Egypt. J. Chem. **Vol. 68,** No. 10 pp. 553 - 572 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Influence of Enzymatic Treatments on the Rheological and Pasting behaviors of Wheat Flour and Its Impact on Pan Bread Staling

Mohammed S.M. Shebl*, Magdy G. El-Shemy, Hany I. Khalil, and Eman K. Nabih

Food Science Dept., Fac., Agric., Ain Shams Univ., Cairo, Egypt.

Abstract

Bread freshness is rather short during storage, owing to several complex physicochemical alterations that occur, which are collectively referred to as staling, that cause it to gradually lose its pleasant aroma and the crispiness of its crust. The response of different concentrations of fungal β-glucanase (BG) and maltogenic α-amylase (MAA) on extensograph parameters of medium-strength flour (MSF) contained fixed levels of fungal α-amylase, glucose oxidase, phospholipase, and ascorbic acid, was studied by the three-dimension polynomial quaternary model compared with strong flour (SF) for predicting the extensogram parameters. The optimum predicting concentrations were verified by using applied extensograph trials and then manufacturing pan bread. Proximate chemical composition and physicochemical characteristics for MSF and SF were studied., also, the effect of enzymatic treatments on pasting behavior was studied. Pan bread was evaluated for physical, texture, and sensory characteristics. The optimal extensograph parameters were selected at predicted MAA (0.29 U/g) and fungal BG (0.04 U/g) levels. MAA showed a clear impact on most pasting parameters by decreasing peak viscosity. Accordingly, the treatment of wheat flour by mixture enzymes (0.29 U/g MAA + 0.04 U/g BG) showed higher impacts. Mixed enzyme-treated bread (0.29 U/g MAA + 0.04 U/g BG) had the greatest effect in reducing hardness after 72 hrs. Pan bread sample that was prepared using treated MSF had significant ($P \leq 0.05$) crumb softness, crumb folding, and mouthfeel scores compared to the control. Generally, MAA and BG, significantly showed higher bread freshness than untreated bread.

Key words: β-glucanase, maltogenic α-amylase, extensogram, medium-strength flour, pan bread.

1. Introduction

Wheat (Triticum aestivum L.) is one of the most important crops and has been used as the main ingredient in bread making. The increasing mechanization in the world of the baking industry and the demand for a wide range of bread types has necessity to modulate the structure and viscoelastic properties of dough. In the modern baking industry, the superior nutritional and sensorial quality of bread needs to use additives like enzymes and ascorbic acid to improve the rheological properties of dough [1].

White pan bread is the predominant among all the baking products. On the other hand, the quality of bread depends on the quality of wheat flour. The quality of wheat varies widely., almost all bakeries need various additives to improve dough characteristics and quality of bread, including greater dough strength to improve crumb strength and improve slicing characteristics which lead to increased loaf volume and better softness as well as maintaining a longer shelf life [2, 3].

However, the shelf life of fresh bread is rather short. During storage, owing to several complex physico-chemical alterations, which are collectively referred to as staling. The staling of bread caused it to gradually lose its pleasant aroma and the crispiness of its crust. In addition, the rigidity of bread crumb increases, crumb resilience deteriorates, and water evaporates from the surface of sliced bread, resulting in a perceived firmer and drier feeling of crumb [4, 5]. Staling is supported by the fact that the staling of bread increased as the storage temperature decreased, which is characteristic for crystallization processes but not commonly applied to chemical reactions [6]. The most important manifestations of staling are an increase in crumb hardness, an increase in crumbliness of the crumb, a decrease in water absorption capacity, deterioration of freshness, and bread flavor [7].

Given that the challenge encountered by food manufacturers is the enhancement of food quality [8], enzymatic treatment of bakery products is considered one of the most important elements that can be used to meet this challenge by preparing a specific combination that suits the type of flour used and improves the quality of the final product [9, 10].

*Corresponding author e-mail: Mohamed6080@agr.asu.edu.eg (Mohammed S.M. Shebl).
Receive Date: 02 January 2025, Revise Date: 31 January 2025, Accept Date: 08 February 2025
DOI: 10.21608/ejchem.2025.350022.11096

The enzymatic treatment of wheat flour is an interesting alternative to generate changes in the structure of the dough and improve the functional properties of the flours. They are generally recognized as safe (GRAS) and do not remain active in the final product after baking [11]. Thus, many studies have praised their effective role in improving dough handling [12] specific volume of bread [13, 14], sensory properties, and color [15], in addition to the possibility of gliadin degradation [16].

Several additives are used to improve the physicochemical shelf-life of bread, with specific amylases, particularly a Bacillus stearothermophilus maltogenic α-amylase, being very effective antistaling agents [17, 18, 19]. The use of commercial enzymes to improve the quality of flour products has gained increasing attention recently. Enzymes (microbial source) are much safer and more efficient in comparison to chemical additives [20, 21]. Since enzymes with different biochemical activities could induce synergistic effects on dough behavior or product quality. Thus, enzymatic treatments improve the rheological behavior of doughs and the quality of the final product [22, 23]. Various types of enzymes can be used as alternatives to chemical improving agents, such as some hydrocolloids and emulsifiers. Many enzymes occur naturally in flour, but several enzymes are added, specifically for their beneficial effects on the dough and bread characteristics. Outcomes include increased dough handling and hydration, improved volume and/or crumb texture, reduced rate of staling, or improved nutritional qualities. Commonly used exogenous enzymes include xylanase, phytase, and amylases [24]. Enzymes most frequently used in breadmaking are the α-amylases from different origins (cereal and microbial), although they have different properties [25, 26]. Modification of initial structures of wheat non-starch polysaccharides through enzyme addition usually brings positive effects on dough and bread characteristics [27]. Cellulases and hemicellulases are used to improve the quality of bread, that leads to an increase in the bread volume and better crumb porosity in whole wheat bread [28, 29]. Maltogenic amylases have the best affinity toward cyclodextrins (CD), compared to starch and pullulan. Moreover, they hydrolyze CD and starch mainly to maltose and produce panose from pullulan [30]. The bakery industry seems to be one of the most promising industrial applications of amylases owing to its ability to reduce the degree of retrogradation phenomenon responsible for bread stalling based on their limited and specific hydrolytic action on flour starch [31, 32].

The maltogenic and fungal α-amylase turned out to be an ideal antistaling enzyme in bread, causing to clear anti-firming effects and maintain the elastic recovery levels, which can be ascribed partly to its intermediate thermal stability [33]. Biodegradation of cellulose with the formation of soluble sugars is catalyzed by a specific multi-enzyme cellulase system composed of endo-1,4-β-glucanase (EC 3.2.1.4), exo.1,4-β-glucanase (EC 3.2.1.91), exo.1,4-β-glucosidase (EC 3.2.1.74), and cellobiase (EC 3.2.1.21) [34, 35].

The main component of the cellulases is endo.1,4-β-glucanase which plays a critical role in the action of the multi-enzyme system. This enzyme is the first to attack cellulose, hydrolyzing internal β-1,4-glycosidic bonds at sites remote from the ends of the polymer chain to form cello-oligosaccharides such as mono-, di-, and tri-saccharides [36], While the endoglucanases randomly attack the internal chain of cellulose to produce cello- oligosaccharides [37]. However, cellulase did not significantly alter the farinographic or extensographic properties of whole wheat dough or the specific volume or final moisture content of whole wheat bread [38, 39].

β-glucanase can act both as exo- as well as endo-hydrolase., endo-β-glucanase randomly slices β-glucan chain internally while exo-β-glucanase acts on the non-reducing ends where they release glucose residues. Based on the type of glycosidic linkage cleaved, they are further classified as 1,3-β-glucanases, 1,4-β-glucanases, and 1,6-β-glucanases [40]. The known specificity and mechanism of the enzyme activity within the immense substrate range would allow us to realize that the β-glucanases are diverse from cellulases. Based on their action patterns, they are divided into two types: exo and endo-hydrolases. Exo-hydrolase catalyzes the hydrolysis of the non-reducing ends of the β-glucan chain and sequentially releases glucose residues as the sole hydrolysis product. Endo-hydrolase cleaves β-glycosidic linkages at random sites laterally in the polysaccharide chains and releases smaller oligosaccharides [40, 41]. Others [42, 43, 44] reported that the presence of carbohydrases (cellulase, xylanase, and β-glucanase) during breadmaking led to an improvement of the specific bread volume and better crumb texture. In addition, carbohydrases induced a retardation of the bread staling by reducing the initial crumb firmness and the kinetics of the firming process during storage.

Overall, the rheological properties of doughs are considered one of the most important indicators of the quality of the final product [45]. The primary objectives of rheological measurements are to get a quantitative description of the material's mechanical properties, to gain information related to the molecular structure and composition of the material, to guess the material's performance during processing, and for quality control [46]. Therefore, many rheological tests are used to predict end-product quality, such as mixing behavior, sheeting, and baking performance [47]. Therefore, the current study aims to improve the rheological properties of medium-strength flour (MSF) by enzymatic treatments to simulate the rheological properties of strong flour (SF) by using different concentration levels of β-glucanase and maltogenic α-amylase to obtain the suitable levels that cause to improve the extensograph parameters of MSF compared to SF and the extent of the effect of these enzymatic treatments on the physical and sensory properties of the pan bread.

2. Materials and methods

2.1. Materials

Medium-strength flour (Semi-hard commercial Russian wheat flour, 72 % ext.) was obtained from Amoun for Milling Company, Giza, Egypt. Strong strength flour (Commercial Australian wheat flour, 72 % ext.) was obtained from Five Stars Milling Company, Suez, Egypt.

Both purified fungal (Aspergillus oryzae) α-amylase, glucose oxidase and phospholipase were purchased from Novozymes Company, Denmark. Purified fungal (Aspergillus niger) β-glucanase was obtained from Escaut Valley BVBA Company, Belgium. Bacillus stearothermophilus maltogenic α-amylase was obtained from AB Enzymes Company, Germany. Ascorbic acid (purity > 99 %). All chemicals used in this study were analytical grade.

2.2. Methods

2.2.1. Physicochemical properties

Wheat flour (72% ext.) was chemically analyzed for moisture and ash according to standard methods AACC [48]. Crude fiber, total lipids and crude protein (Nx5.7) contents were carried out according to the methods described in AOAC [49]. Total carbohydrates were calculated by differences. Falling number, starch damage using Chopin SD Matic instrument, wet and dry gluten were determined according to AACC [48].

2.2.2. Enzymatic activity measurements

2.2.2.1. Alpha-amylase activity

Wheat flour sample was prepared by adding 0.5 g of the flour sample to 100 ml of buffer solution (phosphate buffer, pH 6.9) then, incubated at 25 °C in a water bath and allowed the enzyme to extract over 10 min with occasional mixing. Extracted amylase and fungal α-amylase activities were determined according to the method of Liu et al. [50] using soluble starch as substrate. The mixture was contained of 1 mL from prepared sample or enzyme solution (1mg/mL), and 2 mL of soluble starch (1% starch in 100 mM Tris buffer) then incubated at 25 °C for 3 min. The reducing sugar generated by the hydrolytic activity of the enzyme was determined by 3,5-dinitrosalicylic acid (DNS) method [51]. One unit (U) of α-amylase activity was defined as the amount of enzyme capable of producing 1μ mol of maltose per minute and monitoring the absorbance at 540 nm. Optical density at 25 °C and pH 6.9 for 3 min. A standard curve of maltose was used to calculate the amount of maltose and α-amylase activity was expressed as μ mole of maltose liberated in min at pH 6.9 and 25 °C.

2.2.2.2. Maltogenic α-amylase assay

Maltogenic amylase activity was assayed at 55°C in 50 mM sodium citrate pH 6.0, using of 1% soluble starch (Showa Chemicals Inc.) as substrate [52]. The reducing sugar generated by the hydrolytic activity of the enzyme was determined by the 3,5-dinitrosalicylic acid method [51]. One unit of enzyme activity was defined as the amount of enzyme giving an increase in absorbance at 575 nm of 1μ mol of maltose equivalent per min when the reaction was carried at 55°C and pH 6.0 for 30 min. A standard curve of maltose was used to calculate the amount of maltose and maltogenic α-amylase activity was expressed as μ mole of maltose liberated in min at pH 6.0 and 55 °C.

2.2.2.3. Phospholipase assay

Phospholipase activity was assayed according to Kurioka and Liu [53] by using lecithin sol (U-sol) as substrate where the Inorganic phosphate generated by the hydrolytic activity of the enzyme was measured spectrophotometrically at 500 nm. Optical density by the method of Horecker et al. [54]. One unit of enzyme activity was defined as the amount of enzyme which releases 1 μmol of pi (inorganic phosphorus) per min at 37 °C and pH 7.2 for 30 min. A standard curve of phosphate was developed to calculate the amount of inorganic phosphorus.

2.2.2.4. Glucose Oxidase assay

Glucose oxidase activity was determined by oxidizing 3,5,3',5'-Tetramethyl benzidine (TMM) through a peroxidase-coupled system [55]. One unit of enzyme activity (U) was defined as the amount of glucose oxidase which will oxidize 1.0 μ mole of β-D-glucose to D-gluconic acid and H2O2 per min at 25 °C and pH 6.0 [56]. A standard curve of glucose was used to calculate the amount of glucose and Glucose Oxidase activity was expressed as μ mole of glucose oxidized in min at pH 6.0 and 25 °C.

2.2.2.5. β-glucanase assay

β-glucanase activity was assayed by the reaction system containing 250 μl of a laminarin solution (1%) dissolved in 50 mM sodium acetate buffer (pH 5.0) and 0 -125 μl of enzyme solution. Reaction was allowed to proceed for 30 min at 37 °C and stopped., The reducing sugar formed was then determined spectrophotometrically at 550 nm by the DNS method [51] One unit of enzyme (U) was defined as the amount of enzyme produced one micromole of reducing sugar per min at 37 °C and pH 5.0 for 30 min [57]. A standard curve of β-1.3-D-glucan with alcian blue dye was used to calculate the enzyme activity.

2.2.3. Pasting properties

Pasting properties of wheat flour for control and prepared samples were estimated [48], by using a Rapid Visco Analyzer, RVA 4500 (Perten instruments a PerkinElmer com., Australia) Standard profile. The studied properties were Test Peak viscosity, Trough viscosity, Breakdown, Final viscosity, Setback and Pasting Temp of all investigated samples were recorded.

2.2.4. Rheological properties

Rheological properties of wheat flour dough prepared from the various treatments were evaluated by using Brabender farinograph and extensograph (Brabender GmbH, Duisburg, Germany) [58].

2.2.5. Preparation of pan bread

Pan bread was prepared by the method of Cauvain et al. [59] with some modifications, according to the straight-dough procedure for making pan bread with open top. The recipe used to prepare control pan bread treated flour was 1 Kg (72% ext.). Farinograph water absorption plus 20-40 g that depends on optimum consistency, 20 g dry yeast, 10 g sugar and 10 g salt [48].

2.2.6. Specific volume

According to AACC [48], loaf weight (g) was recorded after cooling at 25±2 ºC for 2 h while, the loaf volume (cm^3) was measured with measurement apparatus (SIMSEK LABORTEKNIK LTD. STI., Turkey) by rapeseed displacement method. On the other hand, the specific volume (cm^3/g) of pan bread was calculated. Specific volume (cm^3/g) was calculated with formula:

Specific volume = Loaf volume (cm^3) / Loaf weight (g)

2.2.7. Texture profile analysis

Texture profile analysis (TPA) of pan bread was carried out by using TA-XT2i Texture Analyzer (Mult-test 1d, Mecmesin, Food Technology Corporation, Slinfold, W. Sussex, UK) according to Bourne [60].

2.2.8. Sensory evaluation

The quality attributes of prepared pan bread are evaluated after cooling for 2 h from baking at room temperature 25 ± 2 °C (zero time) then, every 24 h till 72 h. Sensory evaluation was carried out by ten experienced panelists from the staff of Amoun for Milling Co. Crust color, symmetry, crumb color, pore size, uniformity of pore size, taste and overall acceptability were evaluated according to the 1–9 hedonic scale. The scale had verbally anchored with nine categories as follows: like extremely, like very much, like moderately, like slightly, neither like or dislike, dislike slightly, dislike moderately, dislike very much and dislike extremely [61]. The quality attributes of treated pan bread were evaluated in comparison with the control samples. Organoleptic characteristics of pan bread were evaluated for Crumb softness, Crumb folding and Mouth feeling as staling characteristics. According to Matveeva et al. [62] staling parameters defined as follow:

Crumb softness: Judgment of the overall crumb softness, while touching the bread slice by hand.

Crumb folding: Judgment of the fold ability, while folding+ the bread slice by hand.

Mouth feeling: Judgment of the overall eating quality.

2.2.9. Statistical analysis

Predicting individual extensograph parameters (Y) was assumed by quadratic (Eq. 1) or cubic (Eq. 2) polynomial regression model for the independent variables (maltogenic α-amylase concentrations or β-glucanase concentrations (X)) to optimize the extensograph parameters (Y) used regression analysis. The model proposed for response of (Y) as follows:

$$\boldsymbol{Y = y_{\circ} + ax + bx^2} \quad [\mathbf{Eq.\ 1}]$$

$$\boldsymbol{Y = y_{\circ} + ax + bx^2 + cx^3} \quad [\mathbf{Eq.\ 2}]$$

Where, ($y_{\circ}$), (a), (b) and (c) are intercept, linear, quadratic and cubic regression coefficient terms, respectively and (X) is independent variable. The data were statistically analyzed using the Statistical Analysis System software [63] by one-way analysis of variance (ANOVA). Differences among mean values were compared using Duncan's Multiple range test at a significance level of 95% ($P \leq 0.05$). Results followed by different alphabetical letters significantly differed. Three-dimension contour plot was used as a method to study the response surface of different extensograph parameters (Y) as dependent variables with maltogenic α-amylase concentration and β-glucanase concentration (X and Z) as independent variables. The response surface methods were applied using Sigma Plot Program [64] to locate the optimum maltogenic α-amylase and β-glucanase concentrations to obtain the optimum extensograph parameters that can produce high quality pan bread. The model proposed for Three-dimension response surface of Y as follows:

$$\boldsymbol{Y = y_{\circ} + ax + bz + cx^2 + dz^2 + ex^3 + gz^3 + hx^4 + iz^4 + jx^2z^2 + kx^3z^3 + lx^4z^4} \quad [\mathbf{Eq.\ 3}]$$

3. Results and discussion

3.1. Physicochemical properties of wheat flour

The proximate chemical composition of used wheat flour (72% ext.) including Medium-strength flour and strong wheat flour was estimated and the obtained results are presented in Table 1. The results showed that there were no significant differences for moisture content between Medium-strength flour and strong wheat flour (P≤0.05). Whereas, the strong wheat flour showed the highest values of ash, protein, crude fiber (0.54, 13.90 and 0.49% respectively) with significant differences compared with Medium-strength flour. The highest values of total carbohydrates content (74.10%) recorded by Medium-strength flour. These results are similar with Šramková et al. [65], they studied the chemical gross of wheat flour and reported that the protein content of wheat flour may vary between 10% - 18% of the total dry matter. While Beshir et al. [66] stated that wheat flour contained 14.20% moisture, 0.47% ash, 10.40% protein, 0.45% crude fiber, 1.00% ether extract and 87.68% total carbohydrates. Others [67] found that wheat flour contained 10.90% protein, 1.22% fat, 0.45% crude fiber and 86.40% total carbohydrates.

The quality of wheat flour including gluten content, falling number and damaged starch has an important impact parameter on the end products [68]. Where during wheat milling a portion of the starch granules sustains mechanical damage, the level of which depends on the wheat hardness and milling technique [69], consequently the parameters were estimated, and the obtained results are shown in Table 1. The lower values of wet and dry gluten (26.1 and 12.7% respectively) were observed by medium-strength flour with statistical differences (P≤0.05), whereas the strong wheat flour recorded the lowest value of gluten index with no significant difference, where the gluten quality and quantity affect the functional properties of flour and the quality of bakery products [70, 71]. On the other hand, wheat flour with gluten index in the range of 75-95% caused the optimal bread making quality [72]. The medium-strength flour recorded the highest value of falling number (461.0 sec.) compared to 343.0 sec. for the strong wheat flour with significant differences. Struyf et al. [73] mentioned that the falling number (FN) is an important quality characteristic of cereal products. For instance, the flours with a high FN (>350 sec.) which have a reduced capability to form fermentable sugars [74], meanwhile, the flours with a low FN (<250 s) caused to produce dough that is difficult to handle and bread with a sticky crumb [75]. Thus, FN should be adjusted to 250-300 sec to avoid the lower quality [76]. In contrast, the highest value of damaged starch (5.6%) was significantly recorded by the strong wheat flour where, hard and soft wheat fracture were varied during the milling process which caused to difference in damaged starch levels and particle size [77]. However, the difference in damaged starch ratios had occurred during milling caused some starch granules are mechanically damaged, the level of damage may vary with the severity of grinding and the hardness of the kernel [78, 79]. Our results agreed with other findings [1, 80, 81].

Table 1: Physicochemical properties of wheat flour (on dwb)

Physiochemical parameters	Medium-strength flour	Strong flour
Moisture (%)	14.00 ^{a} ± 0.20	14.00 ^{a} ± 0.10
Ash (%)	00.52 ^{b} ± 0.01	00.54 ^{a} ± 0.01
Protein, N= 5.7 (%)	10.20 ^{b} ± 0.30	13.90 ^{a} ± 0.20
Crude fiber (%)	00.42 ^{b} ± 0.02	00.49 ^{a} ± 0.02
Total lipids (%)	01.18 ^{a} ± 0.03	01.03 ^{b} ± 0.07
Total carbohydrates (%)	84.10 a ± 0.30	80.53 ^{b} ± 0.25
Wet gluten* (%)	026.1 ^{b} ± 00.1	032.5 ^{a} ± 00.1
Dry gluten* (%)	012.7 ^{b} ± 00.1	013.4 ^{a} ± 00.1
Gluten index (%)	093.0 ^{a} ± 02.0	090.0 ^{a} ± 04.0
Falling number* (sec.)	461.0 ^{a} ± 11.0	343.0 ^{b} ± 11.0
Damaged starch (%)	005.4 ^{b} ± 00.1	005.6 ^{a} ± 00.1

- Means in the same row with different letters are significantly different (P≤0.05).
- Data means ± SD; n = 3.

* 14% moisture base.

3.2. Activities of investigated enzymes

The activities of enzymes were 2239, 1714, 1541, 7452, 24249 and 7753, Unit/g/min for MSF, Fungal α-amylase, BG, GOX, Phospholipase and MAA, respectively according to the mentioned methods for determined the enzymes activities.

3.3. Effect of maltogenic α-amylase activity on the extensograph parameters

The effect of maltogenic α-amylase (MAA) activity on the extensograph parameters for medium-strength flour (MSF) that contained fixed levels of fungal α-amylase, glucose oxidase, phospholipase and ascorbic acid compared with strong flour (SF) are presented in Table 2.

Table 2: Effect of maltogenic α-amylase on the extensograph parameters

Level of addition (U/g)	Extensograph parameters					
	Energy (cm^2)	Relative resistance (BU)	Extensibility (mm)	Max. resistance (BU)	Ratio no.	Ratio no. (max)
SF	$123^{a} \pm 10$	$909^{d} \pm 37$	$103^{ab} \pm 11$	$1014^{bc} \pm 42$	$8.8^{b} \pm 0.8$	$9.8^{b} \pm 0.5$
MSF	$121^{a} \pm 07$	$868^{d} \pm 11$	$103^{ab} \pm 05$	$967^{c} \pm 04$	$8.4^{b} \pm 0.6$	$9.4^{b} \pm 0.4$
0.16	$142^{a} \pm 07$	$977^{bc} \pm 43$	$109^{a} \pm 10$	$1066^{bc} \pm 15$	$8.9^{b} \pm 0.2$	$9.7^{b} \pm 0.6$
0.31	$122^{a} \pm 18$	$1009^{a} \pm 19$	$94^{b} \pm 07$	$1088^{ab} \pm 40$	$10.8^{a} \pm 0.6$	$11.6^{a} \pm 0.6$
0.47	$147^{a} \pm 12$	$1115^{a} \pm 21$	$102^{ab} \pm 05$	$1181^{a} \pm 14$	$10.9^{a} \pm 0.4$	$11.5^{a} \pm 0.4$
0.62	$126^{a} \pm 23$	$926^{bcd} \pm 26$	$101^{ab} \pm 15$	$1040^{bc} \pm 23$	$9.3^{b} \pm 1.0$	$10.5^{ab} \pm 1.0$

- Means in the same column with different letters are significantly different (P≤0.05).
- Data means ± SD; n = 3.

SF= strong flour, MSF= medium strength flour with fixed mix.

According to the statistical data, there were no significant differences (P≤0.05) between SF and MSF or other samples which contained different maltogenic α-amylase activity (0.16, 0.31, 0.47 and 0.62 U/g) in relation to dough energy. The lowest values of relative resistance (868 and 909 BU) were obtained by MSF and SF, respectively. However, MAA at activities 0.31 and 0.47 U/g significantly increased the relative resistance of MSF (1115 and 1009 BU, respectively), then decreased at high MAA activity (0.62 U/g) to record lower relative resistance (926 BU) with no significant differences compared to SF. Also, there were no significant differences between all flour samples in terms of extensibility except the sample contained (MAA activity 0.31 U/g) which recorded the lowest value of extensibility (94 mm). On the other hand, there were no significant differences between all treated samples which recorded the maximum resistance values except for the sample treated with (MAA at activity 0.47 U/g) which recorded the highest values of the maximum resistance (1181 BU) and MSF which recorded the lowest maximum resistance value (967 BU).

The highest values of ratio number were (10.8 and 10.9) obtained by MSF treated with 0.31 and 0.47 U/g MAA, respectively with no significant differences between them and with significant differences in comparison with other samples. While the highest values of max. ratio number were (11.6, 115 and 10.5) by adding 0.31, 0.47 and 0.62 U/g of MAA respectively with no significant differences between them, where the MSF recorded the lowest value of max ratio number (9.4) followed by treatment contained 0.16 U/g (9.7) and 9.8 for strong wheat flour with no significant differences between them. These results are in a harmony with Gerrard et al. [82], they stated that, maltogenic amylase exhibits minimal influence on the rheological properties of dough, primarily due to its limited activity at temperatures below 35 °C. Its optimal enzymatic activity is observed at the temperature associated with starch gelatinization, where it effectively hydrolyzes the glycosidic bonds present in gelatinized starch during the baking process.

Results of quadratic and cubic polynomial trends are illustrated in Figure 1. The models were tested for adequacy by analysis of variance. Where, the maltogenic α-amylase concentration was the independent for variables ranged between 0 to 80 ppm (0.62 U/g). Maltogenic α-amylase ratios had a significant (P ≤ 0.05) effect on the extensograph parameters. However, the obtained equation 4 (r^2 = 0.2256) presented quadratic trend, and it was predicted that no effect of maltogenic α-amylase on the Energy behavior.

$$\boldsymbol{Y = 123.0286 + 0.6321\,x - 0.0070\,x^2} \quad (\mathbf{4})$$

Meanwhile, the resistance increased from 868 to 1115 BU with increasing the ratio of maltogenic α-amylase from 0.0 to 80 ppm (0.62 U/g). Equation 5 (r^2 = 0.8881) presented cubic trend, which was used to predict the resistance behavior. Where the resistance dramatically increased from 868 BU at 0.0 ppm of maltogenic α-amylase to the maximum level which was recorded 1090.3 BU at 55.3 ppm (0.428 U/g) of maltogenic α-amylase, then declined to the minimum value recorded 489 BU with 100 ppm (0.78 U/g) of maltogenic α-amylase.

$$\boldsymbol{Y = 875.4286 + 0.9155\,x + 0.1793\,x^2 - 0.0023\,x^3} \quad (\mathbf{5})$$

Equation 6 (r^2 = 0.1558) presented quadratic trend, and it was predicted that no effect of maltogenic α-amylase on the Extensibility behavior.

$$\boldsymbol{Y = 105.2857 - 0.1836\,x + 0.0016\,x^2} \quad (\mathbf{6})$$

Equation 7 (r^2 = 0.8786) presented cubic trend, and it was predicted the Maximum resistance behaviour. Maximum resistance was increased from 967 BU with 0.0 ppm maltogenic α-amylase to the maximum level which was 1157.9 BU with 55.8 ppm (0.433 U/g) maltogenic α-amylase and lowering to the minimum value 772.4 BU with 100 ppm (0.78 U/g) maltogenic α-amylase.

$$Y = 973.4714 + 1.5220\,x + 0.1232\,x^2 - 0.0016\,x^3 \quad (7)$$

Equation 8 (r^2 = 0.9697) presented cubic trend, and it was used to predict the Ratio number behaviour. Where, the Ratio number rose from 8.4 at 0.0 ppm maltogenic α-amylase to the maximum level which was recorded 11.12 with 56.7 ppm (0.439 U/g) maltogenic α-amylase and then lowering to the minimum value 3.46 with 100 ppm (0.78 U/g) maltogenic α-amylase.

$$Y = 8.3529 - 0.0064\,x + 0.0028\,x^2 - 3.2292e^{-5}\,x^3 \quad (8)$$

Equation 9 (r^2 = 0.9221) presented cubic trend, and it was considered as predicted the Ratio number (max) behaviour. Ratio number (max) was increased from 9.4 at 0.0 ppm maltogenic α-amylase to the maximum level was 11.8 with 58.1 ppm (0.45 U/g) maltogenic α-amylase and was decreased to the minimum value 5.94 with 100 ppm (0.78 U/g) maltogenic α-amylase.

$$Y = 9.3329 - 0.0039\,x + 0.0023\,x^2 - 2.6042e^{-5}\,x^3 \quad (9)$$

For instance, the obtained predicted equations showed that the adequate dough behaviour contained different levels of maltogenic α-amylase was suitable for dough to obtain the ideal extensograph parameters from Medium-strength flour to prepare pan bread in the presence of ascorbic acid, fungal glucose oxidase, fungal α-amylase and fungal phospholipase to obtain the optimum conditions for preparing the pan bread from Medium-strength flour.

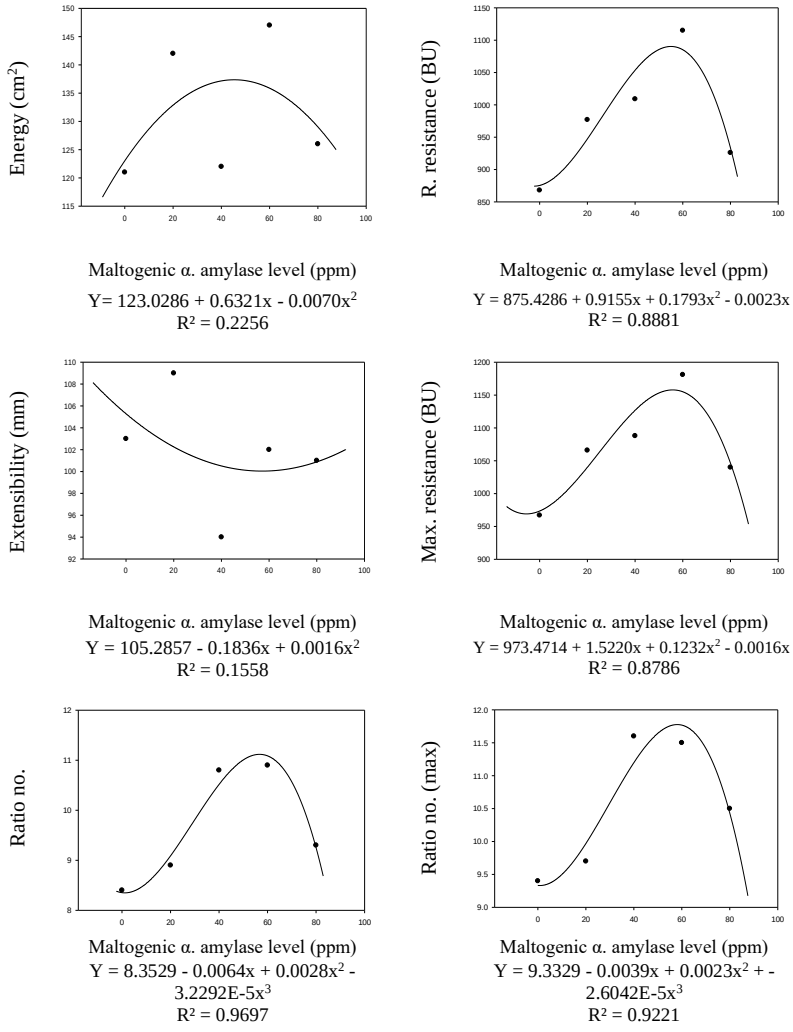


Fig. 1. Polynomial quadratic and cubic trends of maltogenic α-amylase levels (ppm) versus the extensograph parameters.

3.4. Effect of β-glucanase activity on the extensograph parameters

The effect of β-glucanase (BG) activity on the extensograph parameters of medium-strength flour with fixed levels of fungal α-amylase, glucose oxidase, phospholipase and ascorbic acid (MSF) compared to strong flour (SF) are shown in Table 3.

The obtained results indicated that the lowest BG activity (0.015 U/g) caused a positive impact on MSF energy by increasing the rates of BG from 121 cm2 for MSF to 144 cm2 with significant differences between them and other samples

except for the sample containing 30 ppm of BG (0.046 U/g activity). Relative resistance also showed the highest value which was (1036 BU) for the sample contained (0.015 U/g) with significant differences on comparing with other tested samples. However, there were no significant differences between all investigated samples regards the extensibility parameter. Results also showed that there were no significant differences between all tested samples in terms of maximum resistance except the sample contained (0.015 U/g) which recorded the highest value (1158 BU). while the highest values of ratio number (9.6 and 8.8) were observed by MSF containing (0.015 U/g) and strong wheat flour respectively with no significant differences between them, in contrast to the lowest Ratio number values (7.1, 7.6 and 7.7) were recorded in the presence of 0.062, 0.046 and 0.031 U/g respectively. Also, the same trend was observed in the case of maximum ratio number.

Figure 2 illustrates the quadratic and cubic polynomial trends. The models were obtained for adequacy by analysis of variance. The ratios of β-glucanase were in the range between 0 to 40 ppm (0.062 U/g). The different ratios of β-glucanase had a significant ($P \leq 0.05$) effect on the extensograph parameters. Equation 10 ($r^2 = 0.3934$) indicated that cubic trend, and it was predicted with no effect of β-glucanase on the Energy behaviour.

$$Y = 122.7286 + 2.7060\,x - 0.1479\,x^2 + 0.0021\,x^3 \quad (10)$$

Equation 11 ($r^2 = 0.5533$) presented cubic trend and used to predict the resistance behaviour. Resistance was increased from 868 BU with 0.0 ppm β-glucanase to the maximum level which was 978 BU with 10.12 ppm (0.016 U/g) β-glucanase and then decreased to the minimum value which was 794 BU with 34.56 ppm (0.053 U/g) β-glucanase and then increased again.

$$Y = 882.4571 + 22.7452\,x - 1.5557\,x^2 + 0.0238\,x^3 \quad (11)$$

Equation 12 ($r^2 = 0.8666$) showed quadratic trend and used to predict the Extensibility behaviour. Extensibility increased from 103 to 116.6 mm with increasing the level of β-glucanase from 0.0 to 50 ppm (0.077 U/g).

$$Y = 103.3143 + 0.3371\,x + 0.0014\,x^2 \quad (12)$$

Equation 13 ($r^2 = 0.7312$) presented cubic trend, and it was used to predict the Maximum resistance behaviour. Maximum resistance increased from 967 BU with 0.0 ppm β-glucanase to the maximum level was 1115 BU in the presence of 10.13 ppm (0.016 U/g) β-glucanase and then declined to the minimum value 917 BU with 34.33 ppm (0.053 U/g) β-glucanase and then increase again.

$$Y = 977.5857 + 30.5345\,x - 1.9736\,x^2 + 0.0301\,x^3 \quad (13)$$

Equation 14 ($r^2 = 0.8079$) presented cubic trend and used to predict the Ratio number behaviour. Ratio number dramatically increased from 8.4 at 0.0 ppm β-glucanase to the highest level which was 9.23 with 8.1 ppm (0.012 U/g) β-glucanase and decreased to the minimum value 6.97 with 35.25 ppm (0.054 U/g) β-glucanase.

$$Y = 8.5014 + 0.1932\,x - 0.0146\,x^2 + 0.0002\,x^3 \quad (14)$$

Table 3: Effect of β-glucanase on the extensograph parameters

Level of addition (U/g)	Extensograph parameters					
	Energy (cm^2)	Relative resistance (BU)	Extensibility (mm)	Max. resistance (BU)	Ratio no.	Ratio no. (max)
SF	123 ^{b} ± 09	909 ^{bc} ± 37	103 ^{c} ± 11	1014 ^{bc} ± 42	8.8 ^{ab} ± 0.8	9.8 ^{bc} ± 0.5
MSF	121 ^{b} ± 07	868 ^{cd} ± 11	103 ^{c} ± 05	967 ^{c} ± 04	8.4 ^{bc} ± 0.6	9.4 ^{c} ± 0.4
0.015	144 ^{a} ± 06	1036 ^{a} ± 23	108 ^{bc} ± 10	1158 ^{a} ± 10	9.6 ^{a} ± 0.8	10.7 ^{a} ± 0.6
0.031	124 ^{b} ± 11	819 ^{d} ± 47	107 ^{bc} ± 04	976 ^{c} ± 13	7.7 ^{cd} ± 0.5	9.1 ^{cd} ± 0.3
0.046	134 ^{ab} ± 06	866 ^{cd} ± 30	114 ^{b} ± 06	972 ^{c} ± 48	7.6 ^{cd} ± 0.9	8.5 ^{d} ± 0.8
0.062	126 ^{b} ± 11	814 ^{d} ± 14	114 ^{b} ± 10	956 ^{c} ± 07	7.1 ^{d} ± 0.6	8.4 ^{d} ± 0.7

- Means in the same column with different letters are significantly different ($P \leq 0.05$).
- Data means ± SD; n = 3.

SF= strong flour, MSF= medium strength flour with fixed mix.

Equation 15 ($r^2 = 0.8122$) presented quadratic trend and used to predict the Ratio number (max) behaviour. Ratio number (max) decreased with increasing the adding ratios of β-glucanase. The lowest Ratio number (max) was 7.1 with 50 ppm (0.08 U/g) of β-glucanase. while the highest Ratio number was 9.82 with 4.18 ppm (0.006 U/g) of β-glucanase.

$$Y = 9.5714 - 0.0073\,x - 0.0006\,x^2 \quad (15)$$

For instance, the obtained predicted models for the adequate dough behaviour with different levels of β-glucanase to prepare suitable dough with ideal extensograph parameters from Medium-strength flour to prepare pan bread with addition of ascorbic acid, fungal glucose oxidase, fungal α-amylase and fungal phospholipase in a trial to obtain the optimum conditions to prepare pan bread from Medium-strength flour.

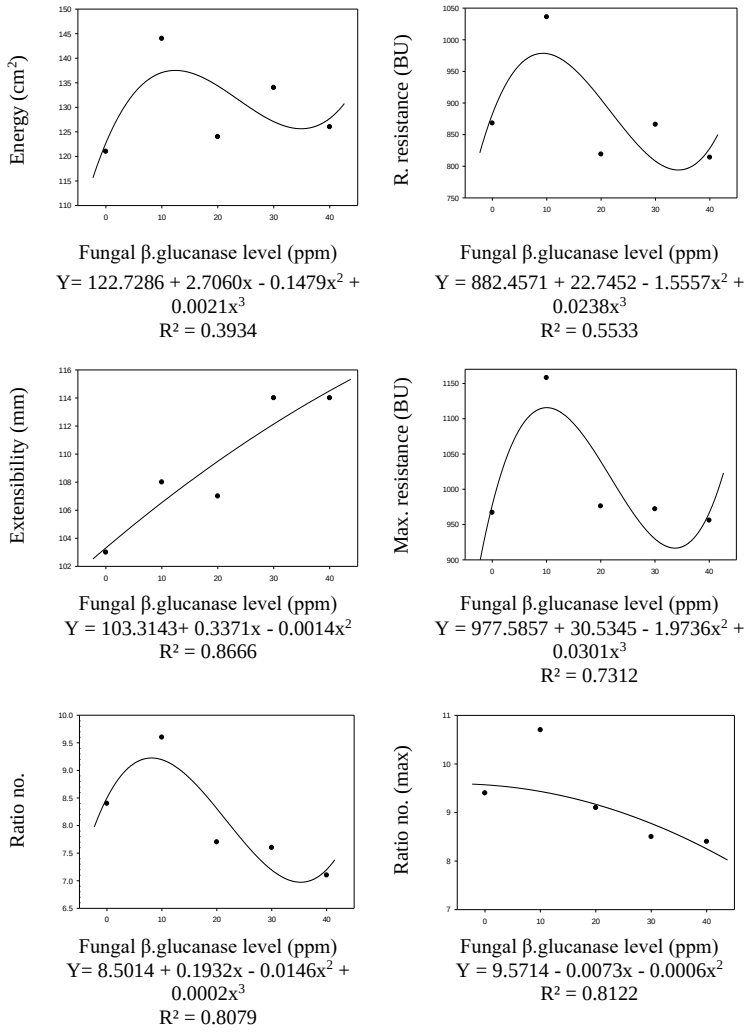


Fig. 2. Polynomial quadratic and cubic trends of β-glucanase levels (ppm) versus the extensograph parameters.

3.5. Effect of interaction between maltogenic α-amylase and β-glucanase on extensograph parameters

The three-dimension response surface study was necessary to optimize the combined ratios of maltogenic α-amylase and β-glucanase. The plot in Figure 3 shows the response surface of extensograph parameters as observed in the presence of maltogenic α-amylase and β-glucanase at different levels for MSF. The equations obtained relation between MSF contained mixing of maltogenic α-amylase and β-glucanase.

The best energy value for mixing maltogenic α-amylase and β-glucanase at different levels was 120.6 cm^2. The quaternary model (Eq. 16) showed the best between maltogenic α-amylase and β-glucanase levels to obtain the high-quality dough with (r^2=0.6955) as follows:

Y=130.3133+0.2974X+0.5540Z-0.0071X2-0.0081Z2-0.0009X3-6.6448E-5Z3+2.5370E-5X4+8.4346E-Z4-4.7265E-5X2Z2+3.8096E-8X3Z3-7.5121E-12X4Z4 **[Eq. 16]**

The predicted model (Eq. 17) had a high determination coefficient (r^2=0.8925). From output data where, the best predicted resistance for that equation was 946.6 BU.

Y=902.2945+11.6375X+9.1768Z-0.4383X2-0.1170Z2+0.0015X3-0.0008Z3-0.0002X4+1.1535E-5Z4-0.0001X2Z2+ 3.7210E-8X3Z3-3.1024E-12X4Z4 **[Eq. 17]**

According to the response surface, the optimum extensibility was 103.1 mm predicted from the model (Eq. 18) with high determination coefficient (r^2=0.9177) as follows:

Y=106.3198-0.2845X-0.1684Z+0.0059X2+0.0011Z2-0.0008X3+3.2401E-6Z3+4.3603E-5X4-6.6691E-8Z4-1.5826E-5X2Z2+1.7856E-8X3Z3-4.0455E-12X4Z4 **[Eq. 18]**

The response surface showed a high determination coefficient (r^2=0.8020) between the maltogenic α-amylase and β-glucanase combination at maximum resistance. The same observation was noticed in the response surface for resistance data. Predicted model (Eq. 19) fits the relationship of maltogenic α-amylase and β-glucanase levels versus maximum resistance. The optimum maximum resistance was 999.6 BU.

$Y=1009.3270+13.2421X+7.4579Z-0.4703X^2-0.0951Z^2-0.0017X^3-0.0007Z^3-4.1104E-5X^4+9.2452E-6Z^4-0.0002X^2Z^2+ 1.5048E-7X^3Z^3-2.5891E-11X^4Z^4$ **[Eq. 19]**

The ratio number obtained from the output data from the predictive model (Eq. 20) with high determination coefficient (r^2=0.8975). According to that output data it could be concluded that the optimum ratio number for pan bread preparation with mixing of maltogenic α-amylase and β-glucanase was 9.3.

$Y=8.3371+0.2151X+0.1239Z-0.0092X^2-0.0015Z^2+ 8.7240E-6X^3-9.1211E-6Z^3-3.0040E-7X^4+1.3137E-7Z^4-4.7623E-7X^2Z^2-3.8808E-10X^3Z^3+1.4763E-13X^4Z^4$ **[Eq. 20]**

At the same time the ratio number (Max) gave similar trend from three-dimension response surface plot of ratio number. The ratio number (Max) was 9.8. Equation 21 was used to plot the maximum ratio number data depending on the variables of maltogenic α-amylase and β-glucanase levels with high determination coefficient (r^2=0.9261) as follows:

$Y=9.3755+0.2186X+0.1070Z-0.0090X^2-0.0012Z^2-7.8572E-6X^3-7.8478E-6Z^3-2.2747E-8X^4+1.0936E-7Z^4-1.4745E-6X^2Z^2+ 4.2953E-10X^3Z^3-1.3221E-14X^4Z^4$ **[Eq. 21]**

Figure 3 helped to predict the optimum maltogenic α-amylase and β-glucanase levels with extensograph parameter values. The determined optimum maltogenic α-amylase and β-glucanase levels were (37.5 ppm [0.29 U/g] and 26.5 ppm [0.04 U/g], respectively). These levels can be used to obtain the suitable MSF for making pan bread harmony with strong flour.

3.6. Verification of predictive Extensograph parameters

Table 4 showed that MSF contained a mixture of 37.5 ppm (0.29 U/g) of maltogenic α-amylase with 26.5 ppm (0.04 U/g) of β-glucanase owing to the predictive values obtained from the former equations. Where the extensograph parameters for the Medium-strength flour contained mixture of 37.5 ppm (0.29 U/g) maltogenic α-amylase and 26.5 ppm (0.04 U/g) β-glucanase were 116, 931, 102, 1016, 9.1 and 10 for Energy (cm^2), Relative resistance (BU), Extensibility (mm), Max. resistance (BU), Ratio number and Ratio number (max), respectively. While the extensograph parameters for strong flour were 123, 909, 103, 1014, 8.8 and 9.8 for energy (cm^2), relative resistance (BU), extensibility (mm), max. resistance (BU), ratio number and ratio number (max), respectively.

Table 4: Extensograph parameters for MSF contained optimal ratios of additives, fungal BG and MAA

Optimum ratio for additives	Extensograph parameters					
MAA 37.5 ppm (0.29 U/g) BG 26.3 ppm (0.04 U/g)	**Energy (cm^2)**	**Relative resistance (BU)**	**Extensibility (mm)**	**Max. resistance (BU)**	**Ratio no.**	**Ratio no. (max)**
Verified values	116	931	102	1016	9.1	10
SF (Target)	123	909	103	1014	8.8	9.8

BU = Brabender unit

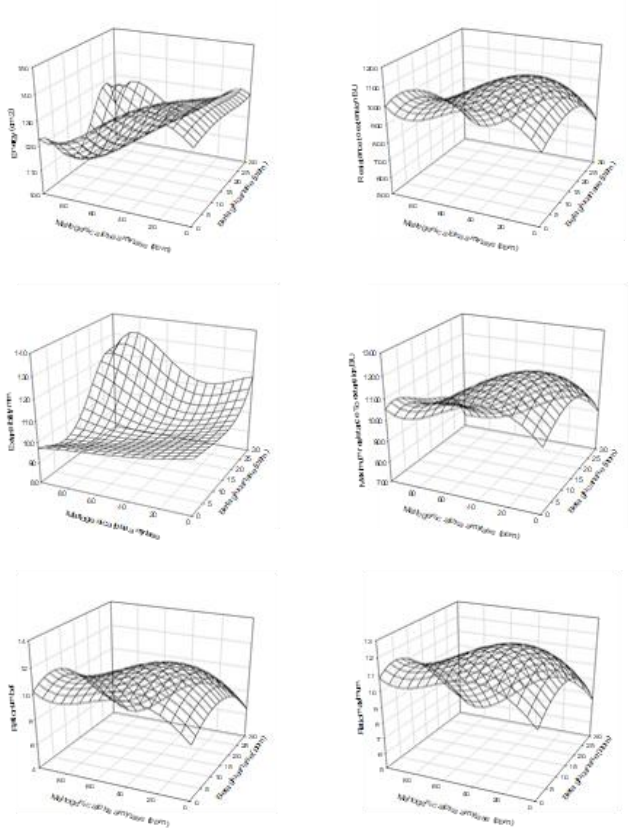


Fig. 3. Three-dimension regression plot to predict the extensograph parameters against different fungal β-glucanase and maltogenic α-amylase levels.

3.7. Pasting properties

The pasting behaviour is influenced by specific starch attributes, including swelling capacity, the extent of gelatinization, and the reassociation of amylose and amylopectin during the cooling phase following starch cooking. The term setback viscosity is often used interchangeably with retrogradation, referring to the increase in paste viscosity that occurs as the starch paste cools. The heating of pre-gelatinized starch results in a reduction in viscosity, attributed to the thinning of the slurry [83]. Data recorded in Table 5, showing the pasting properties of medium-strength flour with fixed additives for (MSF) and other additives with 0.63 U/g maltogenic α-amylase (MAA), 0.034 U/g β-glucanase (BG) and mixture of them (0.29 U/g MAA + 0.04 U/g BG) were estimated. According to the obtained data, MAA showed a clear impact on most pasting parameters by decreasing peak viscosity (1448 cP), trough viscosity (556 cP) and final viscosity (1296 cP) while, setback viscosity (742 cP) with significant differences compared to MSF. The reason for decreasing viscosity by maltogenic α-amylase, and other α-amylase are primarily linked to the reduction of short outer amylopectin chains, which subsequently inhibits retrogradation by decreasing the recrystallization of amylopectin [84]. Furthermore, the amylolytic action on starch granules results in observable surface alterations [85].

However, the connection between these modifications and the property of starch remains inadequately explored [86]. Meanwhile, adding BG with (0.034 U/g) caused to increase the same parameter (1634, 812, 1672 and 863 cP, respectively) with significant differences compared to the control sample. Accordingly, the wheat flour contained a mixture of enzymes (0.29 U/g MAA + 0.04 U/g BG) and showed higher values (1630 and 887 cP) of peak viscosity and breakdown respectively. Data also indicated that there were no significant differences between all tested samples concerning pasting temperature. These results agreed with Ferry et al. [87] they reported that, at the same level of amylases addition the decrease in viscosity was. Also, Barrera et al. [88] studied the effect of maltogenic α-amylase on dough rheology, and they mentioned that maltogenic α-amylase led to decrease peak viscosity, breakdown, setback and pasting temperature. Moreover, amylases decrease the dough viscosity and improve the quality of the technological process which caused an improvement of the quality of the bread by reducing the aging process and increasing volume. Others [89, 90, 91] found that maltogenic α-amylase had a positive impact on bread freshness by decreasing peak viscosity and increasing breakdown viscosity compared to untreated flour.

Table 5: Pasting properties

Pasting parameters	Samples			
	Control	MAA (0.63 U/g)	BG (0.034 U/g)	MAA (0.29 U/g) +BG (0.04 U/g)
Peak viscosity (cP)	$1598^{b} \pm 9.0$	$1448^{c} \pm 10$	$1634^{a} \pm 14.0$	$1630^{a} \pm 11.0$
Trough viscosity (cP)	$757^{b} \pm 6.0$	$556^{d} \pm 12.0$	$812^{a} \pm 7.0$	$746^{c} \pm 5.0$
Breakdown viscosity (cP)	$841^{b} \pm 10.0$	$893^{a} \pm 13.0$	$825^{c} \pm 9.0$	$887^{a} \pm 22.0$
Final viscosity (cP)	$1605^{b} \pm 15.0$	$1296^{d} \pm 11.0$	$1672^{a} \pm 17.9$	$1576^{c} \pm 5$
Setback viscosity (cP)	$846^{b} \pm 5.0$	$742^{d} \pm 11.0$	$863^{a} \pm 13.0$	$832^{c} \pm 9.0$
Pasting Temp. °C	$68.6^{a} \pm 0.1$	$68.6^{a} \pm 0.3$	$68.6^{a} \pm 0.1$	$68.6^{a} \pm 0.5$

- Means in the same row with different letters are significantly different ($P \leq 0.05$).
- Data means ± SD; n = 3.

MAA= maltogenic α.amylase , BG=β-glucanase

3.8. Specific volume of Pan Bread

The effect of enzymes activity on specific volume of pan bread was estimated compared to MSF bread (produced from MSF contained fungal α-amylase, phospholipase, glucose oxidase and ascorbic acid). The obtained results in Table 6 illustrated in Fig. 4, indicated positive effect of enzymatic treatments on specific volume with significant differences compared to the control bread. Results indicated that MSF contained the mixing of MAA and BG had the highest specific volume (5.7 cm^3/g) followed by MSF contained MAA, BG and control, 5.40 cm^3/g, 4.90 cm^3/g and 4.60 cm^3/g, respectively. Generally, the ability of MAA and BG activity to increase the specific volume of bread and enhance its water retention can be attributed to their distinct effects on the structure and properties of dough. Where, MAA hydrolyses starch, breaking down these large molecules into smaller dextrins and oligosaccharides. This partial hydrolysis softens the dough by reducing starch rigidity, which allows for better gas retention during proofing and baking. Consequently, this enhances the dough's expansion, leading to an increase in bread volume [92, 93]. Also, the degradation of β-glucans reduces dough viscosity, enhancing the dough's extensibility and elasticity. This allows the dough to expand more easily, improving gas retention and increasing bread volume [94].

Table 6: Specific volume

Pan bread samples	Specific volume (cm^3/g)
MSF	$4.60^{d} \pm 0.04$
MAA (0.63 U/g)	$5.40^{b} \pm 0.02$
BG (0.034 U/g)	$4.90^{c} \pm 0.01$
MAA (0.29 U/g) + BG (0.04 U/g)	$5.70^{a} \pm 0.02$

- Means in the same column with different letters are significantly different ($P \leq 0.05$).
- Data means ± SD., n = 3.

MAA= maltogenic amylase, BG=β-glucanase

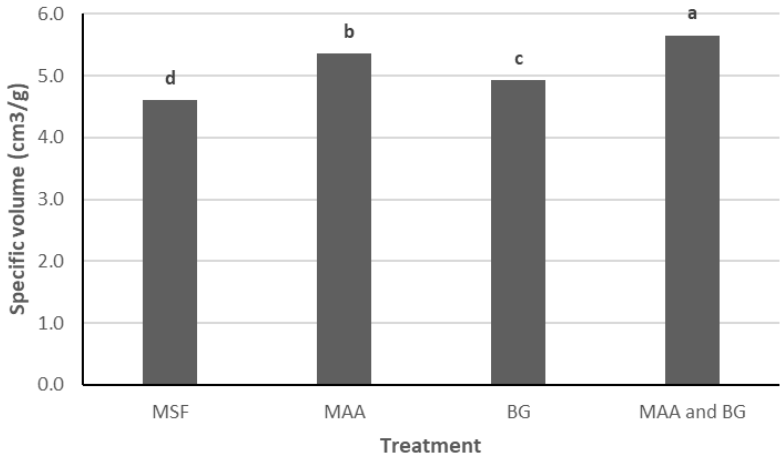


Fig. 4. Specific volume of pan bread.

3.9. Texture profile analysis of pan bread

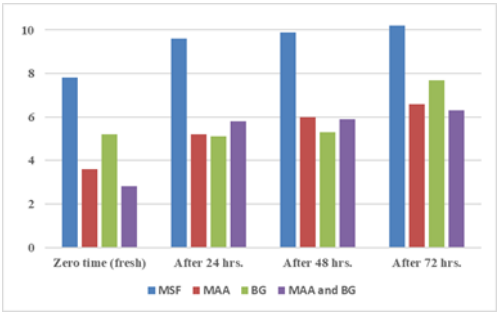
The texture profile analysis results, obtained by means of double cycle compressions evidenced the structural changes that affected the samples during storage and clearly differentiated control from the various enzyme-added samples [95]. Therefore, the effect of maltogenic α-amylase (MAA) and β-glucanase (BG) activity on the texture profile of pan bread during storage period (zero time, after 24, 48 and 72 hrs.) was investigated and the obtained results are presented in Fig 5. Results indicated that the enzymatic treatments were strongly influenced for the reduction of hardness at the initial time and during storage durations (24, 48, and 72 hours) on comparing to the control sample. Meanwhile, the mixed enzymes-treated flour used to make pan bread (0.29 U/g MAA + 0.04 U/g BG) had the greatest effect in reducing the hardness after (72 hrs.) of storage compared to MSF contained each enzyme lonely. On the other hand, the springiness also showed a positive effect by adding enzymes activity (MAA or BG) after storage at (24, 48 and 72 hrs.) compared to untreated flour (control), where the highest values of springiness were 0.79, 0.67 and 0.60 mm, respectively after (24, 48 and 72 hrs.) of storage which recorded by mixed enzymes followed by 0.69, 0.62 and 0.50 mm, respectively for bread samples which caused 0.62 U/g MAA. While MSF contained BG at activity 0.034 U/g recorded the lowest values of springiness during storage (0.64, 0.57 and 0.41 mm, respectively). However, MAA activity caused the highest value of cohesiveness after 72 hrs. Data also indicated that the control bread recorded the highest values of chewiness and gumminess during all storage periods up to 72 hrs. compared to other treated bread. Enzymatic approaches can mitigate starch retrogradation by altering the distribution of amylopectin chain lengths. MAA is frequently employed in these enzymatic strategies [96, 97]. Specifically, MAA is utilized in bread formulations to prolong freshness and delay staling [98]. Thus, MAA proved to be effective in decreasing crumb firmness over time. A positive correlation between the crumb firmness and the amount of recrystallized amylopectin implies that crumb hardening might be caused by the increasing amount of amylopectin retrogradation [92]. Additionally, Aoki et al. [99] evaluated the texture of breads with MAA after six days of storage and suggested that MAA treatment increased the relative content of amylopectin short chains in starch leading to reduced hardness during all storage periods.

3.10. Sensory evaluation

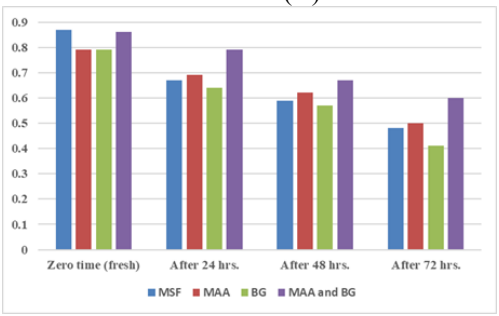
Sensory evaluation of pan bread prepared by using optimum levels of maltogenic α-amylase (0.29 U/g) and (0.04 U/g) β-glucanase presented in Figure 6. the sensory parameters of pan bread made with adding enzymes to MSF used to make pan bread at zero time. Statistically, analysis showed that there were no significant differences between MSF contained fixed additives (control) bread and other treatments with different activity of maltogenic α-amylase (MAA) and β-glucanase (BG) or mixing of them regards to the symmetry, pore size, taste and overall acceptability scores. While the highest crust color scores (5.6 and 5.9) were recorded by bread samples which contained 0.63 U/g MAA and others treated with 0.29 U/g MAA + 0.04 U/g BG, respectively with no significant differences between them and significant differences were noticed between other bread samples. The same trend was observed in uniformity of pore size parameter, where the increasing levels of reducing sugars promote the generation of Maillard reaction products [100, 101], which intensify bread flavor and crust color. However, the crust character mainly depends on the Maillard reaction [102].

Staling characteristics of pan bread.

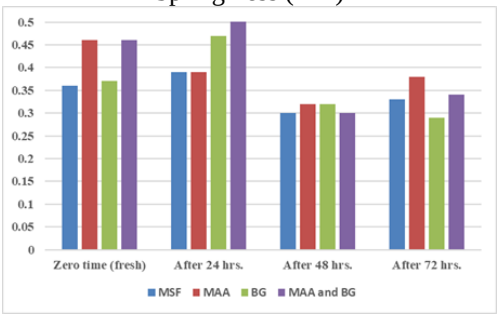
Pan bread was stored for 24, 48 and 72 h in sealed polyethylene bags at room temperature (25 ± 2 °C). The thickness of sample slice was 1 cm. A part of 2.5 cm must be cut and discarded from the bread from the front and end of the loaf. Stalling characteristics during 72 h of pan bread prepared with MSF contained optimum levels of maltogenic α-amylase (0.29 U/g) and β-glucanase (0.04 U/g) are presented in Figure 7. The effect of enzymatic treatments on bread stalling characteristics was sensory evaluated during different storage periods (zero time, after 24, 48 and 72 hrs.). According to the hedonic scale, the control bread sample had recorded score of 5.0 for all storage periods. Thus, during zero time the control bread significantly recorded the lowest scores of crumb softness, crumb folding and mouth feeling, conversely, the highest score of crumb softness significantly recorded by MAA (8.60) followed by 7.40 for samples contained BG then 7.00 for beard sample that contained a mixed enzyme (0.29 U/g MAA + 0.04 U/g BG). However, MSF contained a mixture of enzymes and showed a positive effect regards the crumb folding to record the highest score (8.80) with significant differences compared to other bread samples. Whereas there were no significant differences between all treated MSF which is used to prepare pan bread in terms of mouth feeling.



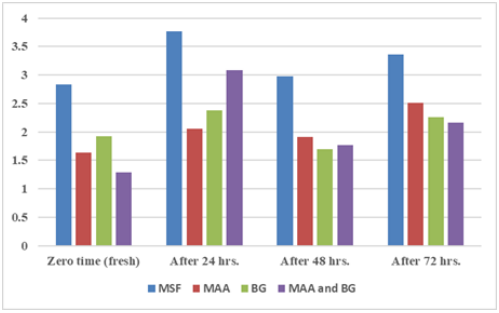
Hardness (N)



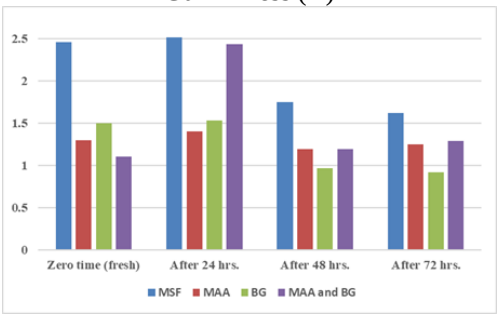
Springiness (mm)



Cohesiveness



Gumminess (N)



Chewiness (N.mm)

Fig. 5. Texture profile analysis of pan bread.

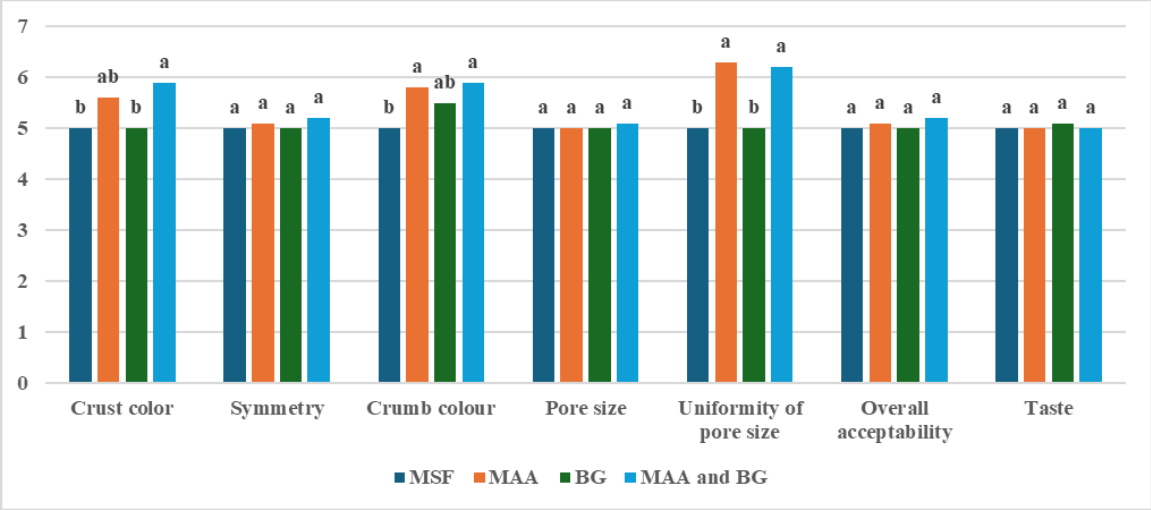
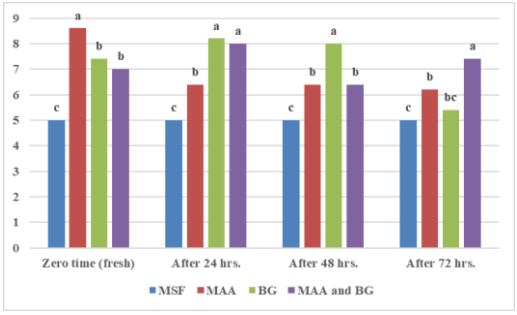
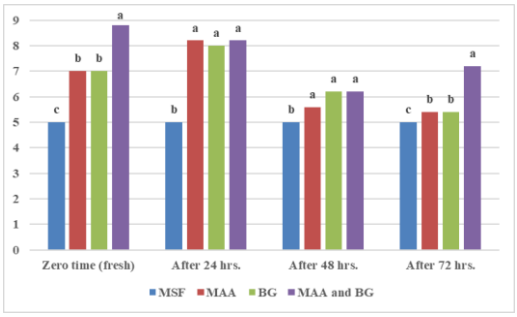


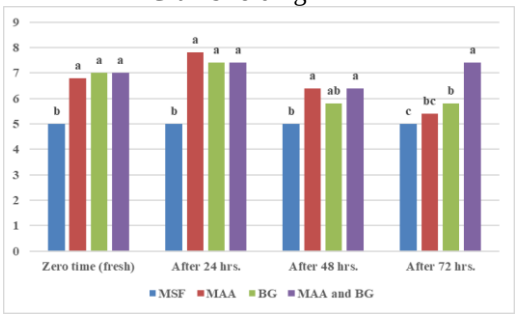
Fig. 6. Sensory evaluation of pan bread.



Crumb softness



Crumb folding



Mouth feeling

Fig. 7. Staling characteristics of pan bread.

Pan On the other hand, after 24 hrs. of storage, the control (MSF contained fixed additives, fungal α-amylase, phospholipase, glucose oxidase and ascorbic acid) bread recorded the lowest scores for all sensory parameters, according to the statistical data no significant differences between all treated bread samples regards crumb folding and mouth feeling, while the flour treated with enzymes (0.034 U/g BG) significantly showed lower score of crumb softness compared to other treated samples. However, after 48 hrs. the highest scores of crumb softness and crumb folding were recorded by flour treated with MAA 0.63 U/g and mixed enzymes respectively, with significant differences compared to the control bread and BG 0.034 U/g treatment. However, mixed enzymes presence of flour caused to obtain the highest score after 48 hrs. regards mouth feeling score with significant differences compared to other bread samples. After 72 hrs. of storage periods, a lower score (5.50) of crumb softness was recorded by bread sample prepared with flour contained 0.034 U/g BG with significant differences compared (7.20 and 7.40) which recorded by bread samples which treated with 0.63 U/g MAA and other treated with mixed enzymes activity (0.29 U/g MAA + 0.04 U/g BG) respectively. Meanwhile, the highest scores of crumbs folding and mouth feeling after 72 hrs. were significantly recorded by mixed enzyme activity (7.20 and 6.40, respectively). It can be concluded that pan bread sample that prepared using treated MSF had significant ($P \leq 0.05$) crumb softness with high mean values compared to the control storage up to 72 hrs. of storage. The crumb folding and mouth feeling values were higher in treated pan bread than control with mean values. Also, Bread prepared without enzyme showed a more open structure, and a dry and opaque crumb, with rigid and fragile features typical of retrograded starch [103].

Generally, it could be observed that the treated pan bread with MAA and BG had significantly higher characteristic values for staling, which caused to delay the stalling.

4. Conclusion

In conclusion, MAA showed a clear impact on most pasting parameters by decreasing peak viscosity. Accordingly, the treatment of wheat flour by mixture enzymes (0.29 U/g MAA + 0.04 U/g BG) showed positive impacts by decreasing hardness and increasing the springiness values of treated bread during different storage periods compared to the control bread. However, treating MSF with maltogenic enzyme improved the freshness and volume of pan bread to a greater extent than treating with β-glucanase, while the combination of both maltogenic α-amylase and β-glucanase had a greater effect than the effect of each enzyme alone.

5. References

[1] Paucean, A., Man, S. M., & Socaci, S. A. (2016). Wheat germ bread quality and dough rheology as influenced by added enzymes and ascorbic acid. Studia Universitatis Babes-Bolyai, Chemia, 61(2), 103-118.

[2] Kim, Y. A., Ko, J. Y., Yoo, S. R., Jang, S. J., Kang, S. H., Han, D. W., ... & Seo, J. H. (2018). Quality characteristics of white pan bread with different water types. Culinary science and hospitality research, 24(3), 104-112.

[3] Chen, A., Pan, C., & Chen, J. (2024). Comparative analysis of bread quality using yeast strains from alcoholic beverage production. Microorganisms, 12(12), 2609.

[4] Correa, M. J., Burbano Moreano, J. J., Guardianelli, L. M., Weisstaub, A. R., Zuleta, A., & Salinas, M. V. (2021). Garlic: A natural bread improver for wheat bread with a high level of resistant starch. Journal of Food Processing and preservation, 45(6), e15519.

[5] Dong, Y., & Karboune, S. (2021). A review of bread qualities and current strategies for bread bioprotection: Flavor, sensory, rheological, and textural attributes. Comprehensive Reviews in Food Science and Food Safety, 20(2), 1937-1981.

[6] Rayas-Duarte, P., & Murtini, E. S. (2020). Bread staling. In breadmaking (pp. 561-585). Woodhead Publishing.

[7] Tao, H., Huang, L. J., Li, S. Q., Lu, F., Cai, W. H., & Wang, H. L. (2024). Insight into the promoted recrystallization and water distribution of bread by removing starch granule-surface and-associated proteins during storage. Food Chemistry, 446, 138829.

[8] Singer, AF, Hussein, AMS, Abdel-Rahman, E.A. and Mostafa S. (2024). Enhancement the biological value of corn flakes using defatted sunflower meal. J. Food and Nutr., 10:1-15.

[9] Harshitha, T., Parinitha, A., Gawali, P. P., Somya, A., & Yannam, S. K. (2024). Grain processing and baking technology. In frontiers in food biotechnology (pp. 91-119). Singapore: Springer Nature Singapore.

[10] Mesta-Corral, M., Gómez-García, R., Balagurusamy, N., Torres-León, C., & Hernández-Almanza, A. Y. (2024). Technological and nutritional aspects of bread production: An overview of current status and future challenges. Foods, 13(13), 2062.

[11] Saadi, S., Saari, N., Ghazali, H. M., Abdulkarim, S. M., Hamid, A. A., & Anwar, F. (2022). Gluten proteins: Enzymatic modification, functional and therapeutic properties. Journal of Proteomics, 251, 104395.

[12] Mohammadi, M., Zoghi, A., & Azizi, M. H. (2022). Effect of xylanase and pentosanase enzymes on dough rheological properties and quality of baguette bread. Journal of Food Quality, 2022(1), 2910821.

[13] Dahiya, S., Bajaj, B. K., Kumar, A., Tiwari, S. K., & Singh, B. (2020). A review on biotechnological potential of multifarious enzymes in bread making. Process Biochemistry, 99, 290-306.

[14] Bock, J. E. (2024). Enzymes in breadmaking. In improving and tailoring enzymes for food quality and functionality (pp. 217-239). Woodhead Publishing.
[15] Hmad, I. B., Ghribi, A. M., Bouassida, M., Ayadi, W., Besbes, S., Chaabouni, S. E., & Gargouri, A. (2024). Combined effects of α-amylase, xylanase, and cellulase coproduced by Stachybotrys microspora on dough properties and bread quality as a bread improver. International Journal of Biological Macromolecules, 277, 134391.
[16] Mostafa, S., Faheid, S. M., Rizk, I., Ragab, G. H., & Kishk, Y. (2022). The impact of different conditions on protease hydrolysis for gliadin degradation. Egyptian Journal of Chemistry, 65(132), 165-169.
[17] Ruan, Y., Xu, Y., Zhang, W., & Zhang, R. (2021). A new maltogenic amylase from Bacillus licheniformis R-53 significantly improves bread quality and extends shelf life. Food Chemistry, 344, 128599.
[18] Liu, P., Ma, L., Duan, W., Gao, W., Fang, Y., Guo, L., ... & Cui, B. (2023). Maltogenic amylase: Its structure, molecular modification, and effects on starch and starch-based products. Carbohydrate Polymers, 121183.
[19] Wang, Y. C., Ma, J. W., Liu, H. J., Jiang, Z. Q., & Li, Y. X. (2024). Simultaneous improvement of thermostability and maltotriose-forming ability of a fungal α-amylase for bread making by directed evolution. International Journal of Biological Macromolecules, 264, 130481.
[20] Vermelho, A. B., Moreira, J. V., Junior, A. N., da Silva, C. R., Cardoso, V. D. S., & Akamine, I. T. (2024). Microbial preservation and contamination control in the baking industry. Fermentation, 10(5), 231.
[21] Noshirvani, N., & Abolghasemi Fakhri, L. (2024). Advances in extending the microbial shelf-life of bread and bakery products using different technologies: A Review. Food Reviews International, 1-26.
[22] Lensky, N., Saidov, A., Kalitka, D., Eseeva, G., & Balguzhinova, Z. (2024). Study of industrial enzyme improvers of the rheological properties of baking flour and the quality of finished products. In BIO Web of Conferences (Vol. 82, p. 02018). EDP Sciences.
[23] Ussembayeva, Z., Dautkanov, N., Yerbulekova, M., & Dautkanova, D. (2024). revealing the influence of sorghum flour on the rheological and baking properties of dough, the quality and nutritional value of bread. Eastern-European Journal of Enterprise Technologies, 130(11).
[24] McKelvey, S. M., & Murphy, R. A. (2017). Biotechnological use of fungal enzymes. Fungi: biology and applications, 201-225.
[25] Ashaolu, T. J., Malik, T., Soni, R., Prieto, M. A., & Jafari, S. M. (2025). Extremophilic microorganisms as a source of emerging enzymes for the food industry: A Review. Food Science & Nutrition, 13(1), e4540.
[26] Radadiya, H., Patel, R., & Kothari, R. (2025). The use of microbial enzymes in the food industries: a global perspective. Microbial Enzymes: Production, Purification and Industrial Applications, 1, 207-224.
[27] Bieniek, A., & Buksa, K. (2023). Properties and functionality of cereal non-starch polysaccharides in breadmaking. Applied Sciences, 13(4), 2282.
[28] Bala, A., & Singh, B. (2017). Concomitant production of cellulase and xylanase by thermophilic mould Sporotrichum thermophile in solid state fermentation and their applicability in bread making. World Journal of Microbiology and Biotechnology, 33, 1-10.
[29] do Nascimento, G. K. S., dos Santos, T. M., Andressa, I., Benassi, V. M., de Andrade Neves, N., & Schmiele, M. (2024). Exploring the use of purple corn cob flour as a new fiber source in pan bread improved by commercial hemicellulases. Recent Progress in Nutrition, 4(3), 1-17.
[30] Zhou, J., Li, Z., Zhang, H., Wu, J., Ye, X., Dong, W., ... & Cui, Z. (2018). Novel maltogenic amylase CoMA from Corallococcus sp. strain EGB catalyzes the conversion of maltooligosaccharides and soluble starch to maltose. Applied and Environmental Microbiology, 84(14), e00152-18.
[31] Woo, S. H., Shin, Y. J., Jeong, H. M., Kim, J. S., Ko, D. S., Hong, J. S., ... & Shim, J. H. (2020). Effects of maltogenic amylase from Lactobacillus plantarum on retrogradation of bread. Journal of Cereal Science, 93, 102976.
[32] Korompokis, K., Deleu, L. J., De Brier, N., & Delcour, J. A. (2021). Investigation of starch functionality and digestibility in white wheat bread produced from a recipe containing added maltogenic amylase or amylomaltase. Food Chemistry, 362, 130203.
[33] Zhang, L., Li, Z., Qiao, Y., Zhang, Y., Zheng, W., Zhao, Y., ... & Cui, Z. (2019). Improvement of the quality and shelf life of wheat bread by a maltohexaose producing α-amylase. Journal of cereal science, 87, 165-171.
[34] Taipakova, S. M., Smekenov, I. T., Saparbaev, M. K., & Bissenbaev, A. K. (2015). Characterization of Aspergillus niger endo-1, 4-β-glucanase ENG1 secreted from Saccharomyces cerevisiae using different expression vectors. Genet Mol Res, 14(2), 6439-6452.
[35] Thapa, S., Mishra, J., Arora, N., Mishra, P., Li, H., O′ Hair, J., ... & Zhou, S. (2020). Microbial cellulolytic enzymes: diversity and biotechnology with reference to lignocellulosic biomass degradation. Reviews in Environmental Science and Bio/Technology, 19, 621-648.

[36] Akram, F., ul Haq, I., Imran, W., & Mukhtar, H. (2018). Insight perspectives of thermostable endoglucanases for bioethanol production: a review. Renewable Energy, 122, 225-238.
[37] Gavande, P. V., & Goyal, A. (2023). Endo-β-1, 4-glucanase. In glycoside hydrolases (pp. 55-76). Academic Press.
[38] Altınel, B., & Ünal, S. S. (2017). The effects of certain enzymes on the rheology of dough and the quality characteristics of bread prepared from wheat meal. Journal of Food Science and Technology, 54, 1628-1637.
[39] Tebben, L. R. (2019). Effect of enzymes, hydrocolloids, and emulsifiers on qualities of dough and bread made from whole grain wheat flour (Doctoral dissertation).
[40] Edison, L. K., Shiburaj, S., & Pradeep, N. S. (2018). Microbial beta glucanase in agriculture. In Advances in Microbial Biotechnology (pp. 53-72). Apple Academic Press.
[41] Pradeep, N. S., & Edison, L. K. (Eds.). (2022). Microbial beta glucanases: Molecular structure, functions and applications. Springer Nature.
[42] Dai, Y., & Tyl, C. (2021). A review on mechanistic aspects of individual versus combined uses of enzymes as clean label-friendly dough conditioners in breads. Journal of Food Science, 86(5), 1583-1598.
[43] Kostyuchenko, M., Martirosyan, V., Nosova, M., Dremucheva, G., Nevskaya, E., & Savkina, O. (2021). Effects of α-amylase, endo-xylanase and exoprotease combination on dough properties and bread quality. Agronomy Research. 19 (S3), 1234-1248.
[44] Özgen, Ö., G., Şahinbaş, D., Düzel, A., Düzdemir, G. E., Altınel, B., Tuluk, K., & Sargın, S. (2024). Production of xylanase intended to be used in bread-making: laboratory scale and pilot scale studies. Biocatalysis and Biotransformation. 42 (4), 509-523.
[45] Sun, X., Wu, S., Koksel, F., Xie, M., & Fang, Y. (2023). Effects of ingredient and processing conditions on the rheological properties of whole wheat flour dough during breadmaking-A review. Food Hydrocolloids, 135, 108123.
[46] Münstedt, H. (2021). Rheological measurements and structural analysis of polymeric materials. Polymers, 13(7), 1123.
[47] Cecchini, C., Bresciani, A., Menesatti, P., Pagani, M. A., & Marti, A. (2021). Assessing the rheological properties of durum wheat semolina: A review. Foods, 10(12), 2947.
[48] A.A.C.C. (2012). International Methods approved of the American Association of Cereal Chemists, 11th Ed., AACC, INC., St. Paul: Minnesota, USA.
[49] A.O.A.C. (2012). Official Methods of the Association of Official Analytical Chemists 19th Ed. Association of Official Analytical Chemists, Arlington, Virginia, USA.
[50] Liu, L., Y. Cen., F. Liu., J. Yu., X. Jiang and X. Chen (2015). Analysis of α-amylase inhibitor from corni fructus by coupling magnetic cross-linked enzyme aggregates of α-amylase with HPLC-MS. J. Chromatography B. 995-996, 64-69.
[51] Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for the determination of reducing sugar. Anal. Chem. 31(3), 426-428.
[52] Cha, H.J., H.G. Yoon., Y.W. Kim., H.S. Lee., J.W. Kim., K.S. Kweon., B.H. OH and K.H. Park (1998). Molecular and enzymatic characterization of a maltogenic amylase that hydrolyzes and transglycosylates acarbose. Eur. J. Biochem. 253, 251 – 262.
[53] Kurioka, S. and P.V. Liu (1967). Improved assay method for phospholipase C. Appl. Microbiol. 15(3), 551-555.
[54] Horecker, B.L., T.S. MA and E.J. Hass (1940). Note on the determination of microquantities of organic phosphorus. J. Biol. Chem. 136,775-776.
[55] Josephy, P.D., T.E. Ling and R.P. Mason (1982).The horseradish peroxidase-catalyzed oxidation of 3, 5,3',5'-tetramethylbenzidine.Free radical and charge transfer complex intermediates. J. Biol. Chem. 277,3669-3675.
[56] Ampon, K., W.Z.W. Yunus and B. Salleh (1994). A spectrophotometric method for the determination of glucose with glucose oxidase [E.C. 1.11.1.7] using titanium (IV)-4-(2'-pyridylazo) resorcinol reagent. Pertanika J. Sci. and Technol. 2(1), 47-53.
[57] Marco, J.L.D and C.R. Felix 2007. Purification and characterization of a β-glucanase produced by Trichoderma harzianum showing biocontrol potential. Brazilian Archives of Biol. Technol. 50 (1), 21-29.
[58] I.C.C. Standards (2012). Standard methods of the International Association for Cereal Science and Technology, International Association for Cereal Science and Technology, Marxergasse 2, Vienna, AT, Austria.
[59] Cauvain, S.P. and Linda S. Young (2007). Breadmaking process. In: Technology of Breadmaking. PP. 28–33, Chap. 2, 2nd Ed. Springer, New York.
[60] Bourne, M.C. (1978). Texture profile analysis. Food Technology, 32(7), 62–66 and 72.
[61] Larmond, E. (1970). Methods for sensory evaluation of food. Food Research Institute, Canada Dept. Agric. Publ. 1284. Ottawa.
[62] Matveeva, Irina., I. Samakidis and G. Bellido (2015). Unlock the full potential of flour – A comprehensive handbook of enzymatic solutions for the milling industry. Novozymes A/S, Bagsvaerd, Denmark.
[63] SAS (2004). “Statistical Analysis System”. SAS User's Guide (Version 8.1). SAS institute Inc., CARY, NC, USA.

[64] Sigma Plot Program (2014). Version 13.0, Build 13.0.0.83, Systat Software Inc. LEAD TOOLS, DUNDAS SOFTWARE Ltd., WP cubed Gmbh, Germany.
[65] Šramková, Z., E. Gregová and E. Šturdík (2009). Chemical composition and nutritional quality of wheat grain. Acta chimica slovaca. 2(1), 115-138.
[66] Beshir, E.O., M. Khallaf., A. El-daim., R. Awad and M. A. A. Kamel (2021). Sweet lupin and whey protein concentrate as supplementants for utilizing in semi–hard biscuit and its chemical properties. Egyptian Journal of Chemistry, 64 (12), 7517-7527.
[67] Hussein, A., G. Ibrahim., M. Kamil., M. El-Shamarka., S. Mostafa and D. Mohamed (2021). Spirulina-enriched pasta as functional food rich in protein and antioxidants. Biointerface Res. Appl. Chem. 11, 14736-14750.
[68] Liu, C., L. Li., J. Hong., X.L. Zheng., K. Bian., Y. Sun and J. Zhang (2014). Effect of mechanically damaged starch on wheat flour, noodle and steamed bread making quality. International J. of Food Sci. and Technol., 49(1): 253–260.
[69] Guttieri, M.J., D. Bowen., D. Gannon., K. O'Brien and E. Souza (2001). Solvent retention capacities of irrigated soft white spring wheat flour. Crop science. 41(4), 1054-1061.
[70] Nabih, E.; M.G. El-Shemy; E.I. Yousif and N. Yasin (2019). Effect of alpha-amylase and xylanase activities on properties of frozen dough prepared from flours of different wheat varieties. Arab Univ. J. Agric. Sci. 27(4), 2139-2154.
[71] Ferrari, M., M. Clerici and Y. Chang (2014). A comparative study among methods used for wheat flour analysis and for measurements of gluten properties using the Wheat Gluten Quality Analyzer (WGQA). J. Food Sci. Tech. 34, 235- 242.
[72] Curic, D., D. Karlovic., D. Tusak., B. Petrovic and J. Dugum (2001). Gluten as a standard of wheat flour quality. Food Technol. Biotechnol. 39(4): 353–361.
[73] Struyf, N., J. Verspreet and C. Courtin (2016). The effect of amylolytic activity and substrate availability on sugar release in non-yeasted dough. J. Cereal Sci. 69, 111-118.
[74] Codina, G. and A. Leahu (2009). The improvement of the quality of wheat flour with a lower content of alpha-amylase trough the addition of different enzymatic products. Ser. J. Agron. 52, 629-635.
[75] Every, D. and M. Ross (1996). The role of dextrins in the stickiness of bread crumb made from pre-harvest sprouted wheat or flour containing exogenous alpha amylase. J. Cereal Sci. 23, 247-256.
[76] Goesaert, H., L. Slade., H. Levine and J.A. Delcour (2009). Amylases and bread firming – an integrated view. J. Cereal Sci. 50: 345–352.
[77] Simmonds, D.H. (1974) Chemical basis of hardness and vitreosity in the wheat kernel. Bakers Digest. 48:16–29.
[78] Hoseney, R.C. (1994) Principles of Cereal Science and Technology, 2nd Ed. The American Association of Cereal Chemists, Inc., St. Paul, MN, Pp. 81–101, 125–145, 229–273.
[79] Kornbrust, B.A., T. Forman and I. Matveeva (2012). Applications of enzymes in breadmaking. In "Breadmaking, Improving Quality." 2nd ed. Stanley P. Cauvain. Woodhead Publishing Series in Food Science, Technology and Nutrition.
[80] Gomes-Ruffi, C.R., R.H. da Cunha., Eveline L. Almeida., Y.K. Chang and Caroline J. Steel (2012). Effect of the emulsifier sodium stearoyl lactylate and of the enzyme maltogenic amylase on the quality of pan bread during storage. LWT - Food Sci. and Technol. 49: 96–101.
[81] Colakoglu, A.S. and H. Ozkaya (2012). Potential use of exogenous lipases for DATEM replacement to modify the rheological and thermal properties of wheat flour dough. J. Cereal Sci. 55, 397-404.
[82] Gerrard, J.A., D. Every., K.H. Sutton and M.J. Gilpin (1997). The role of maltodextrins in the staling of bread. J. Cereal Sci. 26: 201–209.
[83] Sharma, P., H.S. Gujral and C.M. Rosell (2011). Effects of roasting on barley b-glucan, thermal, textural, and pasting properties. Journal of Cereal Sci. 53, 25–30.
[84] Wang, S., C. Li., L. Copeland., Q. Niu and S. Wang (2015) Starch retrogradation: a comprehensive review, Compr. Rev. Food. Sci. F. 14 (5) 568–585.
[85] Chen, Y., S. Huang., Z. Tang., X. Chen., Z. Zhang (2011). Structural changes of cassava starch granules hydrolyzed by a mixture of α-amylase and glucoamylase, Carbohyd. Polym. 85 (1) 272–275.
[86] Chen, X., L. Zhang., X. Li., Y. Qiao., Y. Zhang., Y. Zhao and Cui Z. (2020). Impact of maltogenic α-amylase on the structure of potato starch and its retrogradation properties. International journal of biological macromolecules. 145, 325-331.
[87] Ferry, A.L., J. Hort., J.R. Mitchell., S. Lagarrigue and B.V. Pàmies (2004). Effect of amylase activity on starch paste viscosity and its implications for flavor perception. Journal of Texture Studies. 35(5), 511-524.
[88] Barrera, G. N., G. T. Pérez., P. D. Ribotta and A.E. León (2007). Influence of damaged starch on cookie and bread-making quality. European Food Research and Technology. 225, 1-7.

[89] David, I., A. Rinovetz., G. Bujancă., C. Mişcă., M. Danci., and C. Costescu (2014). The influence of different types of amylases on the bread dough was determined through an alveographic method. Journal of Agroalimentary Processes and Technologies. 20(2), 165-170.

[90] Fadda, C., A.M. Sanguinetti., A. Del Caro., C. Collar., and A. Piga (2014). Bread staling: Updating the view. Comprehensive Reviews in Food Science and Food Safety. 13(4), 473-492.

[91] Mostafa, S.M.T.M. (2020). Production of some functional foods for gluten allergy patients. Ph.D. Sci., Agric. Sci. (Food Science), Fac. Agri., Ain shams University, pp. 82-91.

[92] Chen, Y., S. Eder., S. Schubert., S. Gorgerat., E. Boschet., L. Baltensperger and E. J. Windhab (2021). Influence of amylase addition on bread quality and bread staling. ACS Food Science and Technology. 1(6), 1143-1150.

[93] Rebholz, G.F., K. Sebald., S. Dirndorfer., C. Dawid., T. Hofmann and K.A. Scherf (2021). Impact of exogenous maltogenic α-amylase and maltotetraogenic amylase on sugar release in wheat bread. European Food Research and Technology. 247(6), 1425-1436.

[94] Pérez-Quirce, S., F. Ronda., C. Melendre., A. Lazaridou and C.G. Biliaderis (2016). Inactivation of endogenous rice flour β-glucanase by microwave radiation and impact on physico-chemical properties of the treated flour. Food and Bioprocess Technology. 9, 1562-1573.

[95] Raffo, A., Antonella Pasqualone., F. Sinesio., F. Paoletti., G. Quaglia and R. Simeone (2003). Influence of durum wheat cultivar on the sensory profile and staling rate of Altamura bread. Euro. Food Research and Technol. 218, 49–55.

[96] Grewal, N., J. Faubion., G. Feng., R.C. Kaufman., J.D. Wilson and Y.C. Shi (2015). Structure of waxy maize starch hydrolyzed by maltogenic alpha amylase in relation to its retrogradation. Journal of Agricultural and Food Chemistry. 63(16), 4196–4201.

[97] Zhang, Y., L. Ding., J. Gu., H. Tan and L. Zhu (2015). Preparation and properties of a starch-based wood adhesive with high bonding strength and water resistance. Carbohydrate Polymers. 115, 32–37.

[98] Lombard, V., H. Golaconda Ramulu., E. Drula., P.M. Coutinho and B. Henrissat (2013). The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Research. 42(1), 490–495.

[99] Aoki, N, Umemoto, T., Hamada, S., Suzuki, K., & Suzuki, Y. (2012). The amylose content and amylopectin structure affect the shape and hardness of rice bread. Journal of Applied Glycoscience, 59(2), 75-82.

[100] Drapron, R. and B. Godon (1987). Role of enzymes in baking. In: "Enzymes and their role in cereal technology". Kruger, J.E., Lineback D. and Stauffer C.E. (Eds.) pp.281-324.American Association of Cereal Chemists, St. Paul, Minnesota, U.S.A.

[101] Bowles, L.K. (1996). Amylolytic enzymes. In: Baked goods freshness: Technology, evaluation, and inhibition of staling. Hebeda, R.E. and H.F. Zobel (eds). Pp. 105–129.NY, Marcel Dekker, New York, U.S.A.

[102] Paramithiotis, S., Y. Chouliaras., E. Tsakalidou and G. Kalantzopoulos (2005). Application of selected starter cultures to produce wheat sourdough bread using a traditional threestage procedure. Process Biochemistry. 40: 2813-2819.

[103] Giannone, V., Maria R. Lauro., A. Spina., Antonella Pasqualone., Lucrezia Auditore., Ivana Puglisi and G. Puglisi (2016). A novel α-amylase-lipase formulation as antistaling agent in durum wheat bread. LWT - Food Sci. and Technol. 65: 381–389.