.5 % hydrolysate addition. The addition of bovine collagen was not as effective in reducing whey separation. In fermented goat milk, whether the type and dose of collagen influenced the increase or reduction of syneresis was not unequivocally determined. Adding bovine collagen and hydrolysate reduced whey separation only in goat milk fermented by *L. rhamnosus*. Increasing the dose from 1.5 % to 3.0 % enhanced syneresis in goat milk samples only by adding bovine collagen. In goat milk with hydrolysate, these relationships were not conclusive.

In this study, the analysis of lightness (L^*) values revealed that on the first day of storage, the addition of collagen, both in the form of bovine collagen and hydrolysate, caused darkening of the color in all groups of fermented sheep and goat milk compared to the controls.

Fermented sheep and goat milk exhibited hues of green and yellow. In probiotic sheep milk, a lower gel hardness was observed in samples with bovine collagen compared to their hydrolysate counterparts. Conversely, when analyzing the hardness results for goat milk, it was found that only in

goat milk with hydrolysate KLP3.0H and KLR3.0H was a significant increase in hardness compared to the controls.

For the other groups, no significant effect on the gel hardness of goat milk was noted. After one day of storage, only one case showed that adding hydrolysate reduced milk's springiness (OLA3.0H). The type and dose of collagen did not affect the springiness of the other sheep milk samples or any goat milk groups on the first day of storage. Similarly, after 21 days of storage, there was no observed effect on the springiness of both sheep and goat milk, except for OLA1.5W sheep milk, where gel springiness increased compared to the control group.

Throughout the entire storage period, the number of viable probiotic bacteria cells in all groups of fermented milk remained higher than $8 \log \text{CFU g}^{-1}$. Adding hydrolysate and bovine collagen to goat milk significantly improved the survival of bacteria, particularly noted on the 21st day of storage in milk fermented by *L. casei* and *L. acidophilus*, compared to their control counterparts. In sheep milk fermented by *L. acidophilus* and *L. casei*, more beneficial bacterial survival was observed over the 21-day storage period, with the collagen dose having no impact on the growth and survival of both strains.

Moreover, it was demonstrated that sheep milk containing hydrolysate provided favorable conditions for the growth of *L. casei* even during refrigerated storage.

The addition of collagen to sheep milk enhanced the intensity of the sweet taste due to the presence of sweet glycine. The most intense milky-creamy taste was in sheep milk with hydrolysate fermented by *L. casei*. However, adding collagen (both bovine and hydrolysate) caused a slight off-taste and off-odor in fermented sheep milk. The intensity of the additive and off-tastes in goat milk was influenced by the bacterial strain conducting the fermentation and the storage time. The primary issue associated with using goat milk in dairy product production is its characteristic goat taste and odour. However, adding collagen did not intensify the perception of the goaty taste but instead intensified the milky-creamy and sweet taste.

The effect of milk type and storage time on fermented milk's physicochemical and organoleptic properties was assessed. In all groups of fermented milk, storage time led to an increase in the acidity of the fermented milk. Additionally, higher pH values were observed on the 21st day of storage in all control groups of fermented goat milk compared to fermented sheep milk. The analysis indicated that the production of lactic acid by probiotic bacteria was influenced by both storage time and milk type, as confirmed by ANOVA analysis ($p=0.0000$). On the first day of storage, all control goat milk samples exhibited lower lactic acid content than sheep milk. On the 21st day of storage, only the goat milk samples KLA, KLP, and KLR showed lower lactic acid content compared to their sheep milk counterparts.

The syneresis of milk was influenced by the type of milk used in the study. A higher level of syneresis was observed in fermented goat milk than in sheep milk samples. The highest level of syneresis was found in milk fermented by *L. acidophilus* for sheep and goat milk on both testing days. Conversely,

storage time differentiated the level of syneresis in the OLC, OLP, and OLR samples, where less whey separation was observed, whereas in the KLA and KLR samples, an increase in whey separation was noted. No significant differences due to storage time on the level of syneresis were observed in the other groups.

The analysis of lightness (L^*) values showed that on both the 1st and 21st days of storage, fermented goat milk was darker compared to sheep milk. Additionally, extending the storage time resulted in further darkening of the color of both fermented sheep and goat milk, indicating a significant effect of milk type and storage time on the lightness (L^*). Analyzing the b^* color component concerning milk type revealed a significantly greater presence of yellow color in sheep milk than in goat milk throughout the study period. However, extending the storage time from 1 to 21 days intensified the yellow color only in sheep milk. In contrast, the yellow color in goat milk decreased with storage time. Sheep milk showed a more profound and purer color than goat milk.

An analysis was conducted on how milk type and storage time affect selected texture components (hardness, cohesiveness, and springiness). The data analysis indicated that the gel hardness of fermented milk depends on the milk type. In this case, sheep milk always exhibited a harder gel than goat milk, which is related to the dry matter content. Moreover, storage time did not significantly affect the gel hardness of goat milk. The opposite trend was observed for sheep milk, where extending the storage time to 21 days resulted in a significant increase in the hardness of the fermented milk gel. Goat milk showed higher cohesiveness between particles, except for the OLC and KLC groups, where the opposite correlation was observed. With extended storage time, the cohesiveness of the milk gel did not change significantly in goat milk. Higher springiness values characterized sheep milk. In most cases, storage time increased the springiness of the fermented milk gel, except for KLC milk, where a decrease in springiness was noted.

The type of milk mainly affected the taste of the fermented milk. Off-tastes were more intense in fermented goat milk. In most cases, storage time contributed to an increase in the intensity of sour taste and odor and a decrease in the perception of sweet taste in both types of milk.

In the final stage of the research, *in vitro* digestion was conducted to determine the viability of probiotic bacterial cells under simulated gastrointestinal conditions. The study revealed the lowest survival rate for the *L. rhamnosus* strain during digestion within the model gastrointestinal system, which also exhibited the greatest reduction in the number of viable cells. The addition of bovine collagen had a beneficial effect on the survival of this strain, particularly in goat milk. Furthermore, the survival rate of *L. rhamnosus* varied depending on the type of milk. In goat milk, it ranged from 45.29% in KLR to 64.23% in KLR3.0H, whereas in sheep milk, it ranged from 41.05% in OLR3.0W to 48.49% in OLR1.5H. Goat milk also provided more favorable conditions for the survival of *L. paracasei*, especially with the addition of hydrolysate, compared to sheep milk. The highest survival rate in simulated *in vitro* digestion conditions, relative to the number of cells before digestion, was observed in milk fermented

by *L. casei* and *L. paracasei*. Results showed that, in the small intestine phase, milk fermented by *L. casei* and *L. paracasei* had probiotic counts higher than 5 log CFU g⁻¹, indicating a survival rate above 50%. Meanwhile, *L. rhamnosus* and *L. acidophilus* strains were identified at cell counts greater than 4 log CFU g⁻¹. The addition of collagen and hydrolysate to sheep milk resulted in decreased survival of *L. acidophilus*, whereas the addition of hydrolysate to goat milk increased the survival of this strain. Bovine collagen addition positively influenced the survival of *L. casei* and *L. rhamnosus* under *in vitro* digestion conditions.

Keywords: fermented sheep milk, fermented goat milk, bovine collagen, collagen hydrolysate, probiotic, *in vitro*, survival rate