



Scientific Research

**Investigation the effect of adding alpha-amylase and transglutaminase enzymes on the physicochemical and textural properties of hamburger buns**

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**ABSTRACT**

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The use of whole wheat flour in hamburger bun production, due to its structural characteristics, results in a decrease in the textural quality of the final product. To overcome this challenge, several solutions have been proposed, among which the incorporation of additives, particularly enzymes such as transglutaminase and  $\alpha$ -amylase, is of notable importance. In this study, the effect of adding transglutaminase enzyme at three levels of 0, 0.15, and 0.3% and alpha-amylase at three levels of 0, 0.1, and 0.2% on the physicochemical and textural properties of hamburger bread prepared from whole wheat flour was investigated. The evaluated texture attributes included hardness, cohesiveness, gumminess, and adhesiveness. The results of data analysis showed that the use of transglutaminase enzyme significantly increased protein, moisture, firmness, cohesiveness and bread gumminess; while the pH value decreased. On the other hand, the addition of alpha-amylase, while increasing the percentage of protein, moisture and cohesiveness of bread, significantly reduced its firmness and gumminess. In general, the results of this study indicated that the simultaneous use of two enzymes, transglutaminase and alpha-amylase, can be used as an effective strategy in improving the textural quality of bread made from wholemeal flour. Among the experimental treatments, the addition of transglutaminase enzyme at a level of 0.15% and alpha-amylase at a level of 0.2% showed the best performance in improving the textural characteristics of bread.

## 1- Introduction

Bread is a staple in the diet of Iranians and many populations worldwide. Wheat bread provides a major source of calories and protein, supplying about 20% of the daily energy and protein needs of 4.5 billion people globally [1]. White wheat flour—used for most white breads—has the bran removed during milling, which reduces its fiber content. Consequently, modern white breads often have low dietary fiber [2]. Wheat bran, however, is a rich source of dietary fiber and contains substantial amounts of protein, carbohydrates, minerals, and vitamins [3].

Producing wholemeal (whole-wheat) bread presents post-baking challenges such as reduced specific volume, decreased softness and crust crispness, poorer dough machinability, a denser crumb, and faster staling. Bran components (e.g., cellulose and arabinoxylan) compete with gluten for water, impair gluten development, and weaken the dough structure. Various strategies can compensate for these defects, the most important being the addition of suitable dough improvers [4]. With the food industry shifting toward healthier products and regulatory limits on chemical additives, food enzymes have gained importance [5]. Because wheat flour's performance depends on gluten's viscoelasticity, some enzymes are incorporated into dough formulations to replace chemical additives, shorten processing time, and slow staling [6].

Alpha-amylase is particularly effective against bread staling and it partially hydrolyzes starch into lower-molecular-weight fragments, preventing amylopectin recrystallization [7]. Beyond anti-staling effects, alpha-amylase improves dough elasticity and crust structure by degrading damaged starch to dextrins that yeast can ferment, increasing loaf volume and improving texture [8]. The small sugars and oligosaccharides (glucose, maltose) produced also enhance Maillard browning, contributing to crust color, aroma, and flavor [9].

Microbial transglutaminase (EC 2.3.2.13) is a transferase that can rejoin weak links within protein networks [10]. It catalyzes acyltransferase reactions that form covalent cross-links between proteins, improving texture and quality and creating proteins with novel properties. These cross-links are stable and resistant to pH and temperature changes, thereby enhancing product quality [11].

Given high per-capita bread consumption and substantial product loss from quality decline, improving breadmaking to reduce reliance on chemical additives and extend shelf life is a key industry challenge. Although many studies have explored nutritional, safety, and quality enhancements using functional ingredients, further innovations are needed to improve dough performance and maintain final product quality. This study therefore investigates the use of microbial alpha-amylase and transglutaminase to improve textural properties and produce wholemeal bread with desirable quality.

## 2- Materials and methods

### 2-1- Raw materials

The flour used for bread preparation was obtained from Darreh Shahr Flour Company (Ilam, Iran). Fungal alpha-amylase enzyme with an activity of 120,000 SKB/min and microbial transglutaminase enzyme with an activity of 100 units per gram were obtained from Arteen Chemical Company (Karaj, Iran). Also, baker's yeast (Nabmayeh) was purchased from Razi yeast and alcohol Company. (Tehran, Iran).

### 2-2- Preparation of wholemeal hamburger buns

Bread dough samples were produced by direct dough method using the method of Jooyandeh et al. with slight modifications [12]. The dough was prepared according to the formula including 1000 g whole wheat flour, 12 g dry yeast, 10 g shortening oil, 10 g salt, and 10 ppm improver (potassium bromate). Alpha-

amylase enzyme at levels of 0, 0.1 and 0.2% and transglutaminase enzyme at levels of 0, 0.15 and 0.3% (each based on flour weight) were added to the formula. The dough was prepared using a dough mixer and after 20 minutes of storage and initial fermentation, the dough was punched into 70 g pieces. The dough pieces were placed in a warm room at a temperature of 30 to 32 °C and a relative humidity of about 85% for 50 minutes to rest. The baking process was carried out at a temperature of  $250 \pm 15$  °C for 25 minutes. After cooling, the resulting breads were packaged in polyethylene bags and stored at room temperature for further tests [12].

### 2-3- Evaluation of physicochemical properties

The physicochemical properties of flour and bread, including moisture, ash, fat and protein, were measured according to the Iranian National Standard Nos. 2705-1, 2706-10700, and 19052, respectively [13, 14, 15, and 16]. The pH of the samples was also measured using a Metrohm-780 pH meter made in Switzerland. For this purpose, 10 g of the sample was mixed with 100 ml of distilled water, and after 30 minutes, without filtering, the pH of the upper solution was measured [17].

### 2-4- Evaluation of texture characteristic

TPA (texture profile analysis) was performed to evaluate various characteristics of the produced hamburger buns, such as firmness, cohesiveness, adhesiveness, and gumminess. For this purpose, a Stable Micro System texture analyzer (model TA.XT.PLUS, made

in England) was used. Initially,  $2 \times 2 \times 2$  cm cubes were cut from the center of the bread. For the test, a probe with a cylindrical cross-section of 36 mm in diameter and a moving speed of 1 mm/s was used. Also, a 5 kg load cell was used to apply pressure and the samples were compressed to 45% of the initial height of the samples [18].

### 2-5- Statistical analysis

This study was conducted as a factorial in a completely randomized design with three replications. In this study, the effect of alpha-amylase enzyme at three levels (0, 0.1 and 0.2%) and transglutaminase at three levels (0, 0.15 and 0.3%) on the desired factors was investigated. Data analysis was performed using SPSS software and comparison of means was performed using Duncan's test at a confidence level of 95%.

## 3- Results and discussion

### 3-1- Physicochemical properties

The results of the physicochemical properties of the flour used in bread production are given in Table 1. As can be seen, the flour used contains 12.5% protein and pH is 6.3. Also, the results of the analysis of variance of all the factors studied are shown in Table 2. Based on the results obtained, the effect of both alpha-amylase and microbial transglutaminase enzymes on the ash and fat content of the treated bread samples was not significant ( $p > 0.05$ ).

**Table 1.** Physicochemical properties of whole wheat flour

	Moisture (%)	Ash (%)	Protein (%)	pH
Whole wheat flour	9.31	1.6	12.5	6.3

The results of the analysis of variance of the bread samples produced are reported in Table 2. As it is clear, increasing the concentration of transglutaminase enzyme in breads produced from wholemeal flour had a

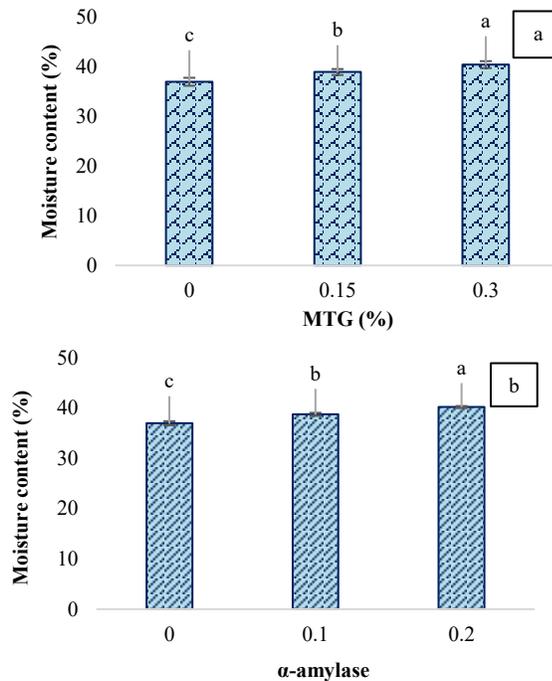
significant effect on the moisture content of the bread samples ( $p < 0.001$ ) and increased the moisture content of the bread samples (Figure 1-a). However, as can be seen, there is no significant difference between the control

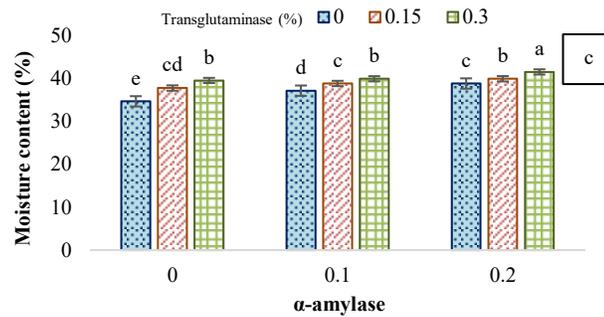
sample and the sample containing 0.15% transglutaminase. The transglutaminase enzyme plays a role in the formation of a protein network and the creation of protein chains with high molecular weight, which trap water in the bread texture and prevent water from migrating from the core to the crust, thereby slowing down the process of bread staleness [19].

In the study on the addition of the enzyme transglutaminase to gluten-free bread, Poursmaeil et al. reported an increase in moisture content with increasing enzyme levels in the formulation [20]. The cross-linked network between the amino acids glutamine and lysine, which is formed by the action of the transglutaminase enzyme, has the ability to trap water and hence increases the water retention capacity. On the other hand,

the increase in water absorption capacity can be due to the deamidation of glutamine (deamidation by the action of the enzyme) and its conversion to glutamic acid, which reduces the hydrophobicity of the environment and consequently increases water absorption [21].

Babakhani et al., in their study of the physicochemical and sensory properties of cake enriched with fish protein powder and transglutaminase enzyme, reported that the moisture content in treatments impregnated with transglutaminase enzyme increased significantly compared to the control treatments, such that moisture content also increased with increasing enzyme percentage [19].





**Figure 1.** The effect of microbial transglutaminase (MTG) (a),  $\alpha$ -amylase (b) and their interaction (c) on the moisture content of bread samples

### The results of the analysis of variance

of the produced bread samples in Table 2 show that the addition of alpha-amylase enzyme also had a significant effect on the moisture content of the bread samples ( $p < 0.001$ ) and with increasing the amount of this enzyme, the moisture content of the samples also increased (Figure 1-b); however, no significant difference was observed between the control sample and the sample containing 0.1% alpha-amylase. In fact, the presence of alpha-amylase enzyme in the dough formulation can increase the amount of damaged starch in wheat flour, which has a positive effect on the water absorption rate of the dough and the moisture content of the final product. Dehghan Tanha and Karimi (2016) investigated the application of psyllium gum and fungal alpha-amylase enzyme on the physicochemical and sensory properties of donuts. The results showed that with increasing the amount of psyllium gum and fungal alpha-amylase enzyme in the donut formulation, the moisture content of the

samples also increased [22]. Purlis and Salvadori (2008) also reported an increase in moisture content as a result of adding alpha-amylase enzyme to cake formulation [23]. Nejatbakhsh et al. (2018) also showed in a study that adding alpha-amylase enzyme and lactic acid sourdough increased moisture, fiber, ash and decreased fat and protein content of toast bread samples compared to the control sample [24]. The interaction effects of adding both alpha-amylase enzymes and microbial transglutaminase are shown in Figure 1-c. According to this graph, adding different ratios of transglutaminase and alpha-amylase enzymes to flour significantly increased the moisture content of the produced bread compared to the control sample. As can be seen, the highest moisture content was found in the sample containing 0.3% transglutaminase enzyme and 0.2% alpha-amylase. The lowest moisture content was observed in the control sample (Figure 1-c).

**Table 2.** Analysis of variance for the effect of  $\alpha$ -amylase and microbial transglutaminase (MTG) on the physicochemical and textural properties of bread

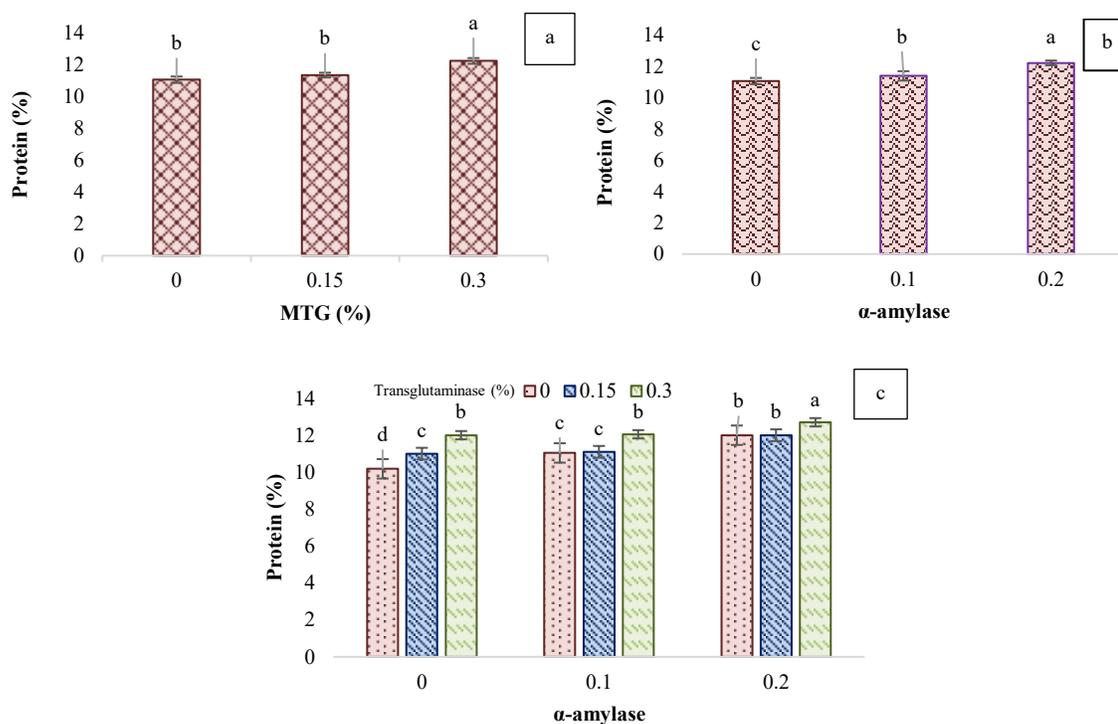
Variable sources	Mean square									
	df	Moisture	Ash	Fat	protein	pH	Hardness	Cohesiveness	Adhesiveness	Gumminess
$\alpha$ -amylase	2	8.44**	0.003 <sup>ns</sup>	0.036 <sup>ns</sup>	37.53***	0.001 <sup>ns</sup>	584.07***	0.000**	851.48 <sup>ns</sup>	214.55***
MTG	2	8.61**	0.002 <sup>ns</sup>	0.197 <sup>ns</sup>	41.104***	0.008***	243.93***	0.001*	515.11 <sup>ns</sup>	88.42***

$\alpha$ -amylase $\times$ MTG	4	9.23**	0.001 <sup>ns</sup>	0.001 <sup>ns</sup>	9.29***	0.003*	86.860***	0.005 <sup>ns</sup>	513.30 <sup>ns</sup>	26.54**
Error	18	0.25	0.00	0.00	0.03	0.002	1.06	0.01	5.74	3.06
CV (%)	-	5.10	1.5	3.86	4.21	1.4	11.54	8.4	1.6	13.96

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

The results of the analysis of variance (Table 2) show that the addition of microbial transglutaminase enzyme to breads produced from wholemeal flour had a significant effect on the protein content of hamburger bread samples ( $p < 0.001$ ). Also, samples containing transglutaminase enzyme had a higher protein content than the control sample. Of course, as can be seen in Figure 2-a, there was no significant difference between the control sample and the 0.15% transglutaminase enzyme, but this difference became significant in the 0.3% sample. Steffaloni et al. (2010) stated that the presence of transglutaminase in bread dough causes a decrease in albumin and globulin and an increase in gliadin levels. Because albumin and globulin do not play a role in dough formation, transglutaminase

binds the aforementioned proteins together and precipitates them, and this action has a beneficial effect on the quality of the product. The beneficial effects of transglutaminase are due to the binding of glutenin subunits with high molecular weight. Transglutaminase, having protein binding properties, also causes the repair of damaged wheat grains [25]. Saeidi et al. (2017) stated that transglutaminase had no significant effect on the protein of the samples [26]. Also, Aziz Mohamadi and Bolandi (2017) reported that the amount of steak protein increased significantly under the influence of the transglutaminase enzyme [27].



**Figure 2.** The effect of microbial transglutaminase (MTG) (a),  $\alpha$ -amylase (b) and their interaction (c) on the protein percentage of bread samples

The results of the analysis of variance (Table 2) show that the addition of microbial transglutaminase enzyme to breads produced from wholemeal flour had a significant effect on the protein content of hamburger bread samples ( $p < 0.001$ ). Also, samples containing transglutaminase enzyme had a higher protein content than the control sample. Obviously, as can be seen in Figure 2-a, there was no significant difference between the control sample and the 0.15% transglutaminase enzyme, but this difference became significant in the 0.3% sample. Steffolani et al. (2010) stated that the presence of transglutaminase in bread dough causes a decrease in albumin and globulin and an increase in gliadin levels [25]. Because albumin and globulin do not play a role in dough formation, transglutaminase binds the aforementioned proteins together and precipitates them, and this action has a beneficial effect on the quality of the product. The beneficial effects of transglutaminase are due to the binding of glutenin subunits with high molecular weight. Transglutaminase, having protein binding properties, also causes the repair of damaged wheat grains [25]. Saeidi et al. (2017) stated that transglutaminase had no significant effect on the protein of the samples [26]. Also, Aziz mohamadi and Bolandi (2017) reported that the amount of steak protein increased significantly under the influence of the transglutaminase enzyme [27].

With the increase in the amount of alpha-amylase enzyme, the protein percentage of the bread samples also increased significantly ( $p < 0.001$ ). As can be seen in Figure 2-b, there is no significant difference between the control sample and the sample containing 0.1% alpha-amylase enzyme, but a slight increase in protein is observed in the sample containing 0.2% alpha-amylase. Nejatbakhsh et al. (2018) showed in a study that the addition of alpha-amylase enzyme and lactic acid sourdough caused a decrease in the

protein content in bread samples compared to the control sample, which was inconsistent with the results of this study [24].

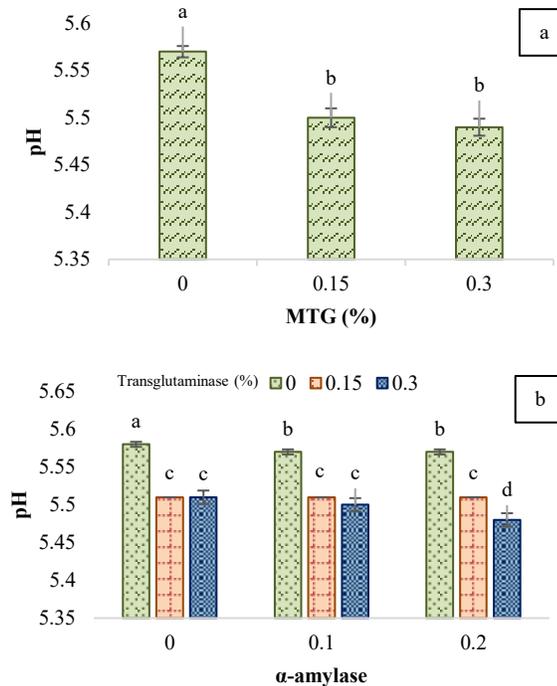
Figure 2-c shows the interaction effects of transglutaminase and alpha-amylase enzymes. As can be seen, adding different ratios of transglutaminase and alpha-amylase enzymes to bread produced from wholemeal flour increased its protein compared to the control sample. The highest protein content was observed in the sample containing 0.3% transglutaminase enzyme and 0.2% alpha-amylase, and the lowest protein content was observed in the control sample.

Regarding the pH factor, with the increase in the amount of transglutaminase enzyme, the pH of the bread samples decreased significantly compared to the control sample ( $p < 0.001$ ) (Table 2, Figure 3-a). The effect of different amounts of transglutaminase enzyme on the changes in the pH of the bread samples is also shown in Figure 3. Based on the results obtained, no statistically significant difference was observed between the samples containing 0.15 and 0.3% transglutaminase enzyme ( $p > 0.05$ ). In general, the pH range of the samples was between 5.5-5.17. The decrease in pH was probably due to the deamidation of the amino acid glutamine and the production of glutamic acid by this enzyme, which caused a decrease in the pH of the resulting breads [28]. Saeidi et al. (2017) also reported on the optimization of gluten-free cake formulation enriched with pomegranate seed powder and transglutaminase enzyme that the addition of transglutaminase enzyme reduced the pH of cake samples [26].

Unlike transglutaminase, the addition of alpha-amylase to the produced samples did not have a significant effect on the pH of the produced breads ( $p > 0.05$ ). As Table 2 shows, the interaction effects of adding transglutaminase and alpha-amylase on the pH were significant ( $p < 0.01$ ). In general, it can be

said that the highest pH was related to the control sample and the lowest pH was related to the sample treated with 0.3% transglutaminase enzyme and 0.2% alpha-amylase (Figure 3-b). Movahhed et al. (2011) reported in a study that alpha-amylase enzyme, by producing metabolites such as

dextrin, increased the substances participating in fermentation, which again led to a decrease in pH, which was inconsistent with the results of this study [29].



**Figure 3.** The effect of microbial transglutaminase (MTG) (a) and the interaction between MTG and  $\alpha$ -amylase (b) on the pH of bread samples

### 3-2- Textural characteristics

The addition of transglutaminase enzyme had a significant effect on the firmness, cohesiveness and gumminess of the bread samples ( $p < 0.001$ ). However, as can be seen in Table 2, this enzyme did not have a significant effect on adhesiveness ( $p > 0.05$ ).

As can be seen in Figure 4-a and 4-c, with an increase in the percentage of transglutaminase enzyme in the produced breads, the firmness and cohesiveness of the samples increased significantly. However, this increase was not significant between the control sample and the sample treated with 0.15% transglutaminase enzyme, but the

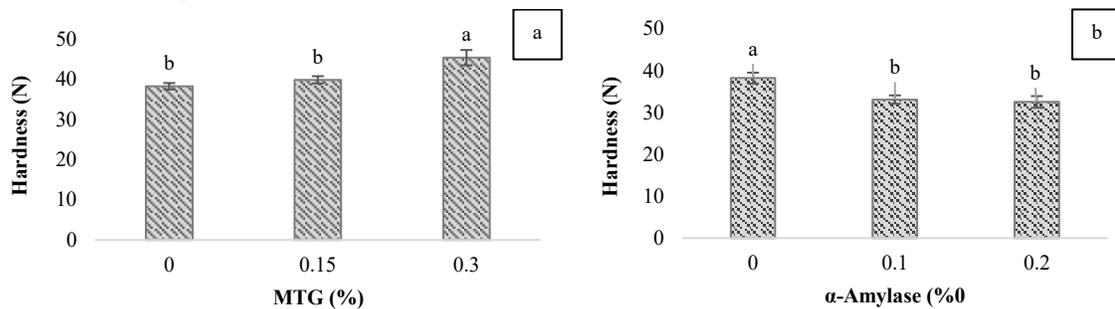
firmness of the samples treated with 0.3% transglutaminase enzyme increased significantly. Also, the addition of transglutaminase enzyme increased the gumminess parameter of the bread samples (Figure 4-e).

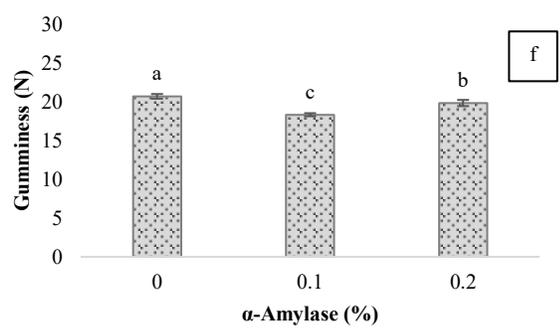
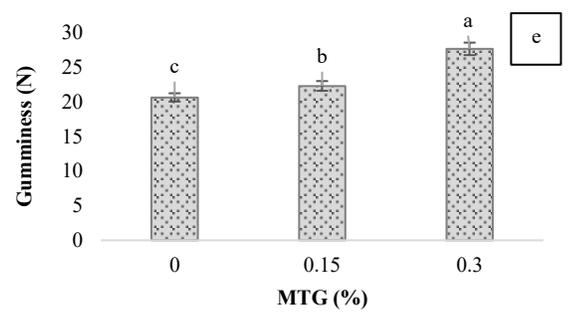
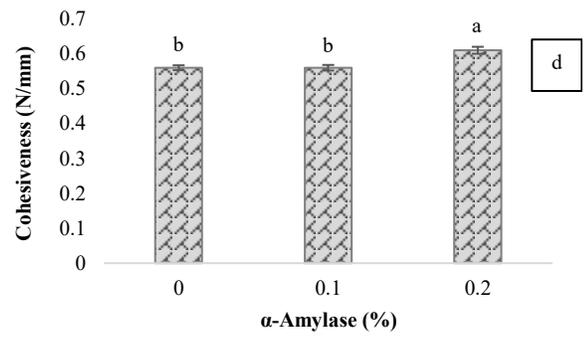
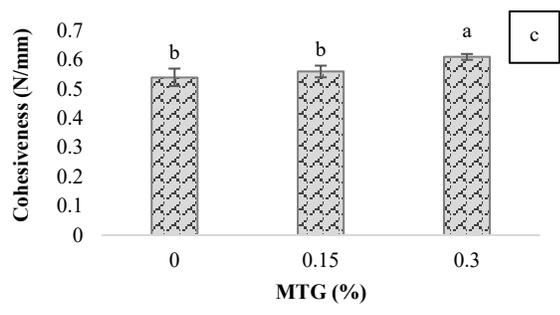
Transglutaminase affects the properties of the dough and the resulting bread through different mechanisms. This enzyme directly causes cross-linking between proteins. Also, adding transglutaminase by deamidating glutamine and lysine is effective in improving water retention capacity and inducing elasticity to the bread texture and involves greater cohesiveness of the protein (gluten) network [30]. Also, by creating cross-links

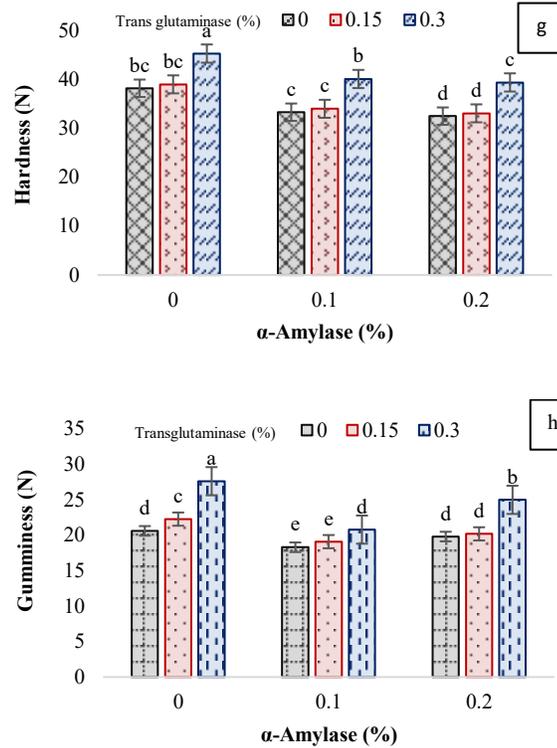
and forming a strong gluten network and increasing gluten strength, the transglutaminase enzyme traps water within the bread texture and in fact increases the water absorption of gluten and reduces the available water of starch, thus reducing retrogradation. By creating stronger links, the bread texture becomes firmer and denser, and more force is required to compress the bread texture. Therefore, the amount of enzyme added to the formulation should be controlled as much as possible [31]. Onyango et al. (2010) investigated the effect of transglutaminase enzyme on dough rheological properties and bread quality prepared from pregelatinized cassava starch and sorghum flour. The results of this study indicated that with increasing enzyme concentration, the resistance to deformation in the dough decreases and the firmness and cohesiveness of the bread texture increases [32]. Dłużewska et al. (2015) showed that transglutaminase enzymatic treatment with 1 unit of enzyme (per gram of protein) reduced the firmness of gluten-free bread, while transglutaminase enzymatic treatment with 10 units of enzyme significantly increased the firmness of the product [33]. The effect of

transglutaminase enzyme on the firmness of bread texture is explained by the increase in the molecular weight of proteins and the formation of larger proteins [34]. The results of Jooyandeh and Mortazavi (2018) also showed that with increasing the concentration of transglutaminase enzyme, the firmness, consistency, and cohesiveness of yogurt increased significantly [35].

The gumminess is defined as the energy required to prepare food for swallowing, and a low value is considered soft and a high value is considered hard or brittle with cell breakage [36], which was consistent with the results of the hardness of the produced breads. Hosseini Ghaboos et al. (2018) also reported that the two characteristics of chewability and gumminess of cakes containing pumpkin powder and transglutaminase enzyme increased significantly compared to the control [37]. Majzoubi and Vosoughipour (2014) reported an increase in gumminess due to the increase in the protein content of the formulation by adding soy protein isolate to the sponge cake formulation [38].







**Figure 4.** The effect of microbial transglutaminase (MTG),  $\alpha$ -Amylase and their interactions on the textural properties of bread samples

The results of the analysis of variance of the texture of bread samples in Table 2 show that the amount of alpha-amylase enzyme added to breads produced from wholemeal flour had a significant effect on the firmness, cohesiveness and gumminess of the bread samples ( $p < 0.001$ ). However, as can be seen, this enzyme did not have a significant effect on adhesiveness ( $p > 0.05$ ).

As can be seen in Figure 4-b and 4-f, with an increase in the percentage of alpha-amylase enzyme, the firmness and gumminess of the bread samples decreased significantly; however, as can be seen, there was no significant difference between the samples treated with 0.1 and 0.2 percent alpha-amylase. Also, the addition of alpha-amylase enzyme increased the cohesiveness parameter in the bread samples (Figure 4-d).

The results of this study are consistent with the findings of Lagrain et al. (2008), who attributed the decrease in bread texture hardness in the presence of alpha-amylase to increased yeast activity, resulting in the expansion of CO<sub>2</sub> gas bubbles and increased porosity in the bread texture [39]. Goesart et al. (2009) stated that the effect of amylase on reducing bread hardness is due to the breakdown of glycosidic bonds in starch into dextrins with lower molecular weight, which causes a delay in the formation of amylopectin double chains and prevents the cross-linking of amylopectin molecules. As a result, it weakens the three-dimensional network formation and ultimately causes a decrease in the firmness of the bread [40]. In the studies of Caballero et al. (2007), similar results were reported regarding the addition of amylase enzyme on the decrease in the firmness of frozen bread [41]. In the study of Sozer et al.

(2011), cakes prepared containing modified starch, alpha-amylase enzyme, gum and emulsifier had lower firmness than the control samples and a significant decrease in their firmness was observed [42]. Bedoya-Perales and Steel (2014) studied the effect of different concentrations of maltogenic alpha-amylase and fat on the technological and sensory quality of cakes. The results of these researchers showed that the use of alpha-amylase enzyme reduced the firmness of the product; therefore, this enzyme can be an appropriate alternative for reducing the fat level of these products [43]. The reduction in the gumminess of bread samples with the addition of alpha-amylase enzyme has also been reported by Rosell et al. (2001), which was consistent with the results of this study [44].

According to the results reported in Table 2, the interaction effects of adding transglutaminase and alpha-amylase enzymes on the firmness and gumminess of breads have been significant ( $p < 0.001$ ). According to Figure 4-g, with increasing the amount of transglutaminase enzyme, the firmness of the bread increased, and with increasing the amount of alpha-amylase enzyme, the firmness of the bread decreased, so that the highest firmness (3.45 N) was related to the sample without alpha-amylase and 0.3% transglutaminase, and the lowest firmness (5.32 N) was related to the sample without transglutaminase and 0.2% alpha-amylase. Also, as can be seen in Figure 4-g, no significant difference was observed between the levels of 0.15 transglutaminase enzyme and the control in terms of firmness. Also, with increasing the percentage of alpha-amylase enzyme, the gumminess of the samples decreased, and with increasing the amount of transglutaminase enzyme to 0.15%, this factor increased; although it showed a significant decrease at the level of 0.3% (Figure 4-h). As a result, as shown in Figure 4-h, the highest gumminess was found in the sample treated with 0.3% transglutaminase and without alpha-amylase, and the lowest

gumminess was found in the sample treated with 0.1% alpha-amylase and without transglutaminase. Significant increases in the firmness, cohesiveness, and gumminess or mechanical properties of foods as a result of enzymatic treatment at high levels of transglutaminase have been reported in various products such as white cheese [45], yogurt [46], and edible film [47].

#### 4- Conclusion

Bread is a staple food worldwide, particularly in the Middle East. Its fiber supports digestive health by preventing constipation and promoting regular bowel movements, helps regulate blood sugar and cholesterol, and increases satiety, which can aid weight management. High-fiber diets are also associated with lower risk of heart disease, type 2 diabetes, and certain cancers. However, bran can reduce dough elasticity and extensibility and impair gas retention during fermentation, producing smaller loaves with a denser crumb. Because whole-wheat flour forms a weaker gluten-viscoelastic network, enzymes can be added to dough to shorten preparation time, slow staling, replace chemical additives, and improve baking performance.

Alpha-amylase is particularly effective at reducing bread staling. It breaks down damaged starches into lower-molecular-weight dextrans that yeast can ferment, which increases loaf volume and improves crumb texture. Microbial transglutaminase (a transferase) reconnects weak bonds in the gluten network by catalyzing acyl-transfer reactions that form covalent crosslinks between proteins. These covalent bonds are highly stable and resistant to pH and temperature changes, enhancing texture and product quality.

In this study, adding transglutaminase increased protein content, moisture, firmness, cohesiveness, and gumminess, while decreasing pH. Addition of alpha-amylase

increased protein content, moisture, and cohesiveness and significantly reduced other textural parameters. Based on these results, use of 0.2% alpha-amylase and 0.15% transglutaminase is recommended to produce hamburger buns with optimal texture and quality.

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### Data Availability

The data used to support the finding of this study are available from the corresponding author upon request.

### Conflict Of Interest

The authors have no conflicts interest to report.

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### 5-References

- [1] Mardani Toroghia, P., Bassiri, A., Milani, E., and Golimovahed, Gh. 2016. The effect of wheat bran addition and extrusion process parameters on the functional properties of wheat-based instant powder. *Innovative Food Technologies*, 3(3): 13-23. (In Persian)
- [2] Gomez, M.F. 2002. Effect of dietary fiber on dough rheology and bread quality. *European Food Research Technology*, 216: 51-56.
- [3] Mardani Ghahfarrokhi, A., and Yarmand, M. S. 2016. Investigation the effect of bran content on the rheological properties and the quality characteristics of Barbary bread. *Journal of Food Science and Technology*, 50(13): 11-21. (In Persian)
- [4] Hance, M. and Dmoor, A. 2012. Flat bread: ingredients and fortification. *Qual. Assur. Saf. Crops Foods*. 4: 2-8.
- [5] Fallahi, P., Habte-Tsion, H. M., and Rossi, W. 2018. Depolymerizing enzymes in human food: bakery, dairy products, and drinks. In *Enzymes in human and animal nutrition*. (pp. 211-237). Academic Press
- [6] Dahiya, S., Bajaj, B. K., Kumar, A., Tiwari, S. K., and Singh, B. 2020. A review on biotechnological potential of multifarious enzymes in bread making. *Process Biochemistry*. 99: 290-306.
- [7] Chang, Y., Yang, J., Jiang, L., Ren, L., and Zhou, J. 2019. Chain Length Distribution of  $\beta$ -amylase Treated Potato Starch and Its Effect on Properties of Starch Nanoparticles Obtained by Nanoprecipitation. *Starch-Stärke*. 71(9-10): 1800321.
- [8] Grewal, N., Faubion, J., Feng, G., Kaufman, R. C., Wilson, J. D., and Shi, Y. C. 2015. Structure of waxy maize starch hydrolyzed by maltogenic  $\alpha$ -amylase in relation to its retrogradation. *Journal of Agricultural and Food Chemistry*. 63(16): 4196-4201.
- [9] Shafi Soltani, M., Salehifar, M., and Hashemi. M. 2013. The effect of using fungal origin alpha-amylase on the qualitative characteristics of dough and toast bread. *Journal of Innovation in Food Science and Technology*, 6(2): 43-54. (In Persian)
- [10] Habibi, S.A., & Jooyandeh, H. 2024. Investigation on the Effect of Persian Gum and Transglutaminase Enzyme on Some Physicochemical and Microstructural Characteristics of Low-Fat Ultrafiltrated Iranian White Cheese. *Food Science & Nutrition*, 12(11): 9810-9821.
- [11] Torabi, F., Jooyandeh, H., & Noshad, M. 2021. Evaluation of physicochemical, rheological, microstructural, and microbial characteristics of synbiotic ultrafiltrated white cheese treated with transglutaminase. *Journal of Food Processing and Preservation*, 45: e15572: 1-11.
- [12] Jooyandeh, H., Minhas K.S. & Kaur A. (2009). Sensory Quality and Chemical Composition of Wheat Breads Supplemented with Fermented Whey Protein Concentrate and Whey Permeate. *Journal of Food Science and Technology*, 46(2): 146-148.
- [13] Institute of standard and industrial research of Iran. NO. 2705-1. 2025. Cereals and cereal products – Determination of moisture content – Part 1: Reference method. (In Persian)
- [14] Institute of standard and industrial research of Iran. NO. 2706. 2025. Cereals, pulses and by-products- Determination of ash yield by incineration. (In Persian)
- [15] Institute of standard and industrial research of Iran. NO. 10700, 1<sup>st</sup> edition. 2008. Animal feeding stuffs – Determination of fat content. (In Persian)
- [16] Institute of standard and industrial research of Iran. NO. 19052. 2015. Cereals and pulses – Determination of the nitrogen content and calculation of the crude protein content – kjeldahl method. (In Persian)
- [17] Aalipour Hafshejani, F., Mahdavi Hafshejani, F., Aalipour Hafshejani, M., and Mohammadi, R. 2020. Determination of the pH, Salt, Sodium and Potassium Content in the Traditional Bread in

- Western Iran. *Journal of Nutrition Fasting and Health*, 8(2): 135-140.
- [18] Feili, R., Abdullah, W.N.W., and Yang, T.A. 2013. Physical and sensory analysis of high fiber bread incorporated with jackfruit rind flour. *Food Science and Technology*, 1(2): 30-36.
- [19] Babakhani, S., Moghaddaszadeh-Ahrabi, S., and Gharakhani, M. 2019. Physicochemical and Sensory Properties of Cake Enriched with Fish Protein Powder (FPP) and Transglutaminase Enzyme. *Journal of Food Science and Technology*, 16(95):179-196. (In Persian)
- [20] Poursmaeil, N., Azizi, M.H., Abbasi, S., and Mohamadi, M. 2011. Formulation of Gluten Free Bread Using Guar and Microbial Transglutaminase Enzyme. *Food Research Journal*, 21(1): 69-81.
- [21] Danesh, E., Goudarzi, M., and Jooyandeh, H. 2018. Transglutaminase-mediated incorporation of whey protein as fat replacer into the formulation of reduced-fat Iranian white cheese: physicochemical, rheological and microstructural characterization. *Journal of Food Measurement and Characterization*, 12, 2416–2425.
- [22] Dehghan Tanha, L., and Karimi, M. 2016. Application of Plantago gum and fungal  $\alpha$ -amylase enzyme on Physicochemical and organoleptic properties of doughnut. *Food Science and Technology (Iran)*, 13(52): 101-110. (In Persian)
- [23] Purlis, E., and Salvadori, V.O. 2008. Bread baking: Analysis of process and quality aspects by simulation. *International Conference of Agricultural Engineering*, 1-4.
- [24] Nejatbakhsh, R., Movahhed, S., and Ahmadi Chenarbon, H. 2018. Effect of Sourdough and  $\alpha$ -amylase on qualitative properties of toast bread. *Iranian Food Science and Technology Research Journal*. 14(2): 259-272. (In Persian)
- [25] Steffolani, M.E., Ribotta, P.D., Pérez, G.T., and León, A.E. 2010. Effect of glucose oxidase, transglutaminase, and pentosanase on wheat proteins: Relationship with dough properties and bread-making quality. *Journal of Cereal Science*, 51(3): 366-373.
- [26] Saecidi, Z., Naschi, B., and Jooyandeh, H. 2018. Optimization of gluten-free cake formulation enriched with pomegranate seed powder and transglutaminase enzyme. *Journal of Food Science and Technology*, 55(8): 3110–3118. (In Persian)
- [27] Aziz Mohamadi, M., and Bolandi, M. 2018. The effect of transglutaminase enzyme on the physicochemical, sensory and textural properties of veal steaks Aziz. *Journal of Food Science and Technology (Iran)*, 14(72), 37-45. (In Persian)
- [28] Gerrard, J.A., and Sutton, K.H. 2005. Addition of transglutaminase to cereal products may generate the epitope responsible for coeliac disease. *Trends in Food Science & Technology*, 16(11): 510-512.
- [29] Movahhed, Sanaz Zharfi, S., and Ahmadi Chenarbon, H. 2014. Investigation of Rheological of Dough and Organoleptical Properties of Toast Breads Containing Banana Flour. *Iranian Food Science and Technology Research Journal*, 9(4): 359-365. (In Persian)
- [30] Boukid, F., Carini, E., Curti, E., Bardini, G., Pizzigalli, E., and Vittadini, E. 2018. Effectiveness of vital gluten and transglutaminase in the improvement of physico-chemical properties of fresh bread. *LWT*, 92: 465-470.
- [31] Pourmohammadi, K., Aalami, M., Shahedi, M., and Sadeghi Mahoonak, A.R. 2011. Effect of microbial transglutaminase on dough rheological properties of wheat flour supplemented with hull-less barley flour. *Food Research Journal*, 21(3): 269-279.
- [32] Onyango, C., Mutungi, C., Unbehend, G., and Lindhauer, M.G. 2010. Rheological and baking characteristics of batter and bread prepared from pregelatinised cassava starch and sorghum and modified using microbial transglutaminase. *Journal of Food Engineering*, (97):4, 465-470.
- [33] Dłużewska, E., Marciniak-Lukasiak, K. and Kurek, N. 2015. Effect of transglutaminase additive on the quality of gluten-free bread. *Foods*. 13(1): 80-86.
- [34] Marco, C., and Rosell, C. M. 2008. Breadmaking performance of protein enriched, gluten-free breads. *European Food Research and Technology*, 227: 1205–1213.
- [35] Jooyandeh, H., and Mortazav, A. 2017. Impact of addition of milk powder and microbial transglutaminase on the sensory and textural properties of set yogurt. *Food Processing and Preservation Journal*, 10(2): 121-136.
- [36] Nasir, S., Allai, F.M., Gani, M., Ganaie, Sh., Gul, Kh., Jabeen, A., and Majeed, D. 2020. Physical, Textural, Rheological, and Sensory Characteristics of Amaranth-Based Wheat Flour Bread. *International Journal of Food Science*, 8874872, 0-9.
- [37] Hosseini Ghaboos, S.H., Seyedain Ardabili, S.M., and Kashaninejad, M. 2018. Physico-chemical, textural and sensory evaluation of sponge cake supplemented with pumpkin flour. *International Food Research Journal*, 25(2): 854-860.
- [38] Majzoubi, M., and Vosoughipour, Z. 2013. The Effect of Enzyme Addition on the Improvement of Cereal Product Quality. *Proceedings of the 21st National Congress on Food Science and Technology of Iran, Shiraz*. (In Persian)
- [39] Lagrain, B., Wilderjans, E., Glorieux, C. and Delcour, J.A. 2012. Importance of gluten and starch for structural and textural properties of crumb from fresh and stored bread. *Food Biophysics*. 7(2): 173-181.
- [40] Goesaert, H., Slade, L., Levine, H., and Delcour, J.A. 2009. Amylases and bread firming – an

- integrated view. *Journal of Cereal Science*, 50: 345–352.
- [41] Caballero, P.A., Gómez, M. and Rosell, C.M. 2007. Bread quality and dough rheology of enzyme-supplemented wheat flour. *European Food Research and Technology*, 224, 525–534.
- [42] Sozer, N., Bruins, R., Dietzel, Ch., Franke, W., and Kokini, J.L. 2011. Improvement of shelf life stability of cakes. *Journal of Food Quality*, 151-162.
- [43] Bedoya-Perales, N.S., and Steel, C.J. 2014. Effect of the concentrations of maltogenic  $\alpha$ -amylase and fat on the technological and sensory quality of cakes. *Food Science and Technology*, 0101-2061, 760-766.
- [44] Rosell, C. M., Rojas, J. A., and Benedito de Barber, C. 2001. Influence of hydrocolloids on dough rheology and bread quality. *Food Hydrocolloids*. 15(1): 75-81.
- [45] Habibi, S.A., & Jooyandeh, H. 2025. Impact of addition of Persian gum and microbial transglutaminase enzyme on the textural characteristics of semi-fat ultrafiltrated white cheese. *Journal of Food Science and Technology (Iran)*, 21(156): 111- 125. (In Persian)
- [46] Jooyandeh, H., Saffari Samani, S., Alizadeh Behbahani B., and Noshad, M. 2022. Effect of transglutaminase and buffalo milk incorporation on textural parameters and starter cultures viability of strained yogurt. *Journal of Food and Bioprocess Engineering*, 5(2): 195-202.
- [47] Kouravand, F., Jooyandeh, H., Barzegar, H. and Hojjati, M. 2018. Characterization of cross-linked whey protein isolate-based films containing *Satureja khuzistanica* Jamzad essential oil. *Journal of Food processing and preservation*, 42(3): e13557: 1-10.



## تأثیر افزودن آنزیم آلفا-آمیلاز و ترانس گلوتامیناز بر خواص فیزیکوشیمیایی و بافت نان همبرگر

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اطلاعات مقاله	چکیده
<b>تاریخ های مقاله :</b> تاریخ دریافت: ۱۴۰۴/۰۷/۲۴ تاریخ داور: ۱۴۰۴/۰۸/۱۸ تاریخ پذیرش: ۱۴۰۴/۰۸/۲۶	استفاده از آرد کامل در تولید نان همبرگر، به واسطه ویژگی های ساختاری آن، موجب افت کیفیت بافتی محصول نهایی می شود. برای رفع این چالش، راهکارهای متعددی پیشنهاد شده که از جمله مهم ترین آن ها می توان به بهره گیری از افزودنی هایی نظیر آنزیم ها، به ویژه ترانس گلوتامیناز و آلفا آمیلاز اشاره کرد. در این پژوهش، تأثیر افزودن آنزیم ترانس گلوتامیناز در سه سطح ۰، ۰/۱۵ و ۰/۳ درصد و آلفا آمیلاز در سه سطح ۰، ۰/۱ و ۰/۲ درصد بر ویژگی های فیزیکوشیمیایی و بافتی نان همبرگر تهیه شده از آرد کامل مورد بررسی قرار گرفت. ویژگی های بافتی مورد ارزیابی شامل سفتی، پیوستگی، صمغیت و چسبندگی بودند. نتایج حاصل از تحلیل داده ها نشان داد که استفاده از آنزیم ترانس گلوتامیناز موجب افزایش معنی دار پروتئین، رطوبت، سفتی، پیوستگی و صمغیت نان شد؛ در حالی که مقدار pH کاهش یافت. از سوی دیگر، افزودن آلفا آمیلاز ضمن افزایش درصد پروتئین، رطوبت و پیوستگی بافت نان، باعث کاهش میزان سفتی و صمغیت آن به شکلی معنی دار گردید. به طور کلی، نتایج این تحقیق حاکی از آن بود که کاربرد همزمان دو آنزیم ترانس گلوتامیناز و آلفا آمیلاز می تواند به عنوان راهبردی مؤثر در ارتقاء کیفیت بافتی نان حاصل از آرد کامل مورد استفاده قرار گیرد. در میان تیمارهای آزمایشی، افزودن آنزیم ترانس گلوتامیناز در سطح ۰/۱۵ درصد و آلفا آمیلاز در سطح ۰/۲ درصد بهترین عملکرد را در بهبود ویژگی های بافتی نان از خود نشان داد.
<b>کلمات کلیدی:</b> آلفا-آمیلاز، بافت سنجی، ترانس گلوتامیناز، خواص فیزیکوشیمیایی، نان همبرگر.	
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