

Enzymatic Improvers as Natural Alternatives to Chemical Additives in Bread-Making

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ABSTRACT

Wheat flour used in breadmaking must have certain qualities, including gluten strength, viscosity, and other properties. Therefore, bakers often add various chemicals to improve the properties of dough and bread. Therefore, the aim of this review is to highlight the most important enzyme improvers as additives during bread making, which are considered generally recognized as safe (GRAS) and natural alternative to chemical improvers. The mixture of α -amylase and glucose oxidase improves dough elasticity and increases bread volume. Adding commercial enzyme blends that feature α -amylase and lipase activity to bread samples produced using the straight dough method improves the bread's shelf life and results in a more thermally stable amylose-lipid complex. The transglutaminase-catalyzed glutamine-lysine crosslink leads to the formation of high molecular weight polymers, altering the functional characteristics of proteins and enhancing the rheological and textural qualities of bread. Xylanase converts hemicellulose that is not soluble in water into a soluble form, allowing it to retain moisture in the dough, which results in larger bread volume, enhanced crumb texture, reduced dough firmness, and minimized stickiness. Lipases improve the dough stability and the gluten structure, which improves the properties of dough management and processing by machines. In pan bread, the enhanced dough flow properties from protein hydrolysis may aid in filling the loaf pans, thereby improving the shape of the loaves.

Keywords: enzymes improvers, bread volume, amylases, cross-linking, oxidizing enzymes.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an essential ingredient in numerous food items. This cereal is characterized by its gluten content, which creates a viscoelastic structure that can hold gases generated during the fermentation of dough. This trait greatly affects the form and structure of fermented bread. As a result, this cereal's wheat flour serves as the foundation for various bakery goods (Cauvain, 2012). Wheat flours characterized by high gluten quality are predominantly utilized in breadmaking since they can hold the gases generated during the fermentation of the bread dough (Ortolan et al., 2017). The difference in the quality of wheat flour utilized in bread production arises from the natural discrepancies in the quality of the wheat grains that produce the flour. Consequently, the industry employs wheat flour enhancement agents to create a product that aligns more closely with consumer expectations.

The proteins that form gluten are key to the viscous and elastic of the dough, so agents affecting this fraction significantly influence and determine its properties. These substances can influence the gluten proteins and starch found in the flour. They can be divided into primary categories: (a) additives, including oxidants, emulsifiers, and hydrocolloids; (b) processing aids, like enzymes; and (c) ingredients, such as vital gluten (Gioia

et al., 2017). The main role of these additives, processing aids, and ingredients is to homogenize the quality of wheat flour and enhance its technological properties. The bakeries can incorporate these listed enhancers into wheat flour to adjust its rheological properties (Ferreira, et al., 2025).

Historically, a diverse assortment of improvers has been utilized in the baking sector to address different deficiencies of raw ingredients, ensure uniform quality, and extend shelf-life. Improvers might be required for acceptable quality in products (Tebben et al., 2018). The addition of additives in food goods, such as baked products, is regulated by the Food and Drug Administration (FDA) in the United States. The FDA sets defined limits on the amount of each additive allowed in a product and offers directions for labeling products that include additives. Producers of bread might choose to exclude an ingredient despite its approval in certain nations (like potassium bromate being allowed in the United States) but prohibited elsewhere, if they predict negative consumer views of their product because of that ingredient (Shanmugavel et al., 2020).

Enzymes have emerged as a preferred option for alternative enhancements (Shelke, 2020), yet there is limited understanding of consumer perceptions regarding their application. In the United States, certain enzyme preparations have been deemed Generally

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Recognized as Safe (GRAS), while others are identified as food additives, and a specific fungal α -amylase utilized in flour is even included in a food standard. Enzymes are frequently utilized alongside other enhancers like vital wheat gluten or ascorbic acid (Shelke, 2020; Tebben et al., 2020). The chemical analysis of enzyme improvers can differ, comprising amylases, proteinases, lipases, hemicellulases, among others. These enzymes are categorized as "processing aids" instead of food additives, signifying that their main role is to alter the characteristics of dough during baking (Lensky, et al., 2024). Enzyme improvers can be divided into two groups depending on their source: natural and synthetic. Chemical enzyme improvers are artificially created compounds that influence particular characteristics of dough, including oxidation, reduction, or emulsification. They can influence the composition of flour proteins, enhance the size and texture of products, and extend freshness and longevity. Natural enzyme improvers come from plant or animal origins and can include minerals, antioxidants, prebiotics, probiotics, and various other advantageous elements. These elements aid in enhancing dough rheology, boosting the fragrance and flavor of bread, elevating its nutritional content, and promoting microbiological stability (Lensky, et al., 2024).

As bread evolved into a primary staple in Western Nations and other areas, individuals started looking for nutritional benefits that extended beyond mere calories. This change in viewpoint highlights the important function these products serve in not only providing energy but also offering essential nutrients and reducing calorie intake (Dai, and Tyl, 2021). The demand for a new range of healthier food items is increasing, including those that are low in calories, high in fiber, and abundant in antioxidants. In addition to that, they should also exhibit outstanding sensory quality. Thus, a typical approach includes the use of additives such as oxidants, enzymes, emulsifiers, and hydrocolloids. These additives fulfill different roles, improving the bread-making process, assisting in ingredient handling, and offsetting variations in raw materials, guaranteeing uniform quality, and maintaining freshness along with other beneficial food characteristics (Vinay, et al., 2025). The main objective of this review is to explain the role of enzyme improvers that are often added to wheat flour to improve the quality of bread and extend its shelf life.

Enzymatic improvers used in bread-making

The application of enzymes in commercial uses has grown in recent years as consumers seek bakery items with more natural-sounding components. Different kinds of enzymes can serve as substitutes for chemical enhancing agents, including certain hydrocolloids and

emulsifiers, and those utilized in baking can all be referred to with the single term "enzymes," a term that many consumers view as natural and clean label in contrast to additives identified by their chemical names. The use of enzymes has grown in recent times due to a rise in naturally-derived ingredients. Wheat enzymes are mainly located in the germ and aleurone layers. Enzymes serve as substitutes for chemical agents like hydrocolloids, emulsifiers, and others. Certain enzymes naturally occur in wheat flour, while others are added. Enzymes enhance the volume of the loaf and boost the properties of the dough. The elements influencing enzyme activity include temperature, pH, water activity, and enzyme concentration (Gioia et al., 2017).

Employing enzymes that enhance the gluten structure presents a compelling option due to regulatory limitations on chemical additives and the market movement towards more natural components and "clean label" offerings. Essential enzymes that improve gluten characteristics comprise glucose oxidase, transglutaminase, and pyranose oxidase (Dai and Tyl, 2021). Numerous enzymes are found naturally in flour, but additional enzymes are incorporated, particularly for their positive impact on dough and bread properties. Effects involve enhanced dough manipulation and moisture, better volume and/or crumb consistency, slower staling process, or superior nutritional properties.

1. Amylolytic enzymes

Amylases derived from different origins (bacterial, fungal, or malt) are utilized to accomplish two primary outcomes: producing sugars that yeast can ferment and minimizing starch retrogradation. Certain enzymes that break down large biopolymers like amylose and amylopectin favor internal connections (endoacting enzymes), whereas others mainly target bonds located at or near the ends of a chain (exoacting enzymes) (Goesaert et al., 2009). Amylase enzymes are categorized as processing aids by the European Union since they do not have a functional impact on the end product. The application of these enzymes in baked products is governed by Regulation (EC) No. 1332/2008 and Regulation (EC) No. 1169/2011.

• α -amylase

α -amylases (EC 3.2.1.1) hydrolyze α -1,4-glycosidic linkages of amylose and amylopectin in a random endoacting manner, and their application can enhance gas production and loaf volume in bread (Goesaert et al., 2009; Tebben et al., 2018). Moreover, the produced dextrins do not retrograde and may even postpone retrogradation (Wang et al., 2015). Like endo-acting amylases, exoacting amylases can supply sugars for yeast and enhance loaf volume. Moreover, maltogenic α -amylases are especially efficient in combating bread staling caused by starch retrogradation, as noted by

Goesaert et al. (2009), who analyzed the impacts of two endo- and one exo-acting (maltogenic) α -amylases. Table (1) demonstrated that the effect of a mixture of α -amylase with non-starch polysaccharide-degrading enzymes on bread quality properties.

The small molecular weight products from starch hydrolysis cannot undergo retrogradation, and these diminished saccharides also hinder the retrogradation of gelatinized starch (Matsushita et al., 2017). Additionally, these saccharides disrupt starch-protein interactions in the stale bread, which reduces firming. α -amylase maintains its activity at the beginning of baking and can break down gelatinized starch, and this partially degraded starch has a slow rate of retrogradation.

The researchers investigated utilizing malt flour in bread instead of incorporating purified α -amylase. Malt flour is frequently utilized as an enzyme additive due to its high α -amylase content, along with maltose, minerals, proteins, and flavoring agents. These elements change the color, taste, and moisture preservation of the bread (Boz, et al., 2010). Evaluation by a trained panel utilizing semi-structured scales established that the enzyme enhanced the elasticity and "eatability" rating of wheat bread, along with improving the crumb texture, flavor, and general acceptance of both whole wheat and white breads.

Table 1. A compilation of previous research results on the effect of α -amylase with non-starch polysaccharide-degrading enzymes on bread quality characteristics

Flour type	Improving enzymes	Concentrations	Effect on bread quality	References
Wheat flour with resistant starch	α -amylase	(0-8 mg/100 g flour)	Decreased resistance to extension	Altuna et al., (2015)
Wheat flour with cassava flour	α -amylase and xylanase	(2500 U/g; 10 ppm) and (235 U/g; 40 ppm)	Increased loaf volume	Serventi et al. (2016)
Wheat flour With whole barley flour	α -amylase and xylanase	(5000 U/g; 10 ppm) and (2500 U/g; 80 ppm)	Increased loaf volume, decreased hardness and staling	O'Shea et al. (2016)
Whole wheat flour	α -amylase and hemicellulase	(1500 U/g; 1000-2000 ppm) and (14,000 U/g; 1000-2000 ppm)	Increased gas retention, loaf volume, decreased hardness and staling	Matsushita et al. (2017)
Wheat flour with wheat bran	α -amylase, cellulase, and xylanase	(2500 U/g; 2-10 ppm), (700 U/g; 10-60 ppm) and (500 U/g, 20-120 ppm)	Decreased development time and resistance to extension; increased stability and softening	Liu et al. (2017)
Refrigerated dough	α -amylase and xylanase	(10,000 U/g; 5-15 ppm) and (12,500 U/g; 5-15 ppm)	Increased loaf volume, decreased hardness	Tao et al. (2018)
Whole wheat bread	α -amylase and hemicellulase	(1500 U/g; 1000-2000 ppm) and (14,000 U/g; 1000-2000 ppm)	Increased gas retention; decreased moisture and decreased staling	Matsushita et al. (2019)
Fresh wheat flour	α -amylase and xylanase	(748 U; 20 & 100 ppm) and (3.5 U; 20 & 100 ppm)	Increased loaf volume, decreased hardness and staling	Kim and Yoo (2020)

- **β -amylase**

β -Amylase (EC 3.2.1.2) is an exoenzyme that hydrolyzes the disaccharide maltose from the non-reducing end of amylose and amylopectin, and is a crucial enzyme in maltose production, which yeast uses during fermentation. This enzyme exists in mature grain in two types, free and bound. β -amylase is the exoenzyme that decomposes dextrin into maltoses; subsequently, maltase from yeast sources converts maltose into glucose. α - and β -Amylase exclusively hydrolyzes the α -1,4-glycosidic bonds in starch. α -amylase targets the α -1,4 linkage randomly, while β -amylase focuses on the α -1,4 linkage at the nonreducing sugar end. Consequently, merely 50–60% of the amylopectin transforms into maltose because of insufficient hydrolysis. Due to its slightly branched structure, the highest level of hydrolysis in this case is between 75–90% (Miguel et al., 2013). Amylose plays an essential role in bread production, as the specific volume of gluten-free loaves is reliant on the rising amounts of amylose present in rice bread (Aoki et al., 2020). Increasing the amount of β -Amylase makes the dough unworkable due to its stickiness (Gioia et al., 2017).

- **Amyloglucosidase**

Amyloglucosidase (EC 3.2.1.3), commonly referred to as glucoamylase, facilitates the liberation of glucose units from the non-reducing terminals of oligo- and polysaccharides like starch, functioning on both α -1,4 and α -1,6 glycosidic connections. (Cauvain, 2012). Nonetheless, amyloglucosidase has a milder impact on fermentable sugar concentrations compared to α -amylase (Struyf et al., 2017) but can shorten baking duration and improve bread crumb color, because the produced glucose participates in the Maillard reaction. Glucoamylases come from the fungus *Aspergillus oryzae* and can target α -1,6 glycosidic bonds to generate a reducing sugar. Amylase derived from fungal source shows reduced temperature stability in comparison to the thermostable amylase from *Bacillus subtilis*. Bacterial amylase demonstrates residual activity in the bread even following the baking process. To tackle this issue, an α -amylase exhibiting moderate thermostability is utilized as a potent antistaling agent in baked products (Chandrasekaran, 2016). Amyloglucosidase operates effectively in wheat bread recipes, enhancing sweetness by boosting sugar levels and elevating yeast performance. Frequently, sweeteners like honey, molasses, corn syrup, and brown sugar are incorporated into the recipe, yet amyloglucosidase generates additional glucose in the dough, facilitating fermentation and enhancing crust color through Maillard browning. Based on the level of usage,

amyloglucosidase either lowered or raised the resistance to extension of whole wheat dough. The alteration became noticeable only after the dough rested for 135 minutes, indicating that the enzyme needs a prolonged duration to take effect (Altinel & Unal, 2017). Similar to the control, the resistance grew with the duration of rest. The addition of amyloglucosidase may postpone the hydration of the dough, as the enzyme-catalyzed reaction competes with flour for available water. This delay was demonstrated by the higher resistance of the dough. Amyloglucosidase also reduced moisture loss during the baking of whole wheat bread, viewed as a beneficial trait, but it led to a decrease in specific loaf volume (Altinel & Unal, 2017).

From the above, it can be concluded that the importance of adding amylase enzymes to bread production can be recommended, as it leads to the release of simple sugars such as maltose and glucose, which are of great importance as food for yeast during the dough fermentation stage, resulting in a larger volume in the final product. Also amylase enzymes are important in producing the desired brown color in bread through the Maillard reaction between monosaccharides and amino acids. Amylase enzymes also help increase freshness, extend shelf life, and reduce crumb staling by forming dextrans that are incapable of retrogradation and do not cause staling of the bread. One of the strengths of previous researches is the use of a mixture of amylase with xylanase or hemicellulase in almost all studies to achieve high bread quality, such as increased volume and softness, reduced staling rate, and thus extended shelf life.

2. Enzymatic improvers with a cross-linking influence

- **Glucose Oxidase (GOX)**

Glucose oxidase (EC 1.1.3.4) operates as an oxidoreductase, serving as a substitute for oxidizing agents, a highly efficient rapid oxidizing enzyme. The enzyme is primarily generated from *Aspergillus* species. The enzyme facilitates the conversion of β -D-glucose into gluconic-D-lactone when oxygen is available, as illustrated by Figure (1). Then, it changes into gluconic acid and hydrogen peroxide. Hydrogen Peroxide acts as a substitute for calcium peroxide and oxidises the gluten sulfhydryl group to disulfide bonds indirectly (Bock, 2015). It demonstrates a dehydrating impact on bread dough. GOX minimizes resistance in whole wheat dough, akin to white dough. Glucose oxidase functions as a chemical oxidant: it decreases the extensibility of dough, enhances the resistance of dough, and boosts the volume of bread. GOX functions through the oxidation of the water-soluble sulfhydryl group and the oxidative gelation of pentosane (El-Rashidy et al., 2015). Glucose oxidase is being utilized more frequently in lieu of

chemical oxidants like ascorbic acid and azodicarbonamide in the baking sector, preferred for its quick effect that improves dough hydration and produces a non-stick surface. GOX facilitates the oxidation of β -D-glucopyranose in an oxygen-containing environment, yielding hydrogen peroxide (H_2O_2) and D-glucose- δ -lactone. The latter undergoes spontaneous hydrolysis into D-glycolic acid when in contact with water. The positive impacts of GOX on dough characteristics mainly stem from the hydrogen peroxide generated, which oxidizes the free sulfhydryl (-SH) groups in gluten proteins to create disulfide bonds (-S-S-), thus enhancing the gluten network (Renzetti & Rosell, 2016; Tang et al., 2014).

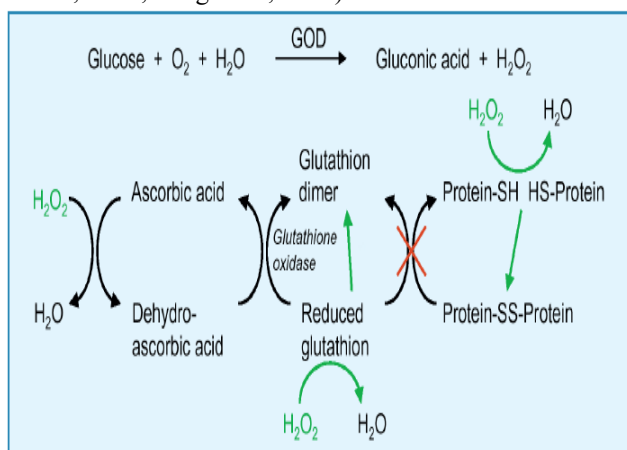


Fig.1. Reactions carried out by glucose oxidase to strengthen bread dough as an alternative to chemical oxidants.

Source: Popper, et al., (2006)

Research has shown that GOX usage decreases dough extensibility (Amiri et al., 2016), boosts elasticity (Meerts et al., 2017), and enhances stability and handling characteristics in bread production (Liu et al., 2018). These enhancements are linked to a rise in glutenin macropolymers, resulting in lower free sulfhydryl levels and greater dough strength. As a result, bread produced with GOX shows increased volume and decreased hardness (Tang et al., 2014).

• Transglutaminase (TG)

Transglutaminase (EC 2.2.13) is part of a group of transferase enzymes. An enzyme found outside the cell that is generated by *Streptomyces mobaraensis*. A transglutaminase (TGase) as shown in Figure (2), is a glutamine g-glutamyltransferase enzyme that facilitates an acyl transfer reaction involving a γ -carboxamide of protein-bound glutamine or peptide by forming a covalent cross-link between amines, peptides, proteins, and other deaminated derivatives of glutamine (Vinay, et al., 2025). The glutamine-lysine crosslink catalyzed by TGase results in the production of high molecular

weight polymers, which changes the functional properties of proteins and improves the rheological and textural attributes of food items. Transglutaminase is an essential enzyme utilized to enhance gluten quality in conjunction with glucose oxidase. TGase promotes the development of covalent cross-links by catalyzing interactions between the ϵ -amino groups of lysine and the γ -carboxamide groups of glutamine in gluten proteins (Aja et al., 2007; Renzetti and Rosell, 2016). This enzyme mainly focuses on HMW-GS, modifying the protein network composition and consequently improving dough characteristics (Dai and Tyl, 2021).

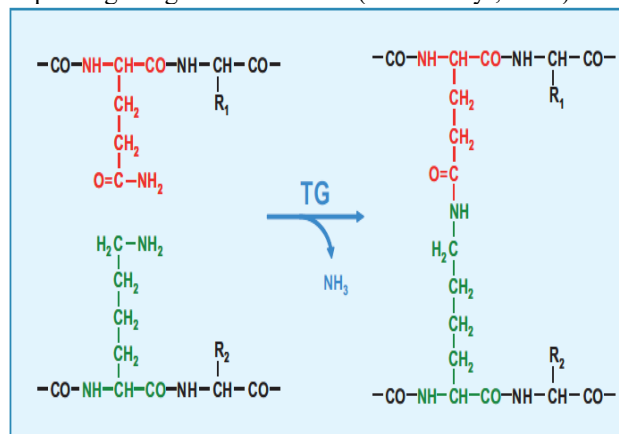


Fig.2. Cross-linking reaction of flour protein molecules by transglutaminase.

Source: Popper, et al., (2006)

The effect of TGase on dough depends on its level and the quality of the employed wheat flour. Research indicates that doughs containing added TG demonstrate decreased extensibility (Bardini et al., 2018) along with prolonged development times, stability, and resistance (Meerts et al., 2017; Niu et al., 2018). Additionally, TG-infused bread exhibits enhanced water retention, along with advancements in loaf volume, structure, and texture (Boukid et al., 2018). Table (2): showed that the effect of GOX and TGase on bread quality.

Conflicting findings exist regarding the impact of transglutaminase on bread staling and loaf volume, and the outcomes likely vary based on the amount used and the type of flour (Basman et al., 2002). The inclusion of transglutaminase in gluten-free rice bread was observed to enhance specific loaf volume and reduce staling (Renzetti and Rosell, 2016), while Collar et al. (2005) reported that it improved sensory evaluations for chewiness and softness in refined or whole wheat breads, in comparison to those without enzyme supplementation; however, excessive use resulted in reduced loaf volume. Despite the hypothesis that transglutaminase could contribute to celiac disease (Lerner & Matthias, 2015), it continues to be widely used in food applications (Mostafa, 2020), though the

seriousness of possible outcomes necessitates additional investigation. Typically used transglutaminase levels in baked goods were demonstrated not to elevate the amount of immunotoxic deamidated gliadins (Scherf et al., 2018). The extra addition of peptidase might be a practical method to break down gluten fragments that transglutaminase could otherwise link (Scherf et al., 2018).

• Laccase (LAC)

Laccase (EC 1.10.3.2) is an enzyme with copper ions that facilitates the oxidation of diverse phenolic compounds by withdrawing one electron, which produces reactive phenolic radicals. Its substrates consist of tyrosine residues in proteins and ferulic acid esters associated with arabinoxylan. LAC promotes the oxidation of tyrosine and peptides containing tyrosine, resulting in the polymerization of these substances. Additionally, LAC has the capacity to transfer phenoxyl radicals to SH groups, resulting in the formation of SH radicals and potentially functioning through SH/SS

exchanges. Laccase oxidizes phenolic substances, including ferulic acid and (more slowly) tyrosine, through a radical-mediated mechanism (Selinheimo et al., 2006), thereby facilitating the cross-linking of polymers in wheat flour, especially arabinoxylans (Labat et al., 2000). Thiols like glutathione or cysteine can reduce phenoxy radicals, resulting in the formation of disulfide bonds (Selinheimo et al., 2007).

Like GOX and transglutaminase, laccase has been noted to enhance the resistance of bread dough to stretching and reduce its extensibility (Selinheimo et al., 2006). Nonetheless, Primo-Martin et al. (2003) discovered that laccase reduced the elasticity of bread wheat dough. The diverse effects caused by laccase could be linked to the different dosages and types of enzymes utilized in these research studies. Laccase has been shown to enhance loaf volume and crumb tenderness in bread produced from refined wheat flour (Selinheimo et al., 2007). LAC treatment typically leads to greater dough firmness and reduced extensibility.

Table 2. A compilation of some previous research results on the effect of Glucose oxidase and transglutaminase on bread quality

Flour type	Improving enzymes	Concentrations	Effect on bread quality	References
Wheat flour	Glucose oxidase	(40 U/100 g flour)	Increased elasticity & specific volume and decreased hardness	Tang et al., (2014)
Wheat flour with resistant starch	Glucose oxidase and Transglutaminase	(0-5 mg/100 g flour) and (0-1 mg/100 g flour)	No significant changes	Altuna et al., (2015)
Wheat gluten	Glucose oxidase	(20, 40, 80, 120 and 160 ppm)	Increased gluten yield and maximum resistance to extension	Amiri et al., (2016)
Strong and weak wheat flour	Glucose oxidase and Transglutaminase	(0.03, 0.6, 1.8 and 3.6 U/g flour) and (0.5, 2, 5 10 U/g flour)	Increased elasticity and strain-hardening behavior of dough	Meerts et al., (2017)
Whole wheat flour	Glucose oxidase	(40 and 80 µg/g flour)	Increased glutenin content acropolymer (GMP)	Liu et al., (2018)
Whole wheat flour	Glucose oxidase and Transglutaminase	(0.5, 1.5, 3.0 and 6.0 U/g flour)	Increased water availability for gluten, stability, and resistance to mixing	Niu et al., (2018)
Weak, medium and strong wheat flour	Transglutaminase	(0.05, 0.1 and 0.2 g/100 g flour)	Increased specific volume, color, texture and water activity	Pizzigalli et al., (2018)
Wheat flour	Transglutaminase	0, 0.05, 0.1 and 0.2% flour	Increased dough resistance and decreased dough extensibility	Bardini et al., (2018)

The strength of dough treated with LAC diminishes as time passes, likely because of the depolymerization of the arabinoxylan network catalyzed by radicals generated by LAC. The thermal inactivation of LAC following the gel formation of water-extractable arabinoxylan halts free radical generation and stabilizes gel firmness, resulting in bread with enhanced specific volume (Vinay, et al., 2025).

The impact of LAC mainly comes from the crosslinking of ferulic acid residues that are esterified to the arabinoxylan part of the dough, leading to a strong arabinoxylan network. The variation in firming between flour-based doughs and those made from hydrated gluten indicates that arabinoxylan, present in greater quantities in flour dough compared to gluten dough, is the favored substrate for LAC. Nonetheless, although LAC mainly functions as an oxidoreductase on arabinoxylans, it may also influence gluten quality (Vinay, et al., 2025).

It can be concluded that oxidizing enzymes can be used instead of oxidizing chemicals, which are often prohibited from being added to bread due to the health risks they can cause to the human body. Oxidizing enzymes decrease the extensibility of dough, improve the resistance of dough, and increase the pan bread volume.

3. Non-starch polysaccharides degrading enzymes

• Hemicellulases

The non-starch polysaccharides found in the cell walls of bran and germ contribute to the low breadmaking quality of whole wheat flour (Autio, 2006). While dough is mixed, arabinoxylans vie with gluten for water (Li et al., 2012). Hemicellulases are a wide range of hydrolytic enzymes that break down hemicellulose, including arabinoxylan, xylan, xylobiose, and arabinogalactan, among others. Arabinoxylan is notably crucial in bread production, making it the central focus for enzymatic activity. Xylanase, also known as endo-1,4- β -xylanase (4- β -D-xylan xylanohydrolase), is a commonly utilized enzyme among hemicellulases that breaks down 1,4- β -D-xylosidic bonds in xylan and arabinoxylan (Dhiman and Mukherjee, 2018).

Hemicellulases are enzymes that promote the hydrolysis of polysaccharides that are not starch. Among these enzymes, endoxylanases are the most frequently utilized in bread production. Hemicellulases like xylanase are recognized for enhancing the characteristics of refined wheat bread, offering advantages such as dough softening, increased loaf volume, improved crumb structure, and reduced staling rate (Jiang et al., 2005). The impact of hemicellulases is

particularly significant for whole wheat bread, which contains more insoluble arabinoxylans compared to refined wheat bread.

Hemicellulase activity enhanced gas retention capacity (Matsushita et al., 2017). Hemicellulases (mainly endoxylanase) reduced the resistance to stretching of whole wheat dough. It made the dough more pliable, with lower enzyme levels causing notable alterations only after 135 min of rest, while higher enzyme levels showed results more quickly (45 min) (Altinel & Unal, 2017). In white bread replaced with whole wheat flour, hemicellulases (like xylanase) enhanced loaf specific volume and reduced bread staling, likely through the breakdown of arabinoxylan. The hydrolysis of insoluble arabinoxylans produces smaller polysaccharides that disrupt starch-protein interactions, consequently preventing staling (Matsushita et al., 2017)

• Xylanase

Xylanases break down the xylan backbone of water-unextractable arabinoxylan, decreasing their molecular size and water-holding ability. This enables improved gluten hydration, leading to enhanced gluten matrix formation and breadmaking capacity. Xylanase transforms water-insoluble hemicellulose into a water-soluble state that retains moisture in the dough, leading to increased bread volume, improved crumb consistency, decreased dough firmness, and lowered dough stickiness (Butt et al., 2008). Using xylanase alongside other enzymes produces better results than xylanase by itself.

Xylanase application impacts the secondary protein structures in bread dough and enhances the ratio of β -turns (Jiang et al., 2018), suggesting a more hydrated gluten network. A favorable correlation was shown between the volume of bread loaves and β -turn content, as evidenced in refined winter wheat (Cao et al., 2017). Jiang et al. (2018) also reported a rise in glutenin macropolymer. Glutenin macropolymer, a crucial element of gluten, is a highly polymerized glutenin structure that affects the dough's rheological characteristics and the bread's loaf volume. Xylanase is said to liberate free water, reducing the quantity of water needed for the dough. On the other hand, a research on whole wheat dough indicated that a mixture of hemicellulases, primarily endoxylanase, did not result in any notable alteration in the farinographic characteristics of whole wheat dough (Altinel & Unal, 2017). Although xylanases improve the extensibility of doughs, a common disadvantage noted is the rise in stickiness (Matsushita et al., 2019), occurring due to the hydrolysis of water-unextractable arabinoxylans (WU-AX), which reduces their ability to retain water.

Moreover, hydrolysis of WEAX must be prevented, as they might beneficially serve to improve the stability of foams (Courtin & Delcour, 2002).

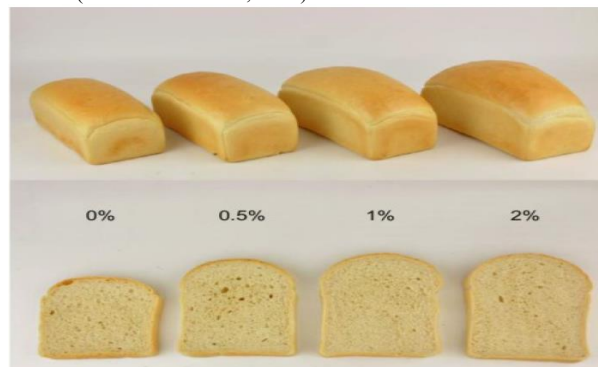


Fig.3. Loaves of bread containing increasing dosages of a pure enzyme improver (amylase and xylanase), added as percentages of flour weight.

Source: Scanlon, et al., (2018)

Many studies have shown that adding xylanase leads to greater loaf volume as shown in Figure (3) and Table (3) (Altinel & Unal, 2017; Driss et al., 2013; Ghoshal et al., 2013; Jaekel, et al., 2012; Kumar & Satyanarayana, 2014). Incorporating xylanase reduces the flour's water uptake, resulting in improved gluten hydration and network development, thus increasing dough rise during fermentation (Ghoshal et al., 2013; Jaekel et al., 2012; Shah et al., 2006). Kumar and Satyanarayana (2014) found increased levels of reducing sugars and soluble proteins in bread made with xylanase. Additionally, the movement of water from pentoses to gluten may cause a reorganization of the gluten network during dough fermentation, facilitating increased rise and larger bread volume (Ghoshal et al., 2013).

Table 3. Impact of xylanase in combination of cross-linking enzymes on wheat dough and bread properties

Flour type	Improving enzymes	Concentrations	Effect on dough and bread quality	References
Wheat flour	Xylanase and laccase	(2500 U/g; 40 ppm) and (1552 U/g; 500 ppm)	Increased dough extensibility, gluten index and decreased elasticity	Primo-Martin et al. (2005)
Wheat flour	Xylanase and laccase	(7954 U/g; 122 ppm) and (15,000 U/g, 12.2-24.4 ppm)	Increased loaf volume, crumb softness and decreased hardness	Selinheimo et al. (2006, 2007)
Wheat flour	Xylanase and transglutaminase	(2500 U/g; 60 ppm) and (100 U/g, 5000 ppm)	Increased loaf volume and decreased hardness	Caballero et al. (2007)
Wheat flour	Hemicellulase and glucose oxidase	(6000 U/g; 50 ppm) and (10,000 U/g; 2-6 ppm)	Increased water absorption; dough elasticity and loaf volume and decreased extensibility	Dagdelen and Gocmen (2007)
Wheat flour with soybean flour	Xylanase and transglutaminase	(2500 U/g; 5-251 ppm), and (100 U/g; 500-3850 ppm)	Increased dough stickiness; extensibility and loaf volume and decreased elasticity	Ribotta et al. (2010)
Wheat flour with millet flour	Xylanase and transglutaminase	(150 ppm)	Increased loaf volume and firmness; and more uniform crumb pores	Schoenlechner et al. (2013)
Wheat flour with resistant starch	Xylanase, glucose oxidase and transglutaminase	(60,000 U/g; 0-10 ppm), (10,000 U/g; 0-50 ppm), and (100 U/g; 0-80 ppm)	Increased gas cell stabilization and stickiness of dough; decreased elasticity and increased loaf volume, darker crust	Altuna et al. (2015)
Wheat flour	Xylanase and glucose oxidase	(150 ppm) and (150 ppm)	Decreased dough elasticity / extensibility ratio	Konieczny et al. (2020)
Whole wheat flour	Xylanase and glucose oxidase	(2500 U/g; 800 ppm) and (10,000U/g; 40, 80 ppm)	Increased dough elasticity and viscosity, decreased elasticity and viscosity	Yang et al. (2021)

Jaekel et al. (2012) noted that loaf volume increased with rising xylanase dosage from 0 to 8 g/100 kg flour, followed by a drop at 12 g/100 kg flour. Enhancement in loaf volume may additionally stem from hydrolyzed (lower molecular weight) hemicellulose, which is less capable of disrupting gluten network development (Matsushita et al., 2017). Altinel and Unal (2017) proposed that the increase in volume resulted from the transformation of water-unextractable arabinoxylan into water-extractable arabinoxylan, enhancing the gas retention capability in the dough.

It can be noted that many previous studies have examined the effect of cellulose-degrading enzymes on bread quality characteristics. It was concluded that the most commonly used enzymes are xylanase and hemicellulase, usually in combination with other enzymes. These studies found that incorporating xylanase reduces the flour's water uptake, resulting in improved gluten hydration and network development, thus increasing dough volume during fermentation. Hemicellulases enhanced loaf specific volume in whole wheat flour, and reduced bread staling, likely through the breakdown of arabinoxylan.

4. Lipolytic enzymes (lipases)

Lipases, (EC 3.1.1.3) known as triacylglycerol acyl hydrolases, catalyze the hydrolysis of triacylglycerol, resulting in the formation of monoacylglycerol (MAG), diacylglycerol (DAG), glycerol, and free fatty acids as shown in Figure (4). Although cereal grains have lipases, the amount is minimal and does not cause the flour to become rancid. The use of lipases has emerged relatively recently when contrasted with the application of proteases and α -amylases (Melis et al., 2019; Giannone et al., 2016). Lipases improve dough stability and the gluten network, which enhances the qualities of dough handling and machine processing. Lipases additionally enhance loaf volume, firm the dough, and refine the crumb texture (Dahiya et al., 2020).

Lipases may be categorized as triacylglycerol lipases, phospholipases, and galactolipases, with their uses in breads and cakes examined and assessed by Gerits et al. (2014). Lipases produce surface-active mono- and diacylglycerols, thereby altering the endogenous lipid composition. It is widely recognized that nonpolar lipids reduce bread loaf volume, whereas polar lipids enhance it and act similarly to several frequently used surfactants (Melis and Delcour, 2020).

First-generation lipases were developed in 1990, and currently, third-generation lipases are in use. Third-generation lipases are more efficient in rapid dough mixing and instant dough preparation.

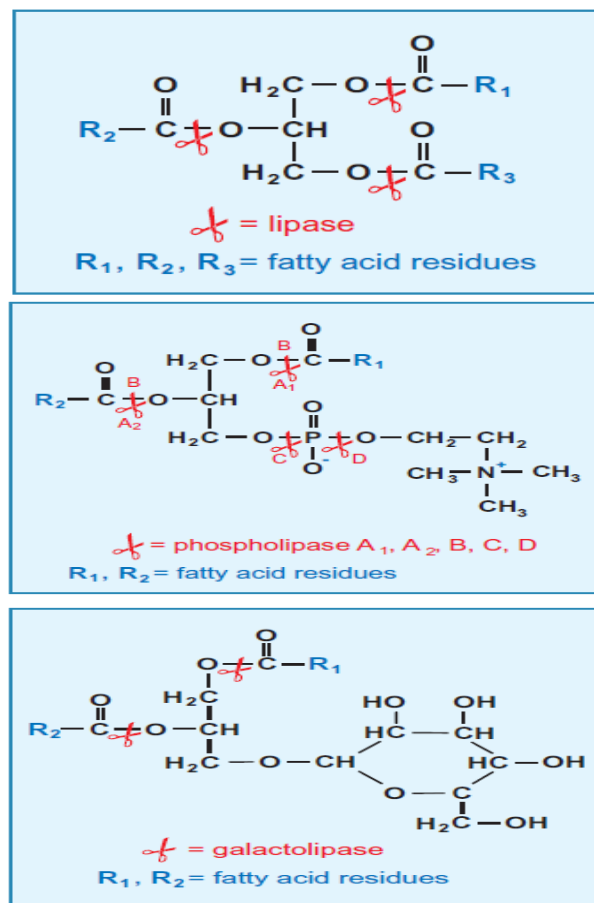


Fig. 4. Action of lipase on fats (triglycerides) molecules, action of phospholipases on lecithin (phosphatidyl choline) and action of galactoolipases on monogalactocyle diglyceride

Source: Popper, et al., (2006)

Additionally, they generate fewer short-chain free fatty acids, which decrease the likelihood of off-flavor development during extended storage of the baked products (Dura and Rosell, 2017). A portion of emulsifiers may be substituted with lipases, which is the reason lipases are utilized as antistaling agents (Bock, 2015)

Certain research indicates that the incorporation of lipase enhances the volume of bread (Gerits et al., 2014), attributed to greater levels of polar lipids, including phospholipids and galactolipids, compared to bread made without lipase (Melis et al., 2019). Melis and Delcour (2020) outlined the features of lipases relevant to effective bread baking and emphasized the significance of attaining a balanced lipid composition. The food industry can utilize various types of lipases, which are divided into three main categories: triacylglycerol lipases (the “true” lipases), phospholipases, and galactolipases (Gerits et al., 2014).

External lipases could enhance the function of internal lipases. The monoglycerides released by both types of lipases can associate gluten proteins and reduce their hydrophobicity, resulting in alterations in dough characteristics. The bonding of gluten by released monoglycerides could influence the relationships between gluten and starch. The formation of surface-active agents from the hydrolysis of both polar and nonpolar lipids has been proposed as the mechanism responsible for the functionality of lipase in dough (Colakoglu & Ozkaya, 2012). Phospholipases facilitate the breakdown of phospholipids. This enzyme is said to enhance the elasticity and extensibility of dough while boosting loaf volume. The addition of phospholipase along with hemicellulase enhanced the rheological properties of whole wheat dough by reducing both the resistance to extension and the resistance/extensibility ratio. A greater specific loaf volume was achieved, likely due to the enzymes enabling more expansion of the dough (Altinel & Unal, 2017).

It be concluded that lipase can be used in bread making, where it produces surface-active mono- and diacylglycerols, thereby altering the endogenous lipid composition. It also improves and extends the dough's stability and gluten network formation, which improves the quality and handling behavior of the dough during manufacturing. It also increases bread volume and softness of the bread crumb.

5. Proteolytic enzymes (Proteases)

Protease enzymes (EC 3.4.x) find commercial applications in the baking industry. Protease lowers the consistency of dough, shortens mixing duration, enhances dough uniformity, and positively affects the flavor and texture of the bread. As illustrated in Figure (5), proteases break down peptide bonds and decrease gluten elasticity, which minimizes dough or paste shrinkage following moulding and sheeting (Miguel et al., 2013). Strong wheat flours can experience advantages from shorter dough mixing times (Cauvain, 2012), achievable through the hydrolysis of gluten-forming proteins by proteases. This reduces resistance to dough stretchability and enhances the softness and elasticity of the bread crumb (Caballero et al., 2007). In pan bread, the improved dough flow characteristics resulting from protein hydrolysis might facilitate filling the loaf pans and consequently enhance the loaves' shape (Gioia et al., 2017). Malt proteases are primarily utilized in low-strength flours to break down gluten proteins and enhance dough softness. *Aspergillus oryzae* provides a fungal protease that is sensitive to heat, restricting its activity to the dough stage only. Bacterial proteases, mainly sourced from *Bacillus subtilis*, show significant activity and are solely used in the United States to reduce the strength of rusk dough. In laminated

dough (used for crackers), bacterial proteases assist in avoiding cracks, curling, and the formation of gas bubbles (Gioia et al., 2017).

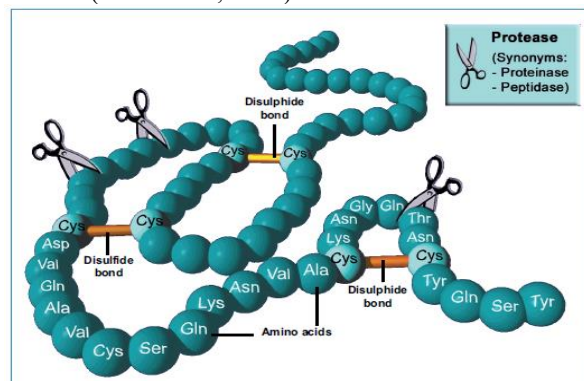


Fig.5. Illustration showing how proteolytic enzymes break down protein molecules.

Source: Popper, et al., (2006)

An enzyme generates an amino acid via proteolysis that interacts with reducing sugar at baking temperatures, resulting in the Maillard reaction, which creates the intended crumb color and flavor in bread. The application of 50 µg of protease from *Aspergillus oryzae* to rice flour led to bread with a high specific volume, attributed to dough swelling, increased gas retention, alterations in dough viscosity, and protein breakdown (Aoki et al., 2020). Thermoase from *Bacillus stearothermophilus* was employed to process dough at 25°C for 16 hr. Glutelins and prolamines are broken down by the thermase enzyme, leading to a product with a high volume, appealing crumb structure, and soft texture. Throughout the storage duration, the rate of staling and the hardness of the crumb in the bread were significantly reduced. Thorough protein hydrolysis should be avoided because it may cause structural collapse of the product and lead to excessive browning, as the released amino groups could engage in the Maillard reaction (Gioia et al., 2017).

It can be concluded that protease enzymes are only added to strong flours, which contain high protein content and a strong gluten network, in order to reduce the strength of the flour and then use it in the manufacture of products such as cakes, biscuits, and pastries. It reduces resistance to dough stretchability and improves the softness and elasticity of the bread crumb.

3. Health applications of enzymes used in bread making

Enzymes are advantageous in baking, and they also offer numerous health advantages to the human body. These enzymes assist in digestion and other essential bodily functions as follow:

- 1- Promotes gut health: Enzymes support the preservation of a healthy equilibrium of beneficial

bacteria in the gut, assisting in digestion and enhancing the immune response.

- 2- Elevated energy levels: Enzymes aid in digesting food more effectively, enabling the body to transform nutrients into energy more rapidly, thereby enhancing vitality and endurance.
- 3- Enhanced digestion: Enzymes such as amylase and protease assist in breaking down carbohydrates and proteins, enhancing effective digestion and nutrient uptake.
- 4- Decrease in inflammation: Some enzymes, including protease, possess anti-inflammatory characteristics that aid in decreasing swelling and enhancing recovery from injuries or long-term conditions.
- 5- Improved Nutrient Uptake: Digestive enzymes help the body to absorb vital nutrients such as amino acids, vitamins, and minerals more efficiently, promoting overall wellness.

CONCLUSIONS

Enzymes that break down (amylases, lipases, and peptidases) or cross-link (transglutaminase and some oxidoreductases) flour components are effectively applied in breads to enhance loaf volume, texture qualities, and shelf stability. Nonetheless, using just a single type of enzyme may not be adequate to attain a satisfactory outcome, whereas mixing various improvers could allow producers to more effectively alter the dough's rheology and improve the quality of baked goods like bread. Results from previous studies illustrated that the use of a combination of lipase and α -amylase, or amylase alongside xylanase, enhances product parameters that indicate bread staling. Synergistic impacts on dough rheology and bread volumes have been noted when xylanase is combined with oxidants or cross-linking agents. Nonetheless, the majority of this research so far has centered on wheat, leaving it to be investigated how a broader variety of flours would react to the application of these combinations. Recommendations for future research are additional effort is required to improve the forecasting of the correct mix of bread enhancers at ideal dosage amounts, particularly in unconventional product systems that use blends of wheat and alternative flours. Dough conditioner combinations typically focus on various flour components, yet further investigation is required to clarify why strong and weak wheat flours are frequently influenced in distinct ways, along with which flour molecular traits most significantly affect product results.

It is recommended that future studies optimize enzyme blends for mixed-grain or gluten-free bakery products.

Conflict of interest

The authors declare no conflict of interest.

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