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Original research

Improving the rheological properties of 18% wheat flour as affected by transglutaminase enzyme

Rahim Rahebi Bardi[a], Mahsa Tabari[b,*], Hamid Tavakolipor[a]

[a] *Agriculture Engineering-Food Sciences and industries, Islamic Azad University, Tehran North Branch, Tehran, Iran*
[b] *Department of Food Sciences and Technology, Faculty of Agriculture, Islamic Azad University, Lahijan Branch, Lahijan, Iran*

A B S T R A C T

Enzymes are useful to modify wheat proteins to preserve the gas better and to correct the rheological properties of the dough of weak flour and bread. Gluten proteins are highly impacting the quality of various gluten-based products, and transglutaminases (TGs) leading to the strengthening, stability and constancy of the dough as well as the improvement of the volume, texture and storage time of the bread. In the present study, the effect of transglutaminase enzyme on physicochemical and rheological properties of 18% wheat flour was examined as well as polymerization was achieved at the optimum mixing time. The use of transglutaminase (TG) has grown in popularity as they promote specific cross-linking between residues of glutamine and lysine in proteins and significantly increased the dough water absorption compared to the control sample. The results of bread staling evaluation by an instrumental or by instron method showed that the required amount of compression of bread was significantly lower than other treatments during the third and fifth days ($p < 0.01$). The results of the evaluation of gluten includes the use of heating and shear forces, which may impact gluten dough-forming ability, showed that the factors of form and shape, characteristics of the surface of bread, porosity, and bread score (qualitative number) were significantly higher than other treatments. Thus, increased understanding of the interplay of gluten functional and the impact of the TG origin in gluten dough functional properties is highly applicable in food industry.

Keywords: Transglutaminase enzyme, Barbari bread, Farinograph, dough, Crosslinking

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1. Introduction

Optimization of dough characteristics and improvement of the quality and durability of the final product are in the top priority in the bread industry (AACC International, 2000). In the past, oil and then various chemicals were used as dough enhancers, but today, natural ingredients and enzymes have been used to fixing bread defects with their specific effects (Aalami & Leelavathi, 2008; Armero & Collar, 1996). The bakery industry has been using yeasts and enzymes for many years to produce many of its products (Babaei Aminlooie & Salehifar, 2017). Most wheat produced in Iran have low quality of gluten due to the type of soil and geographical condition of the country. In such cases, a suitable combination of improvers such as enzymes, emulsifiers, hydrocolloids (gums) can be used to improve the rheological properties of bread dough (Basman et al., 2002; Bauer et al., 2003). Enzymes also play a role in delaying staling because they affect macromolecular compositions of the dough and cause them to decompose and, on the other hand, produce substances that can delay the staling (Feyzipour et al., 2004). Recently, the microbial transglutaminase enzyme has been studied more as a potential enzyme to improve the dough properties and quality of final products, including the effects of MTG on the properties of dough, enhancement in the extensibility, adhesion, water availability and consequently baking quality (Ortolan & Steel, 2017). The results of various studies have shown that the addition of transglutaminase enzymes significantly increases the quality parameters of bread (Rajab Zadeh, 1996; Mohtarami et al., 2015), reported in their study that the transglutaminase enzyme has a positive and significant effect on extensiogram parameters and improves the properties of dough. In another study, Babaei Aminlooie and Salehifar (2017) reported that adding the transglutaminase enzyme

*Corresponding author.
E-mail address: ma.tabari@gmail.com (M. Tabari).
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to the treatments significantly increased the gluten index of the samples and increased the water absorption, the strength of the dough stability and the quality number of the farinograph. Therefore, in this study, it was tried to mix 18% wheat flour and TG in different proportions. Having used the unique function of the transglutaminase enzyme and its effect on the binding of wheat proteins, it could be possible to increase the quality of bread.

2. Material and Methods

2.1. Specifications of consumed raw materials

The flour used in this study was Barbari flour extraction rate bought from Alborz Flour Company. Instant yeast was bought from Razavi Yeast Company. The crystallized and packaged salt was bought from the QOM refined salt company. The transglutaminase enzyme was bought from ORBA Turkey, the German Mullen Chemie Company, and the used wheat bran was bought from Alborz Flour Company.

2.2. Specifications of machines and practical equipment

Farinograph and extensograph (HABELT, Germany) was used to measuring the rheological properties of the dough. Instron Device, Model M350-10CT, was utilized for evaluating the bread's staleness and falling number device was provided from PERTEN company.

2.3. Specifications of treatments

The characteristics of treatments have been shown in Table 1. In this study, the direct method was used to prepare the dough. In this method, the flour and all the raw materials were poured into the mixing container and having a specific amount of water and the dough was prepared in one-step. Mixing the components of the dough continued to reach a desirable consistency of 15 min (500 g), and rounding dough and its fermentation was continued for 15 minutes at 30°C and 75-85% relative humidity. After the fermentation, the dough was divided into 500 g slices being left for 10-15 min. The duration of secondary fermentation in the oven lasted for 45 min at 30-35°C, and the bread was baked in an industrial oven at 260°C for 1 min. The temperature of the oven was measured by the thermocouple. Based on the research method, with 9 treatments and 2 replications, physicochemical tests were performed on the control (treatment 1) including wet content, ash, gluten, gluten index, protein, falling number, zeleny and particle size, as well as rheological tests including extensograph and farinograph tests. Physico-chemical and rheological tests were performed on other samples treated with different enzymes. Then, the best treatments were selected and a baking test was performed on them. Finally, the breads were examined in terms of sensory and staling tests. 5 trained students were used in the sensory tests. The bread staling method was done in the first, third, and fifth days by the means of an artificial instrument. Finally, statistical analysis of the data was done. Physico-chemical tests including protein, zeleny, falling number and rheological tests including extensographic and farinographic tests were conducted at the rheology department of Alborz Flour Company. Other physicochemical tests including wet content, ash, gluten, gluten index and particle size were performed in Chemistry Lab and Alborz Flour Quality Control Company. Bread was baked in traditional Barbari bakery. The tests related to sensory evaluation of bread were performed by 5 qualified judges. Bread staling was evaluated in the Agricultural Faculty of Tehran University.

2.4. Flour chemical characteristics methods

Protein measurements were carried out according to AACC standard No. 16-46 (National Standard No. 2863) by the Kjeldahl method. Gluten moisture was analyzed in accordance with AACC Standard No. 11-38 (National Standard No. 9639-2). Furnace method was conducted according to AACC Standard No. 01-08 (National Standard No. 103) to detecting Ash. Moisture was performed according to oven method and AACC standard No. A 16-44 (National Standard No. 3681). Measuring Zelleny number was carried out in accordance with AACC Standard No. 11-56 (National Standard No. 3681). Measuring the falling number was carried out in accordance with AACC Standard No. B 81-56 (National Standard No. 4175). Particle size was measured according to National Iranian Standard No. 103. Gluten index was carried out in accordance with AACC standard A12-38.

2.5. Rheological tests of dough

2.5.1. Farinographic test

It was carried out in accordance with AACC No. 21-54 (National Standard of Iran, No. 1-3246), by Habelt Farinograph Device.

2.5.2. Extensographic test

It was carried out in accordance with AACC No. 10-54 (National Standard of Iran, No. 2-3246), by Habelt Extensograph Device.

2.5.3. Organoleptic (sensory) tests

This test is a kind of ranking, in which a numerical scale is used so that the numbers are at intervals. The numerical scale may be a polar (zero number on one side of the scale) or bipolar (two opposite traits on both sides). This method can be used to evaluate the severity of some of the physical properties of a product.

2.5.4. Staling test

In order to evaluate the bread, different tests and methods can be used. In this research, it was tried to use Evaluation methods for organoleptic testing and briefly described in order to evaluate the quality of bread from two sensory and machine aspects and Sensory evaluation of staling Bread staling test was performed according to AACC No. 30-74.

2.6. Instrumental methods of Staling (Bread compressibility test with Instron)

Instron has been used for mechanical test of bread. This device is designed to study the stress-tension properties of materials, being able to carry out a wide range of common tests of tensile,

compression, shear and so on. To determine the staling, bread packs were placed in polyethylene bags after cooling and were stored at room temperature. Bread strength was measured on days 1, 3, and 5 using Instron machine and the required amount of energy (Newton) was measured to condense bread loaves. A pressure test with 50 kg load cell and a plate probe with an advancing speed (decreasing speed) of 60 mm / min or 1 ml/s was considered, the initial height of the sample was considered according to AACC standard No. 7409, 25 × 25 mm. The pressure value was 40% and the initial speed and compressibility of the samples were about 10 mm. Finally, the highest point was read on the resulting curve (stiffness).

Table 1. Treatments used in the research.

Code	Characteristics
T0	Control sample (no transglutaminase enzyme)
T1	Sample containing 1ppm transglutaminase enzyme in 100 g flour
T2	Sample containing2 ppm transglutaminase enzyme in 100 g flour
T3	Sample containing3 ppm transglutaminase enzyme in 100 g flour
T4	Sample containing4 ppm transglutaminase enzyme in 100 g flour
T5	Sample containing5 ppm transglutaminase enzyme in 100 g flour
T6	Sample containing6 ppm transglutaminase enzyme in 100 g flour
T7	Sample containing7 ppm transglutaminase enzyme in 100 g flour
T8	Sample containing8 ppm transglutaminase enzyme in 100 g flour

Table 2. Results obtained from flour's physicochemical properties tests.

Sample test	Ash (%)	Wet gluten (%)	Gluten index	Falling number (s)	Zeleny number (mm)
T0	0.80±0.00b*	25.10±0.00^{a}	60.11±0.05^{d}	316.55±1.50^{a}	20.00±0.00^{b}
T1	0.80±0.00^{a}	25.15±0.05^{a}	63.80±0.28^{c}	310.00±1.50^{b}	20.00±0.00^{b}
T2	0.80±0.00^{a}	25.18±0.05^{a}	64.17±0.04^{c}	304.00±1.50^{c}	20.00±0.00^{b}
T3	0.80±0.00^{a}	25.20±0.20^{a}	65.13±0.05^{bc}	298.00±1.50^{d}	20.00±0.00^{b}
T4	0.80±0.00^{a}	25.20±0.20^{a}	66.38±0.37^{bc}	292.00±1.50^{e}	20.00±0.00^{b}
T5	0.80±0.00^{a}	25.35±0.25	65.69±1.69^{b}	286.00±1.50^{f}	20.00±0.00^{b}
T6	0.80±0.00^{a}	25.35±0.25^{a}	66.43±1.65^{ab}	280.00±1.50^{g}	20.25±0.50^{ab}
T7	0.80±0.00^{a}	25.65±0.05^{ab}	66.06±0.00^{ab}	247.00±1.50^{h}	20.50±0.25^{ab}
T8	0.80±0.00^{a}	25.75±0.05^{a}	67.65±0.64^{a}	267.00±1.50^{i}	21.00±0.50^{a}

*The different small letters are indicative of the significant difference in the column ($p < 0.05$).

Table 3. Results obtained from the farinographic test of the dough containing various amounts of trans-glutaminase enzyme.

Sample	Farinograph's quality number	Dough's loosening degree after 20 min (Brabender)	Dough's loosening degree after 12 min (Brabender)	Dough's resistance time (min)	Dough's expansion time (min)	Dough's water absorption (%)
T0	52.55±1.25b*	2.55±0.05^{b}	2.55±0.02^{c}	147.00±14.00^{a}	158.18±0.00^{a}	33.50±10.50^{c}
T1	52.80±0.40^{ab}	2.55±0.05^{b}	2.90±0.01^{bc}	143.00±7.50^{a}	154.00±12.00^{a}	38.00±2.00^{b}
T2	52.65±0.25^{ab}	2.60±0.40^{b}	3.00±0.02^{bc}	149.00±21.00^{a}	152.00±27.00^{a}	37.50±1.50^{b}
T3	53.15±0.55^{ab}	2.75±0.40^{ab}	3.05±0.02^{bc}	137.00±5.00^{a}	146.80±8.00^{ab}	36.00±1.00^{b}
T4	53.56±0.35^{ab}	2.85±0.55^{ab}	3.15±0.01^{bc}	129.00±7.00^{ab}	138.00±5.50^{ab}	37.00±3.00^{b}
T5	53.70±0.50^{ab}	2.85±0.55^{ab}	3.20±0.01^{ab}	126.00±20.00^{ab}	131.00±20.00^{ab}	39.50±3.50^{b}
T6	53.80±0.90^{ab}	2.95±0.15^{ab}	3.45±0.01^{ab}	105.00±18.00^{bc}	115.00±21.00^{bc}	38.00±5.00^{b}
T7	53.85±0.15^{ab}	3.20±0.10^{a}	2.55±0.02^{c}	104.13±15.50^{bc}	114.00±17.50^{bc}	42.00±2.00^{a}
T8	53.95±0.85^{a}	3.30±0.10^{a}	2.90±0.01^{bc}	86.50±7.50^{c}	91.00±10.00^{c}	43.50±2.50^{a}

*The different small letters are indicative of the significant difference in the column ($p < 0.05$).

Table 4. Results obtained from the doughs' stretching resistance based on Brabender for 45, 90 and 135 min.

	Time (min)		
Sample	45(min)	90(min)	135(min)
T0	239.00±10.00^{eA}	280.00±4.00^{dC}	309.00±4.00^{dB}
T1	258.00±8.50^{dB}	324.00±9.50^{cC}	364.00±18.00^{cB}
T2	262.00±0.50^{dcA}	350.03±6.50^{bB}	368.00±14.00^{cC}
T3	257.00±8.00^{dA}	358.00±6.00^{bC}	375.00±7.00^{cB}
T4	272.00±1.50^{bcdB}	378.00±8.00^{bC}	366.00±1.00^{cC}
T5	270.00±4.50^{bcd}	375.00±10.50^{bA}	390.00±3.00^{bC}
T6	277.00±16.00^{bcB}	354.00±10.00^{bC}	393.00±6.00^{bB}
T7	285.00±2.50^{bA}	354.00±9.00^{bC}	415.00±0.50^{aB}
T8	301.00±12.00^{aA}	363.00±6.50^{abA}	415.00±2.50^{aB}

*The different small letters are indicative of the significant difference in the column ($p < 0.05$).
**The different capital letters are indicative of the significant difference in the line ($p < 0.05$).

2.7. Statistical analysis of data

The experiment was conducted in a completely randomized design with 9 treatments in 2 replications. First, one-way analysis of variance and then a comparison of means (of Duncan's range) were conducted at a significant level of 0.05%. Statistical analysis was performed using SPSS, version 16, software and Microsoft Office Excel program.

3. Results

3.1. Ash of flour

The results of variance analysis of samples' ash showed that the effect of treatment on flour's ash containing different levels of transglutaminase enzyme was not significant ($p > 0.05$). In addition, although enzyme level was increased, the results of comparison of the means in samples did not show any statistically significant amount of ash ($p > 0.05$) (Table 2).

3.2. Wet gluten of flour (in percent)

The results of the analysis of wet gluten's variance showed that the effect of treatment on wet gluten containing different levels of transglutaminase enzyme was not significant ($p < 0.01$). In addition, the results of the comparison of the wet gluten's average showed that although the increase in the enzyme level increased the wet gluten of the samples, it was not statistically significant ($p > 0.05$) (Table 2).

3.3. Gluten index of flour

The results of the analysis of variance in gluten index showed that the effect of treatment on gluten index of dough containing different amounts of transglutaminase enzyme was significant ($p < 0.01$). In addition, the results of the comparison of the average gluten index showed that with increasing the enzyme, the gluten index of the samples increased significantly ($p < 0.01$). Although the highest gluten index was observed in T6, T7 and T8 treatments, there was no statistically significant difference between the treatments. The lowest gluten index was in the control sample ($p < 0.01$) (Table 2).

3.4. Falling number of flour

The results of the analysis of variance of the falling number in samples showed that the effect of treatment on the falling number of dough was significant ($p < 0.01$). In addition, the results of the comparison of the average of the falling number of samples showed that by increasing the amount of enzyme, the number of falling number in the samples decreased significantly ($p < 0.01$). Therefore, the lowest falling number was observed in T8 treatments and highest falling number was in control sample ($p < 0.01$) (Table 2).

3.5. Zeleny number of flour

The results of the analysis of Zeleny number's variance showed that the effect of treatment on the Zeleny number of the dough containing different amounts of transglutaminase enzyme was significant ($p < 0.01$). In addition, the results of the comparison of zeleny number's average in the samples indicated that with increasing enzymes, Zeleny number of samples increased significantly so that the highest zeleny number was observed in treatments T6, T7 and T8 ($p < 0.01$) (Table 2).

3.6. Farinograph test

The results of the analysis of variances showed that the effect of treatment on water absorbtion levels of dough content was not significant ($p > 0.05$). In addition, the results of comparison of samples' average showed that the lowest percentage of water absorption was observed in the control dough. On the other hand, with increasing the enzyme, water absorption increased, although this increase was not significant statistically ($p > 0.05$). Addition of the transglutaminase enzyme significantly increased the dough water absorption only when compared to the control sample ($p < 0.05$) (Table 3).

3.7. Dough development time (min)

The results of the analysis of variance of samples showed that the effect of treatment on development time of dough containing different amounts of transglutaminase enzyme was not significant ($p > 0.05$). Also, the results of the comparison of samples' averages showed that with the increase in enzyme, the samples' dough development time increased, although this increase was significant at the extensibility time of some treatments ($p > 0.05$), so there was a statistically significant difference between treatments T7 and T8 compared to T0, T1, T2 ($p < 0.01$) (Table 3).

3.8. Dough's stability time test

The results of variance analysis showed that the effect of treatment on the stability time of dough containing different amount of transglutaminase was not significant ($p > 0.05$). The results of the comparison of averages showed that with increase in enzyme, the resistance time of the dough increased and a significant difference was observed between treatments T6, T7 and T7 compared to other treatments ($p < 0.01$) (Table 3).

3.9. Degree of loosening the dough after 12 min (Brabander)

The results of the analysis of variance of samples showed that the effect of treatment on the degree of loosening of the dough was significant ($p > 0.05$). The results of comparison of averages showed that by increasing the amount of enzyme, the degree of loosening the dough (after 12 min) decreased, although this decrease was significant in some of the treatments ($p < 0.01$). There was a statistically significant difference between treatments T7, T6 and T8 compared to treatments T0, T1, T2 and T3 ($p < 0.01$) (Table 3).

3.10. Degree of loosening the dough after 20 min (Brabander)

The results of variance analysis showed that the effect of treatment on the degree of loosening the dough containing different

amounts of transglutaminase enzyme (after 20 min) was significant ($p > 0.05$). The results of the comparison of the mean of samples showed that the degree of loosening the dough (after 20 min) decreased with the increase in the enzyme; this reduction was significant for some of the treatments ($p < 0.01$). There was a statistically significant difference between treatments T7, T6 and T8 compared to treatments T0, T1, T2, T3, T4, T5 ($p < 0.01$) (Table 3).

Table 5. Results obtained from maximum resistance to the dough containing various amounts of transglutaminase enzyme.

	Time (min)		
Sample	45(min)	90(min)	135(min)
T0	241.00±10.00^{fA}	283.00±3.50^{dC}	370.00±5.00^{eB}
T1	259.00±3.00^{dA}	326.00±9.00^{cC}	376.00±17.00^{dB}
T2	264.00±9.00^{deB}	351.00±6.50^{bB}	370.03±13.00^{dC}
T3	268.03±7.50^{cdeA}	359.00±6.00^{bc}	376.50±7.00^{cdB}
T4	278.00±2.00^{bcdB}	354.00±10.50^{bC}	376.50±7.00^{cdB}
T5	278.00±3.50^{bcdA}	354.00±10.50^{bc}	390.00±3.00^{bcC}
T6	284.00±18.00^{bcB}	365.00±6.00^{abC}	393.00±6.50^{bB}
T7	292.00±5.50^{abA}	379.00±9.00^{aC}	419.00±2.00^{aB}
T8	303.00±13.50^{aA}	378.00±10.00^{aA}	419.00±3.00^{aB}

*The different small letters are indicative of the significant difference in the column ($p < 0.05$).
**The different capital letters are indicative of the significant difference in the line ($p < 0.05$).

Table 6. Results obtained from the elasticity of the doughs containing various amounts of transglutaminase enzyme.

	Time (min)		
Sample	45(min)	90(min)	135(min)
T0	115.00±0.50^{eA}	99.50±8.50^{dC}	92.00±1.00^{cB}
T1	120.03±4.00^{deA}	103.00±0.50^{bcA}	93.50±0.45^{cB}
T2	122.00±4.50^{dcB}	105.00±1.00^{cdB}	93.00±3.00^{cB}
T3	122.00±1.50^{dcB}	105.00±0.50^{cC}	99.00±4.50^{cC}
T4	126.00±0.00^{bcA}	106.00±2.00^{cA}	100.00±4.00^{bB}
T5	127.00±1.50^{bcA}	108.00±0.24^{cdC}	100.00±4.00^{bB}
T6	128.00±4.50^{bB}	113.20±0.35^{abC}	101.00±3.50^{bB}
T7	131.00±4.00^{abA}	113.20±0.35^{abC}	102.00±2.50^{bC}
T8	135.00±0.50^{aB}	116.20±0.14^{aC}	116.00±1.00^{aB}

*The different small letters are indicative of the significant difference in the column ($p < 0.05$).
**The different capital letters are indicative of the significant difference in the line ($p < 0.05$).

Table 7. Results obtained from ratio number of dough containing various amounts of transglutaminase enzyme.

	Time (min)		
Sample	45(min)	90(min)	135(min)
T0	1.90±0.10^{eA}	2.40±0.10^{eC}	3.55±0.05^{dB}
T1	2.00±0.00^{deB}	2.85±0.15^{dB}	3.55±0.05^{dB}
T2	2.20±0.10^{bcA}	2.85±0.15^{dB}	3.65±0.25^{dc}
T3	2.10±0.10^{dcA}	3.30±0.10^{bcC}	2.65±0.05^{eB}
T4	2.10±0.00^{dcB}	3.30±0.10^{bc}	3.75±0.05^{cdB}
T5	2.10±0.00^{dcA}	3.45±0.05^{abA}	3.95±0.15^{cC}
T6	2.15±0.05^{bcB}	3.60±0.10^{aA}	4.20±0.00^{bB}
T7	2.25±0.05^{bA}	3.55±0.15^{aB}	4.50±0.00^{aB}
T8	2.45±0.05^{aA}	3.65±0.25^{aA}	4.50±0.20^{aB}

*The different small letters are indicative of the significant difference in the column ($p < 0.05$).
**The different capital letters are indicative of the significant difference in the line ($p < 0.05$).

Table 8. Results obtained for the energy of dough containing various amounts of transglutaminase enzyme (in cm^2).

	Time (min)		
Sample	45(min)	90(min)	135(min)
T0	44.50±2.50^{dA}	50.00±0.14^{dC}	47.50±1.50^{aB}
T1	51.03±0.50^{cA}	53.50±5.00^{dC}	52.50±4.50^{aB}
T2	52.000±3.00^{cB}	55.00±0.50^{cdC}	55.00±1.00^{aC}
T3	54.00±2.00^{cA}	56.00±1.00^{bcB}	54.50±2.50^{aB}
T4	57.50±1.50^{abA}	55.00±1.00^{bcC}	54.50±0.50^{aB}
T5	58.00±5.00^{abB}	57.50±1.50^{abA}	54.50±2.50^{aC}
T6	58.00±3.00^{abA}	58.00±1.50^{abC}	54.50±1.00^{ab}
T7	58.50±3.00^{abA}	60.00±1.00^{aA}	54.50±1.50^{aC}
T8	60.00±0.50^{aB}	60.00±1.00^{aC}	55.50±1.50^{aB}

*The different small letters are indicative of the significant difference in the column ($p < 0.05$).
**The different capital letters are indicative of the significant difference in the line ($p < 0.05$).

3.11. *Quality number*

The results of variance analysis of samples showed that the effect of treatment on the quality number of the Farinograph of the dough containing different amount of transglutaminase enzyme (after 12 min) was significant ($p > 0.05$). The results of the comparison of mean samples showed that the quality number of T7 and T8 were significantly higher than other treatments ($p < 0.01$). The lowest quality number was observed in the control sample and there was no significant difference between the other treatments ($p > 0.05$) (Table 3).

3.12. *Stretching resistance of the dough in terms of Brabender at 45, 90 and 135 min*

The results of variance analysis of samples at all-time intervals showed that the effect of treatment on the resistance strength of dough containing different amounts of transglutaminase enzyme (after 12 min) was significant ($p > 0.05$). The results of the comparison of the averages of samples in 45 min interval after fermentation showed that the resistance strength of the T8 was significantly higher and the resistance strength of the T0 dough was significantly lower than other treatments ($p < 0.01$) (Table 4).

The results of the comparison of the samples' averages in the 90 min interval after fermentation showed that the resistance strength of the T1 and T0 were significantly lower than other treatments ($p < 0.01$) and the statistical difference in the stretching resistance of other treatments were not observed. The results of the comparison of the mean of samples in the time interval of 135 min after fermentation showed that the stretching resistance of T7 and T8 was significantly higher and the resistance of T0 was significantly lower than other treatments ($p < 0.01$). Also, in all time intervals, by increasing the amount of transglutaminase enzyme, the amount of resistance to stretching of the dough was increased in terms of the Brabender (Table 4).

3.13. *Maximum stretching resistance to extension in terms of Brabender in the intervals of 45, 90 and 135 min*

The results of variance analysis of samples at all-time intervals showed that the effect of treatment on stretching of the dough was significant ($p > 0.05$). The results of the comparison of mean samples in the 45-minute interval after fermentation, showed that the resistance strength of the T8 treatment was significantly higher and the resistance strength of the T0 dough was significantly lower than other treatments ($p < 0.01$). The results of the comparison of mean samples in the 90 min interval after fermentation indicated that the tensile strength of the dough treatments was significantly higher than the T8, T7 and T6 treatments, and the stretching resistance of T0 was significantly lower than other treatments ($p < 0.01$). The results of the comparison of the average of samples in the time interval of 135 min after fermentation showed that the stretching resistance of T8, T7 and T6 treatments was significantly higher and the T0 stretching resistance was significantly lower than other treatments ($p < 0.01$) (Table 5).

The results of variance analysis of samples at all-time intervals showed that the effect of treatment on elasticity resistance was significant ($p > 0.05$). The results of the comparison of the samples' averages in the 45-minute interval after fermentation showed that the elasticity of the T7 and T8 treatments was significantly higher and the elasticity of T0 was significantly lower than other treatments ($p < 0.01$). The results of the comparison of the samples' average in the 90 min interval after fermentation showed that the elasticity of the dough was significantly higher in the T6, T7 and T8, the T0 elasticity resistance of samples in the time interval of 135 min after fermentation showed that the elasticity of the dough was significantly higher in T8 and the elasticity resistance of the treatments T0, T1, T2 and T3 were significantly lower than the other treatments ($p < 0.01$) (Table 6).

3.14. *Dough ratio number at 45, 90 and 135 min intervals*

The results of variance analysis of samples at all-time intervals showed that the effect of treatment on the ratio number of dough containing different amounts of transglutaminase enzyme was significant ($p > 0.05$). The results of the comparison of the average of samples in the 45 min interval after fermentation showed that the ratio number of the dough in T8 code was significantly higher and the T0 ratio number of the dough was significantly lower than other treatments ($p < 0.01$). The results of the comparison of the samples` average in the 90 min interval after fermentation indicated that the ratio of the dough in T5, T6, T7 and T8 treatments were significantly higher and the T0 ratio of the dough ratio was significantly lower than other treatments ($p < 0.01$) .The results of the comparison of the mean of samples in the time interval of 135 min after fermentation showed that the ratio number of T6, T7 and T8 treatments were significantly higher and the ratio number of the T0, T1, T2 were significantly lower than other treatments ($p < 0.01$) (Table 7).

The results of dough energy in terms of square centimeters in the intervals of 45, 90 and 135 min showed that only in the 45 and 90 min after fermentation, the effect of treatment on the energy of the dough containing the different amounts of transglutaminase enzyme was significant ($p > 0.05$). The results of the comparison of the average of samples in 45 min interval after fermentation indicated that the energy of the dough in T8 was significantly higher and the energy of T0 was significantly lower than other treatments ($p < 0.01$). The results of the comparison of the average of samples in the 90-minute interval after fermentation indicated that the ratio number of the dough in T5, T6, T7 and T8 treatments was significantly higher and the number ratio of the dough in T0 was significantly lower than other treatments ($p < 0.01$). The results of the comparison of the average of samples in the time interval of 135 min after fermentation showed that the ratio number of the dough in T6, T7 and T8 treatments was significantly higher and the ratio number of T0, T1, T2 and T2 dough were significantly lower than the other treatments ($p < 0.01$) (Table 8).

3.15. *Staling evaluations of Barbari bread in an instrumental method containing optimal amounts of transglutaminase enzyme*

The results of variance analysis of samples showed that during all time intervals, the effect of treatment on staling of Barbari breads was significant ($p < 0.01$). The results of the comparison of average of the samples on the first day showed there was no statistically significant difference in the required force to compress the breads containing the optimal amounts of the transglymatease enzyme. At the third and fifth day, the required power for the compression of the T8 and T8 codes was significantly lower than the T0 code ($p < 0.01$). In addition, with time passing from day one

to day five, the required compression force in all breads was significantly increased ($p < 0.01$) (Table 9).

3.16. *Sensory evaluation of Barbari bread containing optimal amounts of transglutaminase enzyme*

The results of variance analysis showed that the effect of treatment on all studied sensory factors was significant except for the characteristics of the lower surface of the breads containing the optimal amounts of transglutaminase enzymes ($p < 0.01$). The results of the comparison of the mean of samples showed that the factors affecting form and shape, characteristics of the surface of bread, porridge and porosity and bread score (qualitative number), the score allocated to T8 treatment were significantly higher than other treatments ($p < 0.01$). In other factors, no statistically significant difference was found in T7 and T8 treatments (Table 10).

Table 9. Results obtained for the samples' staleness by the use of instrumental method (N).

	Force (Newton)		
Sample	Day 1	Day 3	Day 5
T0	2.26 ± 1.13^{bC}	11.76 ± 1.12^{aB}	15.50 ± 1.50^{aA}
T7	3.29 ± 0.56^{abC}	7.72 ± 0.84^{bB}	11.20 ± 1.50^{bA}
T8	3.38 ± 0.49^{abC}	6.50 ± 0.50^{cB}	10.50 ± 1.50^{abA}

*The different small letters are indicative of the significant difference in the column ($p <0.05$).
**The different capital letters are indicative of the significant difference in the line ($p <0.05$).

Table 10. Results obtained from the sensory evaluation of barbari bread containing optimal amounts of transglutaminase enzyme.

Property	T0	T7	T8
Form and shape	6.00 ± 1.26^{B}	6.59 ± 0.89^{B}	9.24 ± 0.78^{A}
Properties of the bread's power surface	3.00 ± 1.22^{B}	3.39 ± 0.41^{B}	4.75 ± 0.43^{B}
Properties of the bread's upper surface	6.47 ± 0.43^{B}	7.51 ± 0.57^{B}	9.11 ± 0.46^{A}
Hollowness and porosity	9.60 ± 2.27^{B}	6.78 ± 1.11^{C}	14.28 ± 1.61^{A}
Chewability	7.02 ± 2.30^{C}	12.80 ± 1.30^{B}	14.39 ± 1.30^{AB}
Hardness and softness of the bread's texture and structure	9.20 ± 2.90^{C}	18.10 ± 2.40^{B}	18.78 ± 2.60^{AB}
Odor and flavor taste of the bread	6.80 ± 4.20^{C}	9.73 ± 1.90^{B}	23.60 ± 1.20^{BC}
Total score/20=bread score (qualitative number)	48.20 ± 5.40^{C}	61.92 ± 6.8^{B}	94.16 ± 8.10^{A}

*The different capital letters are indicative of the significant difference in the line ($p <0.05$).

Table 11. Staling evaluations by the sensory method of Barbari bread containing optimal amounts of transglutaminase enzyme.

	samples' bread staleness		
Sample	Day 1	Day 3	Day 5
T0	4.20 ± 0.50^{aA}	2.50 ± 0.22^{cB}	2.00 ± 0.35^{cC}
T7	4.50 ± 0.20^{aA}	3.20 ± 0.30^{bB}	3.00 ± 0.50^{bC}
T8	4.50 ± 0.19^{aA}	3.70 ± 0.15^{aB}	3.50 ± 0.50^{aC}

*The different small letters are indicative of the significant difference in the column ($p <0.05$).
**The different capital letters are indicative of the significant difference in the line ($p <0.05$).

3.17. *Staling evaluations by the sensory method of Barbari bread containing optimal amounts of transglutaminase enzyme*

The results of the analysis of samples' variance showed that during all time intervals except for day 1, the effect of treatment on staling of Barbari breads was significant ($p < 0.01$). The results of comparing the average of samples on the first day after baking- showed that there was no significant difference in the score allocated to bread's staling. At the third and fifth days after baking, the scores allocated to the T8 bread were significantly higher and the allocated score for the T0 code was significantly lower ($p < 0.01$) (Table 11).

4. Discussion

The results of this study showed that there was no statistically significant difference for ash by increasing enzymes, which can be attributed to the very small amounts of mineral compounds in the enzyme and the use of very low enzymes in this study. The results of this study were in line with the results of studies by Babaei Aminlooie and Salehifar (2017) which stated that, by adding the transglutaminase enzyme and sodium caseinate, water absorption was significantly increased; the effect of this enzyme on the formation of crosslinking in gluten proteins has been attributed to increased gluten strength and water holding capacity. In addition, the results of this study were consistent with the findings of Basman et al. (2002), which showed that by increasing the percentage of the enzyme, the water absorption of dough in both types of flour was significantly decreased.

The results of this study were in line with the findings of Babaei Aminlooie and Salehifar (2017), which stated that, with the increasing levels of transglutaminase and sodium caseinate, the development time of dough significantly increased. In the present study, it can be stated that higher percentages of enzymes have been able to produce stronger dough. Babaei Aminlooie and Salehifar (2017) acknowledged that the resistance of the dough was improved by the addition of transglutaminase and sodium caseinate and it was significantly increased with increasing concentrations, which was in agreement with the results of this study. The results of this study in terms of the degree of loosening of the dough after 12 and 20 min (Brabender) were in line with the results of Babaei Aminlooie and Salehifar (2017), who acknowledged that the addition of the microbial transglutaminase and sodium caseinate, decreased the degree of loosening of the dough after 12 min, significantly. The results of this study on the quality number of the Farinograph were in line with the results of Babaei Aminlooie and Salehifar (2017), which stated that the quality of the Farinograph significantly increased with increasing enzymes. In the present study, it can be stated that the higher percentages of the enzyme have been able to significantly increase the quality of the farinograph due to more and stronger connections and, consequently, a wider protein network. The results obtained from this study in terms of the maximum resistance of elasticity of dough based on Brabender at 45, 90 and 135 min intervals were in line with the results of Mohtarami et al. (2015) who stated that among the studied factors, the interaction between the type of flour and transglutaminase had the highest contribution to increasing the elasticity resistance of the dough. Concerning the results of elasticity -in millimeters- at 45, 90, and 135 min, the results of this study were consistent with the results of studies by Simurina et al. (2014), which observed a linear relationship between transglutaminase and the elasticity of the dough, and on the other hand, the elasticity has increased as the L-ascorbic acid has been added. Concerning the results of dough energy in cm^2 in the 45, 90 and 135 min intervals, the results were in line with the results of Mohtarami et al. (2015) who stated that the transglutaminase enzyme also had a single significant effect on the amount of energy and resulted in an increase in the energy of the dough in terms of square centimeters. Babaei Aminlooie and Salehifar (2017) also reported that with increasing the enzyme and sodium caseinate, the energy needed to change the shape of the dough significantly increased, which was in line with the results of the current study.

5. Conclusion

The results of physicochemical tests of flour containing transglutaminase enzymes showed that with increasing of enzyme, the gluten index and zeleny number of samples increased significantly and the falling number of samples decreased significantly. In addition, the results of test showed that the addition of the transglymatinase enzyme significantly increased the water absorption of dough compared to the control sample. Also, with increasing the enzyme, the extensibility time, and the time of the resistance of the dough and the quality number of the forinograph were increased and the degree of loosening of the dough (after 12 and 20 min) of the samples was decreased significantly. The results of extensographic test in 45, 90 and 135 min intervals were significantly increased with the increase in the amount of enzyme's resistance to extension, maximum elasticity resistance, elasticity, ratio number and energy of dough. The results of texture analysis of the machine method showed that there was no significant difference for force needed to compress the breads containing the optimal amounts of transglutaminase enzyme on day one. On the third and fifth days, the force required to compress the bread was significantly lower than the treatments of T0 and T7 and T8. The results of the evaluation showed that the score allocated to T8 treatment in the factors of form and shape, characteristics of the surface of bread, and porosity and bread score (qualitative number), were significantly higher than other treatments.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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