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Evaluation of the effect of alpha-amylase and transglutaminase enzyme treatment on the rheological properties of dough and some baking characteristics of hamburger buns prepared from whole wheat flour

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ABSTRACT

Bread, as a staple food in many countries, requires quality optimization within the baking industry. White bread is highly popular due to its appealing appearance and soft texture; however, the removal of bran during its production leads to a reduction in fiber, vitamins, and minerals. In contrast, whole wheat flour offers higher nutritional value but results in decreased loaf volume, reduced porosity, and accelerated staling. To address these challenges, this study investigated the effects of two enzymes, α-amylase (at levels of 0%, 0.1%, and 0.2%) and microbial transglutaminase (at levels of 0%, 0.15%, and 0.3%), on the rheological properties of bread dough (including water absorption, dough stability, degree of softening, extensibility, and resistance to extension), as well as on porosity and specific volume of whole-wheat bread. The results showed that α-amylase increased specific volume and porosity, while transglutaminase reduced these parameters ($p<0.001$). Furthermore, farinograph and extensograph tests revealed that transglutaminase enhanced water absorption and reduced dough softening, whereas α-amylase decreased dough stability and increased softening ($p<0.05$). The control sample exhibited the highest extensibility and dough stability. Therefore, by using these enzymes at appropriate levels during bread production from wholemeal flour (0.1% alpha-amylase and 0.15% transglutaminase), it is possible to produce a product with higher nutritional value and acceptable rheological characteristics.

1-Introduction

Despite the development and extensive competition in the production of various foods, bread is still recognized as one of the main pillars of the diet [1]. This product, with its relative richness in nutrients, reasonable price, and wide availability, is mainly prepared from wheat flour in many regions of the world [2]. However, the removal of bran in the flour production process leads to a decrease in the content of fiber, vitamins, and minerals in bread. On the other hand, the use of wholemeal flours, due to the high concentration of phytic acid, makes it difficult to absorb essential elements such as iron, zinc, and calcium, and can lead to the occurrence of some nutritional disorders. In this regard, efforts to improve the nutritional value of bread by adding beneficial compounds to flour continue to attract the attention of researchers and food industry professionals [3-5]. Industrial breads have gained a significant position in daily food supply due to their desirable baking quality, wide product diversity, appropriate shelf life, and complete fermentation stage [6]. Nowadays, the use of food additives in the bakery industry is considered a common method to improve the quality, increase the shelf life and enhance the nutritional properties of bread [7]. These compounds can help produce breads with more diverse flavors, higher nutritional value and more stable quality [3 and 8].

The role of flour in bread quality has always been of interest to artisans in this field, because flour is of particular importance due to its starch and protein compounds. Wheat flour has high baking and bakery properties due to the viscoelastic properties of gluten proteins. Weak gluten causes the gluten network in the dough to fail to retain the gases produced by yeasts well and the dough structure to collapse during fermentation. Therefore, dough strength and elasticity are two key and important parameters for evaluating dough quality. To solve this problem, researchers have studied the effect of adding various compounds to weak flours to determine the effective factors and optimal amount of their use. These improving substances include enzymes, vegetable and microbial gums, oxidizing agents, mono- and diglycerides, phospholipids, and sterols.[9[. One of the effective methods for improving gluten quality is the use of enzymes, which leads to increased volume, improved structure, increased shelf life and improved flavor of bread. Enzymes act as biological catalysts that, unlike chemical catalysts, have a very specialized and targeted function. Enzymes are generally known as natural compounds, and given the increasing trend of the food industry to use natural materials and also the restrictive rules of the Food and Drug Administration regarding chemical additives, the use of enzymes in the food industry has become increasingly important. [10]

The enzyme alpha-amylase improves and increases the volume of bread by creating usable sugars for yeasts and helping to produce more gas in the dough. Amylase hydrolyzes (1-4) bonds.aGlycosidase in starch molecules breaks them down into maltodextrins and oligosaccharides. This process allows yeasts to continuously ferment and produce CO gas.$_2$produce, which ultimately leads to an increase in bread volume and improved internal texture. In addition, the simple sugars and oligosaccharides produced enhance the Maillard reactions, which play an important role in creating the desired brown color of the bread crust and the specific flavor of the final product [11]. The enzyme transglutaminase is also able to modify the functional properties of proteins by creating intermolecular and intramolecular covalent bonds. Studies have shown that isopeptide bonds created by transglutaminase can improve the dough properties of low-protein flours, such as soft wheat flour and gluten-free blends, and ultimately enhance the baking properties of the dough.[12[Research by Meerts et al.[13[and Bagagli et al.[14[It has been shown that the enzymes glucose oxidase and transglutaminase are able to improve the viscoelastic properties of flour by changing the structure of the gluten network, so that the use of these enzymes enhances the elastic properties and increases the strength of the dough.

Nowadays, consumption of bread made from wholemeal flour is recommended due to its high nutritional properties. However, bread made from such flour is of low quality, so that consumers prefer to use bread made from white flour instead. Since the use of polysaccharide-degrading enzymes such as alpha-amylase and transferase enzymes such as transglutaminase is considered as one of the effective strategies for improving the quality of bread, in the present study, the synergistic effect of alpha-amylase and transglutaminase enzymes in improving the physical and rheological properties of dough and bread made from wholemeal flour was investigated.

2- Materials and methods

2-1- Evaluation of dough rheological properties

2-1-1- Farinograph test

The farinograph test was performed to measure the water absorption of flour and to examine the stability and degree of dough loosening using a Brabender farinograph model 816100.00, made in Germany. This device records the mixing of flour with water at constant speed and temperature and measures the dough resistance during formation. The test is continued until the dough reaches the desired consistency (equivalent to 500 Brabender units) and by determining the amount of water absorption over a specified period of time, it provides accurate information about the rheological changes of the dough [15, 16].

2-1-2- Extensograph test

The tensile properties and tensile strength of the dough were evaluated using a Brabender extensograph, model 860704, manufactured in Germany [17]. This device simulates the performance of the dough during the fermentation process and, by measuring the force required to stretch the dough, analyzes its ability to develop and retain gases resulting from the decomposition of sugars and starch. This test allows for a detailed examination of the rheological properties associated with the fermentation process and plays an effective role in predicting the performance of the dough at different stages of production and baking [18].Flour was weighed to 300 g and placed in the mixer of the farinograph. Then, 2% sodium chloride was dissolved in 250 ml of water in an Erlenmeyer flask; the amount of water added was equal to the water absorption of the flour minus 1%. The saline solution was slowly added to the flour through a burette and the mixture was stirred for one minute in the farinograph. After preparation, the sample was placed in the center of the skein and the hook of the device and the timer was set to 45 minutes. Similar steps were performed for the second sample and then both samples were placed in the fermentation chamber along with the holding skein. After 45 minutes of skeining the first sample, the container containing the dough with the hook was placed on the level arm of the extensograph, the recording pen was zeroed and the stretching operation began. After the dough was torn by the hook, the sample was removed and the kneading and rolling steps were repeated on the dough piece and it was placed in the fermentation chamber again for another 45 minutes. The same process was repeated for the second sample and finally the relevant graphs were drawn [16 and 17].

2-2- Hamburger bun evaluation tests

2-2-1- Production of hamburger bun samples

Wholemeal flour (Dareshahr Company) was used to prepare the bread. Bread samples were prepared using the direct dough method.[1]and were produced using the method of Joyandeh et al. with slight modifications [3]. Fungal alpha-amylase enzyme (with an activity of 120,000 SKB/min) at levels of 0, 0.1 and 0.2 percent and transglutaminase enzyme (with an activity of 100 units per gram of protein) at levels of 0, 0.15 and 0.3 percent (each based on flour weight) were added to the formula. The dough was prepared using a dough mixer and after 20 minutes of fermentation, it was punched into 70-gram pieces. The dough pieces were placed in a warm room at a temperature of 30 to 32 degrees Celsius and a relative humidity of 85 percent for 50 minutes to rest. The baking process was carried out at a temperature of 15±250°C for 25 minutes. During the production of the samples, the temperature, thickness and dimensions of the bread were kept constant and uniform. After cooling, the resulting breads were packaged in polyethylene bags and stored at room temperature for further testing [3].

1-straight dough method

2-2-2- Specific volume

In order to conduct this experiment, within a maximum period of two hours after the baking process, pieces of bread with dimensions of 2×2 cm were prepared from its geometric center. The weight of each piece was accurately measured. Then, each piece was placed in a container with a certain volume (V_1) was placed and the remaining empty space was filled with rapeseed seeds. After removing the bread piece, the volume occupied by the rapeseed seeds (V_2) was recorded and the actual volume of bread was determined from the difference between the total volume of the container and the volume of the grains (V_1-V_2) was calculated. Finally, the specific volume of the samples was determined by dividing the volume by the weight [19].

2-2-3- Porosity

Image processing was used to measure the porosity of bread crumb. For this purpose, first 5×5 cm slices of bread crumb were prepared. The image of each piece was taken by placing a digital camera at a fixed distance of 30 cm from each sample, in a black box with 45-degree illumination by fluorescent lamps. Then, the porosity of the samples was calculated using Image-J 1.46r software [20].

2.3- Statistical analysis

To investigate the effect of enzymatic treatments with alpha-amylase and microbial transglutaminase on the rheological properties of bread dough (flour water absorption, stability, degree of loosening, elasticity and tensile strength) as well as the porosity and specific volume of bread samples, the data obtained The experiments were analyzed using SPSS version 19 statistical software. One-way analysis of variance test was used to determine significant differences between the means of treatments. The significance level in all tests was considered equal to $p<0.05$. All experiments were performed in triplicate.

3- Results and discussion

3-1- Characteristics of dough farinograph

3-1-1- Water absorption

The results of the farinograph test are presented in Table 1. According to the results obtained, the addition of alpha-amylase (AA) enzyme reduced the water absorption of the dough. The average water absorption in samples containing 0, 0.1 and 0.2% alpha-amylase was determined to be 64.70, 63.36 and 60.45%, respectively, with no significant difference between the control and the sample containing 0.1%. The decrease in water absorption as a result of increasing the concentration of alpha-amylase is probably due to the activity of this enzyme on amylose and amylopectin molecules, which causes their decomposition into smaller units such as dextrin. This decomposition causes the release of more water into the environment, which ultimately causes the dough to become sticky and reduces its water absorption. These results are consistent with the findings of the study by Sanzpenella et al. [21]. This result is also consistent with the results of the study by Russell [7], although in this study, higher concentrations of the enzyme were tested than in this study. In contrast, transglutaminase (TG) enzymatic treatment increased the absorption. The average water absorption in samples containing 0, 0.15 and 0.3% transglutaminase was determined to be 61.39, 63.13 and 63.99%, respectively, with no significant difference between the sample containing 0.15% and the control and the sample containing 0.3% transglutaminase. The increase in water absorption due to the addition of the transglutaminase enzyme can be attributed to the formation of more and stronger connections, and consequently a wider protein network, by the said enzyme. The formation of a stronger protein network allows water to be trapped more easily and more within the network, increasing water absorption [22] Marco and Russell [23] have also reported an increase in dough water absorption by adding transglutaminase enzyme and attributed the reason to the cross-links created by transglutaminase enzyme. In fact, these links cause the formation of protein polymers with high water retention capacity [24]. The Anyway, as mentioned, the treatment Transglutaminase at the highest the level (0.3%) did not produce a significant difference compared to the lower level (0.15), which is probably due to excessive intra- and extra-molecular connections that can somewhat affect the water absorption rate. [25]. According to Table 1, the lowest water absorption (14.59%) was in the sample containing 0.2%Alpha-amylaseand 0%TransglutaminaseAnd its highest amount

(65.96%) was in the sample containing 0%Alpha-amylase and 0.3%TransglutaminaseIt was determined.

3-1-2- Stability time

The results of this study showed that enzymatic treatment with alpha-amylase enzymes(01/0>p) and transglutaminase(05/0>p) It has caused a significant reduction in the shelf life of the dough.The average dough stability in samples containing 0, 0.1 and 0.2% alpha-amylase was determined to be 3.17, 2.68 and 2.32 minutes, respectively, with a significant difference between samples containing the enzyme. Alpha-amylase It didn't exist. These findings are consistent with the results of the study by David et al.[26[These researchers attributed the decrease in dough strength and stability to the effect of the alpha-amylase enzyme on starch structure and its hydrolysis; by breaking down starch and producing maltose, this enzyme leads to gluten dehydration and the release of more water into the dough environment, and as a result, the dough viscosity decreases. Also, a decrease in dough stability was observed with the addition of transglutaminase enzyme. The average dough stability in samples containing 0, 0.15, and 0.3 percent transglutaminase was determined to be 2.90, 2.85, and 2.42 minutes, respectively, and there was no significant difference between the control and the sample containing 0.15 percent transglutaminase. The decrease in dough stability when using higher levels of transglutaminase enzyme could be due to changes in protein structure and disruption of the gluten network of the dough.[22 and 27]As can be seen in Table 1, the highest dough stability (3.52 minutes) was for the control sample, while the lowest stability (2.29 minutes) was for the sample containing the highest levels of both enzymes.

3-1-3- Degree of softness/looseness of dough

The results of the farinograph test showed that the addition of alpha-amylase enzyme caused a significant increase in(001/0>p) The dough was loose (Table 1).The average dough looseness in samples containing 0, 0.1 and 0.2% alpha-amylase was determined to be 154.7, 186.4 and 210.6 Brabender units, respectively, with no significant difference between different levels of the enzyme. Alpha-amylase It was determined. As mentioned in the previous sections, the alpha-amylase enzyme increases free water due to the hydrolysis of starch and the production of maltodextrin, oligosaccharides, and malt trioses, and subsequently the production of maltose, which results in a decrease in stability and an increase in the degree of dough loosening. These results are in accordance with the study by Pierce et al.[28] In contrast, the addition of transglutaminase enzyme at high levels caused a significant decrease in(001/0>p) The degree of softening of bread dough samples increased in terms of unit weight. The average dough looseness in samples containing 0, 0.15, and 0.3 percent transglutaminase was determined to be 0.195, 202.9, and 153.9 Brabender units, respectively. Here, too, there was no significant difference between the control and the sample containing 0.15 percent transglutaminase. The reason for this is the effect of the transglutaminase enzyme in forming a protein network that can strengthen the dough texture and increase its strength. The reason for the difference in the effect of the enzyme on the degree of dough looseness can be attributed to the dual effect of the enzyme. In small amounts of enzyme Transglutaminase Due to the formation of appropriate cross-links between proteins in the dough and the trapping of water in the network, a gluten network with viscoelastic properties is produced; however, in the presence of a high percentage of this enzyme, these connections become stronger and more numerous, causing mechanical damage to the gluten network and a decrease in elasticity.[29[Qureshi and colleagues[22[reported that adding too much transglutaminase enzyme causes the protein network to become brittle and weakens the gluten structure. In fact, wheat gluten proteins lose their functional properties in the presence of excessive connections due to the addition of the enzyme. Therefore, it can be concluded that this enzyme can increase the water absorption, strength and stability of the dough up to certain levels by creating new connections and stronger and wider networks, but adding higher amounts of the enzyme does not always increase the quality.[30]. As can be seen in Table 1, the highest dough slackness (6.228 Brabender) was found in the sample without enzyme. Transglutaminase and 0.2% enzyme Alpha-amylase while the lowest stability (132.5 Brabender) belongs to the sample containing the highest level of enzyme. Transglutaminase and without enzymes Alpha-amylase It is.

Table 1. Comparative analysis of Farinograph and Extensograph dough properties

Characteristics	0% alpha-amylase			0.1% alpha-amylase			0.2% alpha-amylase		
	0% TG	0.15% TG	0.3% TG	0% TG	0.15% TG	0.3% TG	0% TG	0.15% TG	0.3% TG
Water Absorption (%)	63.53 ± 3.40^{abc}	64.62 ± 1.73^{ab}	65.96 ± 0.87^{a}	61.50 ± 2.11^{abc}	63.82 ± 1.27^{ab}	64.76 ± 1.17^{ab}	59.14 ± 0.98^{c}	60.95 ± 1.30^{bc}	61.26 ± 2.35^{bc}
Stability Time (min)	3.52 ± 0.40^{a}	3.35 ± 0.46^{ab}	2.64 ± 0.33^{bc}	2.87 ± 0.08^{abc}	2.86 ± 0.21^{abc}	2.33 ± 0.17^{c}	2.33 ± 0.45^{c}	2.34 ± 0.24^{c}	2.29 ± 0.24^{c}
Softening (BU)	161.3 ± 3.40^{d}	170.3 ± 3.40^{cd}	132.5 ± 15.90^{and}	195.1 ± 7.34^{abc}	209.1 ± 8.68^{ab}	155.1 ± 16.72^{of}	228.6 ± 3.59^{a}	229.3 ± 12.72^{a}	174.0 ± 12.66^{cd}
Extensibility (mm)	160.2 ± 9.57^{def}	151.2 ± 2.50^{if}	134.6 ± 5.08^{f}	185.0 ± 10.03^{bcd}	191.44 ± 13.46^{bc}	169.5 ± 11.96^{cde}	224.9 ± 14.04^{a}	228.5 ± 2.69^{a}	203.55 ± 21.60^{ab}
Resistance to Extension (EU)	141.5 ± 11.04^{d}	143.7 ± 16.62^{d}	220.2 ± 12.72^{a}	131.9 ± 2.76^{of}	133.2 ± 5.99^{of}	194.0 ± 7.00^{b}	112.8 ± 4.23^{and}	115.1 ± 5.99^{and}	164.2 ± 4.43^{c}

Similar letters within each row are not significantly different at the $p<0.05$ statistical level.

3-2- Characteristics of dough extensograph

3-2-1- Dough elasticity

Based on the results of dough extensography, enzymatic treatmentTransglutaminaseUnlike enzymesAlpha-amylaseIt caused a decrease in dough elasticity. In other words, the addition of alpha-amylase had a significant positive effect on the dough development parameter during proofing.The average dough stretchability in samples containing 0, 0.1 and 0.2% alpha-amylase was determined to be 148.6, 0.182 and 0.219 mm, respectively, with no significant difference between each of the different enzyme levels.Alpha-amylase It was determined.Sanz Penela and colleagues[21[In support of the results of this study, the use of amylolytic and phytate-degrading enzymes was recommended to overcome the detrimental effect of bran on mineral availability (phytase) and also on dough technological performance. The increase in dough elasticity due to alpha-amylase enzyme activity is due to starch hydrolysis and weakening of the dough network structure.

On the other hand, according to the results of this study, it was determined that although enzyme treatment at a level of 0.15% did not have a significant effect on the elasticity properties of the dough, higher levels of enzyme significantly reduced elasticity.AverageElasticityThe dough in samples containing 0, 0.15 and 0.3% transglutaminase was determined to be 0.190, 190.4 and 169.2 mm, respectively, and here too there was no significant difference between the control and the sample containing 0.15% transglutaminase.. The significant decrease in elasticity in the sample containing 0.3% transglutaminase enzyme is attributed to the formation of stronger and more frequent cross-links between proteins. This process leads to a strengthening of the dough structure and, as a result, a decrease in its elasticity, so that the dough ruptures more quickly. In fact, the behavior of such a dough is more similar to a solid than to a viscoelastic system. In this case, due to the weakening of the viscous properties of the dough, the irreversible strain is reduced relative to the reversible strain.[29[.

Based on the extensography results presented in Table 1, the highest elasticity of the dough (224.9)Millimeter) corresponding to the sample without enzymeTransglutaminaseand 0.2% enzymeAlpha-amylase is, while the lowest stability (6.134)Millimeter) to the sample containing the highest level of enzymeTransglutaminaseand without enzymesAlpha-amylase It was awarded.

3-2-2- Tensile strength

Based on the results obtained, alpha-amylase enzyme reduced the dough's tensile strength.The average tensile strength of the dough in samples containing 0, 0.1 and 0.2% alpha-amylase was determined to be 168.5, 153.0 and 130.7 respectively, with no significant difference between each of the different enzyme levels.Alpha-amylaseIt was determined.In general, factors that help strengthen the dough structure can increase its tensile strength; in contrast, factors that reduce dough strength, which are usually caused by weakening the bonds in the dough matrix, reduce this property. The enzyme alpha-amylase, through starch hydrolysis, weakened the dough structure and consequently reduced its tensile strength. In addition, the addition of the microbial transglutaminase enzyme increased the tensile strength of the dough, which is consistent with the findings of the research of Bauer et al.[29[It is aligned.AverageTensile strengthThe dough in samples containing 0, 0.15 and 0.3% transglutaminase was determined to be 128.7, 130.6 and 192.8 Extensograph Units (EU), respectively, and here too there was no significant difference between the control and the sample containing 0.15% transglutaminase.This increase in strength is due to the formation of cross-links between proteins by the enzyme transglutaminase, which leads to the strengthening of the gluten network and improvement of the viscoelastic properties of the dough.[29[It is worth noting that different formulations and process conditions have a significant impact on the final results. In fact, enzyme performance is highly dependent on the process conditions. For example, Laber et al.[31[In a study of the effect of preincubation of skim milk with microbial transglutaminase (3 units per gram of milk protein) at 40°C for 150 minutes and subsequent heat inactivation during yogurt preparation, they reported that the consistency of yogurt increased by about 67% with increasing preincubation time and reached a constant level after 60 minutes. The results of these researchers showed that even very small amounts of casein crosslinking can lead to significant changes in the functional properties of milk proteins.

3-3- Evaluation of bread baking characteristics

3-3-1- Specific volume

The results of the effect of microbial alpha-amylase and transglutaminase enzymes on the specific volume of the prepared breads are presented in Figure 1. As can be seen in Figure a-1, the addition of alpha-amylase enzyme to breads prepared from wholemeal flour had a significant effect on the specific volume of the samples ($p<0.001$). In contrast, the interaction effect of the two enzymes alpha-amylase and transglutaminase on this property was not statistically significant ($p<0.05$). According to the data presented (Figure a-1), increasing the percentage of alpha-amylase led to an increase in the specific volume of the bread (Figure 1). However, no significant difference was observed between the control sample (0.47 ml/g) and the sample containing 0.1% alpha-amylase (0.5 ml/g) ($p<0.05$). The enzyme alpha-amylase breaks down starch into simple sugars such as glucose, maltose, and dextrin, providing nutrients for yeast growth and activity. This process leads to increased production of CO gas.$_2$And as a result, the volume of bread will increase. In fact, the increase in CO production$_2$and improving dough elasticity, both of which work to increase the final volume of bread and reduce its texture hardness [32].

In a study by Benjamin et al. [33], the effect of alpha-amylase, xylanase, and lipase enzymes on the quality characteristics of bread was investigated. The results showed that the use of 30 ppm lipase and 30 ppm amylase led to an increase in bread volume and a decrease in its texture stiffness. Wang et al. [34] also investigated the effect of alpha-amylase, xylanase, glucose oxidase, cellulase, and lipase enzymes on the organoleptic properties of frozen pizza dough. Their findings showed that except for alpha-amylase, other enzymes improved the sensory properties of the dough and obtained a higher score than the control sample. Among them, xylanase had the greatest effect on improving the organoleptic quality. Also, the evaluation of the fermentation process of frozen pizza dough showed that in the presence of xylanase, cellulase, and lipase, the dough volume increased by 33.2, 19.7, and 7.4 percent, respectively. Liu et al. [35] investigated the effect of amylase and xylanase on the rheological properties of bran-enriched bread dough. The results showed that amylase significantly increased dough softening, increased mixing time index, and improved dough expansion ability.

According to the data presented in Figure b-1, the percentage of transglutaminase enzyme had a statistically significant effect on the specific volume of bread samples ($p<0.001$), so that with an increase in the percentage of this enzyme, the specific volume of bread decreased. However, no significant difference was observed between the control sample (0.55 ml/g) and the sample containing 0.15% transglutaminase (0.53 ml/g) ($p<0.05$). This decrease in specific volume can be attributed to the formation of cross-links between proteins. The wheat flour used in this study was of a very strong type with high gluten and a significant gluten index. In such conditions, the transglutaminase enzyme, by strengthening the gluten network and creating more connections between proteins, creates a strong protein structure that prevents the bread from opening and increasing the volume. Therefore, in flours with high protein and high gluten quality, the effect of this enzyme on the volume of bread will be inverse. In contrast, transglutaminase can be useful for flours with low protein quality [36].

Figure 1. Effect of transglutaminase (TG) and alpha-amylase enzymes on the specific volume of bread samples. Different letters indicates the values are not significantly different (p<0.05).

Stefani et al.[37[In a study of the effect of microbial transglutaminase enzyme on the properties of voluminous bread, they reported that the addition of this enzyme caused an increase in the specific volume and a decrease in the firmness of the bread crumb at intermediate levels (0.1%). Based on the findings of Gerard et al.[38[The enzyme transglutaminase can play an effective role in improving the bread production process and has a similar function to improvers and oxidizers. The researchers found that adding this enzyme at a level of 2% had a negative effect on bread quality. This negative effect was attributed to the formation of excessive and intense cross-links in the gluten network, which leads to a stiffer texture. While these cross-links are beneficial to bread structure in moderation, their excessive increase prevents bread volume from increasing and prevents the texture from opening and puffing, which was also consistent with the results of this study.

Pourasmael and colleagues[39[By examining the effect of guar gum and transglutaminase enzyme on the specific volume of bread, they found that adding guar gum at levels of 2 and 3% significantly increased the specific volume of bread compared to the control sample. In contrast, using transglutaminase enzyme and increasing its amount in the bread formulation resulted in a significant decrease in the specific volume compared to the control sample. The mechanism of action of transglutaminase is related to the creation of cross-links between the amino acids glutamine and lysine. These cross-links prevent the growth of gas cells during the fermentation process and consequently reduce the specific volume of bread. Also, Bassman et al.[40[reported a decrease in the specific volume of bread made from wheat flour due to the addition of the enzyme transglutaminase. According to their findings, the use of this enzyme in amounts higher than 1% causes a decrease in the volume of bread and destruction of its crust texture.

3-3-2- Porosity

The results of the porosity test of the produced bread samples are reported in Figure 2. As shown in Figurea-2 It is clear that the addition of alpha-amylase enzyme had a significant effect on the porosity of the bread samples (0.001).>p); so that with increasing the percentage of this enzyme, the porosity of the bread samples increased from 33.06% in the control sample to 36.40% in the sample containing 0.2% alpha-amylase (Figure a-2Enzymes, especially amylases, act as a modifier in the production of bakery products. Alpha-amylase reduces the hardness and softens the texture of baked products by breaking down starch and producing short-chain dextrins as a result of starch hydrolysis.[41[Based on the research findings of Sheikholeslami et al.[19[The presence of alpha-amylase enzyme in the formulation of barbary bread, prepared from wheat flour with different degrees of extraction, had a positive effect on the texture, specific volume, porosity and sensory properties of the final product. In this regard, Zeng et al.[42[evaluated the effect of α-amylase and glucose oxidase as bread improvers on the textural and thermal properties of bread using rapid viscosity analysis and differential scanning calorimetry. It was found that α-amylase and glucose oxidase could improve the specific volume and porosity of bread crumb, delay bread staleness, and increase the ethanol index of flour. Therefore, these researchers suggested the use of α-amylase and glucose oxidase as potential texture modifiers for baked products.

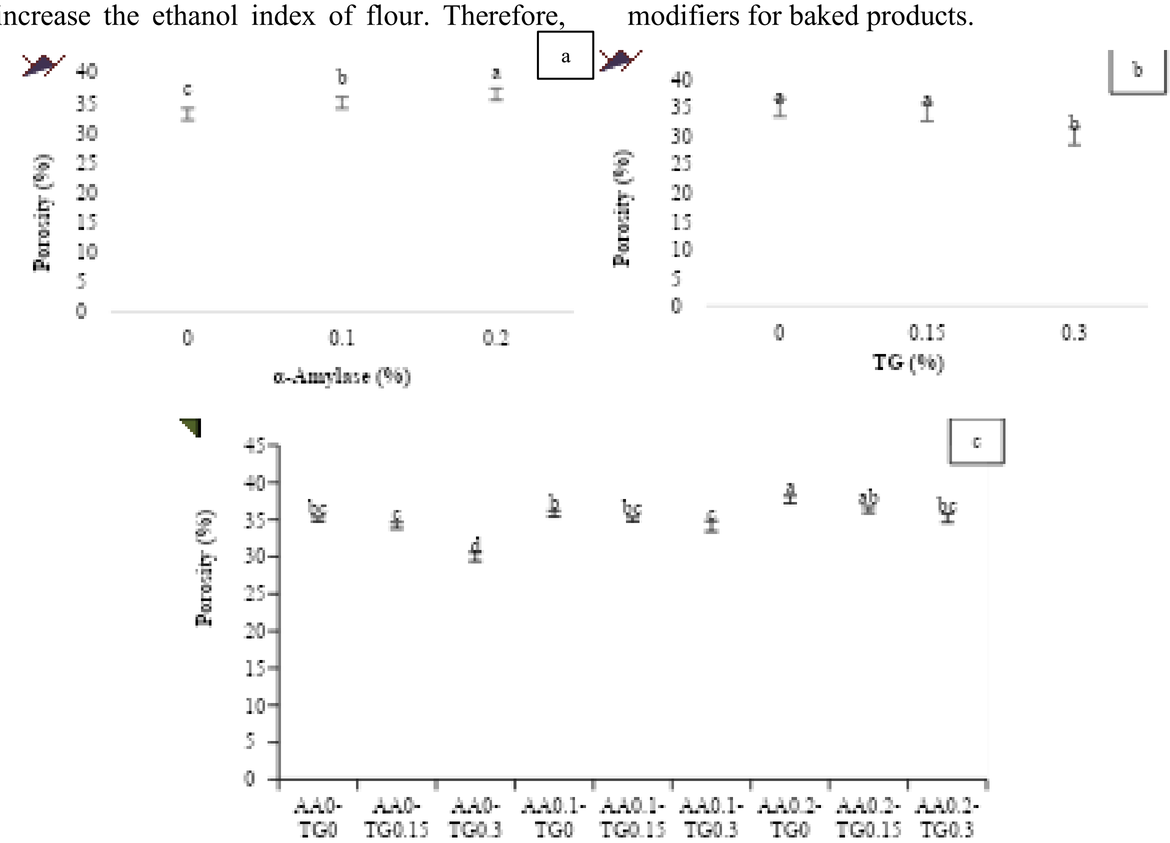

Figure 2. Effect of transglutaminase (TG) and alpha-amylase (AA) enzymes on the porosity of bread samples. Different letters indicates the values are not significantly different ($p<0.05$).

According to Figure b-2, the addition of transglutaminase enzyme to breads made from wholemeal flour had a significant effect on the porosity of the samples ($p<0.001$). With increasing enzyme content, the porosity of the bread samples decreased from 35% in the control sample to 30% in the sample containing 0.3% transglutaminase. However, no significant difference was observed between the control sample and the sample containing 0.15% transglutaminase (34.20%) (Figure b-2). Shin et al. [43], examining gluten-free bread made from rice flour and adding 0.01% transglutaminase enzyme, found that the decrease in specific volume and porosity occurred simultaneously and that both indices decreased under the influence of the enzyme. In another study aimed at investigating the effect of adding different levels of sodium caseinate (0, 3, and 6%) and microbial transglutaminase enzyme (0, 0.75, and 1.5%) on the rheological, physical, and sensory properties of gluten-free bread based on corn flour, it was found that adding the enzyme up to 0.75% significantly reduced porosity [44]. Studies by Delozhovska et al. [45] also confirmed the effectiveness of adding transglutaminase on the quality of gluten-free bread and showed that this enzyme reduces the porosity of the final product.

Figure c-2 shows the interaction effects of both enzymes on the porosity of bread samples; according to this graph, increasing different concentrations of transglutaminase caused a decrease and adding alpha-amylase led to an increase in the porosity of bread samples compared to the control sample. In general, the highest porosity (37.70%) was related to the sample containing 0.2% alpha-amylase without transglutaminase and the lowest porosity (30%) was related to the sample with 0.3% transglutaminase without alpha-amylase.

4- conclusion

Wheat bread, as one of the main food items worldwide, plays an important role in providing daily energy and protein to humans. Among the types of bread, white bread has been more popular with consumers due to its more desirable sensory characteristics such as light color, soft texture, and pleasant taste. The process of producing white bread requires the removal of wheat bran during the milling stage; a measure that, although it helps improve the organoleptic properties of the final product, leads to a significant reduction in the nutritional value of the bread, especially in terms of dietary fiber, vitamins, and minerals. On the other hand, the use of wholemeal flour in bread production, although more nutritionally desirable, is associated with challenges in the final quality of the product; including a decrease in specific volume, a loss of softness and crunchiness of the texture, and an acceleration of the stale process. To overcome these limitations, several solutions have been proposed, one of the most effective of which is the use of biotechnological additives such as enzymes. Meanwhile, microbial transglutaminase and alpha-amylase enzymes play an important role in improving the quality of produced bread by affecting the structure of the gluten network and improving the rheological properties of dough. According to the findings of this study, the addition of alpha-amylase enzyme led to an increase in the parameters of specific volume and porosity of bread, which indicates an improvement in the structure and aeration of the final product. In contrast, the use of transglutaminase enzyme caused a decrease in these two parameters, which could be due to the strengthening of the gluten network and greater compactness of the dough texture. Among the treatments studied, the combination of transglutaminase enzyme at a level of 0.3% and alpha-amylase at a level of 0.2% showed the best performance in terms of physical and rheological properties. For a more accurate evaluation, farinograph and extensograph tests were performed on the selected doughs. The results of these tests showed that the sample containing 0.3% transglutaminase had the highest water absorption and the lowest degree of looseness, which indicates the strengthening of the dough structure and increased water retention ability. In contrast, the sample containing 0.2% alpha-amylase had the lowest water absorption and stability and showed the highest degree of laxity, which could be related to the hydrolytic activity of the enzyme and the relative destruction of the gluten structure. Also, the control sample (without enzyme addition) showed the highest degree of stability and elasticity in the extensograph test, indicating the preservation of the natural structure of the dough without enzymatic intervention.

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Data access

They will be available upon request.

ContradictionBenefits

The authors have no conflicts of interest.

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Authors' Contributions

Ruqayyah Hassan Beigi: Data collection, formal analysis, sources, writing - first draft.

Hossein Joyandeh: Conceptualization, project management, research, visualization, methodology, validation, writing - review and editing.

Mohammad Hojjati: Conceptualization, visualization, methodology, software, validation.

Hassan Barzegar: Research, methodology, software, formal analysis.

6- Resources

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مقاله علمی_پژوهشی

ارزیابی تأثیر تیمار آنزیمی آلفاآمیلاز و ترانس‌گلوتامیناز بر خواص رئولوژیکی خمیر و برخی ویژگی‌های پخت نان همبرگر تهیه شده از آرد کامل گندم

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چکیده

نان به‌عنوان بخش اصلی رژیم غذایی در بسیاری از کشورها، نیازمند بهینه‌سازی کیفیت در صنعت نانوایی است. نان سفید با ظاهر و بافت مطلوب، محبوبیت بالایی دارد، اما حذف سبوس در تولید آن موجب کاهش فیبر، ویتامین‌ها و املاح می‌شود. در مقابل، آرد کامل ارزش تغذیه‌ای بالاتری دارد ولی موجب افت حجم نان، کاهش تخلخل و افزایش سرعت بیاتی می‌گردد. برای رفع این چالش، در پژوهش حاضر تأثیر دو آنزیم آلفاآمیلاز (در سطح ۰، ۰/۱ و ۰/۲ درصد) و ترانس‌گلوتامیناز میکروبی (در سطح ۰، ۰/۱۵ و ۰/۳ درصد) بر ویژگی‌های رئولوژیکی خمیر نان (جذب آب آرد، پایداری، درجه سست شدن، کشش‌پذیری و مقاومت به کشش) و همچنین تخلخل و حجم مخصوص نان تهیه شده از آرد کامل گندم مورد بررسی قرار گرفت. نتایج نشان داد که آلفاآمیلاز موجب افزایش حجم مخصوص و تخلخل نان گردید، در حالی‌که ترانس‌گلوتامیناز این پارامترها را کاهش داد (p<۰/۰۰۱). همچنین، آزمون‌های فارینوگراف و اکستنسوگراف نشان دادند که ترانس‌گلوتامیناز جذب آب را افزایش و درجه سستی را کاهش داد، در حالی‌که آلفاآمیلاز موجب کاهش پایداری و افزایش سستی (p<۰/۰۵) شد. نمونه شاهد نیز بالاترین کشش‌پذیری و پایداری را نشان داد. بنابراین، با به‌کارگیری این آنزیم‌ها در سطوحی مناسب در هنگام تولید نان از آرد کامل (۰/۱ درصد آلفاآمیلاز و ۰/۱۵ درصد ترانس-گلوتامیناز)، می‌توان محصولی با ارزش تغذیه‌ای بالاتر و ویژگی‌های رئولوژیکی قابل قبول تولید کرد.