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Investigation of the Viability of Probiotic Bacteria, Sensory Characteristics and Proteolysis in Probiotic Soy Cheese During Storage

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ABSTRACT: Probiotic soy cheese is a fermented soy milk product that has nutritional and health properties. The aim of this study was to investigate the effect of kind of probiotic culture on the sensory properties and proteolysis in soy probiotic cheese during storage. Probiotic soy cheese was produced using cheese starter (25% thermophilic + 75% mesophilic) as well as probiotic strains of *Lactobacillus acidophilus*, *L. casei* and *Bifidobacterium lactis*. The control sample (without probiotic bacteria) was also produced. These samples were stored for 1 month. Probiotic bacteria added to soy cheese had good survival, therefore after one month storage, the probiotic bacterial count was more than 7 log CFU/g. The quantity of soluble nitrogen and non-protein nitrogen in the samples increased during storage. Treatment 3 (containing cheese starter + *B. lactis*) had a higher rate of proteolysis during storage than the other treatments. Taste, odor, texture and overall acceptance scores of sampels decreased during storage ($p<0.05$) but no change in color score was observed. Treatment 1 (containing cheese starter + *L. acidophilus*) obtained the highest score of sensory overall acceptability and was recognized as the superior treatment.

Keywords: Probiotic Soy Cheese, Proteolysis, Sensory Properties.

Introduction

Nowadays, special attention has been paid to functional food products. One of the most important functional foods is probiotic food product. Probiotics are living microorganisms that, by settling in the intestinal environment, can correct the microbial balance in order to increase their usefulness and prevent the activity of non-beneficial microorganisms and pathogens. Probiotics are usually added to foods as a supplement to offer health properties to consumers such as regulation of immune response or use to improve gastrointestinal disease (Zendeboodi *et al.*, 2020; Pourjavid *et al.*, 2023).

Among the types of food carriers, soybean and its products have received attention because these products contain isoflavones, prebiotic oligosaccharides, all

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essential amino acids, minerals and most water- and fat-soluble vitamins, and this causes soybeans and its products are classified as functional foods (Ahsan *et al.*, 2015). Soy milk, which is obtained from soybeans, increases the growth and activity of probiotic bacteria. This increase is attributed to the activity and viability of probiotic bacteria to the presence of prebiotic oligosaccharides in soy milk such as raffinose and stachyose (Otieno *et al*, 2005; Mital *et al*., 2006). Soy cheese (tofu) is one of the best protein products made from soy milk. Tofu is usually obtained by coagulating soy milk and it is an inexpensive source of highly digestible protein and isoflavones (Dey *et al.*, 2017; Yu *et al*., 2022).

Probiotic soy cheese can be made from soy milk fermentation with cheese starter culture and probiotic bacteria (Liu *et al*., 2006; Zielinska *et al*., 2015).

During cheese ripening, various biochemical reactions such as proteolysis, lipolysis, and glycolysis occur, which are the main cause of texture changes as well as the creation of aroma and flavor in cheese. Proteolysis is the most important phenomenon of cheese ripening. Sensory properties of cheese are changed by the factors influencing proteolysis during ripening. The level of proteolysis during producing and ripening and the extent of casein degradation to peptides and AA can change the body, texture, and color of cheese. Therefore, the evaluation of proteolysis and sensory properties in cheese is very important. Some studies have been carried out about proteolysis and sensory properties in probiotic cheese. In this regard, we can refer to the studies of Natghi (2017) on probiotic cheddar cheese, Effat *et al.* (2018) on probiotic low-salt soft cheese, Yerlikaya and Akbulu (2019) on probiotic izmir tulum cheese, Hamdi *et al.* (2020) on probiotic low-salt feta cheese and Ocampo *et al*. (2020) on probiotic panela cheese. So far, no research has been done on proteolysis in soy probiotic cheese. The aim of the present study was to investigate the effect of probiotic culture on proteolysis and sensory properties of soy probiotic cheese during storage.

Materials and Methods

–*Microbial cultures used to produce probiotic soy cheese*

DVS Mesophilic (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*) and thermophilic (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgarigus*) cheese starters were prepared from Chr.Hansen & LactoProte companies**.** Also, probiotic starters (*Lactobacillus acidophilus*, *L. casei* and *Bifidobacterium lactis*) were prepared in the form of DVS, from LactoProte and Takgene companies.

–*Preparation of soy milk*

Soy milk was prepared according to the method of Ting *et al*. (2009). First, 700 g of cleaned soy beans were soaked in distilled water for 12 h at 25°C in a ratio of 3: 1 (w/w) (ratio of water weight to soy weight is 3: 1). After separating the bean shells and washing them, the beans crushed in a blender at a ratio of 6: 1 (w/w) (ratio of water weight to soy weight is 6:1) with purified boiling water for 5 min. The temperature of the water-soybean mixture was 80°C during crushing until the lipoxygenase enzyme was completely inactivated. Then the mixture was filtered well using a filter cloth and 1% lactose powder was added to the resulting leachate for better activity of the starter bacteria. After heating for 20 min at 121°C, soy milk was quickly cooled to room temperature, and before storage in the refrigerator, the amount of dry matter was adjusted to 12% soluble solids (Brix).

–Preparation of probiotic soy cheese

The temperature of sterilized soy milk was raised to 37°C. Calcium chloride (0.1 g per liter of milk) and a mixture of DVS mesophilic and thermophilic cheese starters were added according to the manufacturer's instructions (40 grams per ton of milk). Also, probiotic starters (*L. acidophilus*, *L. casei* and *B. lactis*) were added according to the manufacturer's instructions (10 grams per ton of milk). The control sample (without probiotic bacteria) was also considerd. Incubation was performed at 37°C until the pH reached 4.5. At this time, the curd was cut with a knife at intervals of 3 cm^3 and the temperature of the sliced curd was raised to 55°C and kept at this temperature for 30 min until the soy proteins were well coagulated. The curd was then transferred to a bag and kept in it for 12 h to remove more water from the curd. Then the curd was transferred to the cheese press machine with a filter cloth and a piston-shaped weight weighing about 2 kg was placed on the cheese. After 6 h, the pressing operation was stopped and the curd was removed from the filter cloth and transferred to the mold. The cheese molds were placed in 4% salt water at 4°C for 3 days and then removed from the salt water and kept in closed glass containers at 10°C. The samples were stored for 1 month (Mashayekh *et al*., 2022).

–Probiotic bacteria counting

To prepare dilutions of cheese samples, 5 g of cheese was weighed and homogenized in sterile zippered bags containing 45 ml of sodium citrate 0.2. A series of dilutions was prepared by adding 1 ml of each dilution to 9 ml of 0.1% sterile peptone water. 1 ml of the desired dilution was cultured in MRS bile agar medium by pour plate method and the plates were incubated at 37°C for 72 h (Oliveira *et al*., 2012; Pourjavid *et al*., 2022).

–Sensory evaluation

Organoleptic properties of tofu samples including: taste, odor, color, texture and overall acceptability characteristics were examined by 12 trained panelists. Ranking method was used (1 worst and 9 best).

–Evaluation of proteolysis

Soluble nitrogen at pH = 4.6 and soluble nitrogen in trichloroacetic acid and non-protein nitrogen of cheese were measured (Kuchroo & Fox, 1982; Ocampo *et al*. (2020)). To measure soluble nitrogen, 30 g samples were homogenized in distilled water and the pH of the samples was adjusted to pH = 4.6 using HCL and NAOH 2 N solutions after resetting the pH, the samples were placed in a 40°C incubator for 30 min and then centrifuged (3500 x*g*). Samples were filtered using Whatman 42 filter paper and soluble nitrogen was measured by kjeldahl method. To 20 ml of the filtered solution was added 5 ml of 60% trichloroacetic acid solution and after 10 min centrifuge at 5000 x*g*, the supernatant was filtered and the amount of non-protein nitrogen was measured by kjeldahl method. Kjeldahl method was used to measure total nitrogen.

–Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the results and statistically significant differences between mean values were determined by Duncan's multi-rangeat a 5% probability level. All experiments were performed in triplicate. SPSS 22 software was used to analyze the data.

Results and Discussion

–*Viability of probiotic bacteria in soy probiotic cheese samples during storage*

According to Table 1, the lowest number of probiotic bacteria was related to treatment 2 (containing cheese starter + *L. casei*). During storage, the viability of probiotic bacteria in all the samples were significantly ($p<0.05$) decreased. However, in all samples, the number of probiotic bacteria were more than 7 log CFU/g. Decreasing the content of fermentable sugars and increasing the amount of acids produced by probiotics and other lactic acid bacteria in the environment during the storage period, caused unfavorable conditions for the growth of probiotic strains and these strains began to be metabolically inactivated and more numbers enter the death phase. Tajik Ahmadabadi *et al*. (2019) investigated the effect of different storage temperatures on the quality of UF probiotic cheese. The probiotic species *B. lactis* was used at two levels of 10^8 and 10^9 CFU/g. The results showed that the survival of probiotic bacteria in the samples decreases during storage, which is consistent with the results of the present study. Similarly, Effat *et al*. (2018) reported that the survival of probiotic bacteria in low-salt soft cheese decreased during 28 days of storage at refrigerator temperature.

–*Sensory characteristics of soy probiotic cheese samples during storage*

According to Figure 1A, during storage, the lowest taste score was related to treatment 4 (control sample). Taste scores of treatment 1 from first day to 30th day, there was no significant difference. In treatment 2 (containing cheese starter + *L. casei*), the taste score in the first and 15th days was not significantly different but on the 30th day compared to the first and 15th days of storage, it decreased significantly ($p<0.05$). Taste scores of treatment 3 (containing cheese starter + *B. lactis*) from first day to 30th day, there was no significant difference. Taste scores of treatment 4 (control sample) from first day to 30th day, there was no significant difference. The most important cause of taste are free amino acids and peptides from the proteolysis process (Ghaemi *et al*., 2010). Inspite of increased in proteolysis intensity in this study, due to the increased production of organic acids by microorganisms such as acetic acid and lactic acid, the taste score decreased. According to Figure 1B, on the first and 15th days, the lowest odor score was related to treatment 4 (control sample). There is no significant difference in the sensory evaluation odor score of treatments 1 (containing cheese starter + *L. acidophilus*) and 3 (containing cheese starter + *B. lactis*) from the first day to the

Table 1. Count of probiotic bacteria (log CFU/g) in probiotic soy cheese samples during storage time (mean ± standard deviation)

Samples	First day	15th day	30th day
T1	8.75 ± 0.05^{Aa}	8.14 ± 0.06^{Ba}	7.49 ± 0.06^{Ca}
T2	7.81 ± 0.03^{Ac}	7.33 ± 0.05^{Bb}	7.01 ± 0.06^{Cb}
T3	8.59 ± 0.03^{Ab}	8.10 ± 0.25^{Ba}	7.59 ± 0.04^{Ca}

Dissimilar capital letters indicate a significant difference in the row ($p<0.05$). Dissimilar small letters indicate a significant difference in the column ($p<0.05$).

T1: The sample containing cheese starter + *Lactobacillus acidophilus*, T2: The sample containing cheese starter + *Lactobacillus casei*, T3: The sample containing cheese starter + *Bifidobacterium lactis*

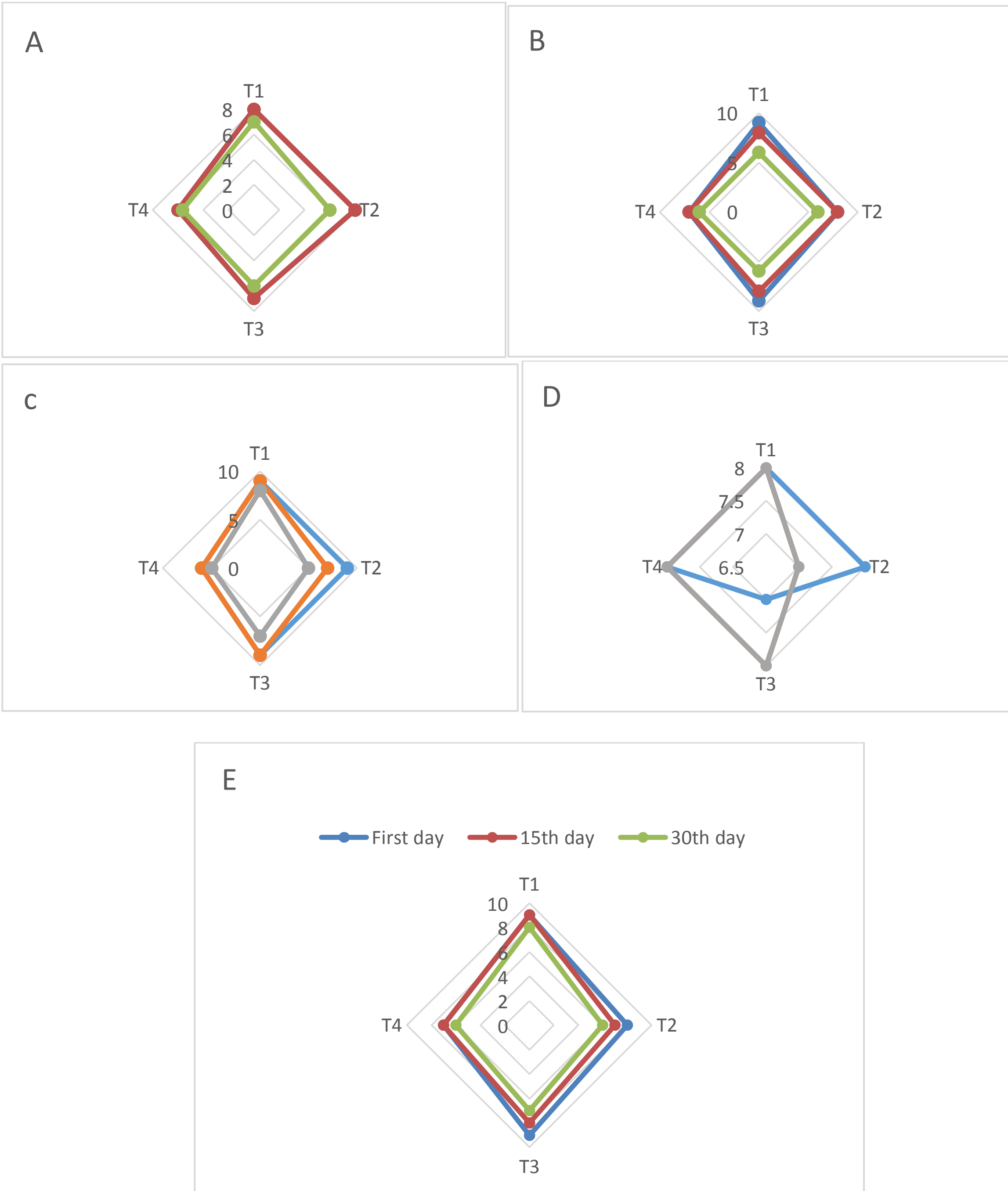

Fig. 1. Sensory features scors of probiotic soy cheese samples during storage. A: Taste; B: odor; C: Texture; D: Color; E: Overall acceptability .
T1: The sample containing cheese starter + *Lactobacillus acidophilus*, T2: The sample containing cheese starter + *Lactobacillus casei*, T3: The sample containing cheese starter + *Bifidobacterium lactis*, T4: Control sample (without probiotic bacteria)

15th day but on the 30th day compared to the first and 15th days of storage, it is significantly reduced ($p<0.05$). There is a significant difference in the sensory evaluation odor score of treatment 2 (containing cheese starter + *L. casei*) from the first day to the 30th day ($p<0.05$) There is no significant difference in the sensory evaluation odore score of treatment 4 (control sample) from the first day to the 30th day. In fact, it can be said that due to the increased production of organic acids by microorganisms such as acetic acid and lactic acid, the odor score decreases. According to Figure 1C, during storage, the lowest texture score was related to treatment 4 (control sample). The texture score of treatment 1 (containing cheese starter + *L. acidophilus*) from first day to 30th day was not significantly different. The texture score of treatment 2 (containing *L. casei*) had a significant decrease from first day to 30th day ($p<0.05$). The texture score of treatment 3 (containing cheese starter + *B. lactis*) was not significantly different in the first and 15th days of storage. On the 30th day there was a significant decrease compared to the mentioned days ($p<0.05$). The texture score of treatment 4 (control sample) from first day to 30th day was not significantly different. The reason for the decrease in texture score is the phenomenon of proteolysis. According to Figure 1D, on the first, 15th and 30th days of storage, there was no significant difference between the color score of the studied samples. Also, no significant difference was observed in the color score of the treatments during storage. According to Figure 1E, on the first day, the lowest overall acceptance score was related to treatment 4 (control sample). On the 15th and 30th days, the lowest texture scores were related to treatment 4 (control sample) and treatment 2. The overall acceptability score of treatment 1 (containing cheese starter + *L. acidophilus*) from the first day to the 30th day was not significantly different. The overall acceptability score of treatments 2 (containing cheese starter + *L. casei*) and 3 (containing cheese starter + (*B. lactis*) from first day to 30th day reduced. The overall acceptability score of treatment 4 (control sample) from the first day to the 30th day was not significantly different. The reason for the decrease in the overall acceptability of the samples is that the score of the studied features such as taste, odor, texture have decreased during thirty days of storage and and thus caused overall acceptability has decreased. Karimi *et al*. (2012) investigated the production of Iranian ultrafiltrated feta cheese containing *L. casei* by relative replacement of NaCl with KCl. Based on the results, during 90 days of ripening time the scores of taste, odour, texture and overall acceptability decreased, which is consistent with the results of the present study. Kazemi *et al*. (2014) evaluated the effect of soy milk addition on sensory properties of probiotic fermented milk. They announced that during 14 days of storage at 5°C, the odor score decreases, that is consistent with the results of the present study. Also they reported that the color score decreased during 14 days of storage at 5°C, which contradicts the results of the present study. Similarly, Hamdy *et al*. (2020) investigated the properties of low-fat feta cheese containing probiotic bacteria. The results showed that the color score did not change during 30 days of storage at 4°C. Yerlikaya and Ozer (2014) in a study of probiotic fresh white cheese stated that during 28 days of storage, the overall acceptabilitys score decreases, which is consistent with the results of the present study.

–*Evaluation of proteolysis in soy probiotic cheese samples during storage*

According to Table 2, on the first day of storage, the highest amount of soluble nitrogen was related to treatment 1 (containing cheese starter + *L. acidophilus*) and the lowest amount of soluble nitrogen was related to treatment 4

Table 2. Proteolysis in probiotic soy cheese samples during storage time (mean± standard deviation)

Parameters	Samples	First day	15th day	30th day
Soluble nitrogen	T1	1.40±0.05^{Ba}	1.43±0.05^{Bb}	1.64±0.04^{Ac}
	T2	0.82±0.04^{Bb}	1.73±0.05^{Aa}	1.72±0.03^{Ab}
	T3	0.75±0.05^{Cb}	1.73±0.03^{Ba}	1.85±0.05^{Aa}
	T4	0.62±0.03^{Bc}	0.99±0.02^{Bc}	1.23±0.02^{Ad}
Non-protein nitrogen	T1	1.21±0.03^{Ba}	1.27±0.05^{Bb}	1.41±0.03^{Ac}
	T2	0.67±0.06^{Bb}	1.68±0.04^{Aa}	1.64±0.01^{Ab}
	T3	0.53±0.03^{Bc}	1.63±0.04^{Aa}	1.71±0.05^{Aa}
	T4	0.47±0.02^{Cc}	0.90±0.03^{Bc}	1.19±0.04^{Ac}
Total nitrogen	T1	3.64±0.04^{Aab}	3.55±0.05^{Aab}	3.40±0.30^{Aa}
	T2	3.53±0.03^{Ac}	3.48±0.02^{Bb}	3.42±0.02^{Ca}
	T3	3.59±0.03^{Ab}	3.50±0.04^{Bb}	3.41±0.02^{Ca}
	T4	3.67±0.02^{Aa}	3.60±0.04^{Ba}	3.53±0.03^{Ca}

Dissimilar capital letters indicate a significant difference in the row (p<0.05). Dissimilar small letters indicate a significant difference in the column (P<0.05).
T1: The sample containing cheese starter + *Lactobacillus acidophilus*, T2: The sample containing cheese starter + *Lactobacillus casei*, T3: The sample containing cheese starter + *Bifidobacterium lactis*, T4: Control sample (without probiotic bacteria)

(control sample). On the 15th day of storage, the highest amounts of soluble nitrogen were related to treatment 2 (containing cheese starter + *L. casei*) and treatment 3. The lowest amount of soluble nitrogen was related to treatment 4 (control sample). On the 30th day of storage, the highest amount of soluble nitrogen was related to treatment 3 (containing cheese starter + *B. lactis*) and the lowest amount of soluble nitrogen was related to treatment 4 (control sample). The amount of soluble nitrogen in all samples increased significantly (p<0.05) during storage. The cause of increased soluble nitrogen is the phenomenon of proteolysis, which is carried out by cheese starters and probiotics. Samy *et al*. (2013) investigated the effect of probiotic bacteria on karish cheese production. These researchers used the species of *L. rhamnosus* and *B. bifidum*, and the results showed that during 28 days of storage, the amount of soluble nitrogen increases, which is consistent with the results of the present study. Effat *et al*. (2018) reported that the amount of soluble nitrogen in probiotic low-salt soft cheese increases during storage, which is consistent with the results of the present study. Moreover, Ocampo *et al*. (2020) reported similar results in probiotic panela cheese.

According to Table 2, during storage, the lowest amount of non-protein nitrogen was related to treatment 4 (control sample). The amount of non-protein nitrogen in the samples increased significantly (p<0.05). The reason for the increase in non-protein nitrogen during storage is due to the proteolytic activity of cheese starter and probiotic bacteria, which lead to the release of more peptides. Dabour *et al*. (2006) investigated the improvement of texture and structure of low- fat cheddar cheese by exopolysaccharide-producing lactococci. The results showed that the amount of non-protein nitrogen increases during storage, which is consistent with the results of the present study. Similarly, Ong *et al*. (2007) evaluated the properties of cheddar cheese produced with *L. acidophilus*, *L. casei*, *L. paracasei* and *Bifidobacterium*. The results showed that the percentage of non-protein nitrogen increases during storage. Moreover, Nateghi (2017) reached similar results in probiotic cheddar cheese. According to Table 2, on the first day of storage, the highest and lowest amounts of total nitrogen, respectively were related to treatments 4 (control sample) and 2 (containing cheese starter + *L. casei*). Total nitrogen values of treatment 1 (containing cheese starter + *L. acidophilus*) did not differ significantly in the studied days. In treatment 2 (containing cheese starter + *L. casei*), total nitrogen levels decreased significantly from first day to 30thday of storage ($p<0.05$). In treatment 3 (containing cheese starter + *B. lactis*) total nitrogen levels decreased significantly from first day to 30thday of storage ($p<0.05$). In treatment 4 (control sample) total nitrogen levels decreased significantly from first day to 30thday of storage ($p<0.05$). Cause of decrease total nitrogen in the samples during the storage period is a proteolysis phenomenon. Shahab Lavasani *et al*. (2011) examined changes in physicochemical and organoleptic properties of Iranian lighvan cheese. The results showed that during 90 days of storage, the amount of total nitrogen decreases, which is consistent with the results of the present study. Similarly, Karimi *et al*. (2012) investigated the production of Iranian ultrafiltrated feta cheese with relative replacement of NaCl with KCl. They reported that during 90 days the ripening time, the amount of total nitrogen decreases. Moreover, Yerlikaya and Akbulu (2019) reported similar results in probiotic izmir tulum cheese.

Conclusion

According to the findings, the survival of probiotic bacteria decreased during storage, but at the end of the storage period in all samples, the microbial count was more than 7 log CFU/g. The amount of soluble nitrogen and non- protein nitrogen in the samples increased during storage. The rate of proteolysis during storage in treatment 3 (containing cheese starter and *B. lactis*) was higher than other treatments. Taste, odor, texture, and overall acceptance scores of stored samples declined ($p<0.05$). However, no change in color score was observed. Treatment 1 (containing cheese starter and *L. acidophilus*) received the highest score for overall sensory acceptance and was deemed superior.

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