The Effect of Different pH, Concentrations of Glutamic Acid and Salt on Non-Protein Nitrogen Compounds, Survival, and Overall Acceptance of Low-Fat Probiotic Cheese

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ABSTRACT: *Today, the demand for low-fat dairy products such as cheese has increased. Reducing fat in cheese reduces the sensory properties of cheese. The use of probiotic bacteria, fat alternatives, and changes in production methods can improve the sensory properties of low-fat cheeses. The aim of this research is to investigate the effect of three independent variables including pH (4.7, 4.9, and 5.1), glutamic acid (1, 2, 3 mg/g), and salt (2, 2.5, and 3%) on the amount of non-protein nitrogen compounds to total nitrogen, survival and overall acceptance of low-fat probiotic cheese during for 30 days' storage. According to the results, the amount of non-protein nitrogen compounds to total nitrogen is increased during the ripening period in all samples, but this increase in the sample containing 2 mg/g glutamic acid, 2% salt, and pH 4.7 is significantly higher than other tested samples. The results are shown that by increasing the amount of glutamic acid and decreasing the amount of salt, the sensory properties and survival of probiotic bacteria are significantly increased. Probiotic bacteria survived in all tested samples for up to 30 days, but this survival is significantly more than in other samples in the treatment containing 3 mg/g glutamic acid, 2% salt, and at pH 4.9. The highest general acceptance score belonged to the treatment containing 3 mg/g glutamic acid, 2% salt, and pH 4.9, which was selected as the best treatment in terms of sensory and nutritional characteristics.*

KEYWORDS*: Low-fat cheese; Non-protein nitrogen compounds; Probiotic survival; General acceptance.*

INTRODUCTION

Cheese is a common name referring to a large group of fermented dairy products produced in a wide range of shapes and flavors around the world [1]. Ultra-filtration (UF) white cheese is one of the most common and famous types of cheese having significant consumption in Iran as the main component of breakfast. Ultra-filtration cheese is produced from milk that has been concentrated by Ultra-filtration method is rich in a variety of proteins, fats, and minerals [2]. Despite the high nutritional value of cheese, consuming high-fat cheese increases cholesterol and cardiovascular disease [3]. The reduction of fat in the cheese causes a firm and rubbery texture, and an unpleasant taste in the cheese [4]. Due to the reduction of fat in cheese, the number of protein increases and

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The protein matrix of the cheese becomes much denser and the texture of the cheese becomes chewable [5]. In order to improve the undesirable properties of low-fat cheeses, the use of adjunct starter cultures such as probiotic bacteria, fat alternatives, and changes in production methods are recommended [6]. Carbohydrate-based fat substitutes cause a soft, creamy texture in low-fat cheese by trapping water mechanically in the cheese texture [7].

Probiotic bacteria are living sources that can improve the microbial balance of the digestive system. Probiotic bacteria in cheese soften and improve the taste of low-fat cheese by increasing the proteolytic activity of the cheese [8]. *Lactic acid* bacteria are the most common probiotic bacteria used in low-fat cheeses [6]. *Nateghi et al.* (2012) optimized the textural properties and formulation of reduced-fat Cheddar cheeses and reported that the use of probiotic bacteria improves the texture of low-fat cheddar cheese [9]. Dairy products, especially cheeses, are good carriers for the transmission of probiotic bacteria t o the human body. The reasons for the high viability of probiotics in cheese compared to fermented milk products are their relatively high pH, solid lattice and compact structure, and high tampon capacity due to its high protein content [10].

But factors such as type of probiotic strain, amount of salt, pH, type of packaging (presence or absence of oxygen), ripening time and storage conditions can affect the survival of probiotic bacteria in cheese [11].

Glutamic acid is an amino acid that is widely existed in free or linked forms in proteins and grocery. Using foods containing high glutamic acid (including tomatoes, mushrooms, and cheese) to get a pleasant taste of food is common. On the other hand, glutamic acid as a prebiotic compound can increase the survival of probiotic bacteria and the precursor of bioactive compounds such as gamma-aminobutyric acid (GABA) in probiotic dairy products [13, 12]. Therefore, *Achachlouei et al*. (2013) investigated the production of low-fat functional cheese by replacing walnut and flaxseed powders instead with milk fat. They reported that white low-fat cheese could be produced by combining walnut or flaxseed powder with better nutritional properties [14].

In another research, *Badri* and *Alizadeh* (2016), investigated the growth and survival of Lactobacillus acidophilus in low-fat mixed yogurt containing Beta-Glucan and reported that Beta-Glucan can be used as a fat substitute in the production of probiotic low-fat yogurt at the level of 0.5% to produce a functional synbiotic food and to provide a new choice for dairy consumers [15].

Since white cheese is prepared by ultra-filtration method is the main industrial cheese in the country in terms of production and is a widely consumed and popular product, the aim of the present study is to the effect of pH, different concentrations of glutamic acid, and salt on non-protein nitrogen compounds, survival, and overall acceptance of low-fat probiotic cheese.

EXPERIMENTAL SECTION

Raw materials

Low-fat milk concentrate, milk protein concentrate, Skim milk, butter, and Ultra-filtrated concentrate were supplied by Pegah Company (Iran), the lactic starter was supplied from Bioprax company (France), enzyme rennet was supplied by CSK Food Enrichment supplies (Netherlands), salt was supplied from Golbahar company (Iran), *Lactobacillus brevis* microorganism was supplied from the Iranian Biological Resource Center (Iran), the Culture medium of MRS-bile agar from Merck Company (Germany), and other used chemicals including 12% trichloroacetic acid, and Trisodium citrate were supplied from Merck company (Germany).

Probiotic low-fat cheese production method

The optimal formulation of *Sharafi et al*. (2019) was used to produce low-fat ultra-filtration cheese with desirable texture properties. Initially, 70% ultra-filtered concentrate of skim milk, 12% milk protein concentrate, and 2% fat milk powder were mixed well with water in a pasteurizer at 50 °C. Then butter (82% fat) was used to adjust the final fat content in low-fat ultra-filtered cheese (9%). All ingredients were then mixed at 60 °C until the complete melt of butter. Next, in order to improve the textural properties of ultra-filtered cheese, 0.1% novel and 0.46% galactomannan were added to the mixture. After that, all the materials were homogenized by homogenizer model Ultra-Turrax, IKA10, Germany T-Basic at 75 °C with 90 bar pressure and then were pasteurized in a pasteurizer (VB-1820J, Faraz Electronic, Iran) at 78 °C for 5 minutes. After pasteurization, milk temperature decreased to 34 °C and lactic starter was added to the milk amount to 0.03% w/w along with

Lactobacillus brevis amount to 10^8 cfum/L and after 30 minutes the rennet enzyme was added to the mixture amounting to 0.05% w/w and stirred well and filled in 100 g molds. After the formation of curd according to the table of treatments, salt amount of 2-3% w/w and glutamate amount to (1-3 mg/g) were sprayed on them. Then, the final pH of the cheese curds was adjusted by sodium hydroxide according to the table of treatments (4.7-5.1) before packing, and then thermal sealing was performed with a layer coated with polypropylene. The samples were stored in an incubator at 8 °C for 30 days [7].

Tests

Non-protein nitrogen (NPN) compounds soluble in 12% Trichloroacetic acid to total nitrogen (TN)

Non-protein nitrogen (NPN) compounds soluble in 12% trichloroacetic acid were evaluated to total nitrogen in three periods (20, 10, and 30 days). 5 g of each cheese sample was grated and mixed well in trichloroacetic acid (12%) solution and in order to sediment the protein compounds, a high-speed refrigerated centrifuge (Sartorius 18.3 K, Sigma, Germany) was used at 3000 g for 30 minutes at 4 °C [6]. The supernatant solution was filtered and its non-protein nitrogen compounds were measured by the Kjeldahl method (AOAC, 1997). Kjeldahl method (AOAC, 1997) was used to measure total nitrogen (TN) [16].

Studying the survival of probiotic microorganisms during storage

10 g of the probiotic cheese sample was transferred to 90 mL of sterile ringer solution under sterile conditions and dilution operation was continued to reach the desired dilution. Then 1 mL of diluted samples was incubated on MRS-bile agar medium for 72 hours at 37 °C by pour plate method [17, 18]. The survival of the probiotic microorganism in the cheese was examined during 10th, 20th, and 30th days.

Sensory evaluation

Cheese samples were randomly evaluated by 10 trained panelists. Cheeses were evaluated from the view of texture, color, taste, and general acceptance based on taste tests by the five-point hedonic method (1 = most undesirable, 5 = most desirable). To each panelist, 15 samples were given in separate containers, with a scoring form and a glass of water [19].

Data analysis method

In order to analyze the results of sensory properties measurement, non-protein nitrogen to total protein (NPN/TN) measurement and studying the survival of all probiotic microorganisms was performed by the analysis of one-way variance Duncan at 95% confidence level.

RESULTS AND DISCUSSION

Studying the NPN/TN changes of probiotic ultra-filtered cheese during storage

The test for measuring the percentage of NPN/TN is a useful technique to evaluate medium to small peptides and amino acids and compounds smaller than ammonium, urea, and amino compounds [6].

The results of NPN/TN amount of probiotic ultra-filtered cheeses containing different concentrations of salt and glutamate at different pHs were shown in Table 1. So on the 10^{th} day, the highest amount of NPN/TN (6.341) belonged to T_4 treatment and the lowest amount of NPN/TN (5.135) was observed in T_9 treatment. At the end of the ripening period and on the 30^{th} day, the highest amount of NPN/TN (11.823) belonged to T_4 treatment, and the lowest percentage of NPN/TN (9.154) belonged o T9 treatment. In fact, by increasing the time and amount of glutamate, the percentage of NPN/TN had an increasing trend, which could be related to the increase in proteolysis properties in the presence of glutamate during the ripening period of cheese. Given that, *Lactobacillus brevis* used in this study had a high proteolytic activity therefore its use in ultra-filtered cheese released more peptides and consequently increased the amount of NPN during storage [6].

According to the results of the present study, the percentage of non-protein nitrogen to total protein in samples containing probiotic starter culture had increased more than in the control sample during the whole ripening time, which might be due to their higher proteolytic activity compared to the control sample. This could be the cause of the breakdown of casein at various junctions to produce peptides and macro peptides. Since the use of rennet and additional starter culture contribute to the formation of soluble peptides and macro peptides, thus, the added culture peptidase and proteinase enzymes were able to hydrolyze effectively and consequently create medium and small peptides [19]. In addition, these soluble

non-protein nitrogen compounds could directly contribute to the taste of cheese [6, 20]. *Dabour et al*. (2006) showed that the amount of non-protein nitrogen to total protein in cheddar cheese samples containing exopolysaccharide had increased significantly during ripening [21].

In another research, Nateghi (2012) reported that the amount of NPN of probiotic cheddar cheese, which was inoculated separately with *Lactobacillus casei* and *Lactobacillus helveticus*, was increased in all samples during 60 days of ripping, but this increase in samples inoculated with *Lactobacillus helveticus* was significantly higher than other tested samples. They reported the effect of different starter cultures on the amount of proteolysis of low-fat cheddar cheese during the storage period. They reported that the concentration of NPN, free and total amino acids were significantly affected by the type of microorganism and storage time. The concentration of total and free amino acids and NPN in low-fat cheeses cultured with additional starter cultures were higher than in control cheese with full fat and without additional starter cultures [6].

It should be noted that increasing pH and more salt in cheese formulation, the activity of probiotic bacteria and starter cultures in cheese was decreased, so the amount of proteolysis also decreased, which led to a decrease in NPN/TN [22]. In addition, *Gobbetti et al*. (1998) confirmed the results of the present study and stated that when the salt level in cheese is more than 4 $g100g^{-1}$, the stability of probiotic strains is significantly reduced. *Karimi et al.* (2012) in their study of the composition of *Lactobacillus casei* in the production of ultra-filtrated feta cheese in which its salt was replaced by KCl stated that by increasing the amount of sodium in the formulation, the growth rate of probiotics was reduced that could be directly related to the reduction of proteolysis and NPN [23].

Studying the probiotic microorganism survival during storage

The most important factor in the use of probiotic bacteria in cheese is their survival during processing and consumption without undesirable effects on the sensory properties of the product [24]. Table 2 shows the survival rate of probiotic microorganisms during 30 days of storage of low-fat probiotic cheese samples. As observed, *Lactobacillus brevis* survival of low-fat ultra-filtered probiotic cheeses containing different concentrations of salt, and glutamate at different pHs during 30 days of storage was decreased, but this reduction between the first to 30th days of storage was significant only in treatments T_7, T_8, T_{10}, T_{12}, T_{14} and T_{15}. There was an inverse relationship between pH and survival. By decreasing pH, the survival process increased. In addition, by increasing the salt percentage, the survival process decreased and by increasing glutamate content, survival increased. On the 10th day, the highest survival (7.95 Log/cfug) belonged to T_7 treatment and the lowest survival (7.35 Log/cfug) belonged to T_9 treatment. On the other hand, on the 30th day, the highest survival (7.28 Log/cfug) belonged to T_4 treatment, and the lowest survival (6.45 Log/cfug) was observed in T_9 and T_2 treatments, in which statistically there was no significant difference. In fact, the growth of probiotic bacteria and increase in acidity, reduction of nutrients and oxygen, competitor microorganisms, bacteriocin compounds, antibiotics, and fermentation conditions are the most important reasons for reducing the survival of probiotic bacteria [25, 26]. According to the results, by increasing the amount of salt in cheeses, the survival of probiotic bacteria showed a reduction trend, which could be due to the intolerance of lactobacillus to the salt consumed in cheese. In this regard, *Zarei et al*. (2018) reported in research that by increasing the pH from 4 to 5 and increasing glutamate from 25 to 250 mg/g the survival process increased. This can be due to the optimal growth and survival conditions of lactic acid bacteria such as *Lactobacillus plantarum* [27].

In general, to maximize the benefits of probiotics, the number of probiotic bacteria in a dairy product should be at least between 10^6 and 10^7 cf/ug at the time of consumption, and this dairy product should daily be consumed 100 grams [25]. According to the results of the survival test during the ripping period given in Table 2, it was observed that on the 30th day of storage there were at least 10^7 cfu/g probiotic bacteria in all tested samples of low-fat ultra-filtered cheese, that this amount was sufficient for the health effects of probiotic products consumption due to the solid network in the cheese that can be effective in preserving probiotic bacteria during the ripening period [28]. *Nomura et al.* (1998) investigated the production of gamma-aminobutyric acid by *Lactobacillus bacteria* during cheese ripening. The results showed that the survival of probiotic bacteria after two weeks of storage was 1.5×10^9 at pH 4.84, 1.91×10^9 at pH 5.21

Table 1: Results of NPN/TN of probiotic UF Iranian white cheese at different pH, glutamate acid, and salt during storage.

Treatment	glutamate acid (mg/g)	Salt (%)	pH	Day 10	Day 20	Day 30
T_1	2	2	5.1	6.137±0.212 ^{abcC}	7.931±0.155 ^{abcdB}	11.132±0.311 ^{abcA}
T_2	1	3.0	4.9	5.217±0.254 ^{bcC}	6.519±0.155 ^{gB}	9.238±0.226 ^{gA}
T_3	3	2.5	4.7	5.941±0.141 ^{abcC}	7.752±0.212 ^{abcdB}	10.722±0.184 ^{bcdA}
T_4	3	2.0	4.9	6.341±0.141 ^{aC}	8.511±0.311^{aB}	11.823±0.240 ^{aA}
T_5	2	2.5	4.9	5.819±0.198 ^{bcaC}	7.532±0.141 ^{bcdefB}	10.441±0.141 ^{cdA}
T_6	1	2.5	5.1	5.711±0.339 ^{abcC}	7.131±0.183 ^{defgB}	^{defA} 10.192±0.240
T_7	2	2.0	4.7	6.218±0.325 ^{abC}	8.341±0.282 ^{abB}	11.618±0.198 ^{aA}
T_8	3	3.0	4.9	5.364±0.297 ^{abcC}	6.701±0.169 ^{fgB}	9.341±0.184 ^{defA}
T_9	2	3.0	5.1	5.135±0.268 ^{cC}	6.341±0.254 ^{gB}	9.154±0.156 ^{gA}
T_{10}	2	2.5	4.9	5.825±0.240 ^{abcC}	7.539±0.367 ^{bcdefB}	10.448±0.127 ^{cdA}
T_{11}	2	3.0	4.7	5.519±0.381 ^{abcC}	6.814±0.297 ^{efgB}	9.519±0.170 ^{efgA}
T_{12}	1	2.0	4.9	6.172±0.169 ^{abcC}	8.163±0.183 ^{abcB}	11.341±0.255 ^{abA}
T_{13}	3	2.5	5.1	5.741±0.155 ^{abcC}	7.254±0.226 ^{cdefgB}	10.276±0.269 ^{deA}
T_{14}	1	2.5	4.7	5.917±0.183 ^{abcC}	7.661±0.198 ^{abcdeB}	10.611±0.240 ^{bcdA}
T_{15}	2	2.5	4.9	5.831±0.226 ^{abcC}	7.535±0.183 ^{bcdefB}	10.445±0.212 ^{cdA}

Different small letters showed significant differences in each column (P≤0.05).
Different capital letters showed significant differences in each row (P≤0.05).

and 1.37×10^9 at pH 5.34 showed an increase in the survival of probiotics by decreasing the pH from 5.34 to 4.84, which was similar to the results of the present study [29]. *Yerlikaya* and *Ozer*, (2014) examined the survival of probiotic bacteria in white probiotic cheese using a combination of *Streptococcus thermophilus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, and *Lactobacillus casei*. They reported that the growth and survival of probiotic bacteria were maintained during 28 days of storage in cheeses and were higher than 7 Log/cf.ug, which is the required number for the probiotic product [30].

Nateghi, (2012) studied the survival of probiotic bacteria (*Lactobacillus casei* and *Lactobacillus helveticus* separately and together) in cheddar cheese for 60 days and reported that samples inoculated with both probiotic bacteria had higher survival compared to other samples that were the result of the synergistic effect of Lactobacillus *helveticus* and *Lactobacillus casei*. They stated that the culture medium of probiotic bacteria and its type can affect the survival of probiotic bacteria [6].

Studying the general acceptance of probiotic ultra-filtered cheese during the storage period

One of the main factors in accepting many products and gaining satisfaction from their consumption is the sensory properties of these products and it is necessary to study and recognize the factors affecting them to achieve the desired sensory properties and also to reduce or prevent undesirable sensory properties [31]. In fact, the general acceptance expresses the general feeling of the judges towards the sample in question. The overall acceptance rate of probiotic low-fat cheese samples is given in Table 3. Based on the results obtained on the 10th day, the lowest total acceptance score of cheese (3.765) belonged to T_9 treatment and the highest total acceptance score of cheese (4.66) belonged to T_4 treatment. On the 30th day, the lowest total acceptance score of cheese (4.115) belonged to T_2 treatment and the highest total acceptance score of cheese (4.86) belonged to T_4 treatment. The amount of salt, glutamate, pH, and shelf life had a significant effect ($p \leq 0.05$) on changes in the overall acceptance of low-fat ultra-filtered probiotic cheese.

Table 2: Results of probiotic survival (Log/cfug) of probiotic UF Iranian white cheese at different pH, glutamate acid and salt during storage.

Treatment	glutamate acid (mg/g)	Salt (%)	pH	Day 10	Day 20	Day 30
T_1	2	2	5.1	7.860±0.127 ^{aA}	7.420±0.212 ^{aA}	7.070±0.240 ^{aA}
T_2	1	3.0	4.9	7.420±0.268 ^{aA}	6.930±0.339 ^{aA}	6.450±0.226 ^{aA}
T_3	3	2.5	4.7	7.850±0.084 ^{aA}	7.550±0.155 ^{aA}	7.100±0.268 ^{aA}
T_4	3	2.0	4.9	7.920±0.028 ^{aA}	7.590±0.212 ^{aA}	7.280±0.127 ^{aA}
T_5	2	2.5	4.9	7.820±0.169 ^{aA}	7.410±0.297 ^{aA}	7.050±0.128 ^{aA}
T_6	1	2.5	5.1	7.630±0.254 ^{aA}	7.110±0.113 ^{aA}	6.620±0.198 ^{aA}
T_7	2	2.0	4.7	7.950±0.024 ^{aA}	7.540±0.183 ^{aAB}	7.160±0.155 ^{aB}
T_8	3	3.0	4.9	7.510±0.226 ^{aA}	7.120±0.127 ^{aAB}	6.640±0.169 ^{aB}
T_9	2	3.0	5.1	7.350±0.155 ^{aA}	6.870±0.084 ^{aAB}	6.450±0.240 ^{aB}
T_{10}	2	2.5	4.9	7.800±0.113 ^{aA}	7.410±0.183 ^{aAB}	6.920±0.254 ^{aA}
T_{11}	2	3.0	4.7	7.560±0.198 ^{aA}	7.150±0.311 ^{aA}	6.670±0.396 ^{aB}
T_{12}	1	2.0	4.9	7.880±0.170 ^{aA}	7.350±0.268 ^{aAB}	6.930±0.113 ^{aB}
T_{13}	3	2.5	5.1	7.750±0.212 ^{aA}	7.210±0.113 ^{aAB}	6.730±0.169 ^{aB}
T_{14}	1	2.5	4.7	7.800±0.099 ^{aA}	7.360±0.240 ^{aAB}	6.870±0.183 ^{aB}
T_{15}	2	2.5	4.9	7.800±0.141 ^{aA}	7.430±0.226 ^{aAB}	6.910±0.084 ^{aB}

Different small letters showed significant differences in each column (P≤0.05).
Different capital letters showed significant differences in each row (P≤0.05).

On the other hand, it was found that the general acceptance rate in probiotic ultra-filtered cheeses containing different concentrations of salt and glutamate at different pHs during 30 days of storage increased with increasing time so by decreasing pH, the overall acceptance increased. In fact, due to proteolytic activity, most samples contain probiotic microorganisms, which can have a significant effect on the better development of taste, flavor, and softer texture, and as a result, the general acceptance of the cheese.

In addition, by increasing salt percentage, overall acceptance decreased and by increasing glutamate content, overall acceptance increased. The absorption of salt in cheese occurs during the production phase and ripening period and usually continues after the lactose fermentation phase is completed. The percentage of salt directly and through the effect on the activity of microflora and existing enzymes influence various properties of cheese, including taste, aroma, texture and biochemical properties, and shelf life of cheese [32]. *Nateghi* (2012) used adjunct starter cultures of *Lactobacillus helveticus*, *Streptococcus thermophilus* and *Lactobacillus casei* to improve the aroma and flavor of low-fat cheddar cheese. She reported that the concentrations of glutamine, methionine, and leucine as major pioneers and participants in enhancing the flavor and flavor composition of cheeses were higher in low-fat cheddar cheese inoculated with *Lactobacillus helveticus* than in low-fat cheddar cheeses inoculated with *Lactobacillus casei* and *Streptococcus thermophilus* and even higher than the control sample of high-fat cheddar cheese [6]. In another research, *Basyigit et al* (2009) produced Turkey Beyaz white cheese containing *Lactobacillus* Plantarum and *Lactobacillus* fermentum probiotic bacteria. They reported that the use of mentioned microorganisms besides the common starter cultures in cheese improves its sensory properties of texture [33].

CONCLUSIONS

In this research, low-fat ultra-filtered cheese was produced using probiotic bacteria under different pH conditions and different amounts of glutamic acid and salt.

Table 3: Results of General acceptance of probiotic UF Iranian white cheese at different pH, glutamate acid and salt during storage

Treatment	glutamate acid (mg/g)	Salt (%)	pH	Day 10	Day 30
T_1	2	2	5.1	4.105±0.120 ^{abcA}	4.340±0.084 ^{abcdA}
T_2	1	3.0	4.9	3.845±0.190 ^{bcA}	4.115±0.077 ^{dA}
T_3	3	2.5	4.7	4.230±0.169 ^{abcA}	4.605±0.0134 ^{abcdA}
T_4	3	2.0	4.9	4.660±0.113 ^{aA}	4.860±0.084 ^{aA}
T_5	2	2.5	4.9	4.190±0.127 ^{abcA}	4.465±0.162 ^{abcdA}
T_6	1	2.5	5.1	3.915±0.134 ^{bcA}	4.270±0.099^{cdA}
T_7	2	2.0	4.7	4.250±0.183 ^{abcA}	4.800±0.127^{abA}
T_8	3	3.0	4.9	4.165±0.148 ^{abcA}	4.405±0.134 ^{abcdA}
T_9	2	3.0	5.1	3.765±0.219^{cA}	4.125±0.148 ^{dA}
T_{10}	2	2.5	4.9	4.175±0.106 ^{abcA}	4.485±0.134 ^{abcdA}
T_{11}	2	3.0	4.7	4.110±0.056 ^{abcA}	4.515±0.162 ^{abcdA}
T_{12}	1	2.0	4.9	4.350±0.099 ^{abA}	4.665±0.120 ^{abcA}
T_{13}	3	2.5	5.1	4.290±0.127 ^{abcA}	4.615±0.134 ^{abcdA}
T_{14}	1	2.5	4.7	3.990±0.127 ^{bcA}	4.295±0.120 ^{bcdA}
T_{15}	2	2.5	4.9	4.195±0.106 ^{abcA}	4.460±0.198 ^{abcdA}

Different small letters showed significant differences in each column (P≤0.05).
Different capital letters showed significant differences in each row (P≤0.05).

The results of this research showed that the percentage of non-protein nitrogen to total protein in samples containing prebiotic starter cultures increased more than in the control sample. Evaluation of ultra-filtered low-fat cheese as a probiotic carrier revealed that although in all samples the number of beneficial bacteria decreased by increasing storage time, However, this product could maintain a good number of *Lactobacillus brevis* bacteria until the end of the 30-day of storage period. pH conditions equal to 4.9, glutamic acid equal to 3 mg/g, and salt equal to 2% for *Lactobacillus brevis* bacteria provided better conditions for the survival of this bacteria. The results of the sensory test also proved that the activity of probiotic bacteria in cheese leads to an increase in the overall acceptance of cheese during storage. Therefore, using the results of this research, the defects related to fat reduction in low-fat cheese production can be improved and produced healthy low calories products.

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