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Production of hamburger buns made from whole wheat flour and investigation the effect of alpha-amylase and transglutaminase enzymatic treatment on the product

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ABSTRACT

Considering the important role of enzymes in improving the quality characteristics of bread, their use in the formulation of bakery products is of great importance. Therefore, the present study was conducted to investigate the effect of microbial α-amylase and transglutaminase enzymes on the sensory quality (overall acceptance) and color parameters (L*, a*, and b*) of hamburger bread, and then to investigate some characteristics of the optimized sample with the control sample during a 5-day storage period. The findings revealed that α-amylase, through starch hydrolysis and the production of simple sugars, intensified the Maillard reaction, resulting in a significant decrease in lightness and a significant increase in redness and yellowness in both the crust and crumb ($p < 0.05$). In contrast, transglutaminase strengthened the gluten network by forming covalent bonds between proteins and reduced the availability of free amino acids, thereby limiting the Maillard reaction. This led to a significant increase in lightness and a reduction in crust and crumb darkness ($p < 0.05$). Based on sensory evaluation, the bread sample treated with 0.1% α-amylase and 0.15% transglutaminase achieved the highest overall acceptance score of 8.2 and was identified as the optimal formulation. The results of the evaluation and comparison of the optimal sample with the control during storage showed that the optimal sample (prepared from whole wheat flour without enzymes) had higher moisture content, water activity, overall acceptability, and lower firmness than the control sample. These findings indicated that simultaneous and purposeful use of these two enzymes can significantly improve the organoleptic and visual quality of bread produced from whole wheat flour.

1- Introduction

Bread is a staple food consumed worldwide and plays a vital role in providing energy, protein, minerals, and vitamins essential for human health. Research shows that bread supplies 42% and 47% of daily energy in urban and rural communities, respectively [1]. Bread quality and the baking process depend largely on the raw materials—flour, yeast, salt, and water—while various additives are used to improve dough formulation, mechanical processing, tolerance to processing, and final product quality.

Since the early 1980s, the use of microbial enzymes (produced by fungi or bacteria) as alternatives to chemical improvers has grown across industries. Enzymes such as protease, α-amylase, cellulase, lipase, xylanase, pectinase, pullulanase, chitinase, and esterase are effective biomolecules with diverse industrial applications [2]. Today, bakers have access to a wide range of enzymes formulated specifically for breadmaking [3], and the bakery industry employs these enzymes to optimize dough properties and improve product quality [4].

Enzymes accelerate biochemical reactions in food processing, enhancing production efficiency and consistency. Key enzymes used in the cereal industry include glucose oxidase, transglutaminase, xylanase, lipase, phospholipase, and α-amylase. α-amylase (EC 3.2.1.1) catalyzes the breakdown of starch into simpler sugars, producing intermediate products such as short oligosaccharides, maltose, and glucose by random hydrolysis of starch chains [5, 6]. It converts damaged or gelatinized starch into dextrins or sugars that yeast can ferment, increasing carbon dioxide production and its retention within the gluten network, thereby improving loaf volume during baking. Overall, α-amylase contributes to bread quality by increasing loaf volume, improving texture, enhancing dough stability, and extending shelf life [5].

Microbial transglutaminase (MTG), a member of the transferase family, is widely distributed in nature. Prior to 1989, transglutaminase was mainly obtained from animal sources such as pig liver; later, microbial production was developed. A bacterial species, *Streptomyces verticillus* (previously *Streptoverticillium*), was identified as a suitable source for MTG production, and its extraction and purification have been achieved. The enzyme from this bacterium has a molecular weight of approximately 40 kDa and an isoelectric point near pH 8.9. Its activity is optimal between pH 4 and 9 and at temperatures of 37–50 °C. MTG catalyzes acyl transfer reactions that form stable covalent bonds between proteins, cross-linking the ε-amino group of lysine in one protein with the γ-carboxamide group of glutamine in another. The resulting crosslinks resemble peptide bonds, are resistant to pH and temperature changes, and do not adversely affect protein nutritional value [7–9]. In breadmaking, transglutaminase can improve the functional properties of flour proteins by forming long-chain, insoluble polymers [3].

This study aimed to investigate the effects of the starch-degrading enzyme α-amylase and the gluten-crosslinking enzyme transglutaminase on the quality of hamburger bread. Sensory attributes and colorimetric parameters were analyzed to assess the impact of these enzymatic treatments. After selecting the optimal formulation, several product characteristics were compared with a control sample (without α-amylase or transglutaminase) during storage.

2- Materials and methods

2-1- Preparation of wholemeal hamburger buns

Doughs were prepared by the direct dough method following Jooyandeh et al. [10] with minor modifications. The formulation (per batch) comprised 1000 g whole-wheat flour, 12 g dry yeast, 10 g shortening, 10 g salt, and 10 ppm potassium bromate improver.

α-Amylase was added at 0, 0.10 and 0.20% (w/w, based on flour) and MTG at 0, 0.15 and 0.30% (w/w, based on flour). Ingredients were mixed in a planetary dough mixer; after 20 min of bulk fermentation, the dough was divided and moulded into 70 g pieces. The pieces were proofed in a climate chamber at 30–32 °C and ~85% relative humidity for 50 min, then baked at 250 ± 15 °C for 25 min. After cooling, loaves were packed in polyethylene bags and stored at ambient temperature until analysis [10].

2-2- Evaluation of bread crust and crumb color

The analysis of the crust and crumb color of bread samples was performed by measuring three color parameters: brightness (L*), redness (a*), and yellowness (b*). The brightness index (L*) ranges from 0 to 100, and the redness (a*) and yellowness (b*) indexes range from -120 to +120 [11].

2-3- Sensory evaluation

Overall acceptability of samples treated with α-amylase and transglutaminase was assessed during 5 days of storage. Ten trained panelists evaluated samples using a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely) following the procedure of Sheikholeslami et al. [12].

2-4- Comparison of some characteristics of the optimal bread and control

2-4-1- Moisture

Moisture was determined according to the Iranian National Standard No. 1-2705 [13].

2-4-2- Water activity

Water activity (a_w) was measured using a Novasina MS1-AW (Axair Ltd., Switzerland). Approximately 1.0 g of finely ground sample was placed into the instrument sample cup and a_w was recorded after instrument equilibration according to the manufacturer's instructions.

2-4-3- Firmness

Bread firmness was measured by texture analysis following AACC method 74-09 using a TA-XT Plus texture analyzer (Stable Micro Systems, UK). Cubic crumb samples (2 × 2 × 2 cm) were cut from the center of each loaf. A 36 mm cylindrical probe compressed each sample at 1.0 $mm \cdot s^{-1}$ to 45% of its original height; peak force was reported as firmness [14].

2-5- Statistical analysis

Statistical analyses were performed using SPSS v.25. Experiments followed a completely randomized design with three replicates. One-way ANOVA was used to identify significant differences among treatments, and mean comparisons were conducted by Duncan's multiple range test at $p<0.05$.

3- Results and discussion

3-1- Bread crust and crumb color characteristics

The ANOVA results for colorimetric measurements are presented in Table 1. Addition of α-amylase to whole-meal breads significantly affected all three color parameters (L*, a*, b*) of both crust and crumb ($p<0.01$). Figure 1 shows the effects of α-amylase and microbial transglutaminase on L*, a* and b*. As shown in Figures 1-a and 1-b, increasing α-amylase concentration caused a significant decrease in L* (lightness) of both crust and crumb ($p<0.05$). Crust L* decreased from 52.22 in the control to 49.30 at 0.20% α-amylase, while crumb L* decreased from 55.90 to 54.23 at 0.20% α-amylase. No significant difference in crumb lightness was observed between the 0.10% and 0.20% α-amylase treatments ($p>0.05$).

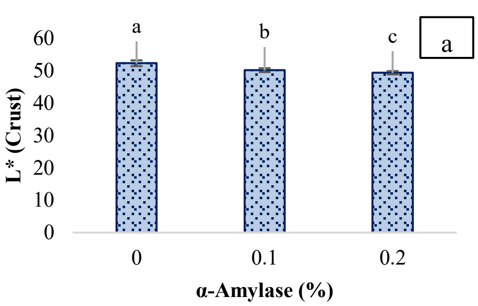
Table 1. Analysis of variance for the effect of α-amylase and microbial transglutaminase (MTG) on the color factors (L*, a* and b*) of bread

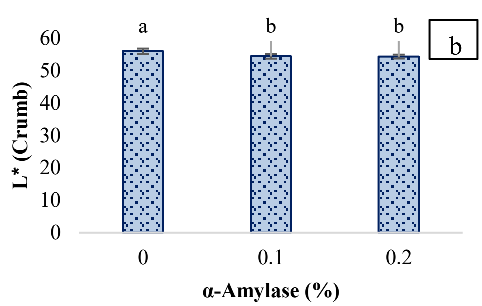
Variable sources		Mean square						
	df	L* (crust)	a* (crust)	b* (crust)	L* (crumb)	a* (crumb)	b* (crumb)	Total acceptability
α-Amylase	2	52.44***	6.43**	7.56**	16.09***	0.22**	2.87***	0.103**
MTG	2	28.28***	8.68**	4.72*	28.34**	0.11ns	0.196ns	0.201***
α-Amylase × MTG	4	29.92***	13.31***	6.81**	2.49**	0.90ns	0.78ns	0.014ns

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

The results of the color factor a* study showed that the increase in α-amylase enzyme increased the amount of this factor in the crust (from 11.03 to 12.7) and the bread crumb (from 4.34 to 5.81). These changes mean an increase in the red color on the surface and the bread crumb of the produced bread (Figures 1-c and 1-d), which is directly related to the intensification of Maillard-related reactions in the sample. In fact, the increase in the intensity of the non-enzymatic browning reaction is completely related to the increase in the percentage of α-amylase in the bread samples. Based on the analysis of the results of the factor b*, which shows the yellowness of the samples (Figures 1-e and 1-f), with the addition of α-amylase enzyme, the amount of this factor on the surface and the bread crumb of the produced bread samples has increased significantly ($p<0.01$), which means an increase in the yellow chroma on the surface and the bread crumb of the produced bread. As can be seen, the amount of b* factor in the crust of bread has increased from 55.8 to 59.7 and in the core of bread from 28.34 to 29.81. In fact, the α-amylase enzyme releases simple sugars such as glucose, maltose, and dextrin due to the breakdown of starch. These sugars participate in the Maillard reaction and reduce brightness and increase the degree of darkness and crispness in the produced breads.

Huang et al. (2020) studied the effect of adding lipase enzyme to improve bread quality. According to the results of this study, in relation to the evaluation of the color of bread samples, the addition of lipase enzyme caused a decrease in the brightness of bread samples [15]. According to these researchers, treatment with lipase enzyme reduces the water-binding capacity of starch and consequently intensifies the Maillard reaction and produces darker bread. The intensity of the Maillard reaction is determined by factors such as temperature, reaction time, pH, material composition, and water activity. Increasing water activity up to a certain level can increase the rate of the Maillard reaction, but with further increase in water activity, the reaction rate decreases [16].





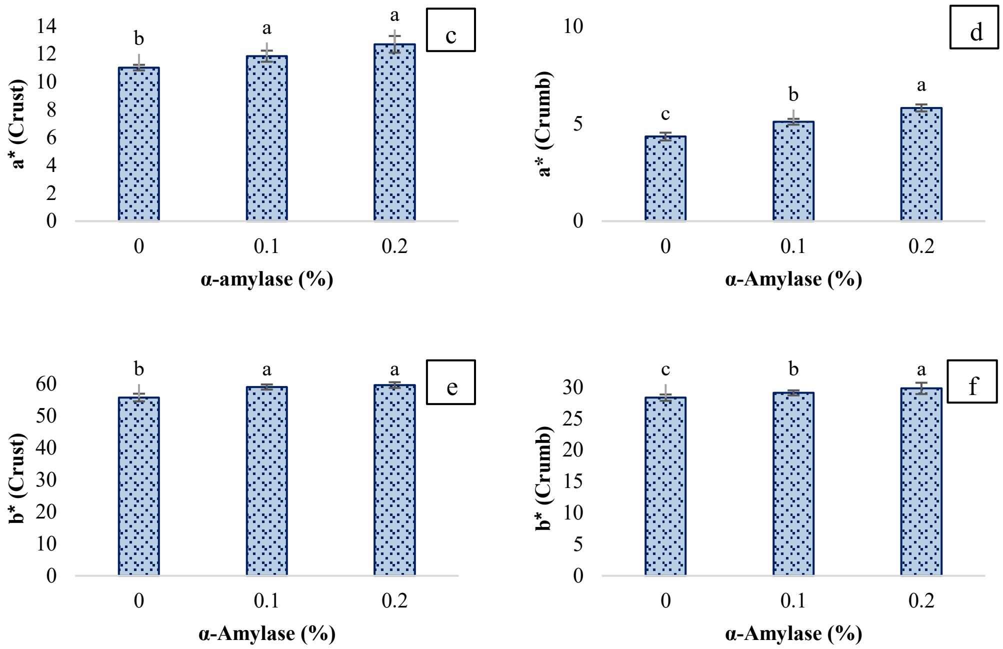


Figure 1. The effect of α-amylase on L*, a* and b* values of crust and crumb of bread samples

Matsushita et al. (2019) reported that by adding the optimal amount of α-amylase enzyme to the bread formulation, the brightness factor of the samples was significantly reduced. The a* and b* factors were also significantly reduced by adding α-amylase enzyme compared to the control sample, which was inconsistent with the results of this study [17]. In another study, Goesaert et al. (2009) observed that α-amylase enzyme increased the concentration of reducing sugars such as glucose and fructose, which resulted in an increase in the Maillard reaction and consequently a decrease in L* in bread samples [18]. A decrease in the amount of L* in the bread crust as a result of adding α-amylase enzyme to the formulation has also been reported by Shafi Soltani et al. [19].

As can be seen in Table 1, the addition of microbial transglutaminase enzyme to breads produced from wholemeal flour had a significant effect on the L* factor in the bread crumb and all three L*, a*, and b* factors on its surface ($p<0.05$). According to Figures 2-a and 2-b, it is observed that with the increase in the amount of this enzyme, the L* factor increased both in the crust (from 52.22 in the control sample to 56.11 in the sample containing 0.30% transglutaminase) and in the bread crumb (from 55.9 in the control sample to 59.77 in the sample containing 0.30% transglutaminase). This phenomenon can be attributed to the decrease in the availability of free amino acids to participate in the Maillard reaction in the presence of the transglutaminase enzyme. This enzyme forms covalent bonds between free amino groups and glutamine residues, leading to the formation of protein complexes with higher molecular weight. As a result, less free amino acids remain available and the Maillard reaction is potentially limited [20], and thus the color of breads treated with this enzyme is lighter. These results were consistent with the findings of Pourmohammadi et al. (2011) on the addition of transglutaminase to wheat bread containing unhusked barley flour [21]. Mahalle and Gharekhani (2018) also found in a study that the color of the crust of breads treated with transglutaminase enzyme was lighter than that of control samples, and they attributed this phenomenon to the reduction in the intensity of Maillard reactions due to the addition of transglutaminase enzyme, and stated that the connections created by transglutaminase can affect the physical structure of bread and change the color of bread [22]

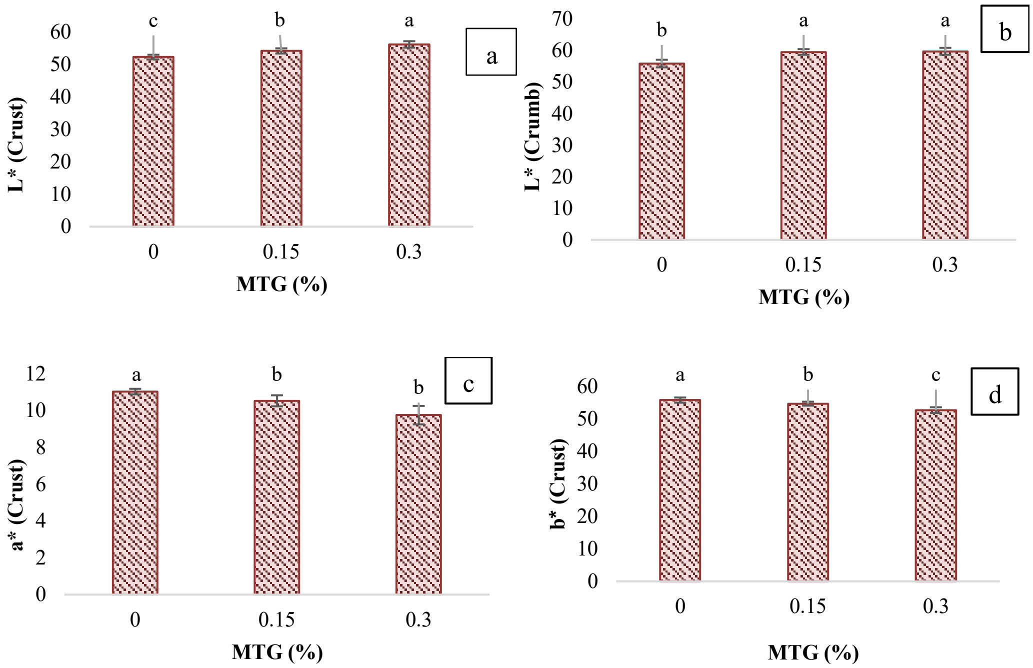


Figure 2. The effect of microbial transglutaminase (MTG) on the L*, a* and b* of bread crust and crumb samples

The results obtained for factor a* in Figure 2-c show that the redness of the crust of bread samples decreased significantly from 11.03 in the control sample to 9.75 in the sample containing 0.30% trans-glutaminase with an increase in the amount of enzyme ($p<0.01$), which is in fact a confirmation of the results obtained in the discussion of brightness; so that the redness of the crust of bread, which also induces a dark color, has decreased due to a possible decrease in the non-enzymatic browning reaction. The results of Bagherzadeh et al. (2018) also showed that breads containing the enzyme transglutaminase had a darker color than the control sample [23]. Factor a* in the core of bread samples was not affected by significant changes ($p>0.05$). Figure 2-d shows the results of factor b*, which examines the yellowness of the crust of bread. As can be seen, by increasing the percentage of transglutaminase enzyme from 0.15% to 0.30%, the b* value decreased significantly from 7.54 to 7.52 ($p<0.01$), although this factor did not show a significant change in the bread crumb ($p>0.05$). As shown in Table 1, the interaction effects of adding α-amylase and transglutaminase on the amount of L* in the crust and crumb of bread were significant ($p<0.01$). Also, the interaction effects of enzyme treatment on the a* and b* factors in the bread crust were significant ($p<0.01$). According to Figures 3-a and 3-b, adding different proportions of transglutaminase enzyme increased L*, and adding α-amylase significantly reduced the brightness factor in the crust and crumb of the produced breads. In general, it can be said that the highest brightness level is related to the sample treated with 0.30% transglutaminase enzyme and without α-amylase enzyme. The lowest brightness level is related to the sample treated with 0.2% α-amylase and without transglutaminase enzyme. Also, adding different ratios of α-amylase increased the redness factor (a*) and adding transglutaminase enzyme reduced this factor on the surface of the produced breads (Figure 3-c). In general, the highest a* level was related to the sample without transglutaminase enzyme and containing 0.2% α-amylase. The lowest a* level was related to the sample without α-amylase enzyme and containing 0.30% transglutaminase enzyme. Regarding the b* factor, as can be seen in Figure 3-d, adding α-amylase to the bread formulation significantly increased the yellowness factor on the surface of the samples. In addition, adding different ratios of transglutaminase enzyme also increased b*. In general, the highest b* value was related to the sample treated with 0.30% transglutaminase enzyme and 0.2% α-amylase enzyme. The control sample also showed the lowest b* value.

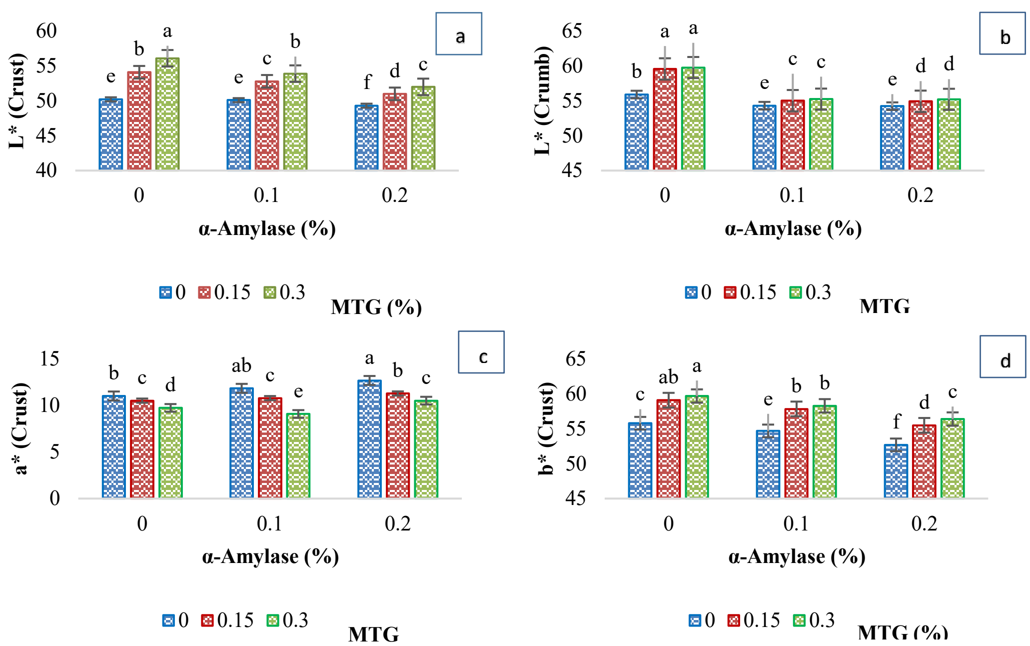


Figure 3. Interaction between α-amylase and microbial transglutaminase (MTG) on the color parameters of bread crust and crumb samples

3-2- Overall acceptance

Adding α-amylase and transglutaminase enzymes had a significant effect on improving the sensory properties of bread samples. The results of the sensory evaluation showed that the enzymatic treatment improved the overall acceptance of the samples. Among the bread samples treated with these two enzymes, the sample that obtained the highest overall acceptance score in the sensory test was selected as the optimal sample. Among the 9 treatments performed, the sample containing 0.10% α-amylase enzyme and 0.15% transglutaminase enzyme with a score of 2.8 was selected as the best sample (Figure 4). Alpha-amylase played an effective role in improving the taste and increasing the browning of the bread surface by breaking down starch and producing simple sugars, while transglutaminase strengthened the gluten network structure by creating cross-links between proteins and made the bread texture more uniform and desirable. These changes led to increased satisfaction of the evaluators in the sensory test. Therefore, it can be concluded that the simultaneous use of these two enzymes is an effective solution for improving the organoleptic quality of bread.

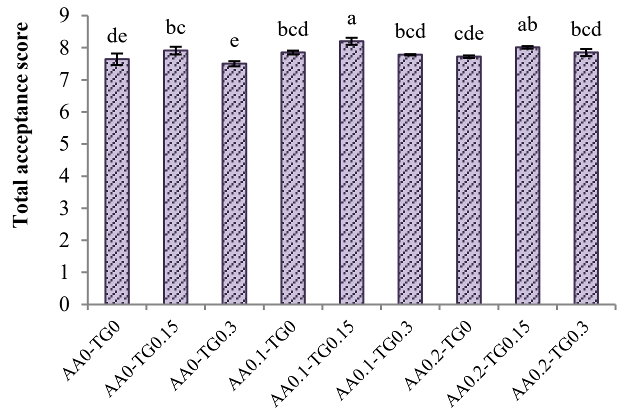


Figure 4. Total acceptance scores of bread samples.

3-3- Comparison of some characteristics of the optimal bread and control

After baking, various physicochemical changes occur in bread crust and crumb, collectively referred to as staling. Delaying staling is an important issue in the baking industry due to its economic implications. During staling, sensory attributes such as aroma, taste, and chewiness change as aromatic compounds and moisture migrate from crumb to crust. Stale bread exhibits reduced springiness and compressibility; loss of crumb moisture leads to diminished crispness and volume and a leathery appearance. The principal changes during staling are an increase in texture firmness and a decrease in moisture [24]. Table 2 presents the ANOVA results for moisture, water activity, firmness, and overall acceptability measured from day 1 to day 5 after baking.

3.3.1 Moisture

Moisture redistribution and migration from crumb to crust strongly influence staling. As shown in Table 2, the optimal sample retained significantly higher moisture than the control on all storage days ($P<0.001$). Moisture decreased significantly over time in both control and optimal samples ($P<0.001$). The highest moisture (38.14%) was recorded for the optimal sample on day 1, and the lowest (31.41%) for the control on day 5.

The transglutaminase used in this study likely contributed to reduced staling by forming protein networks and insoluble polymers that bind water within the crumb, limiting moisture migration to the crust and thus slowing staling [25]. Giannone et al. (2016) reported that a blend of maltogenic α-amylase and lipase acted synergistically to retard staling in durum wheat bread; breads with the blend remained softer and more palatable during storage than those with maltogenic α-amylase alone or the control, with higher moisture and water activity during storage [26].

Table 2. Comparison of the average of the control and optimal samples in terms of shelf life

Storage period (Days)	Sample	Moisture (%)	Water activity	Hardness (N)	Total acceptability (Score)
1	Control	35.41 ±0.21^{C}	0.95 ±0.01^{B}	31.52 ±0.28^{D}	7.62 ±0.28^{B}
	Optimized	38.14 ±0.19^{A}	0.97 ±0.01^{A}	28.15 ±0.30^{F}	8.18 ±0.31^{A}

3	Control	33.75 ±0.28^{D}	0.91 ±0.01^{C}	37.04 ±0.25^{B}	6.55 ±0.33^{C}
	Optimized	37.22 ±0.30^{B}	0.94 ±0.02^{B}	33.52 ±0.41^{E}	7.38 ±0.29^{B}
5	Control	31.41 ±0.33^{E}	0.84 ±0.01^{E}	45.01 ±0.19^{A}	5.25 ±0.32^{D}
	Optimized	35.68 ±0.29^{C}	0.89 ±0.02^{D}	38.97 ±0.32^{C}	6.64 ±0.26^{C}

Similar letters in each column are not statistically significantly different at the $P<0.05$ level.

3.3.2 Water activity

Consistent with moisture results, optimal samples exhibited higher water activity than control samples throughout storage. The lowest crumb a_w (0.84) occurred in the control on day 5, while the highest (0.97) was observed in the optimal sample at the start of storage. Similar findings were reported by Babakhani et al. (2011), who found higher moisture in cakes containing transglutaminase versus control [25], and by Gomez-Ruffi et al. (2013), who observed decreasing crumb a_w over storage in breads containing amylase [27].

3.3.3 Firmness

Table 2 shows that the control sample had higher firmness than the optimal sample on all storage days. Individually, α-amylase and transglutaminase produced opposite effects on firmness (α-amylase reduced firmness; transglutaminase increased it — data not shown), but their combined application resulted in an overall firmness reduction. Firmness increased with storage time: the highest value (45.01 N) was recorded for the control on day 5, and the lowest (28.15 N) for the optimal sample on day 1.

From a sensory perspective, firmness is the force required to compress a sample between the molars and from a mechanical perspective, the force required to achieve a specific shape change. Starch retrogradation is the main factor in bread staleness and amylopectin plays an important role in starch staleness. According to Gomez-Ruffi et al. (2012), firmness and specific volume are directly related in the first day after baking, and higher volume means lower firmness [27]. In this study, the addition of α-amylase enzyme also caused softer bread during storage. According to Gomes, breads containing emulsifiers form complexes with starch molecules, and the enzyme also reduces the molecular weight of starch molecules; as a result, crystal formation (retrogradation) with protein decreases and the firmness decreases [27]. Less firmness of bread compared to the control sample during storage with the addition of α-amylase and transglutaminase has also been reported by Goesaert et al. (2009) [18]. In another study, Kim and Yoo (2020) reported the anti-staleness effect of fungal α-amylase and less firmness of bread compared to the control sample over time [28]. In the study of Chauhan et al. (2023), the addition of α-amylase enzyme softened the bread crumb and prevented its stiffness and improved its consistency and crispness during storage [29]. Goesaert et al. (2009) attributed the anti-stiffening mechanism of α-amylase mainly to the expansion of the destruction of the crystalline component of amylopectin, preventing the formation of a stable amylopectin network during storage and consequently inhibiting retrogradation [18].

3-3-4- Overall acceptability

As can be seen in Table 2, the overall acceptance or acceptability of the control sample (without enzyme) was lower than the optimal sample (containing 0.10% α-amylase enzyme and 0.15% transglutaminase enzyme) on all days of storage. In addition, the overall acceptance of bread samples decreased over time. Improvement in sensory scores, especially aroma and crispness, of bread samples produced using α-amylase enzyme has also been reported by Chavvan et al. (2023) and Hejrani et al. (2013) [29 and 30]. Chauhan et al. (2023) reported that adding 0.05% α-amylase enzyme to frozen semi-baked barbarian bread increased the scores of sensory factors of color and flavor [29]. Hejrani et al. (2014) also reported that adding amylase to frozen semi-baked barbary bread increased the scores in the sensory factors of crust color and flavor [30]. Improvement in sensory properties of bread containing α-amylase has also been reported by Gomez et al. (2012) [27]. In another study, Jalayer et al. [31] showed that replacing barley flour in bread formulation reduced some product properties, including specific volume, sensory properties, and overall quality. However, the addition of xanthan gum and transglutaminase enzyme was able to correct these changes. The results of this study indicated that using a combination of 30% barley flour, 0.4% xanthan gum, and 0.25% transglutaminase enzyme, a product with similar properties to the control bread can be produced [31].

4- Conclusions

Considering the vital role of enzymes in improving the quality of bakery products, their targeted use can lead to the enhancement of the sensory, visual and nutritional properties of bread. In recent years, the focus on functional enzymes such as α-amylase and microbial transglutaminase as new tools in modifying the structure and color of bread has increased. In fact, chemical processes such as the Maillard reaction and protein structure modification play an important role in the final quality of bread, and the use of specific enzymes can control these processes in a targeted manner. According to the results of this study, the addition of α-amylase and microbial transglutaminase enzymes to the hamburger bread formulation has a significant effect on improving the color and sensory properties of the final product. Alpha-amylase, by increasing reducing sugars, intensified browning reactions and improved bread flavor, while transglutaminase, by modifying the protein structure, made the bread texture more uniform and its color brighter. The optimal combination of these two enzymes not only increased consumer satisfaction in sensory evaluation, but also improved the stability of bread quality during storage. Therefore, the simultaneous use of these enzymes at optimal concentrations (0.10% α-amylase and 0.15% transglutaminase) can be considered as an effective solution in the bakery industry to produce breads made from wholemeal flour with quality and acceptable sensory acceptability

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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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مقاله علمی_پژوهشی

تولید نان همبرگر تهیه شده از آرد گندم کامل و بررسی تأثیر تیمار آنزیمی آلفا-آمیلاز و ترانس-گلوتامیناز بر محصول

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چکیده

با توجه به نقش مهم آنزیم‌ها در بهبود ویژگی‌های کیفی نان، استفاده از آن‌ها در فرمولاسیون محصولات نانوایی از اهمیت بالایی برخوردار است. بنابراین، پژوهش حاضر به منظور بررسی تأثیر آنزیم‌های آلفاآمیلاز و ترانس‌گلوتامیناز میکروبی بر کیفیت حسی (پذیرش کلی) و فاکتور-های رنگی (*L، *a و *b) نان همبرگر و در ادامه بررسی برخی ویژگی‌های نمونه بهینه با نمونه کنترل طی مدت ۵ روز نگهداری انجام شد. نتایج نشان داد که آلفاآمیلاز با تجزیه نشاسته و تولید قندهای ساده، موجب افزایش واکنش مایلارد و در نتیجه کاهش معنی‌دار روشنایی و افزایش معنی‌دار قرمزی و زردی در پوسته و مغز نان گردید (p<۰/۰۵). در مقابل، ترانس‌گلوتامیناز با ایجاد پیوندهای کووالانسی بین پروتئین‌ها، ساختار گلوتنی را تقویت کرده و با کاهش دسترسی اسیدهای آمینه آزاد، شدت واکنش مایلارد را محدود ساخت که منجر به افزایش معنی‌دار روشنایی و کاهش تیرگی پوسته و مغز نان شد (p<۰/۰۵). بر اساس نتایج ارزیابی حسی، نمونه نان تیمار شده با ۰/۱ درصد آلفاآمیلاز و ۰/۱۵ درصد ترانس‌گلوتامیناز، با امتیاز پذیرش کلی ۸/۲، به عنوان نمونه بهینه معرفی گردید. نتایج ارزیابی و مقایسهٔ نمونهٔ بهینه با شاهد (نمونهٔ تهیه شده از آرد کامل فاقد آنزیم) طی مدت نگهداری نشان داد که نمونهٔ بهینه از مقادیر رطوبت، فعالیت آبی، پذیرش کلی بیشتر و سفتی کمتری نسبت به نمونهٔ شاهد برخوردار بود. این یافته‌ها نشان داد که استفاده هم‌زمان و هدفمند از این دو آنزیم می‌تواند کیفیت ارگانولپتیک و ظاهری نان همبرگر تهیه شده از آرد کامل گندم را به‌طور قابل توجهی ارتقاء دهد.