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Enhancing Dough Quality: The Effects of Transglutaminase and Glucose Oxidase on Stability and Mixing Resistance

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ABSTRACT

The aim of this study was to investigate the influence of Transglutaminase (TGase) and Glucose Oxidase (GOase) on protein extraction and dough rheological properties in composite flours made of bread wheat, durum wheat, barley, oat, rice, sorghum and Emmer wheat. Significantly variable were the effects of the digestive enzymes on the extraction yield of albumins, globulins, prolamins, and glutenins in the different cereal matrices. The rheological experiments carried out utilizing Amylograph and Farinograph analyze demonstrated enzyme-specific effects on essential properties like peak viscosity, water absorption, dough development duration, and stability. The results highlight an important aspect of enzyme selection towards the modulation of flour functionality and the complexity of interactions between enzymatic activity and cereal protein composition. TGase is also proven to greatly impact dough physical properties, increasing strength and elasticity, while GOase will evidently improve dough stability and decrease stickiness, respectively. The two enzymes act synergistically to enhance the handling properties of the dough, thus serving as useful additives for the industrial baking sector.

Keywords: *Dough Stability; Enzymatic Treatments; Modifying the rheological; Metabolic Control; Quality of wheat-based products.*

1. INTRODUCTION

Several of the wheat proteins are associated with the bread-making of wheat, especially gluten protein, which is critical to baking quality (Rekowski *et al.*, 2021; Souza *et al.*, 2004). Heat and drought

have a strong impact on this protein content. In comparison to frequent high temperatures stress events, drought stress also contributes to an increment of gluten protein levels in wheat grain (Rakszegi *et al.,* 2019; Lan *et al.,* 2022; Lama *et al.,* 2022).

However, the wheat grain processing quality (of bread wheat flour) may vary across different periods of occurrence of drought stress, depending on the severity of stress (Ceresino *et al.,* 2020; Kuktaite *et al.,* 2004; Yaqub *et al.,* 2024; Malik *et al.,* 2011).

In a industrial baking process, wheat flour and water are mixed, which makes dough stability and resistance to mixing stress essential (Jiang *et al.,* 2009). These changes affect the dough rheological properties elasticity, viscosity, and extensibility that are fundamental to estimating the quality of the final product, in particular in diverse growing environments (Gao *et al.,* 2020)

TGase and GOase are commonly used enzymes to enhance the dough rheological property without chemical additives. TGase catalyses the formation of cross-links between glutamine and lysine residues and reinforces the gluten network. GOase oxides glucose, and generates hydrogen peroxide, which facilitates disulfide bond formations in gluten (Jiang *et al.,* 2009; Gao *et al.,* 2020; Yaqub *et al.,* 2024; Cao *et al.,* 2019).

The increasing growing interest in utilizing cereal-based food products with additional functional properties has ignited interest in enzymatic modalities of enhancing dough quality and processing performance (Gao *et al.,* 2020; Cao *et al.,* 2019). Enzymes of interest in this context include Transglutaminase (TGase), which establishes ε-(γ-glutamyl)lysine isopeptide linkages responsible for protein cross-linking, and Glucose Oxidase (GOase), which induces dough strengthening through glucose oxidation and subsequent disulfide linkage formation (Jiang *et al.,* 2009; Gao *et al.,* 2020; Cao *et al.,* 2019). Although TGase and GOase have been recognized as being greatly effective, the functional properties of their applications differ widely among various cereal sources due to glutenin and gliadin's structural and compositional differences (Gao *et al.,* 2020; Cao *et al.,* 2019). Herein, this study systematically investigate the effects of TGase and GOase on protein extraction efficiency and dough rheological properties of a range of cereals (Gupta *et al.,* 2011; Johansson *et al.,* 2002., 2011; Ceresino *et al.,* 2020; Kuktaite *et al.,* 2004 ; Malik *et al.,* 2011). In this research, we focus on unraveling the interactions at the enzyme and cereal-specific levels which dictate the functionality of dough in order to provide insights into the precision improvement of flour for cereal processing, which based on the detailed knowledge at the molecular level and provide targeted approaches to optimize the flour for cereal processing.

2. MATERIALS AND METHODS

Materials

- **Enzymes**

  Microbial Trans-glutaminase :TGase from *Streptoverticillium sp.* (activity: 50 U/g) and GOase (activity: 5000 U/g) were used.

  Glucose Oxidase (GOase): GBK-BG100 Glucose Oxidase was purchased from Baishengyuan, Shush an, District, Hefei, Anhuri Co. China. Its activity was 5000 U/g of crystal powder.

- **Flour preparation:** First, the grains of bread wheat, durum wheat, barley, oat, rice, sorghum emmer wheat were cleaned, washed, and dried at room temperature (around 25ºC), and stored in a cool environment. Moisture content of grains was determined for tempering grains to 15% moisture. The water tempering amount for Kernels was calculated according to standard tempering Table 1. (AACC, Approved Method No.26-95) (AACC, 2000).
- **Water:** distilled water was used to provide consistent hydration to the dough. then were packed in polyethylene packages and incubated overnight with the necessary amount of water added to each sample of cereal grains. Mill All grain samples in a local milling shop in Sulaimania (In the morning). Mill discs were adjusted in a possible range while trying to decrease flour grains extraction rate. Subsequent to milling, the flour was sieved through sieve No.9xx (150 µm). Farine et son sont pesés pour connaitre leurs taux d'extractions
- **Other Ingredients**: Salt (sodium chloride) and sugar (sucrose) were added to the dough formulation at standard levels (e.g., 1.5% salt, 2% sugar based on flour weight). Seven cereal grains were dry-cleaned, tempered to 15% moisture, milled, and sieved (150 µm). Extraction rate was calculated post-sieving.

Protein Extraction and Purification

Protein fractions albumin, globulin, prolamin, and glutenin were extracted based on solubility, using water, salt solution, ethanol, and phosphate buffer, respectively. Protein content was determined spectrophotometrically using the Warburg-Christian method Figure (1).

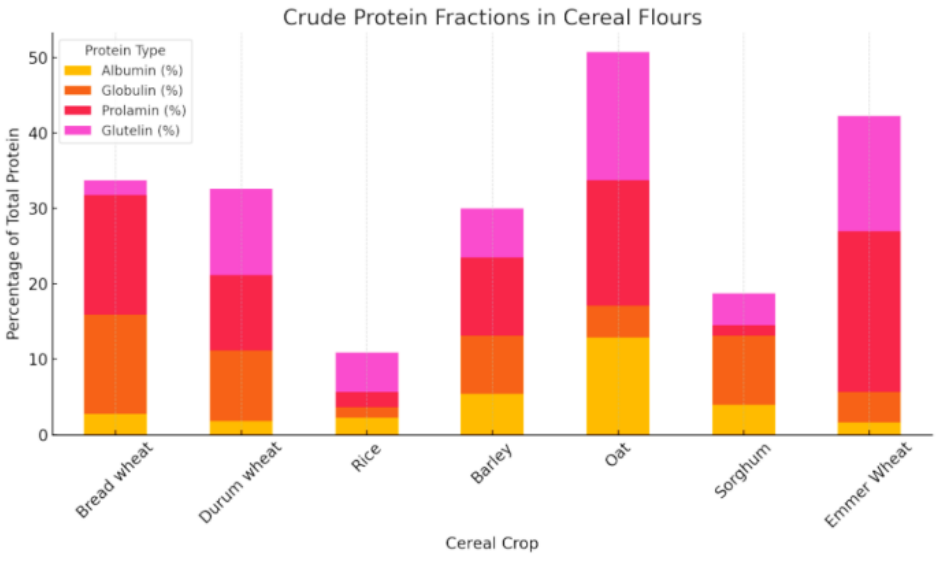

Figure 1. The distribution of crude protein fractions (albomin, globulin,prolamin,and glutein) indifferent cereal flours :

Rheological Analysis

Amylograph and Farinograph tests were conducted (AACC, 2000) to assess gelatinization, viscosity, development time, stability, and mixing tolerance under treatments with TGase and GOase.

Amylograph test

Amylograph was used to measure the rheological properties of flour for cereal grains samples paste with or without Transglutaminase (TGase) and Glucose oxidase (GOase) according to AACC method No.22-12.0.1 AACC, (2000) by adding 450 ml distilled water to 60g of flour in beaker Figure (2). Then it was mixed well and poured to the Amylograph basin. Amylograph was also used to determine the paste viscosity at uncontrolled cooling to (50 °C). The effect of adding 50U/g of TGase and 1U/g of GOase on amylograph parameters for all studied cereal flours were determined. The studied amylograph parameters were: -

1. Pasting temperature°C.; it is the beginning of viscosity increasing.
2. Peak viscosity Units AU. (Or BU); It is the maximum of viscosity at heating.
3. Peak viscosity temperature °C; it is the temperature at Peak viscosity.
4. Hot paste Viscosity AU; it is the viscosity after 20 min. of heating.
5. Break down Viscosity AU; it is the difference between the peak viscosity and hot paste viscosity (1n AU).
6. Cold Paste Viscosity AU; it is the maximum viscosity at cooling to 50°C.
7. Set back viscosity AU; it is the difference between cold paste viscosity and hot paste viscosity.

Figure 2. Amylograph, Brabender® GmbH & Co. KG Germany equipment used for investigating the effects of various ingredients, such as enzymes, on dough properties.

Farinograph test

Farinograph was used to determine the water absorption and rheological properties for all flour samples according to AACC method No.54-21.0.2 (AACC, 2000) by using 300g mixer. Also the effect of adding 50U/g of TGase and 1U/g of GOase on farinograph parameters for studied flour samples was determined. The studied farinograph parameters were prepared as the followings:

1. Water absorption (correct to 500 FU).
2. Development time (min.) that is the time required for the top of the curve to cross the 500 Farinograph Unit (FU) line.
3. Stability (min.) is the time difference between arrival time and departure time.
4. Tolerance index (MTI) FU is the difference in FU's between the peak time and peak time plus 10min.

5. Time to breakdown (min.) is time lapsed until the top of the curve permanently drops below the 500 FU line.
6. Farinograph quality numbers

Statistical Analysis

Data were analyzed using a CRD with three replicates to determine the significance of differences between groups. Means were compared using Duncan's multiple range with a significance level set at ($P \leq 0.05$).

3. RESULTS AND DISCUSSION

Protein Extraction and Purity

Figure (3). showed the protein extraction experiment aimed to isolate the four major types of crude protein albumin, globulin, prolamin, and glutelin from various cereal flours. It is important to note that the objective was not to optimize extraction efficiency, but rather to obtain sufficient quantities of each protein type for subsequent enzymatic cross-linking reactions. Therefore, the observed extraction efficiencies were generally lower than those reported by Ju *et al.* (2001) and Anderson and Lamsal (2011), who achieved efficiencies ranging from 80–96% in rice and corn through targeted optimization.

Despite this, the experiment provided meaningful insights into protein distribution and extractability across different cereals. Notably, the albumin fraction showed considerable variation. Emmer wheat had the lowest albumin content at 1.62% of total protein, while oat presented the highest at 12.88%. These findings are in agreement with Chang (2010), who reported that albumin constitutes approximately 10–20% of total oat protein and around 5.9% in rice, highlighting the unique protein profile of oats.

Similarly, globulin content ranged from 1.38% in rice to 13.18% in bread wheat. Interestingly, these values deviate from those reported by Eliasson and Larsson (1993), who found that globulin made up 10% of rice protein and 6–7% in bread wheat, with a broader range of 3–4% in corn and up to 55% in oats. The discrepancies may be attributed to differences in solvent selection and extraction protocols, which are known to affect protein solubility and yield Table (1).

Table 1. Extraction amount and efficiency of crude protein fractions.

Cereal crop	Albumin %		Globulin %		Prolamin %		Glutelin %		Extraction efficiency % of total protein
	of flour	of total protein	of flour	of total protein	of flour	of total protein	of flour	of total protein	
Bread wheat	0.38	2.79	1.78	13.09	2.16	15.88	0.27	1.98	33.75
Durum wheat	0.27	1.80	1.41	9.40	1.49	9.93	1.72	11.46	32.60
Rice	0.20	2.24	0.12	1.38	0.18	2.02	2.13	5.30	10.94
Barley	0.65	5.41	0.93	7.75	1.24	10.33	0.78	6.50	29.99
Oat	1.34	12.88	0.44	4.23	1.73	16.63	1.77	17.02	50.76
Sorghum	0.59	3.96	1.37	9.19	0.20	1.34	0.64	4.29	18.78
Emmer Wheat	0.26	1.62	0.65	4.06	3.41	21.31	2.45	15.31	42.30

Prolamin fractions were particularly low in sorghum and rice, at 1.34% and 2.02%, respectively. In contrast, higher levels were observed in durum wheat (9.93%) and emmer wheat (21.31%). However, these values remained below those reported by Giuberti *et al.* (2011), who found prolamin to constitute 28%, 39%, and 36% of total protein in barley, corn, and wheat, respectively. Despite lower overall yields, the ratio of gluten to non-gluten proteins in barley, corn, and wheat aligned with Giuberti et al.'s findings, indicating a consistent distribution pattern, if not absolute quantity.

The glutelin fraction showed the highest extractability in oat (17.02%), while bread wheat yielded the lowest (1.63%). This pattern is not necessarily indicative of actual protein content, but rather reflects the limitations of the extraction solvents used. Glutelin, particularly glutenin, is known for its resistance to solubilization, even in the presence of reducing agents—a point emphasized by Kurowska and Bushuk (1988). Thus, solvent efficacy plays a more significant role than protein abundance in determining extraction results.

Overall, total protein extraction efficiency ranged from 10.94% in rice to 50.76% in oat. Although these values are below conventional benchmarks, they were sufficient for the purposes of this study. The results offer valuable insights into the relative solubility and distribution of protein types across cereal grains, providing a foundation for selecting appropriate protein substrates in cross-linking and dough functionalization experiments.

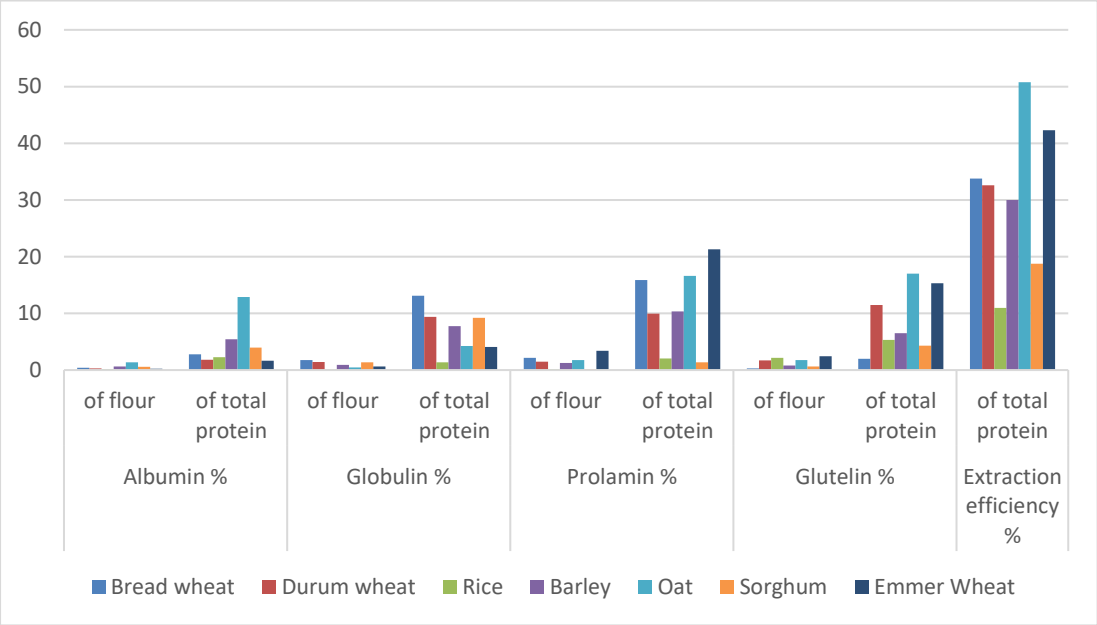

Figure 3. Extraction amount and efficiency of crude protein fractions.

Effect of TGase on Protein Cross-Linking

TGase treatment consistently reduced free amino groups, confirming protein cross-linking. It improved dough thermal stability, specific volume, and reduced crumb hardness in multiple studies Figure 4 and Table (2). (Marco & Rosell, 2008; Huang *et al*., 2010; Caballeroa *et al*., 2005).

Table 2. Effect of TGase and GOase on wheat paste amylograph parameters.

Composite flour	Gelatinization temperature (ºC)	Peak viscosity (AU)	Peak Viscosity Temperature (º`C)
Bread wheat (control)	63.00	350.00	93.00
Bread wheat + Durum wheat without enzyme	67.00	235.00	94.50
Bread wheat + Durum wheat +1g TGase	66.00	295.00	>95.00
Bread wheat + Durum wheat +0.1GOase	72.00	175.00	92.00
Bread wheat +Barley without enzyme	67.50	400.00	>95.00
Bread wheat + Barley +1g TGase	54.00	470.00	>95.00
Bread wheat + Barley +0.1g GOase	64.50	110.00	91.00
Bread wheat + Oat without enzyme	78.00	700.00	95.00
Bread wheat +Oat + 1g TGase	75.00	790.00	92.50
Bread wheat +Oat + 0.1g GOase	81.00	420.00	95.00
Bread wheat + Sorghum without enzyme	76.50	490.00	>95.00
Bread wheat + Sorghum +1g TGase	77.00	505.00	>95.00
Bread wheat + Sorghum +0.1GOase	81.00	380.00	>95.00
Durum wheat+Barley without enzyme	60.00	490.00	94.50
Durum wheat + Barley +1g TGase	63.00	320.00	92.00
Durum wheat + Barley +0.1g GOase	67.00	190.00	93.00
Durum wheat + Oat without enzyme	79.00	700.00	94.50
Durum wheat +Oat + 1g TGase	76.50	730.00	94.50
Durum wheat +Oat + 0.1g GOase	84.00	450.00	94.00
Durum wheat + Sorghum without enzyme	79.00	440.00	> 95.00
Durum wheat + Sorghum +1g TGase	79.50	395.00	>95.00
Durum wheat + Sorghum +0.1GOase	78.0	450.00	5.00

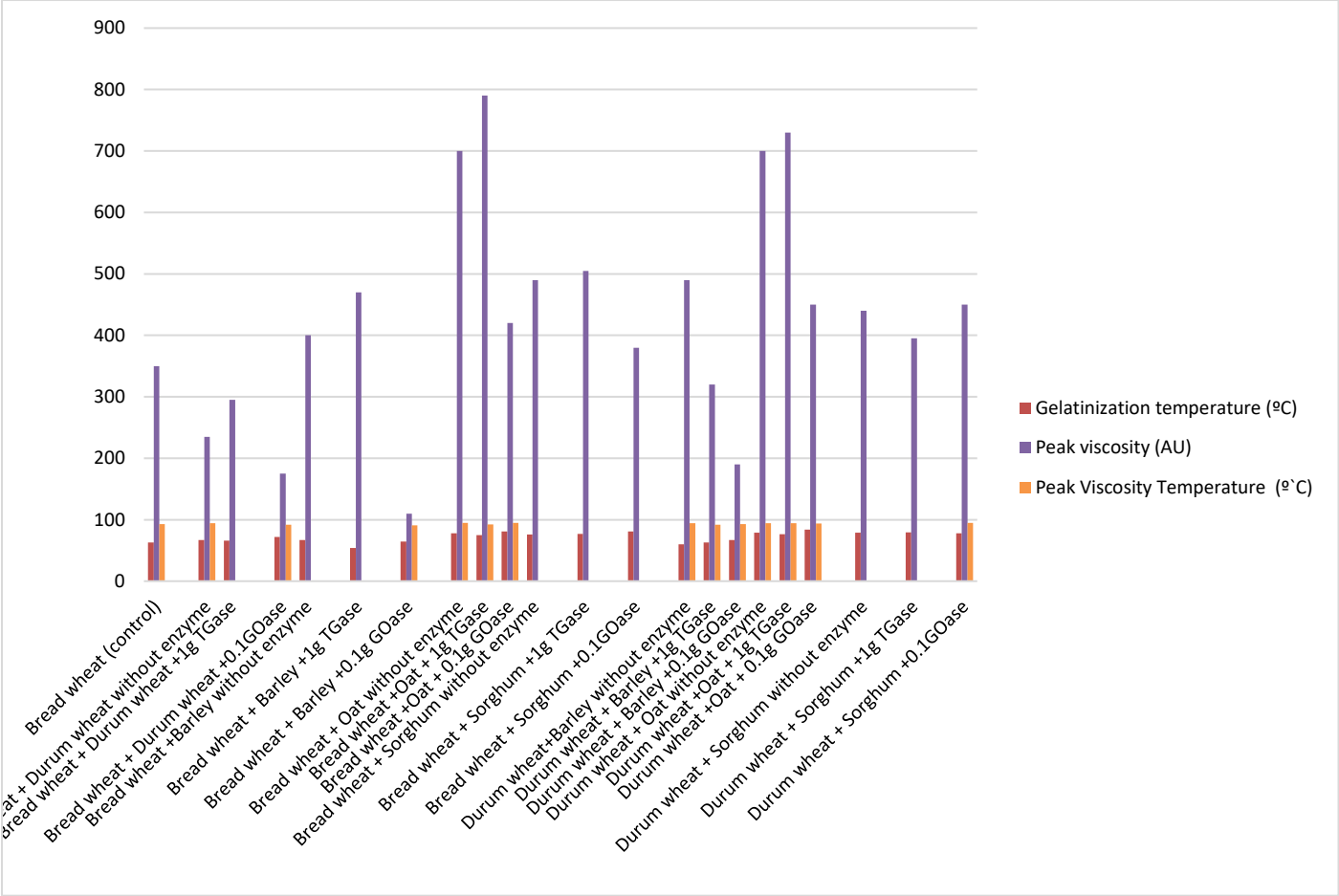

Figure 4. Effect of TGase and GOase on wheat paste amylograph parameters

Rheological Properties – Amylograph Results

Enzymatic treatments significantly modified pasting behavior. TGase increased peak viscosity in most samples, especially barley and oat composites. GOase had mixed effects, often lowering peak viscosity and increasing gelatinization temperature. The combined treatments highlighted differential enzyme interactions with cereal proteins. The amylograph analysis Table 3. revealed that both the type of cereal composite flour and the enzymatic treatments with transglutaminase (TGase) and glucose oxidase (GOase) significantly influenced gelatinization and viscosity characteristics. Gelatinization temperatures (GT) for the cereal flours generally aligned with their expected natural ranges: 60–68°C for bread wheat, durum wheat, and barley, and 70–80°C for sorghum and oat (Wianecki & Kołakowski, 2007; Salih *et al.*, 2018; Tester & Karkalas, 1996).

Peak viscosity (PV), an indicator of maximum starch swelling and paste strength, was notably low (235–490 AU) in all flours except oat, falling below the recommended 600 AU threshold associated with acceptable bread quality (Brabender Amylograph, 2016; Salih *et al.*, 2019). These reduced PV values suggest high amylase activity or starch damage, which compromises loaf volume and crumb structure (Brabender Amylograph, 2016; Salih *et al.*, 2020).

Enzymatic modification showed divergent effects. TGase generally decreased GT and increased PV across bread wheat, durum wheat, barley, and oat composite flours. This may be due to TGase's role in enhancing the protein matrix, potentially forming protective interactions around starch granules that mitigate degradation. Conversely, GOase treatment typically increased GT (by 5–7°C in bread wheat, oat, durum,

and barley) and decreased PV, possibly due to enhanced amylase activity or starch damage. This effect is likely linked to oxidative reactions involving hydrogen peroxide and gluconic acid, by-products of GOase activity that can indirectly affect starch properties (Bankar *et al.,* 2009; Tzanov *et al.,* 2002; Salih *et al.,* 2021; Yaqub *et al.*, 2024).

Interestingly, sorghum flour demonstrated resistance to GOase-induced changes in viscosity, suggesting inherent stability or structural differences in its starch or protein matrix. Peak viscosity temperature (PVT), which reflects the thermal stability of starch granules, ranged from 91 to over 95°C across samples. This suggests the presence of intact starch granules capable of water absorption despite enzymatic and thermal stress, supporting the overall integrity of the starch component under amylograph testing conditions (Brabender, 2010 ; Salih *et al.*, 2025; Omar et al., 2025; Yaqub *et al.*, 2024; Rahman *et al.*, 2021).

Table 3. Effect of TGase and GOase addition on farinograph parameters of composite flour.

Treatments	Water absorption %	Development time min.	Stability min.	Tolerance Mixing Index FU	Time to break min.	Farinograph quality number degree
Bread wheat Aras (control)	71.70	4.70	2.90	23.00	11.90	119.00
Bread wheat + Durum without enzyme	70.20	17.70	8.70	130.00	17.70	177.00
Bread wheat + Durum +1g TGase	67.40	5.70	6.80	121.00	6.20	62.00
Bread wheat + Durum +0.1GOase	68.30	4.70	4.40	66.00	7.30	73.00
Bread wheat +Barley without enzyme	58.90	16.00	22.10	166.00	16.00	160.00
Bread wheat + Barley +1g TGase	62.40	4.90	18.60	157.00	4.90	49.00
Bread wheat + Barley +0.1g GOase	62.00	30.00	28.70	78.00	30.00	300.00
Bread wheat + Oat without enzyme	67.00	5.40	7.30	108.00	5.60	56.00
Bread wheat +Oat + 1g TGase	67.4.00	5.70	6.80	121.00	6.20	62.00
Bread wheat +Oat + 0.1g GOase	63.10	2.70	6.20	61.00	2.80	28.00
Bread wheat + Sorghum without enzyme	58.20	6.90	3.20	6.00	30.00	300.00
Bread wheat + Sorghum +1g TGase	58.00	8.70	8.00	11.00	30.00	300.00
Bread wheat + Sorghum +0.1GOase	58.00	11.00	6.90	12.00	23.70	237.00
Durum +Barley without enzyme	64.70	29.40	14.60	287.00	30.00	300.00
Durum + Barley +1g TGase	62.10	10.20	26.60	225.00	10.50	105.00
Durum + Barley +0.1g GOase	64.80	25.20	14.30	191.00	30.00	300.00
Durum + Oat without enzyme	67.40	5.70	6.80	121.00	6.20	62.00
Durum +Oat + 1g TGase	61.90	6.70	25.10	21.00	29.20	292.00
Durum +Oat + 0.1g GOase	61.40	21.00	13.20	4.00	30.00	300.00
Durum + Sorghum without enzyme	55.40	19.20	24.20	4.00	30.00	300.00
Durum + Sorghum +1g TGase	54.50	12.20	22.00	6.00	30.00	300.00
Durum + Sorghum +0.1GOase	55.50	11.00	12.80	9.00	24.60	246.00

Rheological Properties – Farinograph Results

Table (3). showed the values of farinograph parameters for studied cereal flour indicating that all the addition treatments either the type of cereal flour or cross linking enzymes affect positively or negatively on the farinograph parameters .Water absorption was reduced in composite flours, particularly sorghum blends. TGase shortened dough development time (DT) in durum and barley blends, suggesting accelerated network formation. GOase effects on DT

were inconsistent, indicating specific interactions with protein types (Abdulrahman *et al.,* 2025; Fatah *et al.,* 2025; Hamasalih *et al.,* 2025; Mohammed et al., 2020). TGase and GOase both improved dough stability and reduced the Mixing Tolerance Index (MTI), especially in barley and oat composites.

Enzymatic Specificity and Protein Selectivity

As mentioned, Enzyme Specificity is the property of an enzyme that determines whether it can act on a substrate and catalyze a specific reaction. This property is essential for enzyme activity, and it is dependent on the enzyme's active site structure (2011; Ceresino *et al.,* 2020; Kuktaite *et al.,* 2004 ; Malik *et al.,* 2011). Enzyme binding/cross-linking to cereal proteins was selective in terms of maintaining cross-linking capabilities. TGase preferentially used durum and barley proteins, while GOase preferentially used oat proteins. Such selectivity was reflected by enhancements in dough development time and stability.

4. CONCLUSION

TGase and GOase have opposite effects on cereal dough composition but synergistically strengthen the dough, making it more stable and resistant to mixing stress. TGase and GOase greatly enhance the stability of the dough and the resistance of the dough to mixing stress. Combined, these provide the best catering to dough, which makes it perfect for baking use such as in industry. TGase biosynthetic protein network formations and thermal stability, and GOase promotes dough strength through oxidative cross-linking. The selection of the best enzyme type as well as dosage of that enzyme should be based on flour composition and desired functionality. The knowledge of enzyme-protein interactions in different cereals assists in developing bakeries and functional foods modified as per the needs.

According to the above-mentioned mechanism, TGase promotes gluten network formation by crosslinking while GOase enhances the oxidative status of gluten, thus their complementary actions are exhibited with synergistic functions in baked goods quality. Optimizing enzyme concentrations is a deliberate acclimatization that can maximize these advantages without adversely affecting dough properties.

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