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Evaluation of the effect of oat extract and microbial transglutaminase enzymatic treatment on phenolic compounds, sensory properties and viability of probiotic bacteria in synbiotic yogurt

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ABSTRACT

Functional foods provide important physiological functions by providing bioactive compounds (such as probiotics, prebiotics, and antioxidants) that can contribute to the prevention of common diseases. Oat extract (OE) contains bioactive compounds (fiber beta-glucan, phenols, and tocotrienols) and prebiotics that stimulate the growth of beneficial intestinal bacteria and enhance anti-inflammatory and metabolic properties. However, its addition to yogurt may reduce sensory quality. In the meantime, microbial transglutaminase (MTg) enzymatic treatment is one of the proposed ways to improve the sensory properties of the product by modifying the texture and consistency of yogurt. Therefore, in this study, the effect of enzymatic treatment (0 and 0.02%) of MTg on some properties of synbiotic yogurt containing different concentrations of 0, 10, 20 and 30% OE was investigated during a 21-day storage period. The results showed that addition of OE improved total phenolic content (TPC), probiotic bacteria count and texture score of yogurts, while reducing the color and flavor scores of the samples. TG treatment also increased TPC content and product color and texture scores and reduced the number of probiotic bacteria. With the passage of storage time, although the TPC content increased, the number of probiotics and the sensory properties score of yogurts decreased. However, number of probiotic bacteria was determined to be more than the standard value (7 log CFU/g) after 21 days of storage. Based on the results of this study, the synbiotic yogurt sample treated with 0.02% TG enzyme and containing 20% barley extract was identified as the best yogurt sample.

1- Introduction

Yogurt has become one of the most popular foods in the world due to its unique nutritional value and pleasant taste. Currently, yogurt is divided into two types, non-probiotic and probiotic, based on its probiotic status. Non-probiotic yogurt contains only standard cultures (traditional starters *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*), while probiotic yogurt is fortified with probiotic strains such as Bifidobacterium and *Lactobacillus acidophilus* [1]. A new range of dairy products, including synbiotic yogurts containing probiotics and prebiotics, is gaining popularity. In dairy products, lactic acid bacteria are known as probiotics, which are capable of producing large amounts of bactericidal proteins. As a result, synbiotic yogurt has gained popularity as a functional food that promotes human health [2]. The World Health Organization defines probiotics as live microorganisms that, when consumed in adequate amounts, confer health benefits on the host, such as enhancing the intestinal microbiota, reducing inflammation and oxidative stress, improving metabolism, and enhancing immunity [3]. Probiotics have been shown to be effective in treating intestinal and systemic disorders, reducing lactose intolerance, maintaining serum cholesterol levels, and having anticancer, antimutagenic, and antihypertensive activities [4-6]. In recent years, Lactobacillus and Bifidobacterium species have been used as the main types of probiotics in functional foods [7]. The International Dairy Federation (IDF) recommends that the number of active probiotics should reach at least 10^7 CFU/g to be able to perform their physiological function in the digestive tract [8]. The benefits of probiotics are strongly influenced by the viability of microorganisms, so probiotics must be stable and active both in the product and in the host body. Therefore, probiotics must survive during the production and storage process and pass through the digestive tract, without affecting the physicochemical and sensory properties of the product [9]. However, probiotics are sensitive to pH, bile salts, hydrogen peroxide, storage temperature, etc. [10, 11]. Various methods have been proposed to increase the survival rate of probiotics in dairy products, including the selection of resistant strains, microencapsulation of probiotic strains, addition of micronutrients and prebiotics, and improvement of the buffering capacity of yogurt against pH changes [12-14].

Prebiotics are non-digestible compounds that promote the growth of probiotics in the gastrointestinal tract [15]. In contrast, these compounds do not affect the growth of pathogenic microorganisms [9]. Other benefits of prebiotics for the host include binding to harmful toxins and preventing the adhesion of pathogens to the intestinal epithelium [16, 17]. Glucans, fructans, and mannans are the most commonly used prebiotics in the food

industry [18]. Synbiotics are defined by the International Scientific Society for Probiotics and Prebiotics (ISAPP) as “a mixture of live microorganisms and substrates that are selectively utilized by the host microorganisms and provide health benefits to the host” [19]. Krasaekoopt and Watcharapoka [18] observed an increase in the survival of *Lactobacillus acidophilus* (1.1 log cycle) and *Lactobacillus casei* (0.4 log cycle) encapsulated with galactose oligosaccharides in yogurt. According to these researchers, the addition of prebiotics such as galactooligosaccharides or inulin during the microencapsulation process of probiotics increases the resistance of these microorganisms to the simulated digestive system and enhances the growth of probiotics in yogurt and orange juice.

Among the various types of prebiotic foods, whole oats are considered a potential source of prebiotic compounds that have positive effects on human gut health, including the ability to lower blood cholesterol and antioxidant and anticancer activities [20]. In yogurt, oats are also a suitable prebiotic ingredient because they contain beta-glucans and resistant starches that selectively stimulate beneficial gut bacteria such as Bifidobacterium and Lactobacillus, potentially improving gut health, immune function, and metabolic markers when consumed regularly. Adding oats to yogurt can also improve texture while providing soluble fiber that slows gastric emptying and increases satiety [20]. Several studies have shown that oat-based substrates support the growth and activity of probiotic strains in fermented dairy matrices and can increase the production of short-chain fatty acids during colonic fermentation, which is associated with colonic health and systemic benefits [21-23].

Microbial transglutaminase (MTg) has been approved as a GRAS substance by the US Food and Drug Administration (FDA) since 1998 under the number “GRN 000095” [24]. The addition of this safe enzyme to yogurt formulations can help develop its structure and texture through protein cross-linking reactions [25]. The high flexibility of casein proteins, together with little or no secondary structure, makes these proteins more easily cross-linked by transglutaminase than whey proteins with a dense globular structure. Therefore, transglutaminase induces intermolecular polymerization of caseins and network formation, which leads to significant changes in the functional properties of yogurt [26, 27].

Considering the aforementioned materials and the importance of producing functional products with appropriate functional characteristics, this study evaluated the effects of adding oat extract and applying microbial transglutaminase enzymatic treatment on phenolic compounds, the viability of probiotic bacteria, and the sensory properties of yogurt, with the objective of developing a synbiotic product.

2- Materials and methods

2-1- Raw materials

In this study, whole oat flour produced by Arena 111 Company located in Babolsar, Mazandaran Province was used. To prepare the barley extract, the mixture of barley flour and water in a ratio of 1:5 was mixed well with a mixer and then the resulting extract was separated using a filter cloth. Also, the enzyme transglutaminase (MTg) of microbial origin, produced by Ingredients BDF Natural Company of Spain with an enzymatic activity of 100 units per gram of powder, was used. Yogurt starter powder of DVS type (Chr Hansen, ABY-10, made in Denmark), containing yogurt starter bacteria (*Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus thermophilus*) and probiotic bacteria (*Lactobacillus acidophilus* LA5 and *Bifidobacterium bifidum* BB-12) was purchased from the representative of Christian Hansen Denmark in Iran. Yogurt samples were prepared using fresh low-fat milk (1.5% fat).

2-2- Method for preparing probiotic yogurt samples

Pasteurized low-fat milk with 1.5% fat and 8% dry matter was used to prepare yogurt samples. Initially, the milk was thermally processed at 90°C for 10 minutes. Barley extract was also heated separately under the aforementioned conditions and added to the milk at different levels of 0, 10, 20, and 30%. The mixture was cooled to 45°C. Transglutaminase enzyme was then added at 0.02% (w/v), and a probiotic strain and yogurt starter were each added at 0.05% (w/v) (total 0.10% w/v). In the next step, the yogurts were packaged in 100-gram containers and after incubation for about 3 hours, the samples were transferred to a refrigerator at 5°C and stored overnight. Finally, the yogurt samples were evaluated on days 1, 11, and 21 of storage [28].

2-3- Measurement of phenolic compounds

Measurement of total phenolic compounds of the yogurt samples were performed according to the method presented by Marinova et al. [29]. For this purpose, 2.5 g of yogurt sample was weighed into a centrifuge tube and 2.5 ml of normal hexane was added to it. After mixing with a vortex for one minute, 2.5 ml of methanol-water solution with a volume ratio of 80:20 was added to the mixture and the sample was centrifuged for 5 minutes at a speed of 5000 rpm. The upper oily phase was separated using a syringe and transferred to a new centrifuge tube. 2.5 ml of methanol-water solution was added again and after vortexing for one minute, it was centrifuged as in the previous step. This process was repeated once more and finally, the aqueous and oily phases were collected separately. The resulting aqueous phase was combined in a 50-mL volumetric flask and, after filtering, 2.5 mL of Folin-Ciocalteu reagent was added to it. After 3 minutes, 5 mL of saturated sodium carbonate solution

was added to the mixture and the final volume was made up to 50 mL with distilled water. The sample was kept in the dark at room temperature for one hour and then its absorbance was read at a wavelength of 725 nm relative to the control sample. Finally, the total amount of phenolic compounds was calculated in terms of micrograms of gallic acid equivalents per gram of sample [30].

2-4- Microbial evaluation

MRS agar was used to count lactic acid bacteria. Also, to assess the viability of probiotic bacteria, MRS agar containing bile salts was used. The mixed culture method (Pour plate) was used as the main culture technique. After performing serial dilutions, the plates were placed in an incubator at 37°C and kept in an incubator for 48 hours according to the manufacturer's instructions. The results of the bacterial count were reported as an average and in terms of colony forming units per gram (CFU/g). To perform the dilution, first one gram of each yogurt sample was mixed with 9 ml of physiological saline solution. Then, subsequent dilutions were prepared by transferring one ml of each dilution to 9 ml of physiological saline solution [31].

2-5- Sensory assessment

For sensory evaluation, the most important organoleptic properties of yogurt samples, including color, aroma, texture, and taste, were examined and the samples were compared using a 9-point hedonic test. In this way, the samples were randomly given to the evaluators along with a survey form to evaluate the aforementioned sensory properties, and they expressed their opinions based on the scoring of each of the sensory properties. Before evaluation, the samples were taken out of the refrigerator for 30 minutes and kept at room temperature so that the temperature of all samples was the same during the evaluation and did not affect the sensory results [32].

2-6- Data Analysis

This study was conducted as a factorial study in a completely randomized design with three replications. In order to investigate the effect of enzymatic treatment with microbial transglutaminase at two levels (0 and 0.02%) and oat extract at four levels (0, 10, 20 and 30%) on the variables under study, SPSS statistical software (version 24) was used. Data analysis was performed using Duncan's test at a confidence level of 95%.

3- Results and discussion

3-1- Total phenols

According to the results of analysis of variance (Table 1), the simple effects of microbial transglutaminase enzyme, oat extract and storage time ($p<0.001$) and the interaction effect of enzyme-oat extract-time had a significant effect ($p<0.05$) on the total phenolic content of yogurt samples. However, other interaction effects (enzyme-oat extract, time-enzyme

and time-oat extract) were not significant in this regard ($p<0.05$).

Table 1 Analysis of variance for the effect of Oat extract, Transglutaminase enzyme and time on the physicochemical properties and acceptability of yogurt samples during storage time

Variable sources	Mean square							
	df	Phenol	Odor	Color	Taste	Texture	*B. bifidum*	*L. acidophilus*
Oat Extract	3	0.320^{***}	0.004^{ns}	0.502^{***}	0.223^{*}	0.094^{*}	0.701^{***}	0.427^{***}
Enzyme	1	0.300^{***}	0.027^{ns}	0.179^{**}	0.000^{ns}	0.443^{***}	0.417^{***}	1.868^{***}
Time	2	0.240^{***}	4.256^{***}	2.878^{***}	15.895^{***}	1.970^{***}	0.480^{***}	3.460^{***}
Enzyme*Oat Extract	3	0.000^{ns}	0.014^{ns}	0.006^{ns}	0.015^{ns}	0.001^{ns}	0.014^{***}	0.012^{ns}
Time*Oat Extract	6	0.002^{ns}	0.015^{ns}	0.008^{ns}	0.013^{ns}	0.006^{ns}	0.006^{***}	0.002^{ns}
Time*Enzyme	2	0.000^{ns}	0.075^{ns}	0.126^{**}	0.007^{ns}	0.001^{ns}	0.001^{ns}	0.044^{ns}
Time*Enzyme* Oat Extract	6	0.002^{*}	0.009^{ns}	0.004^{ns}	0.007^{ns}	0.000^{ns}	0.002^{*}	0.006^{ns}
Error	24	0.040	0.068	0.017	0.068	0.025	0.001	0.021
CV (%)	-	2.220	5.010	5.170	5.290	4.860	0.260	0.330

ns, *and ** and *** means non-significant, and significant at 5%, 1% and 0.1%.

The effect of oat extract concentration on the total phenol content of yogurt is shown in Figure 1-a. According to this figure, with increasing the percentage of oat extract in the samples, the total phenol content of yogurt increased significantly ($p<0.001$); so that the application of 10, 20 and 30% oat extract increased the total phenol content of yogurt by 6.72, 16.8 and 26.05%, respectively, compared to the control sample (without oat extract). This observation is generally consistent with the findings reported by Alqahtani et al. [33]. These researchers investigated the effect of adding oat flour to goat milk yogurt on the total phenol content. The results of this study showed that with increasing the amount of oat flour, the total phenol content increased significantly, especially up to the seventh day of storage. As can be seen in Figure 1-b, the total phenol content of yogurt increased by 10.24% compared to the control sample (without enzyme treatment). It has been shown that phenolic compounds can easily bind to proteins. Interactions between phenolic compounds and proteins can be formed through covalent bonds and/or non-covalent interactions through hydrophobic, electrostatic, van der Waals, and hydrogen bonding forces [34]. On the other hand, enzymatic treatment with MTg reduces the availability of amino acids due

to covalent interactions and cross-linking between amino acids (especially lysine and glutamine, which are MTg substrates). Thus, the possibility of amino acids associating and binding with phenolic compounds decreases [35], which can lead to an increase in the concentration of free phenolic compounds (fewer covalent bonds) in yogurt.

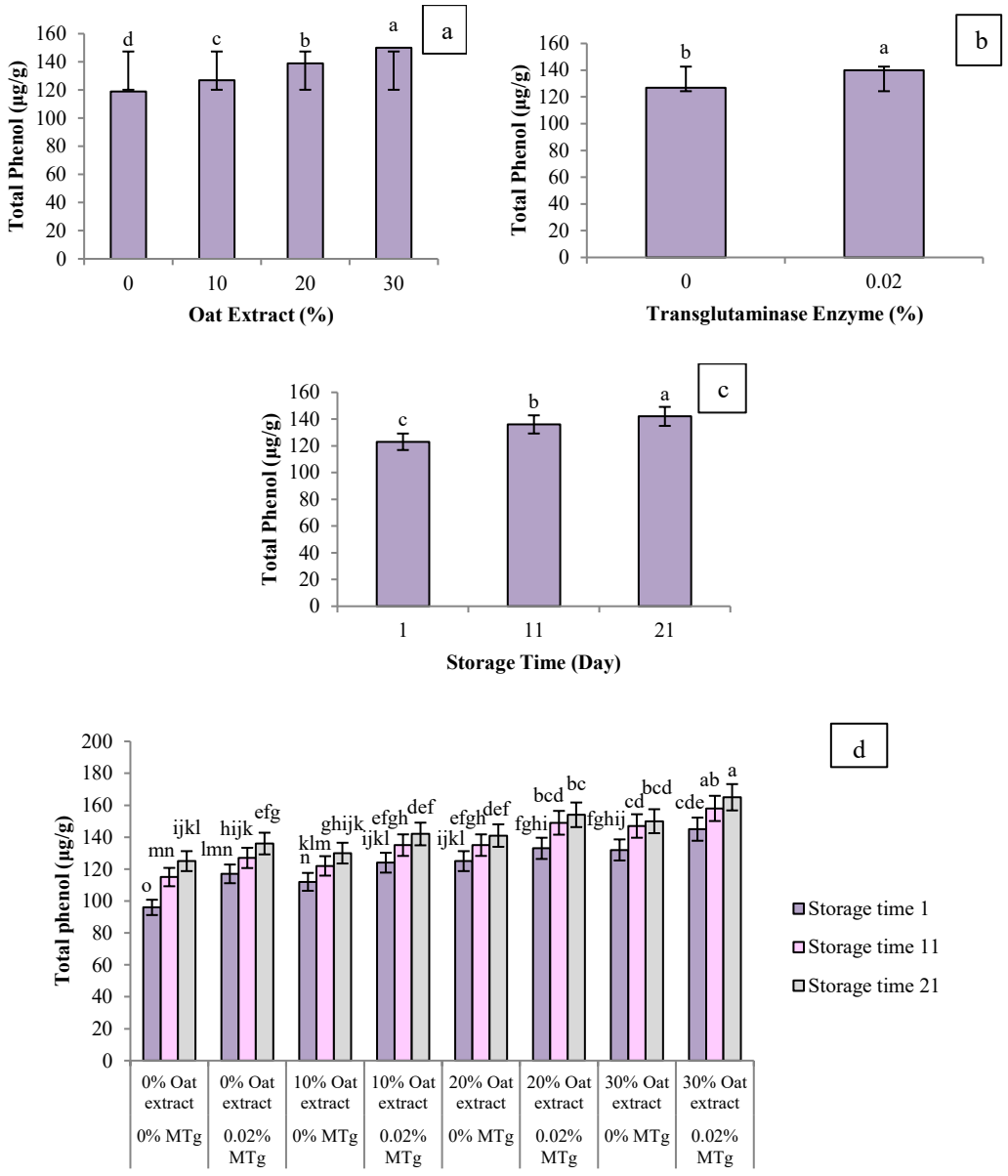


Figure 1. The effect of Oat extract (a), Microbial transglutaminase enzyme (b) Storage time (c) and their interaction (d) on the Total Phenol of yogurt samples

As can be seen in Figure 1-c, the total phenol content of yogurt increased with increasing storage time, with the highest value (142 µg/g) observed on day 21 of storage and the lowest (123 µg/g) observed on day 1 of storage. Overall, from day 1 to day 21 of storage, the total phenol content of yogurt samples increased by 15.45%. The interaction effect of enzyme-oat extract-storage time on the total phenol content of yogurt is shown in Figure 1-d. The highest total phenol (165 µg/g) was observed in the sample containing 0.02% transglutaminase enzyme and 30% oat extract on day 21 of storage, and the lowest (96 µg/g) was observed in the yogurt sample without oat extract and without enzyme treatment on day 1 of storage. In a study conducted by Jooyandeh et al. [36], the total phenolic content of functional yogurt samples containing bell pepper extract was investigated. These researchers reported that the addition of concentrated bell pepper extract significantly increased the total phenolic content of yogurt samples. Also, based on the results of this study, a significant increase in the total phenolic content of samples containing bell pepper extract was observed after 11 days.

3-2- Investigation of probiotic count

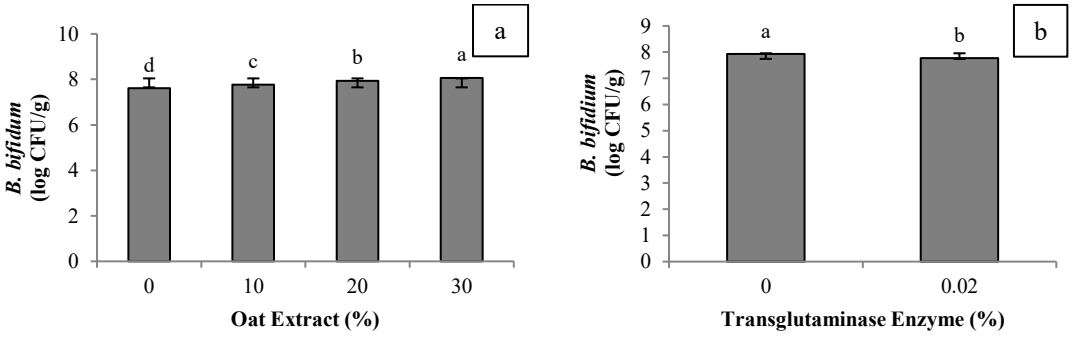
3-2-1- *Bifidobacterium bifidum*

According to the results of analysis of variance (Table 1), all three variables MTg enzyme, oat extract and storage time had a significant effect on the population of *Bifidobacterium bifidum* BB-12 in yogurt. Regarding the interaction effects, the effect of enzyme-oat and storage time-oat ($p<0.001$) and enzyme-oat extract-storage time ($p<0.05$) had a significant effect on the population of Bifidobacterium in yogurt. The interaction effect of storage time-enzyme also had no significant effect on this factor ($p<0.05$).

The effect of oat extract concentration on Bifidobacterium is shown in Figure 2-a. According to this figure, with increasing percentage of oat extract in the samples, the population of Bifidobacterium increased; so that the use of 10, 20 and 30% oat extract increased Bifidobacterium by 1.91, 4.07 and 5.59%, respectively, compared to the control sample. The reason for the increase in Bifidobacterium survival with increasing oat extract concentration is related to the prebiotic property of oat extract. Oat extract can be used as a source of carbon and energy by probiotic bacteria and increases their survival in the product [37]. In accordance with this study, Cichońska and Ziarno [38] stated that the addition of prebiotic and carbohydrate compounds to fermented soy milk improves the growth of lactic acid bacteria. Zhang et al. [39] in another study investigated the antioxidant activity and prebiotic effect of oat phenolic compounds. The results of this study showed that oat phenolic compounds increased the growth of Bifidobacterium and Lactobacillus/Enterococcus species. They also reported that there was a positive correlation between the

antioxidant activity and prebiotic effect of oat phenolic compounds. However, Mårtensson et al. [40] reported that the presence of oat extract did not affect the number of lactic acid bacteria.

Figure 2-b shows the effect of transglutaminase enzyme on probiotic bacteria *Bifidobacterium bifidum*. As can be seen in the figure, the amount of bifidobacterium decreased from 7.92 log CFU/g to 7.77 log CFU/g with the use of the enzyme. Although the amount of bifidobacterium decreased with increasing enzyme concentration, ultimately their remaining number in the enzyme-treated sample was also at an acceptable level and above the standard. According to the defined standard, for the health-promoting properties of probiotic products to affect humans, their number should be 10^6-10^7 CFU per gram of product [41]. Faergemand and Qvist [42] stated that the high level of cross-linking created by the enzyme could possibly reduce the availability of low molecular weight peptides for feeding lactic acid bacteria and consequently reduce their growth. In a study by Farnworth et al. [43], the viability of *Lactobacillus casei*, *Bifidobacterium bifidum*, and *Lactobacillus acidophilus* bacteria in yogurt samples treated with MTg enzyme remained constant during 8 weeks of storage. Ozrenk [44] reported that the growth rate of yogurt bacteria in the presence of transglutaminase enzyme decreased depending on the enzyme concentration. A significant decrease in the number of lactic acid bacteria in cheese due to the use of transglutaminase enzyme was also reported in the article by Bohmid et al. [45].



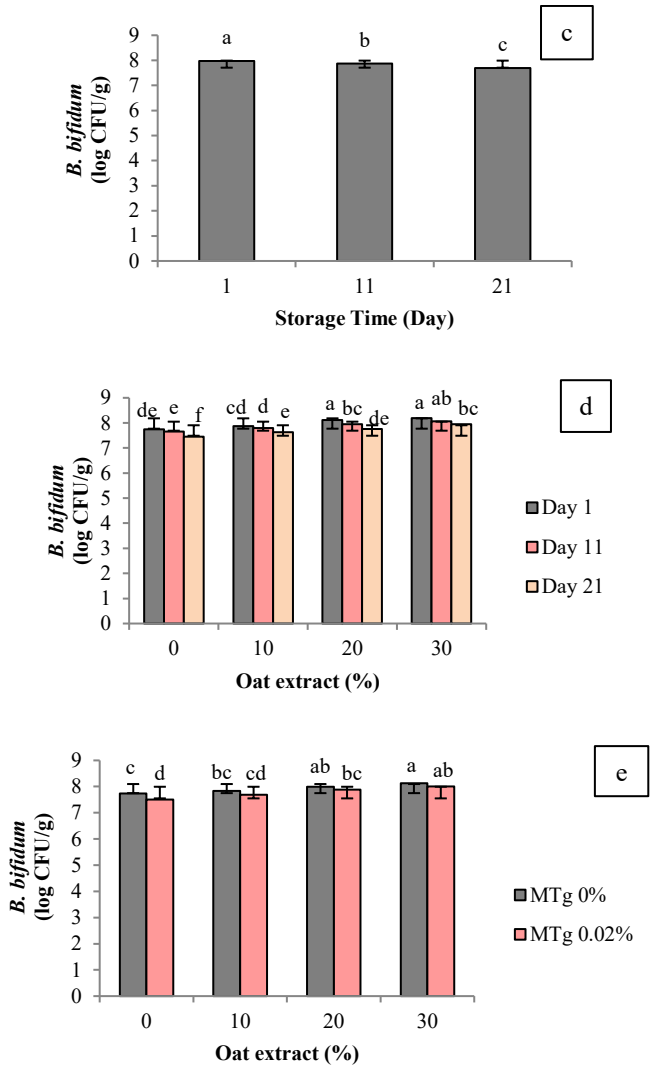


Figure 2. The effect of Oat extract, Microbial transglutaminase enzyme, Storage time and their interaction on the *B. bifidum* count of yogurt samples

The effect of storage time on bifidobacterium is seen in Figure 2-c. According to this figure, with increasing storage time, the amount of bifidobacterium decreased; so that the highest bifidobacterium (log CFU/g 7.98) was on the first day of storage and the lowest (log CFU/g 7.70) was on the 21st day of storage. It should be noted that despite the decrease in bifidobacterium at the end of the storage period, their remaining number in the product was ultimately at an acceptable level. The interaction effect of time-oat extract on bifidobacterium is shown in Figure 2-d. The highest bifidobacterium (log CFU/g 8.18) was related to the sample containing 30% oat extract and the first day of storage, and the lowest bifidobacterium (log CFU/g 7.46) was related to the sample

without oat extract on the 21st day of storage. In general, the amount of Bifidobacterium decreased during storage, and increased with increasing percentage of oat extract. Alqahtani et al. [33] reported that the number of probiotic bacteria *B. bifidum* and *L. acidophilus* remained above the acceptable therapeutic level for probiotics during storage in yogurt samples containing oat flour, and in fact, the addition of oat flour significantly increased the survival of probiotics. These researchers attributed this to the presence of beta-glucan and high nutrients in oats. The interaction effect of enzyme-oat extract on the population of Bifidobacterium is shown in Figure 2-e. The highest amount of bifidobacterium (log CFU/g 8.13) was observed in the sample containing 30% oats and without enzyme, and the lowest amount of bifidobacterium (log CFU/g 50.7) was observed in the sample containing 0.02% enzyme and without oat extract. In general, the amount of bifidobacterium in the samples without enzyme was higher than in the samples containing enzyme.

3-2-2- *Lactobacillus acidophilus*

According to the results of analysis of variance (Table 1), the main effects of all three variables, transglutaminase enzyme, oat extract and storage time, had a significant effect at the 0.1% probability level on the *Lactobacillus acidophilus* LA5 population of yogurt ($p<0.001$). The interaction effects of these variables had no significant effect on the probiotic acidophilus bacteria of yogurt samples ($p<0.05$).

According to Figure 3-a, the highest *Lactobacillus acidophilus* (log CFU/g 8.32) was observed in the sample containing 30% oat extract and the lowest (log CFU/g 7.88) was observed in the sample without oat extract. Glibowski and Skrzypczak [46] stated that oat extract could improve the survival of *Lactobacillus acidophilus* and *Bifidobacterium lactis* bacteria in synbiotic ice cream due to its prebiotic effect; So that the decrease in the number of probiotic cells in samples treated with oat extract was less than the control sample during 90 days of storage. Also, Mårtensson et al. [40] reported in the production of synbiotic yogurt that by adding oat extract to milk, the survival rate of *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* increased. In Figure 3-b, the effect of the transglutaminase enzyme on *Lactobacillus acidophilus* is shown. As can be seen in this figure, the amount of *Lactobacillus acidophilus* decreased from 8.31 log CFU/g to 7.92 log CFU/g with the use of the transglutaminase enzyme. In fact, similar to the effect on the population of bifidobacteria, MTg enzymatic treatment caused a significant decrease in the population of acidophilus compared to the control sample ($p<0.001$). Although the viability of these probiotic bacteria decreased with increasing enzyme concentration, the final amount of these bacteria was also higher than the standard.

In addition, as shown in Figure 3-c, the number of *Lactobacillus acidophilus* bacteria increased until the 11th day and then decreased. The reason for the decrease in the number of *Lactobacillus acidophilus* at the end of the storage period is probably due to the decrease in the nutrients required by these bacteria and the increase in acidity [31].

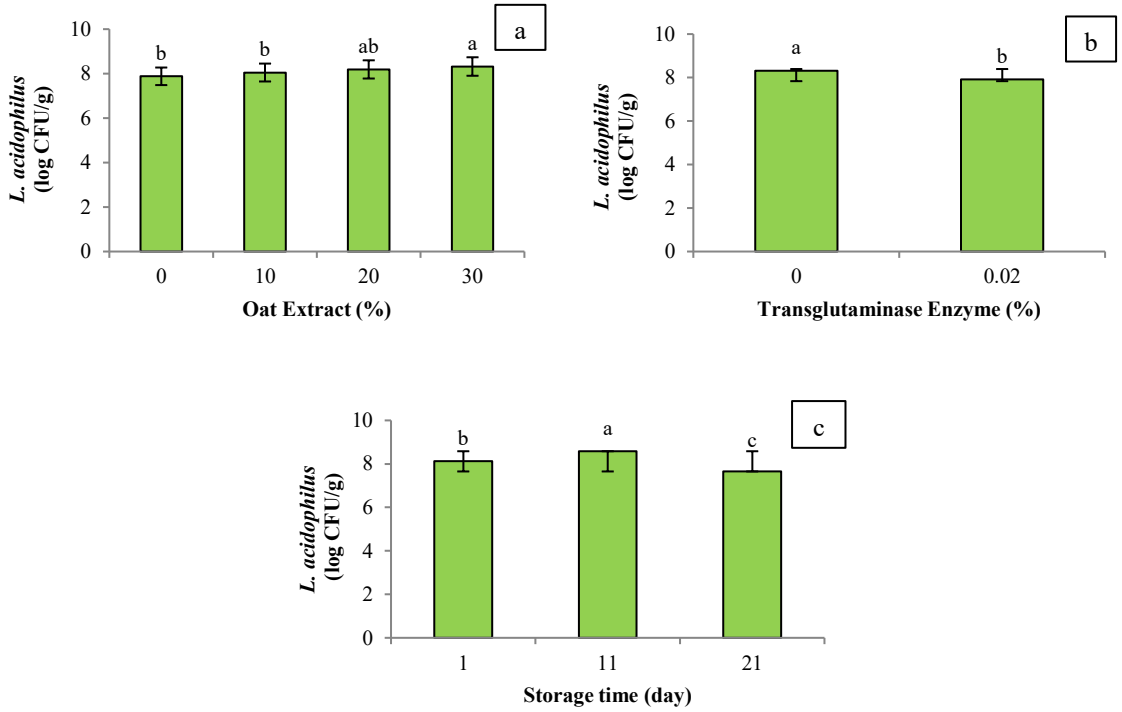


Figure 3. The effect of Oat extract, Microbial transglutaminase enzyme and Storage time on the *L. acidophilus* count of yogurt samples

3-3- Sensory results

3-3-1- Aroma

The results of the analysis of variance (Table 1) showed that in relation to the aroma factor, only the storage time variable had a significant effect ($p<0.001$). None of the other variables and their interactions had a significant effect on the aroma score of the yogurt samples ($p<0.05$). As shown in Figure 4-c, with increasing storage time, the aroma score of the yogurt samples decreased; so that the highest aroma score (8.19) was observed on the first day of storage and the lowest (7.16) was observed on the 21st day of storage.

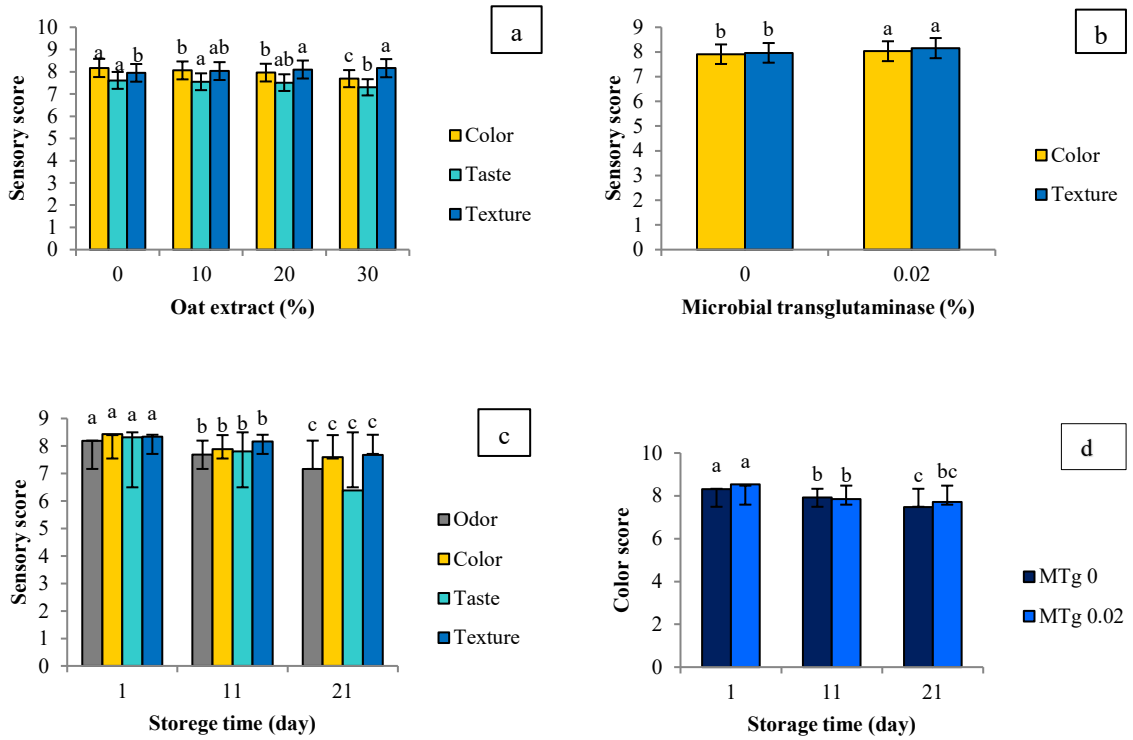


Figure 4. The effect of Oat extract, Microbial transglutaminase enzyme, storage time and their interaction on the sensory properties (odor, color, taste and texture) of yogurt samples

3-3-2- Color

The simple effects of microbial transglutaminase enzyme, oat extract ($p<0.001$) and storage time ($p<0.01$) had a significant effect on the color of yogurt samples (Table 1). Among the interaction effects, only the enzyme-storage time interaction had a significant effect on the color score ($p<0.01$).

According to Figure 4-a, with increasing the percentage of oat extract in yogurt samples, the color score decreased from 17.8 in the control sample to 69.7 in the sample containing 30% oat extract. Alqahtani et al. [33] stated in a study that the color score of samples containing oat extract was not significantly different from the control sample. Also, Demir et al. [47] reported that the color score of yogurt samples decreased with increasing oat extract, which is consistent with the results of this study.

With the use of transglutaminase enzyme, the color score of yogurt samples increased from 7.91 to 8.03 (Figure 4-b). Probably due to the activity of transglutaminase enzyme and the formation of more covalent bonds, a uniform and firm texture was created, resulting in better light reflection. In Figure 4-c, the effect of storage time on the color score of yogurt samples is shown. With increasing storage time, the color

score of yogurt samples decreased. The highest color score (8.43) was observed on the first day of storage and the lowest (7.59) on the 21st day of storage. The interaction effect of enzyme-storage time (Figure 4-d) also showed the highest color score in the sample treated with transglutaminase enzyme and the first day of storage, while the lowest score for the sample not treated with enzyme was recorded on the 21st day of storage.

3-3-3- Taste

The simple effects of oat extract ($p<0.05$) and storage time ($p<0.001$) had a significant effect on the taste of yogurt samples (Table 1). Other variables and interactions were also not significant ($p<0.05$).

Figure 4-a shows the effect of oat extract concentration on the taste score of yogurt samples. According to the figure, with an increase in the percentage of oat extract in the samples, the taste score of yogurt samples decreased from 7.61 to 7.30. Figure 4-c also shows the effect of storage time on the taste score of samples. As can be seen in this figure, with an increase in the storage time until the 21st day, the taste score of yogurt samples decreased from 8.31 to 6.38. At the end of the storage period, due to a significant decrease in pH, an increase in acidity, and some sourness of yogurt samples, the taste acceptance score decreased compared to the first day of storage.

So far, various results have been reported on the taste quality of the product as a result of MTg enzymatic treatment. As mentioned above, in this study, MTg enzymatic treatment did not have a significant effect on the taste of yogurt samples ($p<0.05$). Mahmood and Sebo [48] studied the effect of transglutaminase enzyme on the sensory properties of white cheese. Their results showed that enzyme-treated samples obtained higher taste scores than the control sample. Also, Aloglua and Oner [49] studied goat milk treated with transglutaminase enzyme. Their results showed that the control sample obtained the lowest flavor score and the sample treated with transglutaminase enzyme obtained the highest score. On the other hand, Sanli et al. [50] stated in their study that microbial transglutaminase enzymatic treatment did not have a significant effect on the flavor of yogurt samples. Jooyandeh et al. [51] reported that transglutaminase enzymatic treatment with 2 or 4 units of enzyme had no significant effect on taste acceptance of semi-skimmed ice cream, but samples treated with 6 units of enzyme showed a significant decrease in taste acceptance.

3-3-4- Texture

The addition of oat extract ($p<0.05$), treatment with transglutaminase enzyme and storage time ($p<0.001$) all had a significant effect on the texture factor of yogurt samples. According to Figure 4-a, with increasing the percentage of oat extract in the samples, their texture score

increased from 7.95 in the control sample to 8.16 in the sample containing 30% extract. Hydrocolloids often help in creating a suitable texture due to increasing viscosity, but the change in taste they produce is a limiting factor in this regard. Therefore, choosing the appropriate level of hydrocolloids is considered an important factor in the production of fermented milk products [52]. In Figure 4-b, the effect of transglutaminase enzyme on the texture score of yogurt samples is shown. As can be seen in the figure, with the use of the enzyme, the texture score of the samples increased from 7.96 to 8.16. In fact, increasing cross-linking by transglutaminase improved the texture of yogurt and was more acceptable to the evaluators. The results of the study by Jooyandeh et al. [51] are completely consistent with the results of this study. These researchers, by investigating the effect of transglutaminase enzyme on the organoleptic properties of semi-skimmed ice cream, reported that the enzymatic treatment improved the texture, so that the sample treated with the highest amount of enzyme received the best acceptance from the evaluators. This is while Karzan et al. [53] reported in a study that goat cheese samples treated with transglutaminase enzyme did not differ significantly in terms of color, flavor, and texture scores from the control sample. With increasing storage time, a decreasing trend in the texture scores of yogurt samples is observed (Figure 4-c). In fact, the texture quality of the samples decreased over time; So that the highest texture score of yogurt samples (8.34) was observed on the first day and the lowest (7.67) was observed on the 21st day of storage.

4- Conclusion

In recent years, the consumption of functional foods has gained great importance, especially for the prevention of common diseases. Products enriched with prebiotics, probiotics and bioactive metabolites can improve the gut microbiota, reduce chronic inflammation and metabolic risk factors, and play a role as part of preventive dietary strategies in promoting public health. In the present study, the effect of microbial transglutaminase (MTg) enzymatic treatment on synbiotic yogurt formulated with different amounts of oat extract (OE) was evaluated to determine its effect on the viability of probiotics, sensory characteristics and antioxidant properties (total phenolic content) of the product during refrigerated storage. The results of this study showed that the addition of OE and MTg improved the total phenolic content and consistency of yogurt. In addition, emphasizing the role of functional foods in our diet, this study showed that the addition of prebiotic-rich oat mixtures with selected probiotic strains can improve the viability of probiotic bacteria. Thus, the use of such synbiotic yogurt can enhance gastrointestinal health, modulate metabolic markers, and contribute to

preventive dietary strategies. The results of this study showed that the probiotic bacteria *Bifidobacterium bifidum* and *Lactobacillus acidophilus* were able to maintain their viability above the standard, even over time. However, it is suggested that other methods that help maintain and survive the beneficial bacteria in the food, such as microencapsulation, be used to enhance the probiotic population.

Data availability

Data will be made available on request.

Conflicts of Interest

The authors declare no conflicts of interest.

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Author Contributions

Parwin Kianara: Data collection, Formal analysis, Resources, Software, Writing - original draft.

Hossein Jooyandeh: Conceptualization, Project administration, Investigation, Visualization, Methodology, Validation, Writing – review & editing.

Mohammad Noshad, Mohammad Hojjati and Mohammad Amin Mehrnia: Conceptualization, Project administration, Investigation, Visualization & Validation.

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مقاله علمی_پژوهشی

ارزیابی تأثیر عصاره جو دوسر و تیمار آنزیمی ترانس‌گلوتامیناز میکروبی بر ترکیبات فنولی، خواص حسی و قابلیت زنده‌مانی باکتری‌های پروبیوتیک ماست سین‌بیوتیک

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کلمات کلیدی:

MTg،

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چکیده

غذاهای عملکردی با فراهم‌آوردن ترکیبات بیواکتیو (مانند پروبیوتیک‌ها، پری‌بیوتیک‌ها و آنتی‌اکسیدان‌ها) عملکردهای فیزیولوژیک مهمی را ارائه می‌دهند که می‌توانند در پیشگیری از بیماری‌های رایج نقش داشته باشند. عصاره جو (OE) حاوی ترکیبات زیست‌فعال (فیبر بتا-گلوکان، فنول‌ها و توکوترِی‌نول‌ها) و پری‌بیوتیک‌هایی است که رشد باکتری‌های مفید روده را تحریک کرده و خواص ضدالتهابی و متابولیک را تقویت می‌کنند. در هر حال، افزودن آن به ماست ممکن است سبب کاهش کیفیت حسی آن گردد. دراین میان، تیمار آنزیمی با ترانس‌گلوتامیناز میکروبی (MTg) یکی از راه‌های پیشنهادی برای بهبود خواص حسی محصول از طریق ارتقای کیفیت بافت و قوام ماست است. بنابراین در این مطالعه، تأثیر تیمار آنزیمی MTg (۰ و ۰/۰۲ درصد) بر برخی ویژگی‌های ماست سین‌بیوتیک حاوی غلظت‌های مختلف OE (۰، ۱۰، ۲۰ و ۳۰ درصد) طی یک دوره نگهداری ۲۱ روزه مورد بررسی قرار گرفت. نتایج نشان داد افزودن OE سبب بهبود محتوای فنلی کل (TPC)، شمارش باکتری‌های پروبیوتیک و امتیاز بافت ماست گردید، درحالی‌که امتیاز رنگ و طعم نمونه‌ها را کاهش داد. تیمار TG نیز سبب افزایش محتوای TPC و امتیاز رنگ و بافت محصول و کاهش تعداد باکتری‌های پروبیوتیک شد. با گذشت زمان نگهداری، هرچند محتوای TPC افزایش یافت، اما تعداد پروبیوتیک‌ها و امتیاز تمامی ویژگی‌های حسی ماست کاهش معنی‌داری یافت. درهرحال، مقدار باکتری‌های پروبیوتیک پس از ۲۱ روز نگهداری بیش از مقدار استاندارد (۷ log CFU/g) تعیین شد. براساس نتایج این تحقیق، نمونهٔ ماست سین‌بیوتیک تیمار شده با مقدار ۰/۰۲ درصد آنزیم TG و حاوی ۲۰ درصد عصارهٔ جو به‌عنوان بهترین نمونهٔ ماست مشخص گردید.