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Study on the effect of Persian gum and transglutaminase enzyme on the sensory, color, and microbial characteristics of semi-fat ultrafiltrated white cheese during cold storage

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ABSTRACT

The present study was conducted to investigating the effect of Persian gum (PG) and microbial transglutaminase (MTGase) on sensorial, color and microbial characteristics of ultrafiltrated semi-fat white cheese during 60 days of cold storage. In order to produce semi-fat cheeses, PG was used at three levels of 0, 0.25, and 0.5% and MTGase enzyme at three levels of 0, 0.5 and 1 unit/g of protein. The results revealed that the treatment of cheese samples with PG and MTGase enzyme had a positive effect on the sensory and quality characteristics of the product. In general, the cheese sample containing 0.5% PG and 0.5 units of MTGase enzyme attained the highest sensorial scores. Based on panelists' preference, during the storage time, aroma and texture scores increased while color and appearance attributes decreased. The results obtained from the analysis of color values revealed that the lightness (L∗) of cheeses increased with the addition of PG and MTGase enzyme treatment and decreased with the passage of storage time. Unlike the lightness, PG and MTGase enzyme had no significant effect on a∗ (red-green) and b∗ (yellow-blue) values of the experimented cheese samples. The results obtained from the microbial evaluation showed that the addition of PG increased the viability of lactic acid bacteria (LAB), but it had no effect on the count of mold and yeasts. On the other hand, increasing the concentration of the enzyme decreased the growth and survival of the studied microorganisms. The results of this study showed that PG can be used as a fat substitute along with MTGase enzyme to produce ultrafiltrated low-fat white cheese with favorable technological and sensory characteristics comparable to high-fat cheese varieties, and the best sample of ultrafiltrated semi-fat cheese is obtained using a treatment containing 0.5% PG and 0.5 unit of MTGase enzyme.

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1- Introduction

For the first time, about 8000 years ago, during the agricultural revolution, cheese was produced in areas of Iraq, between the Euphrates and Tigris rivers, known as the Fertile Crescent. In general, cheese is a fermented dairy product rich in nutrients and digestible, which is widely consumed all over the world. However, the effects of cheese consumption on human health is still a controversial issue. On the one hand, cheese as a rich source of high quality proteins (mainly casein), lipids, minerals (such as calcium, phosphorus and magnesium), vitamins (such as vitamin A, K2, B2, B12 and folate), probiotics and bioactive molecules (such as bioactive peptides, lactoferrin and short chain fatty acids). Therefore, its consumption has countless health benefits. On the other hand, cheese contains relatively high amounts of saturated fat and salt, which are considered as undesirable food compounds for the health of the heart and blood vessels [1]. Currently, most dietary guidelines recommend the consumption of dairy products as part of a healthy diet. At the same time, you should avoid consuming a variety of high-fat and high-sodium foods. In any case, whole milk products are not a set of nutrients that can be separated, but have complex physical and nutritional structures. For example, the dairy matrix affects the digestibility and bioavailability of nutrients; As a result, consumption of dairy products can affect human health and disease [2].

On the other hand, fat plays an important role in creating desirable mechanical, sensory and color characteristics of some dairy products, including cheese [3]. In fact, fat acts as a softening factor in the casein matrix and reduces the mechanical strength and softening of the cheese texture [4]. In addition, free fatty acids produced by lipolysis are among the most important flavor compounds of cheese [5]. Therefore, the use of fat substitutes can be a promising solution to improve the structural and mechanical characteristics of low-fat food products [6]. In fact, fat substitutes are a group of food components that can mimic the physical, rheological and sensory characteristics of fat in different food products, but have fewer calories compared to fats [7].

Gums are one of the most important and widely used fat substitutes in a variety of food products, which are also widely used in the dairy industry [7-10]. Persian gum (PG) is an anionic gum extracted from the trunk and branches of the wild almond tree (scientific name: *Amygdalus scoparia*). This hydrocolloid is also known by the names of Zado gum and Shirazi gum. Structurally, Persian gum is a highly branched polysaccharide consisting of galactose and arabinose as the main monosaccharides along with smaller amounts of rhamnose, mannose and xylose [11]. Today, due to the characteristics of this gum and its ability to create consistency, stabilization and emulsification, its use in the food industry has been considered [6]. In a study carried out in this regard, the effect of different concentrations of Persian gum on the syneresis, textural and rheological characteristics of ultrafiltrated (UF) white brined cheese during the ripening period was studied. Based on the obtained results, the use of Persian gum led to a 26-44% reduction in the syneresis of samples containing gum compared to the control sample at the end of the cycle. With the increase in gum concentration, a significant decrease was observed in the amount of syneresis, hardness, adhesiveness, elastic modulus and viscous modulus of cheeses; meanwhile, the springiness of cheeses increased significantly. The samples

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containing gum had the highest hardness, elastic modulus and viscous modulus on the 45th day of ripening, but the cohesiveness and springiness showed a decreasing trend during ripening [12].

In addition, various enzymes are used in the food industry to improve the technological characteristics, increase the nutritional value and the production efficiency of various products. Transglutaminase enzyme (TG, protein-glutamine-ε-glutamyltransferase, EC 2.3.2.13) is one of the most widely used enzymes in the cheese industry. For the first time, this enzyme was isolated from guinea pig liver in 1957. After that, transglutaminase was described in other living organisms such as yeasts, plants, marine organisms, invertebrates, amphibians and birds. Nowadays, microorganisms are the main source of transglutaminase enzyme production. In general, transglutaminases are a family of transferase enzymes that cross-link between γ-carboxyamide groups of glutamine residues and ε-amino groups of lysine residues in polychains. They catalyze the peptide and form ε-(¬γ-glutamyl¬) lysine (G-L) bonds. By forming a protein network structure by creating G-L bonds, it is possible to increase the viscosity of protein solutions or make them gel [13]. Also, one of the efficient strategies in the field of increasing the ratio of moisture to protein in low-fat cheeses is the treatment of milk used in cheese making with transglutaminase enzyme [3]. It has been shown that compared to the cheeses of the control group, the samples treated with microbial transglutaminase enzyme (MTGase) have higher moisture content, production yield and hardness [3, 7]. In addition, the addition of MTGase improves the mouthfeel, textural characteristics and overall acceptability of cheese samples [3, 7]. For example, transglutaminase enzyme treatment with 80 enzyme units (per gram of protein) and a duration of 20 minutes resulted in achieving the highest amount of solids and total protein content in soft cheese [14]. In another similar study, the effect of adding different concentrations of MTGase and different cooking times on the characteristics of analog mozzarella cheese was investigated. The findings of this research showed that the feature of cross-linking by the enzyme has significantly improved the color, texture, microstructure and meltability of cheese at a protein concentration of 2.5 U/g and a cooking time of 20 min. MTGase concentration, cooking time and the combination of these factors significantly affected the color indices (L*, a* and b*) and texture characteristics (hardness, adhesiveness, springiness, gumminess, etc.) , but had no significant effect on pH values [15]. According to the mentioned subjects, the present research was conducted with the aim of investigating the effect of Persian gum and transglutaminase enzyme on some sensory and microbial characteristics of ultrafiltrated low-fat white cheese.

2- Materials and methods

2-1- Raw materials

Ultrafiltrated cheese samples were produced using retentate production at Pegah factory in Khuzestan, located at the 3rd kilometer of Shush-Dezful road. All experiments related to sensory and microbial evaluation were performed in the laboratory of the Department of Food Science and Technology, Agricultural Sciences and Natural Resources University of Khuzestan. Milk protein concentrate powder (containing maximum 5% moisture, minimum 70% protein, 16.5% lactose, 8% ash, and 1.3% fat) was obtained

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from Pegah Khorasan. Farsi gum (PG) was obtained from a local shop in Mollasani and after cleaning and washing, the powder Gum was prepared. Rennilase culture was purchased from Christian Hansen (Copenhagen, Denmark). Microbial transglutaminase (MTGase) enzyme powder with an average activity of 100 units per g of protein was obtained from BDF Natural Ingredients (Girona, Spain). YGC agar and MRS agar culture media were purchased from Merck (Darmstadt, Germany).

2-2- Production of low-fat cheese samples using ultrafiltration method

All samples of ultrafiltrated cheese were produced in Pegah Dairy Factory of Khuzestan according to the method of Nosrati et al. [16]. High quality fresh cow's milk was used to produce cheese. After the milk was received by the factory, its temperature was reduced to about 5 °C by a plate heat exchanger and then it was stored in steel tanks until the process was carried out. The milk was directed from the storage tanks to the pasteurization line and the temperature of the milk increased to 50 °C. After transferring the milk to the creamer and adjusting its fat to 3%, the milk was passed through two bactofugation devices to reduce its microbial load to more than 99%. In the next stage, the milk was pasteurized at a temperature of 72°C for 15 seconds, and after cooling it with a plate heat exchanger to a temperature of about 5°C, the pasteurized milk was stored in tanks. In order to produce ultrafiltrated cheese, pasteurized milk was sent to the cheese production line and its temperature was increased to 50 °C by a heat exchanger. Then, to concentrate the milk, it was transferred to the ultrafiltration system. After passing through the ultrafiltration system, the amount of milk dry matter increased to about 32%. The retentate compounds prepared were: 2.7% lactose, 1.4% ash, 15.35% fat and 12.38% protein.

In the next stage, to prepare semi-fat cheese samples, a solution containing milk concentrate powder (MPC) with dry matter similar to retentate (32%) was added to retentate in an equal amount (V/V) until the amount of fat was halved decrease. After adding Persian gum powder at three levels of 0, 0.25 and 0.5% (W/V), the mixture was homogenized using a homogenizer (Ronghemamachinery, JHG-Q60-P60, China) and heated at 75 °C for 15 seconds. Transglutaminase enzyme (MTGase) was also added at the levels of 0, 0.5 and 1 unit (per gram of undigested protein). Then, retentate was filled in 100 cc containers with cheese and 3% of the starter culture mixture and cheese rennet was added to the prepared retentate mixture. Next, the containers were entered into the coagulant tunnel with a temperature of about 30 °C, and after leaving the tunnel, fat-resistant paper was placed on the surface of the cheese samples and 2% (w/w) salt was added to it. After closing the cheese containers with aluminum foil, at the end, the cheese containers were transferred to a warm room for incubation at temperature of 35-37 °C, so that after 18-24 hours the pH dropped to about 4.8. Finally, the samples were transferred to the cold room with a temperature of 5 °C and sensory, colorimetric and microbial tests were performed on the cheese.

2-3- Sensory evaluation

Sensory characteristics of cheese samples such as color and appearance, aroma and texture were evaluated by 10 trained panelists. Before performing the test, the samples were kept at room temperature for 30 minutes so that during the evaluation,

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the temperature of all the samples would be the same and the sensory evaluation results would not be affected [6].

2-4- Color evaluation

In order to measure the color of cheese samples, Hunetrlab colorimeter (CR-400, Minolta, Japan) was used. The investigated color indices included L* (lightness), a* (red-green) and b* (yellow-blue) [6].

2-5- Microbial tests

2-5-1- Counting lactic acid bacteria (LAB)

In order to dilute the cheese samples, 1 gram of each treatment was added to the first tube containing 9 ml of peptone water and successive dilutions of the sample were prepared. In order to count lactic acid bacteria, MRS agar culture medium was used. After culturing the samples using the pour plate (mixed) method, the plates were kept at a temperature of 37 °C for 48 hours in anaerobic conditions (anaerobic jar). To determine the bacterial concentration, the standard colony counting method was used [17].

2-5-2- Counting mold and yeasts

In order to count molds and yeasts, YGC agar culture medium was used. Cultivation was done by the surface method and after that, the plates were kept at a temperature of 25°C for 48 hours [18].

2-6- Statistical analysis

In this research, according to the different levels of Persian gum (0, 0.25, and 0.5%) and MTGase enzyme (0, 0.5, and 1 unit per gram of insoluble protein), 9 cheese samples were produced. In addition to the low-fat control sample, the factory sample as a high-fat control was compared with 9 low-fat samples. All the tests were done with three repetitions. Sensory and microbial characteristics of the samples were investigated during the first, thirty and sixtieth days of cold storage. The obtained data were analyzed using SPSS software. In order to examine the significant difference between 10 cheese treatments, simple analysis of variance was used, and to examine the interaction effects between treatments and storage time (in semi-fat samples), factorial design was used in a completely random format. The average data were compared at the level of significant difference of 5% ($p < 0.05$). All results were expressed as the mean of three replicates ± standard error.

3-Results and Discussion

3-1- Sensory evaluation of cheese samples

3-1-1- Color and appearance

In relation to many food groups, color can provide consumers with useful information about the sensory characteristics of a product or main characteristics such as premium, natural or healthy. For example, the color of cheese can indicate its shelf-life. This means that as the ripening time increases, by increasing the levels of volatile compounds that make up the taste, its appearance usually becomes darker. Therefore, the lightness of the cheese is an observable sign that, depending on the individual preferences of the consumers, influences their decisions in purchasing the product [19].

The color and appearance of any food item is affected by the reflection, absorption or transmission of light, which in turn depends on the physical structure and chemical nature of that food item. The statistical findings obtained from the effect of the independent variables: Persian gum (PG), MTGase enzyme and storage time on

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the scores of the color and appearance of the cheese samples according to the panelists are shown in figures 1 and 2 and also in the table 1. The results of this study showed that all three variables, PG, MTGase enzyme and storage time have a significant effect on the mentioned parameters ($p < 0.05$); while, statistically, the interaction effects of none of the variables were significant ($p > 0.05$).

The white color of milk is due to the scattering of white light by colloidal particles and milk proteins (casein micelles and whey proteins), fat globules, citrate and phosphate. In relation to cheese, the amount of light scattering depends on the number of holes or the amount of tissue porosity, fat content and also the presence of milk fat substitutes [20 and 21]. Examining Figures 1 and 2 shows that with the increase of PG concentration in the formulation of cheese samples, the score of color and appearance has increased; So that the samples containing the highest amount of gum got the highest score for color and appearance. The amount of color score in semi-fat cheese samples containing 0, 0.25 and 0.5% PG was determined as 7.91, 8.03 and 8.12, respectively, and in terms of appearance was determined as 7.98, 8.07 and 8.21. Probably, an increase in the number light scattering centers in cheese as a result of adding PG, resulted in a higher score for color and appearance [6].

In the samples treated with MTGase enzyme, the highest color and appearance acceptance score was observed in the sample containing 0.5 enzyme units. Therefore, adding Persian gum and transglutaminase enzyme to cheese can successfully increase the acceptance of the color and appearance of this dairy product among consumers. According to the results shown in Table 1, with the increase in storage time, the acceptance of the color and appearance of different cheese samples decreased significantly, and at the end of the storage period, the lower scores of color and appearance were assigned by the panelists.

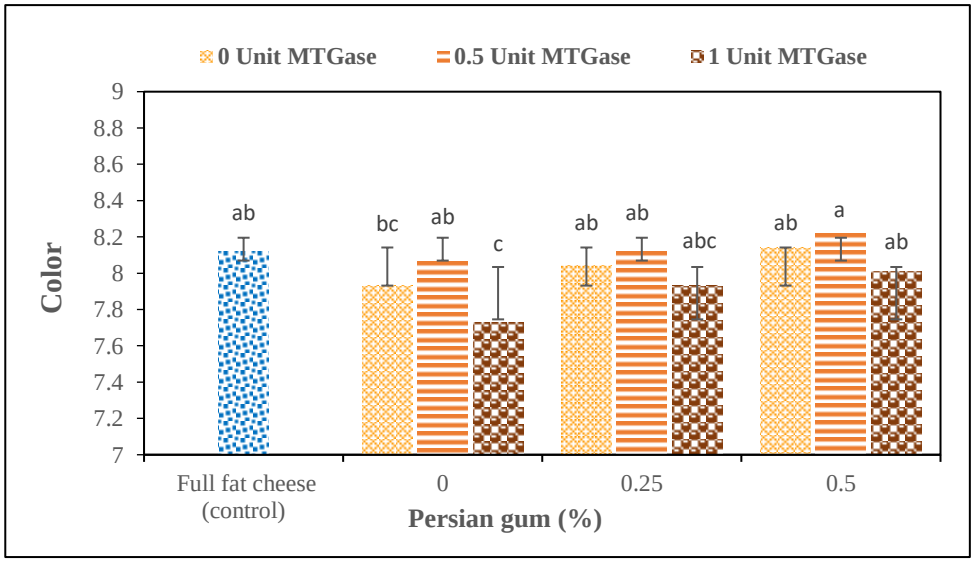

Figure 1. Effect of different concentration of MTGase and PG on the color of cheese samples

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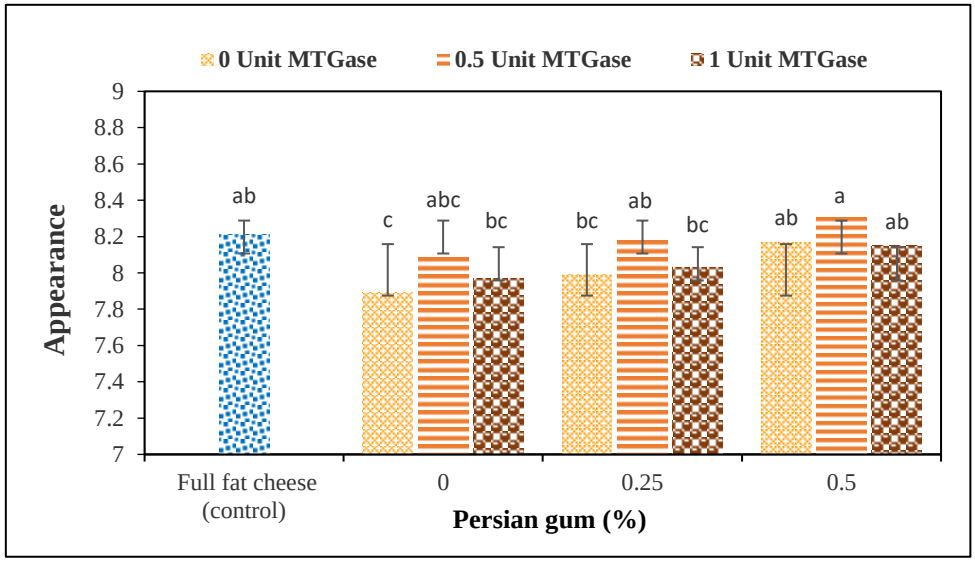

Figure 2. Effect of different concentration of MTGase and PG on the appearance of cheese samples

In another study, the effect of adding lipase and MTGase enzymes on the sensory characteristics of fresh quark cheese was investigated. During the 21 days of cold storage, a significant decrease in the color score reported by the panelists was observed, and in all samples, the lowest color score was on the 21st day of storage. In addition, there was no significant difference between the color score of the treated samples and the control sample, except for the first day of storage in which the color score of the control sample was significantly higher than that of the treated samples. Also, the moisture content of all the cheese samples decreased during the storage period and the samples got a wrinkled appearance, which had a negative effect on the attractiveness of the cheeses' color [17]. In another study, it was found that there was no significant difference between the color of fresh cheeses treated with MTGase enzyme and control samples [22].

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Table 1. The results of analysis of variance (ANOVA) of the effect of different concentration of MTGase and PG on the color and appearance of cheese samples during the cold storage

Parameter	Factor	Storage time (day)		
		1	**30**	**60**
Color	MTGase concentration (U/g protein)			
	0	8.16 ± 0.23^{ab}	8.072 ± 0.200^{a}	7.879 ± 0.276^{ab}
	0.50	8.34 ± 0.14^{a}	8.720 ± 0.289^{a}	7.989 ± 0.196^{a}
	1	8.03 ± 0.22^{b}	7.933 ± 0.201^{a}	7.706 ± 0.260^{b}
	PG concentration (%)			
	0	8.09 ± 0.21^{a}	7.93 ± 0.28^{a}	7.71 ± 0.29^{a}
	0.25	8.16 ± 0.22^{a}	8.00 ± 0.19^{a}	7.93 ± 0.27^{a}
	0.50	8.28 ± 0.22^{a}	8.14 ± 0.87^{a}	7.94 ±0.18^{a}
Appearance	MTGase concentration (U/g protein)			
	0	8.07 ± 0.256^{a}	8.039 ± 0.173^{b}	7.944 ± 0.235^{b}
	0.5	8.20 ± 0.182^{a}	8.194 ± 0.155^{a}	8.183 ± 0.242^{a}
	1	8.18 ± 0.173^{a}	8.056 ± 0.124^{b}	7.911 ± 0.215^{b}
	PG concentration (%)			
	0	8.06 ± 0.19^{b}	7.97 ± 0.15^{b}	7.93 ± 0.16^{b}
	0.25	8.12 ± 0.18^{ab}	8.13 ± 0.14^{a}	7.94 ± 0.24^{b}
	0.50	8.27 ± 0.21^{a}	8.19 ± 0.14^{a}	7.97 ± 0.29^{a}

3-1-2- Aroma, taste and texture

During the following three main reactions, aroma and flavor compounds are formed in cheese: a) metabolism of remaining lactose, lactate and citrate, b) proteolysis, and c) lipolysis. In general, food flavoring compounds are classified into two groups of volatile and non-volatile compounds. Volatile flavoring compounds include alcohols, acids, esters, aldehydes, and ketones, all of which are responsible for the aroma of various foods. On the other hand, non-volatile flavoring compounds include compounds such as organic acids, amino acids, reducing sugars, nucleotides, polypeptides and other small molecules, all of which play a role in creating the taste of food [23].

The results of the statistical analysis of the effect of PG variables, MTGase enzyme and storage time on the acceptance of aroma and flavor of low-fat cheese samples are shown in Table 2. The results showed that all the independent variables investigated had a significant effect on the acceptance of the aroma and taste of ultrafiltrated cheese ($p < 0.001$). In addition, apart from the interaction effect of storage time and PG, no significant difference was observed among other investigated variables ($p > 0.05$). Based on the results shown in Figure 3, the semi-fat cheese sample containing 0.5% PG and 0.5

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units of MTGase enzyme with 8.5 points, like the high-fat control sample with 8.54 points, had the highest aroma and flavor score. According to Figure 3, by increasing the MTGase enzyme concentration to 0.5 units, the taste score increased, but a higher enzyme concentration (one unit) caused a noticeable decrease in the aroma and taste of semi-fat cheese. The amount of aroma and taste score in semi-fat cheese samples containing 0, 0.5 and 1 unit of MTGase enzyme was determined as 7.66, 7.99 and 7.67, respectively. Also, with the increase in PG concentration, the rate of aroma and taste among panelists increased significantly ($p < 0.001$). The amount of aroma and taste score in semi-fat cheese samples containing 0, 0.25 and 0.5% PG was determined as 7.31, 7.79 and 8.22, respectively. Regarding the storage time, the aroma and taste score in the UF semi-fat cheese samples was determined as 7.64, 7.78, and 7.90, respectively, in periods of 1, 30, and 60 days.

Carrying out proteolysis and lipolysis processes during cheese ripening plays an important role in improving sensory characteristics such as aroma, taste, texture and overall acceptance. The secondary changes that occur simultaneously with these processes cause minor and general changes in the sensory characteristics of cheese. The main substrates of such processes include caseins, lipids and soluble compounds in milk. During the storage period, with the change of the microbial flora, the characteristics of the clot also change as a result of the biochemical reactions performed, and for this reason, new characteristics are created in the aroma, taste and texture of the samples [16]. Reducing the amount of fat may affect the perception of the taste of cheese by humans; because the size of fat globules in low-fat cheeses is smaller compared to high-fat types and also they are placed in the protein matrix of cheese [24]. In another study, the results obtained from the effect of free and coated MTGase enzyme on the sensory characteristics of cheese showed that the sample containing 60 ppm of coated enzyme has the most acceptable taste; while the sample with 60 ppm free enzyme has the highest color and odor score [25].

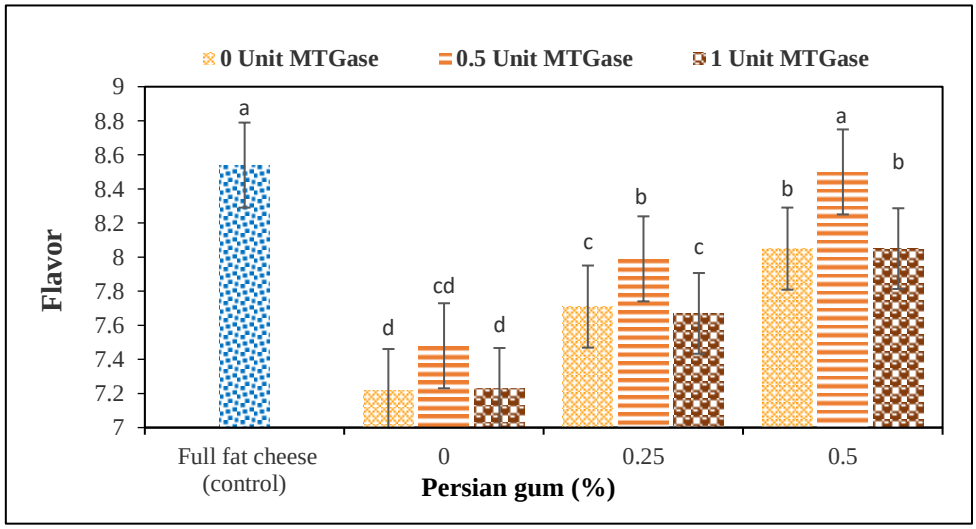

Figure 3. Effect of different concentration of MTGase and PG on the flavor of cheese samples

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Table 2. The results of analysis of variance (ANOVA) of the effect of different concentration of MTGase and PG on the flavor and texture and consistency of cheese samples during the cold storage.

Parameter	Factor	Storage time (day)		
		1	**30**	**60**
Flavor	MTGase concentration (U/g protein)			
	0	7. 54 ± 0.44^{b}	7.64 ± 0.23^{b}	7.80 ± 0.52^{b}
	0.50	7.84 ± 0.53^{a}	8.01 ± 0.36^{a}	8.11 ± 0.56^{a}
	1	7.53 ± 0.41^{b}	7.07 ± 0.25^{b}	7.79 ± 52^{b}
	PG concentration (%)			
	0	7.18 ± 0.26^{c}	7.44 ± 0.25^{b}	7.311 ± 21^{c}
	0.25	7.60 ± 0.21^{b}	7.83 ± 0.29^{a}	7.93 ± 0.23^{b}
	0.50	8.14 ± 0.28^{a}	8.07 ± 32^{a}	8.46 ± 0.30^{a}
Texture	MTGase concentration (U/g protein)			
	0	6.80 ± 0.64^{a}	7.00 ± 0.78^{ab}	7.43 ± 0.65^{a}
	0.5	7.00 ± 0.62^{a}	7.22 ± 0.29^{a}	7.46 ± 0.50^{a}
	1	6.94 ± 0.72^{a}	6.90 ± 0.30^{b}	7.07 ± 0.43^{b}
	PG concentration (%)			
	0	6.25 ± 0.21^{c}	6.41 ± 0.26^{c}	6.74 ± 0.24^{c}
	0.25	6.80 ± 0.25^{b}	6.81 ± 0.18^{b}	7.34 ± 0.24^{b}
	0.50	7.69 ± 0.20^{a}	7.90 ± 0.30^{a}	7.87 ± 0.37^{a}

Figure 4 shows the results of adding PG and MTGase enzyme to the texture of cheese samples and comparing it with the high-fat control sample. Table 2 also shows the results obtained from the effects of independent variables PG, MTGase enzyme and storage time on the texture acceptance of cheese samples. The findings of this research revealed that the addition of gum significantly affected the texture of cheese samples ($p < 0.001$) and the samples containing the highest concentration of PG

obtained the highest texture score, which this may be due to the hydrophilic character of the gums. As a result of the addition of gum, water molecules are largely replaced by the reduced fat and are placed among the protein particles, which, as a result, prevents the protein network from becoming too dense. Another reason for the reduction of cheese texture hardness can be attributed to the interactions between gum and protein chains. These interactions cause the reduction of protein crosslinks in the structure of cheese, and as a result, a weaker protein matrix is created, which makes the texture of the cheese softer [26].

In addition, like the other sensory characteristics investigated, the addition of MTGase enzyme had a positive effect on the acceptance of the texture of the treated cheese samples by the panelists, and in this regard, the treatment containing 0.5 units of the enzyme (7.23) had the highest texture score. In addition, there was no significant difference between the low-fat control sample (7.08 points) and the sample containing 1 unit enzyme (6.96 points). It seems that the use of higher enzyme concentrations causes excessive hardness and decreases the desired quality of cheese texture due to the increase in the formation of intra- and extra-molecular bonds of the casein protein network. Among the different semi-fat treatments under investigation, the highest texture quality with a score of 7.93 was awarded to the low-fat cheese sample containing 0.5% PG

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and 0.5 units of MTGase enzyme, which did not significantly differ from the high-fat sample with a texture score of 7.99 (Figure 4).

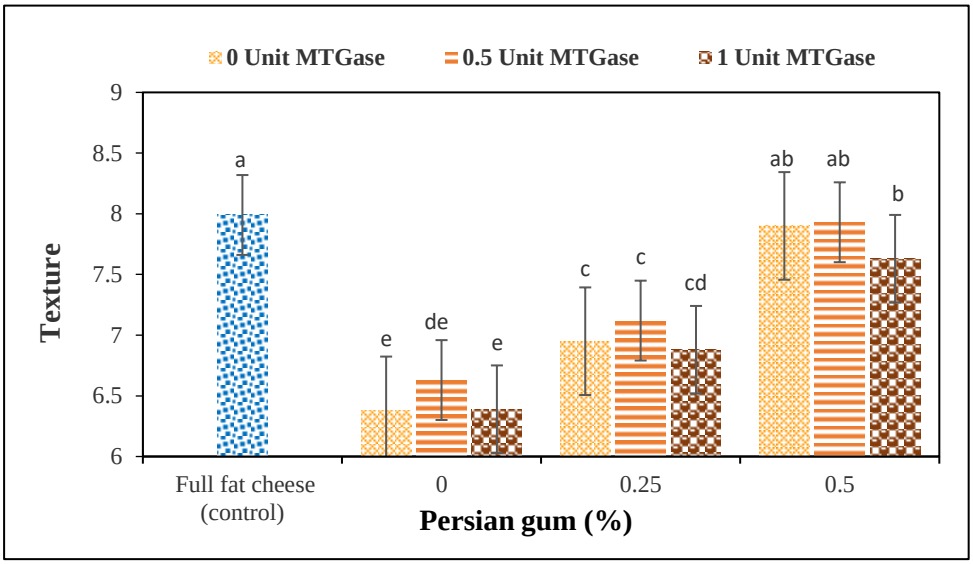

Figure 4. Effect of different concentration of MTGase and PG on the texture of cheese samples

In accordance with the findings obtained from this study, in another study, the highest texture score was obtained for the control and treated cheese samples with low levels of MTGase enzyme. The texture improvement of cheeses treated with appropriate enzyme levels can be due to the increase in moisture content and the formation of crosslinks between protein molecules. However, in the mentioned research, in terms of color, taste and taste, no significant difference was observed between the control cheese samples and the samples treated with MTGase [27].

Similarly, the sensory characteristics of low-fat ultrafiltrated cheeses containing capacarrageenan gum and transglutaminase enzyme were studied during a thirty-day period. The findings of this research revealed that except for smell, capacarrageenan gum had a significant effect on other sensory characteristics such as color, texture and taste of cheese samples. Addition of MTGase enzyme also had significant affects only on the color and texture parameters and no significant effect was observed in relation to smell and taste. In addition, the length of the storage period significantly affected all the sensory characteristics of the samples. The use of gum at the rate of 0.03% improved the texture and taste of low-fat cheeses, but the use of higher concentrations of gum had a negative effect on these factors. In the constant amounts of gum, the samples containing enzyme received a higher score compared to the samples without enzyme by the panelists [26].

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Table 3. Effect of different concentration of MTGase and PG on the brightness of cheese samples during the cold storage

Parameter	Storage period (days)	PG concentration (%)	MTGase concentration (U/g protein)	**Mean ± SD**
Brightness (L*)	1	0	0	**89.07 ± 1.38**
		0	0.5	**90.61± 1.148**
		0	1	**89.16 ± 1.22**
		0.25	0	**90.95 ± 1.57**
		0.25	0.5	**91.97 ± 1.31**
		0.25	1	**91.00 ± 1.60**
		0.5	0	**91.51 ± 1.40**
		0.5	0.5	**92.28 ± 1.17**
		0.5	1	**92.51 ± 0.54**
	30	0	0	**86.78 ± 1.84**
		0	0.5	**88.95 ± 1.58**
		0	1	**87.55 ± 1.43**
		0.25	0	**87.41 ± 1.47**
		0.25	0.5	**88.80 ± 0.71**
		0.25	1	**87.68 ± 0.43**
		0.5	0	**89.30 ± 1.54**
		0.5	0.5	**90.42 ± 1.09**
		0.5	1	**89.63 ± 1.82**
	60	0	0	**84.84 ± 2.16**
		0	0.5	**86.64 ± 2.16**
		0	1	**86.11 ± 2.05**
		0.25	0	**86.58 ± 1.84**
		0.25	0.5	**87.99 ± 1.39**
		0.25	1	**87.56 ± 1.42**
		0.5	0	**87.47 ± 1.63**
		0.5	0.5	**88.76 ± 1.21**
		0.5	1	**88.55 ± 1.02**

3-2- Color of cheese samples

In Table 3, the statistical results obtained from the color index L* (lightness) of the cheese samples during the cold storage period are shown, which shows the significance of the independent variables PG and MTGase enzyme ($p < 0.05$). However, the effect of these variables on a* indices (red-green) and b* (yellowish-blue) values of cheese samples were not significant ($p > 0.05$). According to the results obtained from this study, the brightness of the examined cheese samples decreased during cold storage. This decrease in L* values can be due to the increase in hydration of proteins and the decrease in free water droplets during the storage period, which causes a decrease in light scattering [24]. L^* values of the cheese samples during the 60-day storage period ranged from 84.84 (cheese sample containing 0% gum and 0% enzyme on the 60^{th} day of storage) to 92.51 (treatment containing 0.5% PG and 1 unit of MTGase enzyme on the first day of storage). In accordance with the results obtained from the present study, Torabi et al. [27] showed that the addition of transglutaminase enzyme together with whey proteins increases the number of serum holes and creates a porous texture in cheese. In addition, the brightness (L*) of all samples decreased during the ripening period. In a similar study, it was found that there is a

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slight difference between the color of the samples treated with transglutaminase enzyme and the control samples, which indicates the low effect of MTGase on the color of the cheese samples. In addition, b* values greater than zero were measured; this means that the color of the studied samples was yellow. In general, the samples prepared from whole milk powder had a yellower color compared to the types produced from fresh milk, which may be due to the color of the raw materials. In fact, milk powder has a darker color due to the drying process at high temperature [22].

3-3- Investigating the process and survival rates of lactic acid bacteria (LAB)

Figure 5 shows the results obtained from the effect of different concentrations of PG and MTGase enzyme on changes in growth and survival of lactic acid bacteria (LAB) of cheese samples. The addition of gum significantly ($p<0.001$) increased and the addition of enzyme significantly ($p<0.01$) decreased the growth and survival of LAB. On average, cheese samples containing the highest level of gum (0.5%) had higher growth and viability compared to other treatments. The highest growth rate of LAB was observed in the treatment of semi-fat cheese containing 0.5% gum and without enzyme units with 8.42 Log CFU/g, which was not significantly different from the high-fat control sample with 8.69 Log CFU/g. In addition, the examination of the treated cheese samples during the storage period showed that the growth and survival of lactic acid bacteria increased insignificantly first and then decreased (Table 4).

In accordance with the results obtained from this study, it has been reported that with the addition of transglutaminase enzyme and also with the passage of time, the population of lactic acid bacteria in cheese samples has decreased [22]. MTGase enzyme does not have any toxic effect on LAB, but the reason for this decrease may be the delay in the growth of these bacteria; because peptides with low molecular weight as well as amino acids required for the growth of LAB form cross-links with the transglutaminase enzyme and as a result became out of the reach of these bacteria [7]. In another study, the effect of adding MTGase enzyme and carrageenan gum on the microbial characteristics of UF-low-fat cheese during thirty days of storage was studied. The results of this research revealed that the addition of MTGase and storage time had a significant effect on the population of lactic acid bacteria. However, gum concentration had no significant effect on this factor. After 15 days of sample production, the LAB population increased from 33.7 Log CFU/g to 21.8 Log CFU/g, but on the 30th day of storage, it decreased to 7.21 Log CFU/g [26]. In another study, with the exception of the sample containing 0.3 g/L transglutaminase enzyme and 0.06 g/L lipase enzyme, in which the concentration of lactic acid bacteria was higher than other samples, no significant change was observed in the LAB population of the examined quark cheese samples. In addition, during 21 days, the LAB population increased significantly and its highest rate was reported on the 21st day [17].

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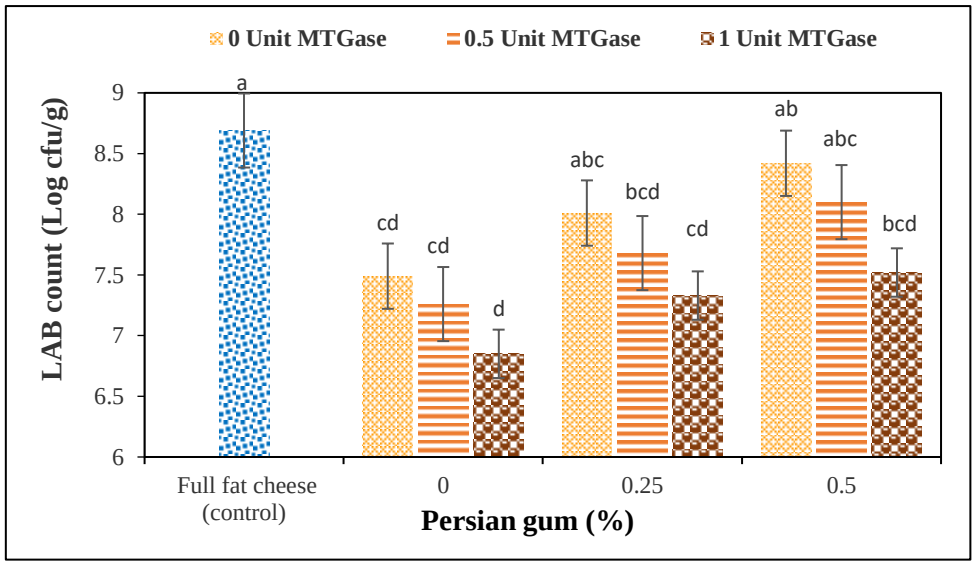

Figure 5. Effect of different concentration of MTGase and PG on the survivability of lactic acid bacteria

3-4- Investigating the growth rate of mold and yeasts

Mold contamination of dairy products may occur during different stages of the production cycle, from livestock farms to dairy production units. Naturally, there are 10^3- 10^5 CFU/mL of mold cells in raw milk, and milking places in livestock farms are one of the important sources of these microorganisms entering milk. Also, the contamination of dairy products by molds can be caused by the environmental air pollution of the production of these products, especially after the pasteurization process. Although molds are usually not considered as the main causes of spoilage of milk and other dairy products, it may lead to visible changes in some cultivated products as a result of the growth of mycelium on the surface of such products, and also cause bad taste through proteolytic activity. Often, moldy spoilage occurs in products such as cheese and butter and causes a bitter taste and a moldy appearance in these products [28].

Table 4. The results of analysis of variance (ANOVA) of the effect of different concentration of MTGase and PG on the survivability of lactic acid bacteria, molds and yeasts of cheese samples during the cold storage

Parameter	Factor	Storage time (day)		
		1	30	60
	MTGase concentration (U/g protein)			
	0	7. 27 ± 0.65^{a}	8.92 ± 0.66^{b}	7.73 ± 0.64^{a}
Lactic acid bacteria	0.50	7.10 ± 0.54^{a}	8.60 ± 0.64^{ab}	7.34 ± 0.67^{ab}
(Log cfu/g)	1	6.58 ± 0.52^{b}	8.16 ± 0.50^{b}	6.94 ± 0.65^{b}
	PG concentration (%)			
	0	6.47 ± 0.41^{b}	8.04 ± 0.62^{b}	7.08 ± 0.80^{a}

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	0.25	7.03 ± 0.51[a]	8.68 ± 0.51[a]	7.31 ± 0.70[a]
	0.50	7.45 ± 0.55[a]	8.97 ± 0.53[a]	7.63 ± 0.55[a]
	MTGase concentration (U/g protein)			
	0	1.08 ± 0.53[a]	1.02 ± 0.64[a]	2.06 ± 0.45[a]
	0.5	0.97 ± 0.46[a]	0.92 ± 0.59[a]	1.79 ±0.56[a]
Molds and yeasts (Log cfu/g)	1	0.83 ± 0.25[a]	0.86 ± 0.76[a]	1.63 ± 0.56[a]
	PG concentration (%)			
	0	0.86 ± 0.56[a]	0.90 ± 0.70[a]	1.47 ± 0.34[b]
	0.25	1.15 ± 0.44[a]	1.12 ± 0.77[a]	1.79 ± 0.34[ab]
	0.50	0.87 ± 0.49[a]	0.78 ± 0.44[a]	0.21 ± 0.63[a]

As can be seen in Figure 6, the findings of this study showed that the addition of PG gum to some extent increased and the addition of MTGase enzyme also partially decreased the growth of mold and yeasts in semi-fat cheese samples ($p > 0.05$). The highest growth rate of fungi was observed in the sample containing 0 units of transglutaminase enzyme and 0.25% Persian gum. The possible reason for the increase in the population of mold and yeasts with the increase in the percentage of gum is that large amounts of water are available to this microorganism, which leads to their higher activity. The decrease in the growth of fungi due to the addition of enzyme can also be due to the formation of cross-links by the enzyme and the removal of the compounds required for the growth of these microorganisms. In addition, during the cold storage period, the growth of mold and yeasts in the samples of low-fat cheese treated with PG and MTGase enzyme first decreased slightly and then increased significantly (Table 4). After 30 days of producing the samples, the count of mold and yeast briefly decreased from 0.96 Log CFU/g to 0.93 Log CFU/g, but on the 60th day, its value was significantly increased to 1.83 Log CFU/g [26]. In another study, it was determined that the duration of twenty-one days of cold storage has a significant positive effect on the growth of molds and yeasts in all quark cheese samples. Among the treated samples, the highest population of molds and yeasts was observed in the sample containing 0.3 g/L of transglutaminase enzyme and 0.06 g/L of lipase enzyme, and after that, the sample containing 0.2 g/L transglutaminase enzyme and 0.04 g/L lipase enzyme and the sample containing 0.1 g/L transglutaminase enzyme and 0.02 g/L lipase enzyme had the highest mold population; while, the lowest mold population was reported in the control sample (4.49 Log CFU/g). The growth rate in the control sample was lower than the treated samples and after 21 days, the growth of yeasts and molds in this sample was reported to be only 1.1 Log CFU/g. In addition, during the storage period, the population of coliforms increased significantly, and in all the examined samples, its highest level was measured on the 21st day of storage [17].

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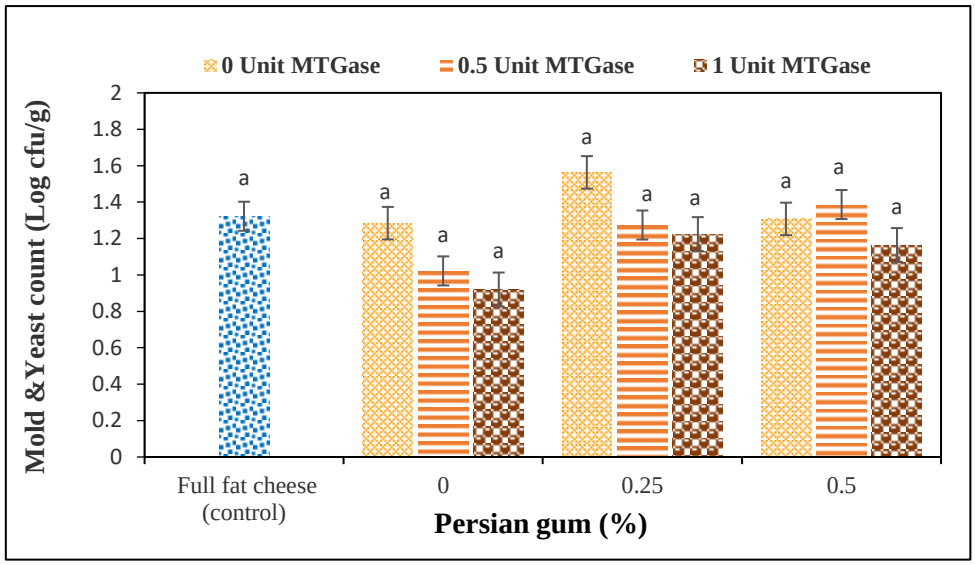

Figure 6. Effect of different concentration of MTGase and PG on the count of molds and yeasts

4- Conclusion

The findings obtained from the present research revealed that adding Persian gum and transglutaminase enzyme to low-fat UF cheese improved the color and appearance of this dairy product. With the increase of the storage period, scores of the color and appearance of different cheese samples decreased significantly and at the end of the storage period, the panelists assigned a lower color and appearance score to the cheese samples. In addition, with the increase in enzyme concentration, the taste score increased, but increasing the enzyme concentration by one unit resulted in a significant decrease in the aroma and taste of semi-fat cheese. Increasing the concentration of PG also caused an increase in the aroma and flavor score of the cheese samples. Addition of gum also significantly affected the texture of cheese samples; so, the highest texture score was assigned to the samples containing the highest concentration of PG. The addition of enzyme also had a positive effect on the texture scores of the treated cheese samples by the panelists, and the treatment containing 0.5 units of enzyme got the highest texture score. The measurement of brightness (L*) of cheese samples showed a decrease in L* values during the cold storage period. The results obtained from the microbial tests showed that the addition of gum significantly increased and the addition of enzyme significantly decreased the growth and survival of LAB. In addition, during the storage period, the growth and survival of lactic acid bacteria increased insignificantly first and then decreased. The addition of gum to some extent caused an increase and the addition of enzyme also partially decreased the growth of mold and yeasts in semi-fat cheese samples. As a result, by using appropriate levels of Persian gum and transglutaminase enzyme, UF cheese with desirable technological characteristics can be produced. Based on the general results obtained in this research, with the help of 0.5% of Persian gum and 0.5 unit of transglutaminase enzyme (per gram of protein) a low-fat cheese with sensory characteristics similar to full-fat cheese could be produce.

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مقاله علمی_پژوهشی

بررسی تأثیر صمغ فارسی و آنزیم ترانس‌گلوتامیناز بر ویژگی‌های حسی، رنگ و میکروبی پنیر سفید فراپالودهٔ نیم‌چرب طی دورهٔ نگهداری سرد

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چکیده

مطالعهٔ حاضر با هدف بررسی تأثیر صمغ فارسی (PG) و آنزیم ترانس‌گلوتامیناز میکروبی (MTGase) بر ویژگی‌های حسی، رنگ و میکروبی پنیر سفید فراپالودهٔ نیم‌چرب طی مدت ۶۰ روز نگهداری در یخچال انجام گرفت. به‌منظور تولید نمونه‌های پنیر نیم‌چرب از PG در سه سطح ۰، ۰/۲۵، ۰/۵٪ و آنزیم MTGase در سه سطح ۰، ۰/۵ و ۱ واحد (به ازای هر گرم پروتئین ناتراوه) استفاده گردید. نتایج مشخص ساخت که تیمار نمونه‌های پنیر با PG و آنزیم MTGase تأثیر مثبتی بر ویژگی‌های حسی و کیفی این فراورده لبنی داشته است. به‌طورکلی، تیمار حاوی ۰/۵٪ PG و ۰/۵ واحد آنزیم MTGase بالاترین امتیازات حسی را توسط ارزیاب‌ها کسب کرد. براساس نظرات ارزیاب‌ها، طی مدت‌زمان نگهداری ویژگی‌های حسی عطروطعم و بافت افزایش یافت و در مقابل امتیازات رنگ و ظاهر نمونه‌های پنیر کاهش یافت. نتایج به‌دست‌آمده از آنالیز شاخص‌های رنگی مشخص ساخت که میزان روشنایی (L^*) نمونه‌های پنیر با افزودن PG و تیمار آنزیمی MTGase افزایش و با گذشت مدت‌زمان نگهداری سرد کاهش یافت. برخلاف پارامتر روشنایی (L^*)، متغیّرهای PG و آنزیم MTGase اثر معناداری بر شاخص‌های a^* (قرمزی-سبزی) و b^* (زردی-آبی) نمونه‌های پنیر مورد آزمایش نداشتند. نتایج به‌دست‌آمده از آزمون‌های میکروبی نشان داد که افزودن PG سبب افزایش زنده‌مانی باکتری‌های اسید لاکتیک (LAB) شد اما تأثیری بر شمارش کپک و مخمّرها نداشت. ازسوی‌دیگر، افزایش غلظت آنزیم سبب کاهش رشد و زنده‌مانی میکروارگانیسم‌های مورد مطالعه گردید. نتایج این مطالعه نشان داد که می‌توان از PG به‌عنوان یک جایگزین چربی به‌همراه آنزیم MTGase جهت تولید پنیر سفید فراپالودهٔ کم‌چرب با ویژگی‌های تکنولوژیکی و حسی مطلوب قابل مقایسه با انواع پرچرب استفاده کرد و بهترین نمونه‌ی پنیر فراپالوده نیم‌چرب با استفاده از تیمار حاوی ۰/۵٪ PG و ۰/۵ واحد آنزیم MTGase به‌دست می‌آید.

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