

Enzyme Applications in Artisan Breads

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Basic enzymatic processes have been used in brewing, alcohol production, and baking since prehistoric times. Enzymes are active proteins found in all living things, including plants, animals, and microbes, that catalyze the biochemical reactions necessary for life. They work by temporarily binding to one or more specific elements of the reaction that they affect. In doing so, they lower the amount of activation energy needed to trigger the reaction and accelerate it. Unlike many chemical alternatives, enzymes are efficient, specific, and biodegradable (10). The use of enzymes can contribute greatly to sustainability and a reduced carbon footprint by reducing waste, energy, and off-specification products.

With the exception of some of the earliest studied enzymes, such as pepsin, rennin, and trypsin, most enzyme names end in "-ase." The International Union of Biochemistry and Molecular Biology (IUBMB) initiated standards of enzyme nomenclature that recommend that enzyme names indicate both the substrate acted upon and the type of reaction catalyzed. The Enzyme Commission number (EC number) is a numerical classification scheme used for enzymes based on the chemical reactions they catalyze:

- EC 1. Oxidoreductase: Oxidoreductases catalyze oxidation reduction reactions. At least one substrate is oxidized and at least one substrate is reduced.
- EC 2. Transferases: Transferases catalyze group transfer reactions—the transfer of a functional group from one molecule to another.
- EC 3. Hydrolases: In hydrolysis reactions, C-O, C-N, and C-S bonds are cleaved by addition of H₂O in the form of OH– and H+ to the atoms forming the bond.
- EC 4. Lyases: Lyases cleave C-C, C-O, C-N, and C-S bonds by means other than hydrolysis or oxidation.
- EC 5. Isomerases: Isomerases rearrange the existing atoms of a molecule, i.e., create isomers of the starting material.
- EC 6. Ligases: Ligases synthesize C-C, C-S, C-O, and C-N bonds in reactions coupled to the cleavage of high-energy phosphate bonds in ATP or other nucleotides.

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https://doi.org/10.1094/CFW-63-2-0067 © 2018 AACC International, Inc. Enzyme activity depends on pH, temperature, water activity, ionic strength, and the presence of different molecules in the medium. Differences in enzyme activity also occur depending on the origin of the enzyme preparation. For example, fungal, cereal, and bacterial amylases exhibit different pH and thermal stabilities. Fungal α -amylase is inactivated after 2–3 min at 65–75°C. Cereal α -amylases are slightly more thermostable and remain active during the early stages of starch gelatinization. Bacterial amylases have even higher thermal stability and may survive baking temperatures (1).

Enzyme Classes Used in Baking

Enzymes are commonly used in flour and dough to improve the quality of finished baked goods by altering the way flour behaves in mixing and the way dough behaves in forming, proofing, and baking. Hydrolyzing enzymes such as amylases, proteases, lipases, and cellulases, which require water to act on polymers (starch, protein, lipids, and fiber), are commonly used in the baking industry, with amylases and endo-xylanases by far the most widely used today (9). Typical effects that can be achieved with baking enzymes include improved dough fermentation, handling, and machinability properties; enhanced mixing tolerance and proofing stability; more intense crust color; increased loaf volume; improved crumb characteristics; and extended shelf life.

- Amylases modify damaged and gelatinized starch in flour, providing more substrate for the yeast, which results in better bread volume and crumb structure. Malto- and tetraogenic amylases create starch structures that slow staling due to starch retrogradation, thereby extending bread freshness (shelf life).
- Proteases hydrolyze the hydrogen bonds between peptides, reducing dough mixing time and improving pan flow.
- Lipases act on flour lipids (polar and nonpolar), improving the emulsifying effects of these lipids and providing increased dough strength and larger loaf volume.
- Arabinoxylanases (pentosanases) act on 5-C sugar-containing arabinoxylans, reducing their water absorption during dough mixing, which improves gluten development and results in better bread quality.
- Cellulases can help to increase water-soluble dietary fiber in whole grain flours and decrease the water-holding capacity of cellulosic material in bran. This provides more water for gluten development during dough mixing, resulting in improved loaf quality.
- Glucose and hexose oxidases are classified as oxidoreductases and are nonhydrolyzing enzymes. In the presence of

glucose (or hexose) and oxygen these enzymes generate hydrogen peroxide, which can play several different roles in dough formation, including

- 1) Oxidizing the sulfhydryl groups in peptides, forming disulfide bonds and providing increased dough strength (11).
- 2) Oxidizing glutathione from yeast, preventing or reducing its dough-weakening effect (13).
- Oxidizing the ferulic acid residues on the arabinose side chain (termed oxidative gelation) and improving the stability of dough during baking (5).
- 4) Forming tyrosine cross-links and strengthening dough (14).
- Transglutaminases (transferases) are used in gluten-free dough to create a better protein network to maintain the structure of the baked bread (12). The enzymes combine with a glutamine residue, releasing ammonia. The combination reacts with the amine group of a lysine residue of another protein, releasing the enzyme to react again, until it either has no more glutamine residues to cross-link with or is inactivated during baking.

Challenges Associated with Artisan Bread Baking

Consumers perceive "artisan-made foods" to be foods made in small batches and prepared with familiar ingredients found in their kitchens. They also perceive "artisan bread" to be bread made by a skilled baker using a lengthy process in which unique aromas, textures, and crumb structures are developed.

Artisan bread has an open cell structure, thick crust, intense flavor, and chewy texture, and no two loaves look exactly alike. Industrial bread producers are looking for formulation options that enable them to make artisan-style breads with the same attributes, but more consistently and faster to reduce labor and waste. Maintaining quality while increasing production is the main challenge to making artisan-style breads on an industrial scale. Enzymes, which are active proteins, can aid in the processing, dough stability, and end quality of artisan breads, whether



Fig. 1. Effects of fungal α -amylase, endo-xylanase, and glucose oxidase on baguette appearance (50 ppm ascorbic acid used in each loaf). (Photo: Novozymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished)

they are produced in the local bake shop, supermarkets, or higher volume bakeries.

Despite a faster rate of consumption than is experienced with packaged bread, bread staling remains a challenge for artisan bread. Bread staling is caused by the gradual transition of amorphous starch to a partially crystalline, retrograded state. The chemical and physical changes of staling in the bread crumb begin as soon as the loaf is removed from the oven. Increased firmness, dryness, and loss of product freshness are prevalent features of stale bread crumb, and all of these changes negatively affect consumer preference. The rate of bread staling depends on flour, formulation, processing, and storage conditions (6). Enzymes can be used in formulations to decrease the rate of staling and improve dough stability and baked bread appearance.

Use of Enzymes to Overcome Artisan Bread Production Challenges

There are several challenges encountered with artisan bread production. Enzymes can be used by bread makers to overcome numerous challenges:

- Small deviations in the quality of raw materials result in inconsistencies in dough and bread quality. Enzymatic flour correction can assist with overcoming these issues.
- Artisan doughs typically have a high hydration ratio and sticky nature, which can cause production issues. Enzymes can be used to improve dough rheology.
- The long fermentations used in artisan dough production result in dough that is more vulnerable to degassing and cell structure damage in automated systems. Enzymes can be used to increase dough strength and improve volume and crumb structure.
- Staling occurs within hours of baking. Enzymes can be used to slow the rate of starch retrogradation, resulting in a softer and more resilient bread at the time of consumption.

Flour Correction. In artisanal bakeries, small deviations in the quality of raw materials can be compensated for by making minor adjustments to process conditions based on the baker's experience. This is termed flour correction and may involve the addition of fungal α -amylase. Addition of fungal α -amylase is useful when variations in flour quality moderately impact dough handling or machinability properties (3).

There are expectations for flour quality as far as processing (i.e., flour economy or water absorption, tolerance to mixing, and tolerance to fermentation) and performance (i.e., bread

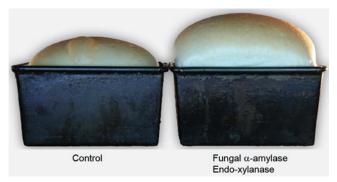


Fig. 2. Effect of fungal α -amylase and endo-xylanase on oven spring of pan bread after 12 min of baking. (Photo: Novozymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished)

volume, crumb color, and crumb structure) are concerned. Because these expectations are the same for every bag of flour, it is important to maintain consistent flour quality (Novozymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished).

The dynamics of individual milling and baking markets are influenced by many factors, not the least of which is the objective to increase yield, which can be achieved by adding fungal α -amylases, endo-xylanases, and glucose oxidases to flour. As illustrated in Figure 1, enzymatic solutions can further help increase loaf yield, if bread is sold per piece. The example shown in Figure 1 demonstrates how enzymatic flour correction can improve baking performance, helping millers satisfy evolving customer needs and achieve cost optimization.

Differences in climates, baking procedures, and dough temperatures can be compensated for by improving fermentation tolerance and oven spring. As shown in Figure 2, flour can be supplemented with fungal α -amylase and endo-xylanase to produce a loaf with better volume as a result of enhanced oven spring.

Dough Rheology Improvement. *Uninhibited Xylanase in Baguettes.* Bread producers desire a dry, stable dough with high tolerance to process variations (2). Uninhibited bacterial xylanase can be used for flour standardization or specialization of breadimprover products, providing improved volume performance, desired texture and appearance, and a dry, balanced dough with the use of one xylanase.

Wheat has naturally occurring xylanase-inhibitor proteins, which also inhibit exogenous xylanases, causing an increase in enzyme dosage used. An uninhibited xylanase works very well under conditions where dough temperature may be higher than 34°C. A tolerance to high temperatures has been seen with this xylanase, which is beneficial under production conditions with fluctuating temperatures (Novozymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished).

This uninhibited xylanase provides improved baguette volume and desired bloom and crust crispiness, even in low dosages with a French flour type (Fig. 3). The dough shown in Figure 3 was processed using traditional methods, with a long proofing time. The dough properties were dry and balanced. A high proofing

Control 20 ppm 30 ppm 40 ppm Endo-xylanase Endo-xylanase Endo-xylanase

Fig. 3. Effect of uninhibited xylanase on baguette quality. (Photo: Novozymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished) tolerance was seen as well, even after 2.5 hr of fermentation, which assisted in securing predictable and stable baguette production.

Cellulase in Whole Wheat Bread. In addition to arabinoxylans, whole wheat or whole grain flours contain other nonstarch polysaccharides, such as cellulose, soluble β -glucans, and glucomannans, that may interfere with gluten development. A multicomponent cellulase can be used to modify these different compounds without compromising product quality (Fig. 4) (7).

Improving Dough Strength and Eliminating Undesirable Ingredients. *Glucose Oxidase in Baguettes.* In breadmaking, a strong gluten network is needed to resist mechanical stress during dough processing and provide gas retention during proofing. This enables the development of oven spring during baking and results in bread with good volume. A strengthening effect is vitally important when flour with weak gluten-forming proteins is used (6).

Enzymatic solutions such as glucose oxidase and fungal α -amylase can help unlock the full strengthening potential of natural gluten in flour. These enzymes improve dough stability and handling characteristics, leading to greater bread volume and improved appearance of baked products. For companies interested in creating azodicarbonamide (ADA)-free formulas, glucose oxidase is a natural processing aid that can be used as an alternative (Novozymes, "Unlock the Full Potential of Flour— A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished).

As illustrated in Figures 5 and 6, both volume and fermentation stability are increased with use of glucose oxidase compared with 1% vital wheat gluten. For French baguettes fermented for

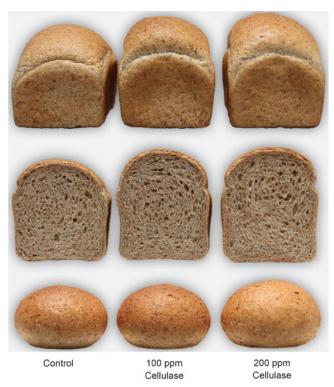


Fig. 4. Effect of a multicomponent cellulase on whole wheat bread quality (60 ppm ascorbic acid and fungal α -amylase used in each loaf). (Photo: Novozymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished)

2 and 2.5 hr, the addition of glucose oxidase resulted in 7 and 17% volume increases, respectively, compared with the addition of 1% vital wheat gluten (Fig. 6).

Polar Lipase in Turkish-Style bread. Recent generations of baking lipases show specificity toward both polar and nonpolar lipids in formulations. This specificity toward polar flour lipids creates more polar substances, increasing their stabilizing capacity (4). By increasing the emulsifying and stabilizing capacity of flour lipids with the appropriate lipase, the level of added stabilizing emulsifiers, such as diacetyl tartaric acid ester of monoand diglycerides (DATEM) and sodium stearoyl-2-lactylate (SSL), can be greatly reduced, if not entirely eliminated (Novozymes, "Unlock the Full Potential of Flour-A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished).

The key benefits of using a polar lipase are improved crumb structure and whiteness (Fig. 7). When used in combination with fungal α-amylase and/or endo-xylanase, polar lipases improve loaf volume without additional dough stickiness, thereby improving the overall quality of the bread.

Due to their activity toward polar lipids during dough processing, lipases with dual specificity contribute to strengthening the



Fig. 5. Effect of glucose oxidase versus 1% vital wheat gluten in French baguettes. (Photo: Novozymes, "Unlock the Full Potential of Flour-A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished)

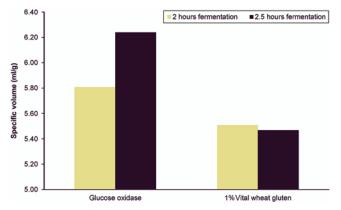


Fig. 6. Specific volume of French baguettes made with glucose oxidase versus 1% vital wheat gluten. (Graph: Novozymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished)

dough. This allows for significant savings in ingredient costs and eliminates or reduces the acidic aroma associated with emulsifiers such as DATEM. Polar lipases can be used in a broad range of baking processes with different grades of flour, and they are particularly well suited for Turkish- and French-style breads (Fig. 8).

In these processes, small dosages of polar lipase resulted in optimum performance, which was fully comparable to the performance of DATEM. In contrast to bread without emulsifiers or lipase, the bread with polar lipase had good volume and a nicely open bloom. In general, polar lipases also show good tolerance to variations in flour quality (Novozymes, "Unlock the Full Potential of Flour-A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished).

Slowing the Rate of Staling. Maintaining bread freshness (soft crumb with crispy crust) is an important quality parameter in artisan breads. Loss of freshness, or "staling," has a significant negative financial impact on bakers because stale returns may account for 10-15% of their production. A major effect of staling is an increase in crumb firmness and loss of fresh crumb springiness or elasticity over time. The mechanism of bread staling is not known with great certainty. However, numerous publications report that these changes in texture are due to modifications in the configuration of highly branched amylopectin molecules-either due to their reversion from a swollen, amorphous, gelatinized state to their native rigid, crystalline state or to an increase in the number of complexes formed



+ 0.15% SSL

Polar lipase

Fig. 7. Effect of polar lipase versus emulsifiers (diacetyl tartaric acid ester of mono- and diglycerides [DATEM] and sodium stearoyl-2lactylate [SSL]) on quality of Turkish-style bread fermented overnight (16.5 hr of fermentation at 24°C; fungal α -amylase and endo-xylanase used in each loaf). (Photo: Novozymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished)

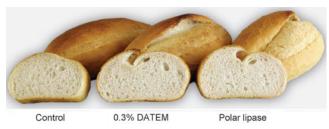


Fig. 8. Effect of polar lipase versus diacetyl tartaric acid ester of monoand diglycerides (DATEM) on quality of Turkish-style bread (75 min of fermentation at 30°C; fungal α -amylase and endo-xylanase used in each loaf). (Photo: Novozymes, "Unlock the Full Potential of Flour-A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished)

with the gluten protein in flour. The starch can be modified with an amylase to alter the way it reconfigures itself (Novozymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished).

A significant reduction in stale returns and associated costs can be obtained by incorporating enzymes that assist in maintaining bread freshness. At temperatures ≥60°C, when most of the starch is available for modification, fungal α -amylase is largely inactivated (8). Maltogenic and malto-tetraogenic α -amylases, in contrast, are active after the starch gelatinization temperature is reached but are functionally inactivated by the time the bread exits the oven. What makes these enzymes particularly unique is the way they modify the starch molecule. Instead of shattering the amylopectin, like thermostable bacterial amylases do, they leave its primary structure intact, generating small dextrins from the ends of the starch molecules. This results in slowing of starch retrogradation, leading to a softer and more resilient bread for an extended time. A comparison of the activity pattern of traditional bacterial α -amylase with that of maltogenic α -amylase on amylopectin is shown in Figure 9.

Maltogenic α -amylase can be used in yeast-raised baking formulations with different flour qualities where crumb freshness is required. Notably, the enzyme does not influence dough properties, bread volume, or crumb structure (Novozymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished). The main benefits of maltogenic α -amylase include

- Maintaining crumb softness due to retardation of starch retrogradation.
- Maintaining crumb elasticity, resulting in improved mouth-feel of stored bread.
- Improvement of crumb softness and elasticity during long periods of bread storage compared with emulsifiers such as monoglycerides.
- Can be used as an enzymatic tool for quality differentiation and brand building for bakeries.
- Reduced costs per unit as a result of rationalized product and distribution efficiencies.

Specialty Applications

Artisan breads can also be produced using ancient grain and rye flours, depending on regional preferences. Enzymes can help improve these bread formulations as well.

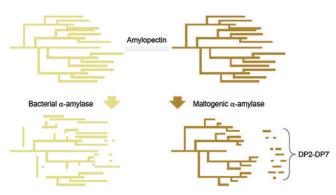


Fig. 9. Comparison of the activity patterns of different α -amylases on amylopectin. (Graph: Novozymes, "Unlock the Full Potential of Flour— A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished)

Rye Bread. Maintaining consistent quality and producing a soft, elastic bread has been an ongoing challenge in rye bread production. In 50:50 rye/wheat bread flour formulations, dough-conditioning enzymes can be used to improve dough stability, bread shape, and crumb structure. As illustrated in Figure 10, adding enzymes can improve volume and crumb structure. In addition to improved stability, the resulting bread has a less dense, fluffier crumb structure that gives the bread more pleasant eating properties. Enzymes can also be used to extend freshness and provide a softer, more elastic bread throughout storage (Fig. 11).

Ancient Wheat Breads. Ancient grains, including emmer, spelt, and kamut (Khorasan) wheats, are a part of the global trend toward healthier bread consumption. These ancient wheat varieties provide very different baking performance than modern wheat. They typically produce breads with lower volume and higher density, which is challenging for bakers who want to produce breads with the soft, elastic, and moist texture and aerated crumb structure that many consumers prefer (Novozymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished). In addition, use of ancient wheat flours produces unstable dough and a final bread that stales faster. Fortunately, enzymes can be used to compensate for many of these challenges and make it possible to produce delicious ancient wheat breads with improved dough stability, volume, and crumb structure.

A variety of enzymes can be used to strengthen both gluten and bread dough, resulting in better dough stability and increased dough volume, as well as improved crust crispiness and bloom and a finer crumb structure (Fig. 12). Additionally, maltogenic α -amylase produces a softer (Fig. 13) and more elastic bread (Fig. 14) that stales more slowly. The result is an excellent an-

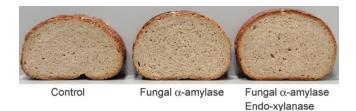


Fig. 10. Effect of fungal α -amylase and endo-xylanase in bread made with 50:50 rye/wheat flour. (Photo: Novozymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished)

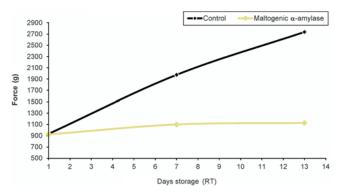


Fig. 11. Crumb softness profile for bread made with maltogenic α -amylase (50:50 rye/wheat flour, including sourdough; pH 4.8 in final bread) stored at room temperature (RT). (Graph: Novozymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished)



Endo-xylanase Glucose oxidase Polar lipase

Fig. 12. Effects of enzymes (fungal α -amylase, endo-xylanase, lipase, and glucose oxidase) on spelt wheat bread properties. (Photo: Novo-zymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished)

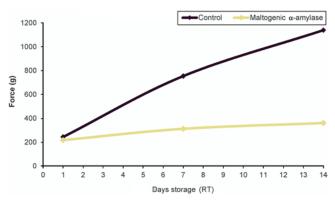


Fig. 13. Effect of maltogenic α -amylase on crumb softness of spelt bread during storage at room temperature (RT). (Graph: Novozymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished)

cient wheat bread with improved softness, moistness, and eating properties throughout storage.

Conclusions

Enzymes have the potential to be key ingredients that can enable the forthcoming industrial artisan-style bread movement by improving the processing, dough stability, and end quality of breads, whether they are produced in the local bake shop, supermarkets, or higher volume bakeries.

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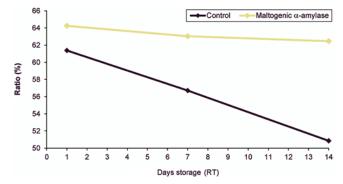


Fig. 14. Effect of maltogenic α -amylase on crumb resilience (elasticity) of spelt bread during storage at room temperature (RT). (Graph: Novozymes, "Unlock the Full Potential of Flour—A Comprehensive Handbook on Enzymatic Solutions for the Milling Industry Baking," unpublished)



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