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The effect of transglutaminase enzyme and carrageenan gum on sensory, textural and microbial properties of low fat ultrafiltrated cheese

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ABSTRACT

Because fat content has a great impact on the sensory properties of the cheese like appearance, taste and texture, its reduction causes fundamental changes in the sensory and structural characteristics of the product. Therefore, by using fat replacers, as well as to produce a healthy cheese, a product with an acceptable quality could be produced. In the present study, low-fat cheese samples (8% fat) were produced thru ultra-filtration (UF) method. To produce the cheese samples, *k*appa-carrageenan gum was applied at three levels (0, 0.03 and 0.06%) and transglutaminase was added at 2 levels (0 and 0.5 unit/g of protein). The low-fat sample without the gum and enzyme addition was considered as the control. All samples were evaluated in terms of sensory, textural and microbial characteristics after 1, 15 and 30 days of production. According to the results, the addition of gum caused a significant reduction of cheese hardness, while enzymatic treatment had adverse effect and it increased this parameter ($p<0.01$). Addition of 0.03% of gum resulted in the higher taste and texture quality, but the sensory attributes of low-fat UF-cheese samples decreased at the higher level, i.e. 0.06%. Furthermore, results showed that enzymatic treatment caused a substantial reduction on lactic acid bacteria (LAB) count, while gum addition had no effect. Overall, as the time of storage increased and until the 15th day of storage, all the sensory scores, the cheese hardness and the count of LAB increased while at the end of 30th day of storage, all these parameters significantly decreased. In conclusion, according to the sensory evaluation and texture results, the low-fat UF-cheese containing 0.03% carrageenan treated with transglutaminase was selected as the best sample.

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1- Introduction

Demand for low-fat cheese has increased globally due to increased public awareness of consumer health issues. The production of low-fat cheese with the same quality as high-fat cheese has been the subject of attention of researchers and cheese industries in the world for years. In any case, fat reduction causes extensive changes in the texture, melting property, as well as the organoleptic characteristics of the food [1 and 2]. Consumers expect low-fat cheeses to have the same characteristics as high-fat cheeses, and of course, if low-fat cheeses with good flavor are produced, the desire to consume them increases [3].

One of the most effective solutions to solve the problems caused by fat reduction in cheese is the use of fat substitute compounds [4]. It can be said that the purpose of using fat substitutes is to change the sensory quality of food as much as possible along with reducing the amount of fat and calories [5]. Among the main challenges in the formulation of low-fat cheese are the excessive stiffness of the texture, the loss of flavor and the unfavorable melting characteristics [6 and 7]. To solve these problems, the product's moisture content can be adjusted by adding materials with high water holding capacity (hydrocolloids) to the cheese. Based on this, several carbohydrate-based fat substitutes, such as microcrystalline cellulose, carrageenan, gum arabic, polyanionic gum, starch, beta-glucan and kathira gum, have been used to produce different cheeses [8 and 9].

Interaction of milk proteins with transglutaminase (TG) is one of the most effective methods to improve the nutritional, technical and biofunctional characteristics of dairy products; On the other hand, transglutaminase reduces production costs by reducing the amount of fat and stabilizer in the final product [10]. This enzyme can form intramolecular and intermolecular covalent crosslinks between two amino acids, lysine and glutamine. Unlike protein cross-linking, which does not cause any change in charge distribution on the surface of proteins, acyl transfer reactions can create new groups in proteins, resulting in changes in molecular charge, hydrophilicity, and protein structure. Therefore, among the advantages of using transglutaminase in dairy products, we can mention the increase of gel resistance, improvement of viscosity and storage modulus [11]. Exposure to temperature changes or physical stress causes separation of serum in casein gel. This problem can be avoided by adding transglutaminase, for example, to yogurt, because this enzyme increases the water holding capacity. It has also been reported that ice creams treated with transglutaminase have better aeration and foam consistency [12]. In fact, by using the transglutaminase enzyme, low-fat ice cream and cheese can be produced with less fat-free solids content. In addition, with the use of this enzyme in the production of dairy products, the moisture content or water retention capacity of the product is increased and tasty products are produced with better textural and rheological characteristics; Meanwhile, production efficiency increases in products such as cheese [13, 14 and 15].

So far, various researches on the simultaneous or separate effect of enzymeTG And different fats or gums have been applied to dairy products, including ultra-refined cheese. In investigating the effect of Persian and almond gums on ultra-refined low-fat cheese, Jovindeh et al. [1] reported the improvement of the physicochemical and rheological properties of the product. Adding the mentioned gums to the cheese increased the porosity and opened the casein network of the cheese. Torabi et al. [16] also showed that enzyme treatmentTG And the addition of inulin and

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water cheese as prebiotic compounds significantly improves the sensory properties and increases the bacterial population (lactic acid bacteria and probiotics) in ultra-refined low-fat cheese. However, so far there is no research on the simultaneous effect of enzyme treatmentTG And kappa-carrageenan gum has not been done on ultra-refined low-fat cheese. Considering the importance of producing low-fat dairy products and also considering the high position that cheese has in the food basket of society, the present research aims to investigate the production of ultra-low-fat cheese using kappa-carrageenan gum and transglutaminase enzyme and the effect of these compounds on sensory characteristics. Texture and microbial analysis of ultra-refined low-fat cheese was carried out.

2- Materials and methods

1-2- Materials

The low-fat processed cheese samples were produced using Natrave in the Pegah factory of Khuzestan, located at the 3rd kilometer of Shush-Dezful road. Rent Kmax (as a coagulant) was obtained from Christian Hansen Dairy Company of Denmark, milk protein concentrate powder from Pegah Khorasan Company, and commercial Kappa-Carrageenan gum (with the brand name Genogel) was obtained from Cipicalco Company of Denmark. Brand name mesophilic startersCHOOZIT 230 contain*Lactococcus lactis* subspecies*Cremoris* And*Lactococcus lactis* subspecies*Lactis* and thermophilic initiatorsYO-MIX 532 The content of the strains*Streptococcus thermophilus* And*Lactobacillus delbruecki* subspecies*Bulgaricus* They were also prepared from Denmark's Danesco company.

2-2- Cheese production method

Milk protein concentrate powder (containing 80% protein and 0.6% fat) was used to produce low-fat cheese (8% fat). In the samples containing gum, kappa-carrageenan powder was used at the levels of 0, 0.03 and 0.06%. After adjusting the amount of natrave fat and adding gum, natrave was homogenized at a pressure of 70 bar using Ronghe machinery homogenizer model JHG-Q60-P60 made in China. After pasteurizing the samples at 75°C for 15 seconds, the temperature of the samples was reduced to 32°C and transglutaminase enzyme was added at two levels (zero and 0.5 enzyme units per gram of protein). Next, Natraveh was transferred to the coagulating line and the solution containing mesophilic and thermophilic cheese and rennet starters was added to Natraveh. Natrava was filled in 400 milliliter cheese containers and then the packages entered the coagulation line to form the cheese curds. Then the cheese packages were kept in a greenhouse at a temperature of 29°C and after the pH of the samples reached 4.8, they were transferred to a cold room at a temperature of 5°C.]16[. Low-fat control samples (without enzyme and gum) were also produced for comparison with other treatments (Table 1). The samples of ultra-refined low-fat cheese were evaluated at 1, 15 and 30 days after production in terms of sensory properties, texture and microbial characteristics.

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Table 1. Different treatments for low-fat ultrafiltrated cheese production

Treatments	Transglutaminase (Unit/g protein)	Carrageenan (%)
1 (Control)	0	0
2	0	0.03
3	0	0.06
4	0.5	0
5	0.5	0.03
6	0.5	0.06

2-3- Sensory evaluation

The preference of the characteristics of color, smell, taste and texture of ultra-refined low-fat cheese samples was done using the 9-point hedonic test. The number of evaluators in the sensory test was 20, and the samples were randomly presented to them after coding with random numbers [16]. All sensory characteristics were evaluated during 30 days of storage in the refrigerator.

4-2- Tissue evaluation test

In order to check the hardness of ultra-processed cheese samples, texture profile test (TPA) by tissue measuring deviceStable Micro System) modelTA.XT.PLUS England) and using prop 5S/P It was done according to Jovandeh method (2009) with some changes. The speed of the probe was set to 1 mm/s and the probe penetrated up to 50% of the initial height of the cheese samples (10 mm depth). The speed of the probe before and after the test was set to 2 and 1 mm/s, respectively. Cheese samples were taken out of the refrigerator before testing and were kept at room temperature for half an hour to reach a constant temperature. The texture test was performed in all cheese samples in three different parts of the cheese and the average results were recorded [17].

5-2- Microbial test

In order to isolate and count lactic acid bacteria, first, 25 grams of cheese sample was transferred to sterile jars with 225 milliliters of 2% weight-volume sodium citrate solution (manufactured by Sigma) and completely homogenized by a shaker for 5 minutes, and then to remove Suspended particles were filtered. In this way, the first dilution^{1-}10 was prepared and other dilutions ($^{2-}$10 to^{7-}10) They were prepared with the help of sterile peptone water of 0.1% weight-volume. Next, for the purpose of mixed culture (pure plate), from tubes containing dilutions prepared at the rate of 0.1 milliliter by sampler, transferred to petridish containers and to those specific culture environments.MRS Agar, made by Merck, Germany, was added and mixed. The plates were incubated at 37°C for 48 hours. Microbial flora was evaluated in 3 periods of 1, 15 and 30 days after production and in 3 repetitions. Counting of the formed plots was done by the plot counting machine]18[.

6-2- Statistical analysis

According to the two variables of transglutaminase enzyme at 2 levels and carrageenan gum at 3 levels, 6 cheese samples were produced and the characteristics of the samples were compared during 30 days of storage in the refrigerator, on days 1, 15 and 30. Therefore, a total of 18 samples were produced in at least two replicates. This research was done in a completely randomized factorial design. Using SPSS 20 software, the effect of gum, enzyme and storage time on the mentioned factors was investigated and averages were compared using Duncan's method with a 95% confidence level.

3. Results and Discussion

3-1- Sensory evaluation

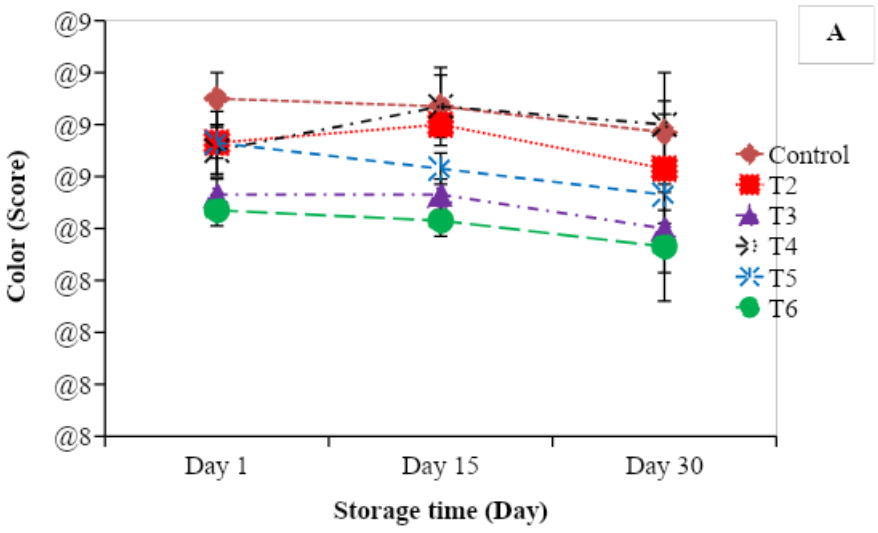
The results related to the variance analysis of the effect of different variables (the amount of kappa-carrageenan gum and transglutaminase enzyme) on sensory properties, texture and the amount of lactic acid bacteria of ultra-refined low-fat cheese samples during 30 days of storage in the refrigerator are

shown in Table 2 and Figure 1.As can be seen in this table, apart from smell, the variable of kappa-carrageenan gum had a significant effect on other sensory evaluation factors (color, texture and taste).01/0p<). The addition of transglutaminase enzyme also had a significant effect only on color and texture factors (05/0p<(and it had no significant effect on the smell and taste factors)05/0p>). The passage of time also showed a significant effect on all sensory evaluation factors (01/0p<).

Table 2. Analysis Variance for sensory, textural and microbial properties of low fat ultrafiltrated cheese

Chang sources	Degree of freedom	Mean square					
		Aroma	Color	Texture	Taste	Hardness	LAB (log cfu/g)
Gum	2	0.005^{ns}	0.62^{**}	0.96^{**}	0.35^{**}	0.04^{**}	0.007^{ns}
Enzyme	1	0.06^{ns}	0.07^{*}	0.19^{*}	0^{ns}	0.03^{**}	4.00^{**}
Time	2	0.34^{*}	0.07^{*}	0.47^{**}	0.13^{**}	0.01^{**}	5.42^{**}
Gum×Enzyme	2	0.20^{ns}	0.001^{ns}	0^{ns}	0.001^{ns}	0^{ns}	0.61^{**}
Gum×Time	4	0.02^{ns}	0.01^{ns}	0.02^{ns}	0.04^{ns}	0^{ns}	0.01^{ns}
Enzyme×Time	2	0.02^{ns}	0.003^{ns}	0.01^{ns}	0.03^{ns}	0^{ns}	0.04^{ns}
Gum×Enzyme×Time	4	0.06^{ns}	0.02^{ns}	0.02^{ns}	0.01^{ns}	0^{ns}	0.01^{ns}
Error	36	0.07	0.01	0.04	0.02	0.001	0.02

ns, * and ** represent non significant, significant at p<0.05 and significant at p<0.01, respectively.



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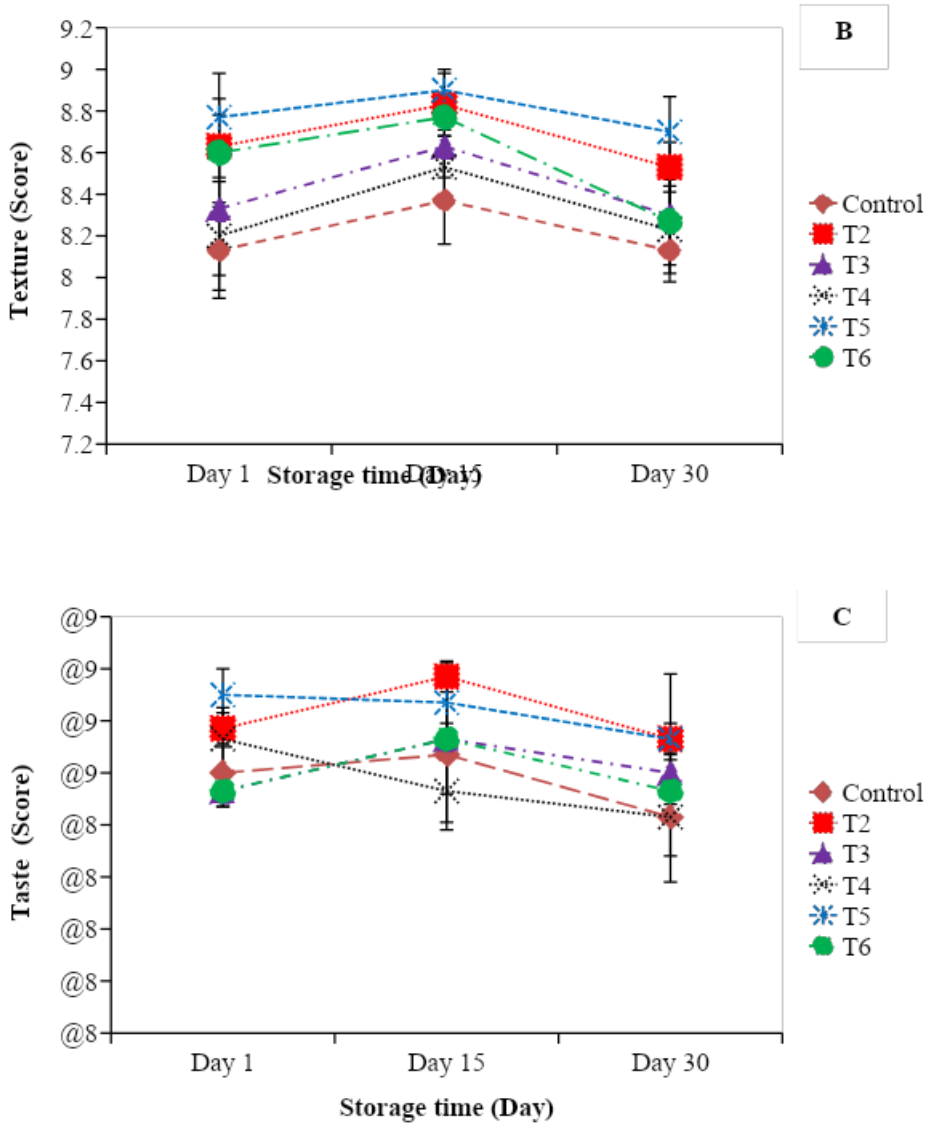

FIG 1. Sensory scores (color, texture and taste) of different treatments of low fat ultrafiltrated cheese during cold storage

The comparison of the averages obtained from the sensory evaluation of ultra-refined low-fat cheese samples is shown in Figure 1. As mentioned above, enzyme treatment, except aroma and taste, and addition of gum, except aroma, had a significant effect on other sensory characteristics of cheese samples. As can be seen in Figure 1, in connection with the evaluation of cheese color, the evaluators gave the lowest color score to the sample containing the highest level of kappa-carrageenan (treatments 3 and 6); While samples without gum (control sample and treatment 4) received the highest color score (Figure 1-A). Contrary to the characteristic of color, increasing the concentration of gum causes a significant decrease in the score of the samples, adding gum up to a concentration of 0.03% improves the taste and texture of the samples; So that this sample obtained the highest taste (8.83) and texture (8.73) scores compared to the control and the sample containing 0.06% gum.(01/0p<). In other words, according to the results recorded by the evaluators, the use of 0.03% gum (treatments 2 and 5) improved the texture and taste of low-fat cheese, but at higher levels (treatments 3 and 6), the texture and taste of cheese decreased. found (Figure 1-B and 1-C).

Based on figures (1-B) at constant amounts of gum, samples containing enzyme received a higher score than samples without enzyme in the texture

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evaluation section. EventuallyAccording to the sensory evaluation results, low-fat cheese treated with transglutaminase enzyme and containing 0.03% kappa-carrageenan (treatment 5) was selected as the best sample according to the evaluators.. Other researchers also reported that the addition of gum improves the texture of low-fat cheese [19] and also improves the sensory characteristics of the cheese [20] due to the increase in the water retention capacity of the product. Rostam Abadi et al. (2017) reported that in the production of low-fat extra-purified Iranian white cheese, with the increase in the amount of Persian gum in the formulation, the scores related to the texture of the samples also increased.]21[. Torabi et al. [16] showed that the optimal sample of synbiotic ultra-refined low-fat cheese containing inulin and cottage cheese compounds and treated with enzymesTG Microbial had higher tissue quality than probiotic and non-probiotic control samples in all storage periods.earned Danesh et al[22] also reported thatBy combining the optimal amountBlue cheese protein concentrateAlong with the enzyme treatment, the sensory characteristics of the low-fat sample improved significantly, and as a result, no significant difference was observed in terms of color and appearance, texture, and overall acceptance between the high-fat sample and the optimal sample.

Also, the results of this research showed that with the passage of storage time up to the 15th day, all the sensory characteristics increased and continued and until the end of the storage time, these scores decreased significantly. Although there was no significant difference in the quality of aroma, color and taste of the product on the 1st and 15th days of storage, these scores decreased significantly at the end of 30 days of storage. Regarding the quality of the appearance and texture of the samples, the results showed that there was no significant difference on the 1st and 30th days of storage, but on the 15th day, this score was significantly higher than other periods of storage. A decrease in the sensory quality of cheese at the end of the storage period has also been reported by other researchers[16 and 23].

2-3- Tissue stiffness

The results of analysis of variance (Table 2) show that all three variables, amount of gum, enzyme, and storage time had a significant effect on the hardness of the samples.(01/0p<). Figure 2 also shows the results related to the texture of low-fat processed cheese samples during different treatments. Based on the obtained results, the addition of gum and enzyme treatment had different effects on the firmness of cheese texture. The addition of gum decreased the hardness (treatments 2 and 3, respectively) and the addition of enzyme resulted in the increase of cheese hardness (treatments 4, 5 and 6). In fact, enzyme treatment by creating intramolecular and intermolecular transverse connections between milk proteins, creates a strong three-dimensional network through casein proteins and provides minimum space to retain water molecules, and thus increases the firmness of the product texture.[24 and 10]. Increasing texture hardness in low-fat cheese]10[Cottage cheese]25[and yogurt]26[It has been reported as a result of treatment with transglutaminase enzyme.

Also, with the passing of the storage time, first the stiffness of the tissue increased slightly ($p>0.05$) and then at the end of the storage time, it decreased significantly ($p<0.05$). LikelyThe main reason for increasing or decreasing the firmness of cheese during storage can be related to the amount of moisture in the cheese. Parallel to the increase in storage time, the moisture content of different samples decreased in the first 15 days and then increased significantly until the end of 30 days of storage (results not shown). According to the trend of cheese moisture changes during the storage period in the present study, Torabi et al. [16] reported similar results in Farapaloude white cheese and Alves et al. [27] reported similar results in synbiotic raw cheese. Javaindeh et al. [23] found a significant decrease in the moisture content of different samples of Iranian white cheese containing salt during the ripening period due to the osmotic pressure caused by the placement of the cheese clot in the brine solution and the diffusion of salt into the cheese matrix and the outflow of water from the cheese tissue. they said. However, these researchers reported that the moisture content of white cheese samples treated with enzymesTG and

containing cheese protein isolate during 60 days of storage significantly decreased and increased the firmness of the cheese. In addition, the decrease in cheese moisture until the middle of the storage period can be due to the increase in syneresis resulting from the increase in cheese acidity [28]. The increase in moisture in cheese samples at the end of the storage period can also be related to the increase in proteolysis reactions and the increase in the hydrophilic properties of cheese proteins and the increase in the water storage capacity [16].

In general, according to histological results, the softest sample was related to treatment 3 (containing 0.06% gum and no enzyme) and the hardest sample was related to treatment 4 (without gum and containing 0.5 enzyme units per gram of protein). Therefore, these results showed well that by adding kappa-carrageenan hydrocolloid, the problem of increasing the stiffness of low-fat cheese texture, which is caused by reducing its fat content, can be well overcome. In fact, by adding hydrocolloids in the cheese formulation, the water retention capacity in the cheese matrix is increased, and in this way, by simulating the role of fat in the product, a low-fat product with a suitable texture can be produced.]20[. In confirmation of these results, Rafiei et al. (2022) reported that the use of rice starch hydrocolloid reduced the hardness of low-fat mozzarella cheese and could also cause favorable sensory properties in low-fat cheese.]29[. Jovandeh et al[30] reported that with the incorporation of fermented whey protein concentrate in feta cheese formulation, due to the entrapment of denatured whey proteins in the casein protein matrix, the moisture level and efficiency of the product increase significantly.

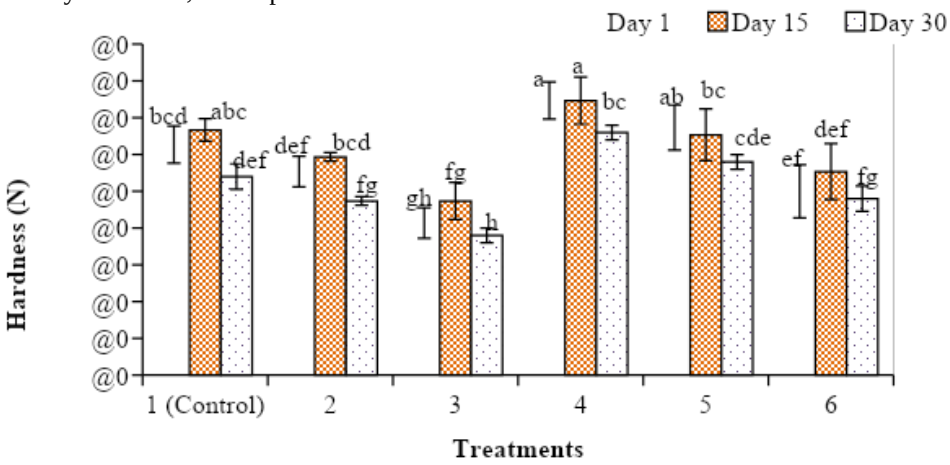

FIG 2. Hardness of different treatments of low fat ultrafiltrated cheese

3-3- Total count of lactic acid bacteria

Based on the results of analysis of variance in Table 2, the addition of transglutaminase enzyme and also the passage of time had a significant effect on the population of lactic acid bacteria (01/0p<); While the amount of kappa-carrageenan had no significant effect on this factor (05/0p>). As in Figure 3-A It can be seen that enzyme treatment has reduced the number of lactic acid bacteria (treatments 4, 5 and 6). After 15 days of producing the samples, the population of lactic acid bacteria fromlog cfu/g 7/33 tolog cfu/g 8/21 increased, but after that and after 30 days of production, the population of these bacteria increasedlog cfu/g 7/21 decreased (Figure 3-B). In accordance with the results of the present study, Pham et al. (2021) also reported that with the addition of transglutaminase enzyme as well as the passage of time, the population of lactic acid bacteria in cheese decreased [31]. Transglutaminase enzyme has no toxic effect on lactic acid bacteria, but it is possible that the reason for this decrease is the delay in the growth of lactic acid

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bacteria; Because peptides with low molecular weight as well as amino acids required by bacteria have crosslinked with transglutaminase enzyme and as a result have become inaccessible to lactic acid bacteria [32].

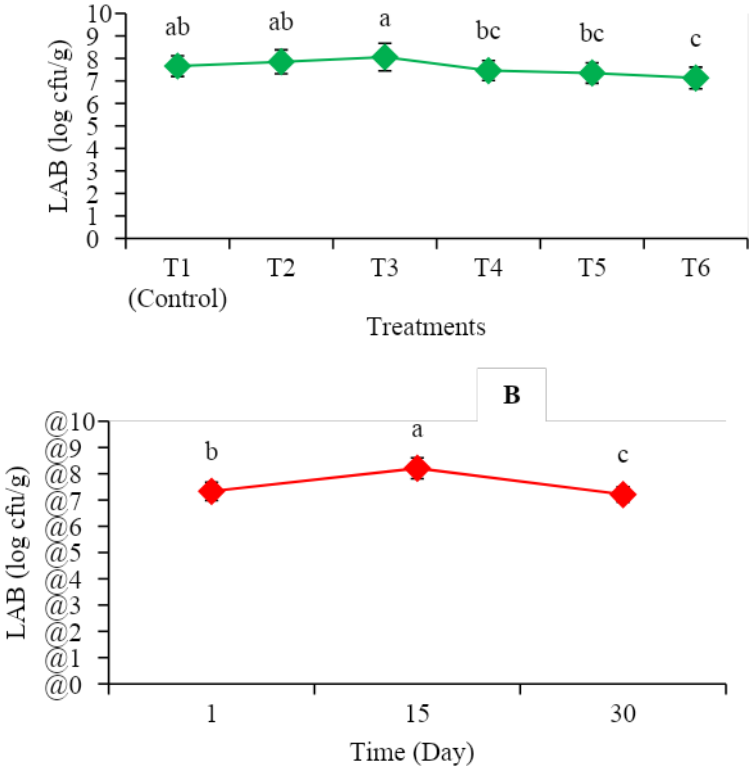

FIG 3. The effect of different treatments (A) and storage time (B) on the count of lactic acid bacteria (LAB)

4 - Conclusion

Since the reduction of fat in dairy products causes negative effects on the appearance, texture and sensory characteristics of these products, the use of an appropriate level of hydrocolloids as a substitute for fat as well as the correct use of transglutaminase enzyme is a very useful solution to produce health-oriented dairy products with sensory and The texture is desirable. In addition, nowadays, the role of the microbial flora of consumed fermented foods, especially fermented dairy products, has been proven to improve people's health and prevent the occurrence of various diseases. The results of this research showed that although enzyme treatment increased the firmness of ultra-refined low-fat cheese, but the combination of enzyme treatment with the addition of gumKappa-carrageenan improved the quality of cheese (texture firmness and sensory characteristics). In addition, although the addition of gum had no effect on the count of lactic acid bacteria, the enzyme treatment caused a significant reduction in the number of proteins by creating cross-links between the proteins and preventing their access to the mentioned bacteria. However, no significant difference was observed between the sample treated with transglutaminase enzyme and containing 0.03% gum and other samples. SoFinally, based on the findings of this research,The sample treated with transglutaminase enzyme and containing 0.03%

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kappa-carrageenan was selected as the best sample of ultra-low-fat cheese in terms of sensory characteristics, texture firmness and microbial results..

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تأثیر آنزیم ترانس‌گلوتامیناز و صمغ کاراگینان بر خواص حسی، بافت و ویژگی‌های میکروبی پنیر فراپالوده کم‌چرب

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چکیده

با توجه به این‌که چربی تأثیر زیادی بر ویژگی‌های حسی پنیر نظیر ظاهر، طعم و بافت آن دارد، کاهش آن موجب تغییرات اساسی در ویژگی‌های حسی و ساختاری محصول می‌شود. لذا با استفاده از ترکیبات جایگزین چربی می‌توان علاوه بر تولید پنیری سالم‌تر، محصولی با کیفیت محصول مشابه پرچرب تولید کرد. در پژوهش حاضر نمونه‌های پنیر کم‌چرب (۸ درصد چربی) به روش فراپالایش تولید شدند. جهت تولید نمونه‌ها از صمغ کاپا-کاراگینان در ۳ سطح (۰، ۰/۰۳ و ۰/۰۶ درصد) و آنزیم ترانس‌گلوتامیناز در ۲ سطح (صفر و ۰/۵ واحد آنزیم به ازای هر گرم پروتئین) استفاده شد. نمونه شاهد کم‌چرب فاقد آنزیم و صمغ به‌عنوان شاهد کم‌چرب در نظر گرفته شد. کلیه‌ی نمونه‌ها در مدت ۱، ۱۵و۳۰ روز پس از تولید، از نظر ویژگی‌های حسی، بافتی و میکروبی مورد ارزیابی قرار گرفتند. براساس نتایج، افزودن صمغ سبب کاهش و در مقابل افزودن آنزیم موجب افزایش سفتی پنیر گردید ($p<0/01$). استفاده از مقدار ۰/۰۳ درصد صمغ سبب بهبود طعم و بافت پنیر کم‌چرب گردید اما در سطوح بالاتر (۰/۰۶ درصد)، خواص حسی پنیر کاهش معنی‌داری یافت. همچنین نتایج نشان داد که تیمار آنزیمی سبب کاهش جمعیت باکتری‌های اسید لاکتیک گردید اما افزودن صمغ تأثیری نداشت ($p>0/05$). به‌طور کلی، با گذشت زمان نگهداری و تا روز پانزدهم، تمامی امتیازات حسی، سفتی پنیر و تعداد باکتری‌های اسید لاکتیک افزایش یافت اما در انتهای مدت ۳۰ روز نگهداری تمامی مقادیر مذکور به‌طور معنی‌داری کاهش یافتند. در نهایت با توجه به نتایج ارزیابی حسی و بررسی بافت نمونه‌ها، پنیر کم‌چرب تیمار شده با آنزیم ترانس‌گلوتامیناز و حاوی ۰/۰۳ درصد کاراگینان به عنوان بهترین نمونه انتخاب شد.