

Enzymes in bread baking

What they are, how they work & solutions to try

novozymes® 



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Chapter 1:

An introduction to enzymes



WHAT ARE ENZYMES

Enzymes are produced by microorganisms and are widely distributed in all ecosystems of nature. They are made up of proteins that are found in all living organisms, including animals, plants and microorganisms. They act as biological catalysts that facilitate chemical reactions.^{1,2}

To date, scientists have identified over 10,000 different enzymes, and it is estimated that many more have yet to be discovered.

Bacteria and fungi regularly produce enzymes for the food industry. These small friends enjoy popularity given their technological applications. Examples include:

- ✓ **Lactic acid bacteria**
- ✓ **Food-grade filamentous fungus**
- ✓ **Yeast (a.k.a. *Saccharomyces cerevisiae*)**

Enzymes in your life

Enzymes are natural, and make the preparation of a wide variety of products, like cheese, yogurt, bread, wine, glucose syrups and beer a possibility.

Whether we acknowledge it or not, we rely on enzymes for our daily activities. From household detergents to pulp and paper processing, and from bakery products to fruit juices, we are always counting on these molecules to do the hard job for us.

How do enzymes work in food?

The main function of enzymes in living organisms is to help convert food. It takes starting material and converts into simpler forms and finally to energy, and new material for cellular repair and growth.^{1,2}

Enzymes have the unique ability to initiate or accelerate the rate of biochemical reactions that would otherwise proceed very slowly, without undergoing any permanent change themselves.

They are not consumed in the reaction and can therefore continue to catalyze a reaction, i.e. modify the starting material, as long as the reactants or substrates, are available.³



Chapter 2:

How Enzymes Can Help

TYPES USED IN BAKING

Enzymes are amazing processing aids and can be used to make products in high-speed bakeries. They can be used in production environments that operate at 100–300 dough pieces per minute at the divider. High-speed bakeries often produce no-time (straight) dough systems which require stability and machinability to yield bread and buns that yield:

- Excellent volume through optimum gas production and gas retention
- Optimum gluten development with superior dough handling properties
- Higher flour hydration with minimal dough stickiness
- Finer crumb structure
- Balanced crust color
- Superior textural qualities over extended shelf life

To obtain these properties, a wide variety of dough improvers, also known as dough conditioners, has been developed. Such dough conditioners include:

- ✓ **Oxidizing agents**
- ✓ **Reducing agents**
- ✓ **Emulsifiers**
- ✓ **Vital wheat gluten (VWG)**

The majority of dough conditioners are currently not accepted as clean label options. Enzymes, on the other hand, represent an alternative that is widely accepted by consumer groups.

Baking solutions

Enzyme	Functionality
Maltogenic alpha amylase	Freshness
Fungal alpha amylase	Flour correction
Asparaginase	Acrylamide reduction
Lipase	Emulsification
Lipoxygenase	Oxidation
Glucose oxidase	Dough strengthening
Transglutaminase	Gluten strengthening
Xylanase	Dough conditioning

Given the right conditions of pH, time, temperature, moisture and concentration, enzymes can replace traditional dough conditioners. Here are their main functions.

Freshness

Freshness and shelf life extension are synonymous to keeping the softness of the product over time. This is especially challenging in producing packaged bread. Consumers expect the freshest product when it comes to the texture and mouthfeel of packaged bread.

Surveys show that consumers value the sensory quality of moistness. Consumer panels that rate a particular bread high in “moistness” and “tenderness” also gave it a higher “like” score. A top reason to throw bread out is “dryness”. Unknown to many, this attribute of “dryness” is not due to the loss of moisture in packaged bread over time, but due to the staling and starch retrogradation in the baked product.⁴

Staling, which involves loss of crumb freshness, has a significant negative financial impact on bakers. Stale returns can eat up portions of the total product manufactured. This is a considerable amount of food waste that is not contributing positively to your bottom line. The staling of bread and cake includes an increased crumb firmness, loss of fresh crumb springiness or resiliency, and a decrease in perceived moistness over time that— all issues that can be solved by enzyme-based solutions.

2 Flour correction

Flour correction is another key function of amylase enzymes. Flour correction enzymes are used in bread improvers/premixes to standardize flour at the mill to compensate for fluctuations in amylase activity.

Flour produced from sound grain contains two types of amylase: α -amylase which is usually scarce and requires supplementation, and β -amylase that is found in abundance. These were among the first enzymes to be used in breadmaking. Today, they are routinely added to flour to modify starch.

Native starch is a polymer made up of amylose and amylopectin. Amylose represents a linear helical structure of glucose units, while amylopectin is a highly branched structure. Amylose makes up 20–25% of the starch content in wheat flour while amylopectin is the remaining 75–80% of starch content.



Yeast needs fermentable sugars due to its inability to readily digest flour's starch. In breadmaking, amylase is needed to generate fermentable sugars from starch so that yeast cells use it to produce carbon dioxide gas and leaven the dough. The sugars produced by amylase activity also contribute to the formation of desirable color and aromas in bread via the metabolic activity of yeast on sugars and non-enzymatic browning (Maillard) reactions.

There are many different types of amylases that can modify starch. For our scope, the ones of interest are fungal α -amylase and amyloglucosidase (glucoamylase).

A major challenge for high-speed bakers is to ensure consistent bread quality, even during wheat crop changeovers. Any variation in gas production will influence dough leavening in the proof box and during oven spring, hence affecting final bread volume. Flour with insufficient amylase levels will yield dough with extended proofing times and loaves with insufficient volume, poor color and an underdeveloped crumb structure.

3 Dough strengthening

Proper dough strengthening can be achieved by using lipases instead of traditional emulsifiers, such as diacetyl-tartaric esters of monoglyceride (DATEM), or the sodium or calcium salts of stearoyl lactylates (SSL/CSL).

Lipases hydrolyze the natural lipids of flour and added shortening at the lipid-air or lipid-water interface and transform them into natural emulsifiers. This yields loaves with improved oven spring, increased loaf volume and bloom, and a homogeneous, silky, white crumb with a fine grain.

Lipases act on triglycerides, phospholipids and glycolipids, all naturally present in flour as substrate for the enzyme. The result of enzymatic hydrolysis of lipids by lipase is the formation of functional mono- and diglycerides that are capable of interacting with gluten, to promote strengthening during mixing.⁵

4 Gluten strengthening

In commercial breadmaking, a strong gluten network is needed to help retain gas during dough extruding, sheeting, proofing and oven spring. The strengthening effect is of vital importance when using low-quality flour with weak gluten-forming proteins.

Gluten structure can be strengthened by oxidizing the sulfhydryl groups in the gluten proteins in order to form disulfide bonds that crosslink the gluten proteins together. This oxidation has traditionally been promoted by aging flour. It can also be obtained by using redox agents such as:

- Potassium bromate
- Azodicarbonamide (ADA)
- Ascorbic acid

The first two compounds in particular are under public scrutiny and have come under food safety review. Potassium bromate and ADA have been banned in the European Union and in many countries of the world.

Alternatives to chemical oxidizing agents include glucose oxidase and hexose oxidase. In the presence of oxygen, GOX oxidizes glucose to gluconic acid, forming hydrogen peroxide (a true oxidizing agent).

5 Dough conditioning

High speed processes require proper dough conditioning so that the dough remains intact and unchanged through the stress points. Dough conditioning improves dough handling properties through:

- Increased extensibility
- Improved elasticity
- Resistance to tear
- Reduced stickiness

Dough conditioners are especially needed in varieties of bread:

- Whole wheat bread
- Rye bread
- Multigrain bread
- Whole grain bread
- Fiber bread
- Raisin bread

Dough conditioning (cont.)

These products have one thing in common. They all bring a heavy load of bran and aleurone layer, which are rich in non-starch polysaccharides (NSPs). NSPs make up about 3% of refined wheat flour and 8% of whole wheat or rye flour. The higher the flour extraction at the mill, the higher the amount of NSPs in the resulting flour. NSPs can bind as much as 10 times its weight in water, becoming responsible for up to 30% of dough's water absorption capacity. The high-water absorption capacity of the NSPs found in flour undermines their role in breadmaking.

Pentosans can be further divided into arabinoxylans and arabinogalactans. Arabinoxylans (AXs) are made up of the five-carbon sugars arabinose and xylose. AXs consist of a linear backbone of D-xylose units that are linked by β -(1-4) glycosidic bonds with single α -L-arabinose units attached to carbon 3 of xylose molecule as branches.⁵ Some of the arabinose units are esterified with ferulic acid.

AXs can be water unextractable (WU-AX) or water extractable (WE-AX) depending on how firmly they are bound to the endosperm cell wall. The water-extractable fraction comprises 1/3 arabinoxylans' total weight, and the water-unextractable 2/3.

Arabinoxylan's water solubility is related to the molecular size and the number of arabinose side chains. The xylose backbone is very insoluble but becomes more soluble as more arabinose is bound. AXs become more soluble as they decrease in molecular size.^{6,7}

Formulations rich in bran and NSPs create doughs that have/are:

- **Competing with the proteins for water absorption, under-hydrating and under-developing functional gluten-forming proteins**
- **More susceptible to overmixing**
- **Poor water distribution**
- **Higher risk of less gas retention as the gluten network is weaker**
- **Weaker gluten film and foam stability are disrupted by the "cutting action" of bran particles**
- **Stiff and exhibits little extensibility**
- **Less tolerance and lower performance through the sheeter and moulder, therefore, less forming pressures must be used**
- **More susceptible to over-proofing**

6

Acrylamide reduction

In April 2002, Swedish researchers discovered that significant levels of acrylamide, a potential carcinogen, can be found in heated, starch-based foods. Out of concern for consumers and their brands, many food manufacturers are looking to reduce acrylamide levels in their products.

Acrylamide forms naturally in food as a product of the Maillard reaction at temperatures above 120°C (248°F) and low moisture. The Maillard reaction creates the golden crust color and delicious flavor of many baked, fried, roasted or extruded products, but also leads to the formation of acrylamide when the amino acid asparagine reacts with reducing sugars.



Chapter 3:

Baked goods & good enzyme solutions



HEALTHIER BAKING

Along with the use of natural ingredients, pre-ferments and sourdough, enzymes are the future of more natural and healthier baked goods — for both yeast- and chemically-leavened goods. As natural processing aids, enzymes are the consumer-friendly choice for meeting these market demands.

High-speed bakers seek more natural product development, with shorter and more familiar ingredient lists while maintaining equivalent shelf-life as regular SKUs. Meeting organic and 100% natural product claims requires:

- ✓ **An understanding of how ingredients interact with each other**
- ✓ **Access to world class & state-of-the-art processing technologies**
- ✓ **Following strict plant sanitation procedures**

Clean label yeast-leavened products must be carefully formulated. Here are some recommendations:

- Nutrition and health claims on label
- Quality and functionality of wheat flour
- Dough scaling weight (will ultimately determine how much flour improvers and dough conditioners needs to be added to the recipe)
- Special scoring patterns and attractive toppings
- Presence of inclusions (e.g. fruits, whole grains, etc.)
- Dough processing technology (e.g. stress-free shaping and moulding)
- Shelf-life (both mold-free and textural)

To successfully and sustainably market such products, bakers need ingredient solutions to:

- Keep bread soft and fresh during its entire shelf-life
- Deal with flour quality and performance variations
- Strengthen and condition the dough to ensure optimum oven spring and desired finished product texture
- Make smart use of processing technology and equipment resources
- Maintain the same quality, batch after batch, consistently

Product freshness

Enzyme freshness solutions offer more operational flexibility to high-speed bakers that can help products stay fresh for longer with the following benefits:

- More time for planning, production and distribution
- Longer production runs which mean less downtime due to cleaning, pan changeover and line adjustments
- More inventory can be held to accommodate for high demand seasons and market demand fluctuations
- Longer distances can be covered, and new markets can be served
- Product on shelf for longer increases chances of being sold

According to scientific research, the mechanisms behind bread staling are due to the reorganization of starch fractions, particularly those of highly branched amylopectin molecules. This is due to their retrogradation from the gelatinized state into their crystalline state. Modifying the starch with an alpha amylase can alter the reorganization it undergoes during bread storage.

Novozymes' Novamyl® product range is based on a unique maltogenic alpha-amylase with intermediate heat stability that helps maintain the eating characteristics of freshly baked bread that consumers desire for an extended period of time. This enzyme solution is unique in the way that it modifies the starch granule. Instead of shattering amylopectin, the maltogenic amylase leaves its primary structure intact, generating small Dp2-Dp7 molecules (mainly maltose and some number of oligosaccharides and small dextrins) from the non-reducing ends of starch chains.

This results in starch granules that have reduced rates of retrogradation and recrystallization, resulting in a softer and more resilient product for a longer time. The maltogenic α -amylase is still active after the temperature for starch gelatinization, but it is functionally inactivated by the time the bread or cake leaves the oven (about 96°C/205°F at sea level).

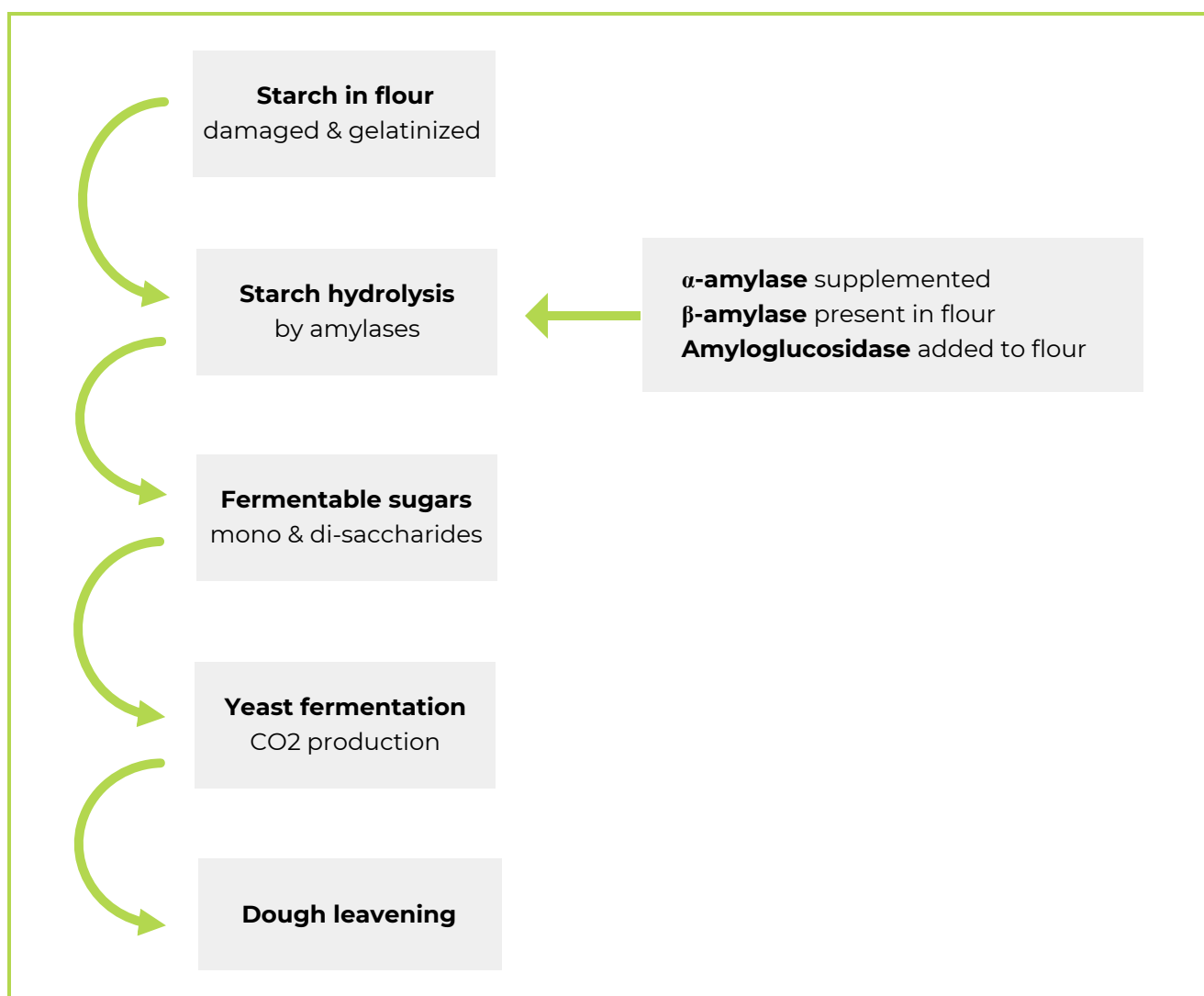
2 Flour correction

For flour correction, flour is often supplemented with an α -amylase, whether from malt or fungal sources, to optimize the amylase activity. Flour-correcting fungal α -amylase Fungamyl® products are an excellent option for adjusting flour by standardizing α -amylase activity.

Novozymes' Fungamyl® products are often seen as the best choice for flour standardization given their benefits:

- Optimized flour amylase activity
- Accelerated fermentation rate
- Enhanced sponge fermentation and gas production
- Increased oven spring
- Pronounced volume-improving effect
- Fine and even crumb structure
- Improved crust color

The following diagram explains how fungal α -amylase and other solutions, such as amyloglucosidase, work on starch.



Enzyme	Action	Products
α -amylase	Randomly cleaves α -1,4 glycosidic bonds of amylose and amylopectin	Oligosaccharides with varying lengths and different dextrins
β -amylase	Successively removes maltose from the non-reducing ends of amylose and amylopectin Cannot cross a branch point (α -1,6 bond)	Maltose from dextrins provided by α -amylase
Amyloglucosidase	Cleaves α -1,4 and α -1,6 bonds in amylose and amylopectin	Can completely degrade starch to glucose

Fungal α -amylases do not work well with the falling number test due to their low thermostability, making it difficult to meet flour specifications. [Fungamyl® Prime](#) is a new fungal α -amylase that consistently reduces the falling number of flour in relation to the dosage, makes it easier to meet flour specifications, offers an alternative to malt flour, and delivers excellent baking performance, improved volume, crust color, and a finer, more even bread crumb, just like the original Fungamyl®.

3 Dough strengthening

Novozymes' [Lipopan® enzymes](#) have good dough-strengthening properties for industrial bread-making. With Lipopan®, bread improver manufacturers have enzymatic options that can cost-efficiently match emulsifier performance in a vast range of baked goods.

Lipopan® enzymes are specially developed lipases which can target not only traditional triglycerides, but also polar lipids, such as glycolipids and phospholipids (both naturally present in the flour, and added in the form of oil, butter and natural emulsifier lecithin).

Lipases modify lipids at the water-fat interface, resulting in improved dough characteristics with better gas-cell stability against bubble coalescence. Polar molecules, such as lysolecithin and galactosyl diglycerides, can interact with glutenin and gliadin molecules promoting protein aggregation (dough strengthening effect). This yields loaves with improved oven spring, increased loaf volume and bloom, and a homogeneous, silky, white crumb with thin-walled porosity.

Due to their activity towards polar lipids, lipases with dual specificity are particularly well suited as substitutes for emulsifiers such as DATEM and SSL. This allows for significant savings on ingredient costs and eliminates or reduces the acidic aroma associated with these emulsifiers.

4 Gluten strengthening

Alternatives to traditional redox agents (e.g. ADA, bromates and calcium peroxide) include gluten strengthening enzymes. Such enzymes are glucose oxidase (GOX) and hexose oxidase (HOX). High-speed bakeries are paying a lot of attention to glucose oxidases mainly due to the need to offer clean label bread and buns that are free from ADA and other chemical additives.

Bread flour from hard wheat contains 11–14% protein. 80–85% of total protein corresponds to gluten-forming proteins, gliadins and glutenins.

Gluten proteins can be strengthened by oxidizing the sulfhydryl (thiol) groups of cysteine residues to form disulfide bonds that crosslink the gluten protein chains together. In the presence of oxygen, GOX catalyses the oxidation of glucose to gluconic acid, and in doing so, produces hydrogen peroxide (H₂O₂). The hydrogen peroxide then oxidizes active SH groups to form disulfide bonds (S-S). The S-S bonds promote protein aggregation, improving dough structure and enhancing gas retention capacity of dough:

**2R-SH (2 cysteine residues from separate protein chains) + H₂O₂ → R-S-S-R
(cysteine molecules link and bring protein chains together) + 2H₂O**

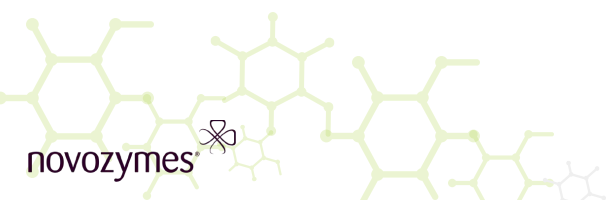
Through oxidation, GOX provides the following benefits:

- Improved dough stability / tolerance
- Reduced dough stickiness
- Enhanced water absorption
- Cost-effective
- Increased loaf volume
- Substitute traditional chemical oxidants
- Reduced vital wheat gluten

Novozymes' Gluzyme® (glucose oxidase), Gluzyme® Mono and Fungamyl® Super BR help unlock natural gluten's full strengthening potential in flour. Gluzyme is a fungal glucose oxidase. Fungamyl® Super BR is a blend of Gluzyme® with Fungamyl. In this enzymatic synergy, α-amylase provides enough substrate for glucose oxidase to perform optimally before oxygen becomes limiting due to normal dough fermentation.

As GOX requires both oxygen and glucose, its strengthening effect on the bulk rheology of the dough occurs mainly during mixing, where oxygen is not limiting. The drying effect on the dough surface by hydrogen peroxide can proceed in later stages of the bread making process, as long as glucose and oxygen are available.

Gluzyme® improves dough stability and dough handling characteristics, leading to higher bread volume and improved final appearance of the baked products. GOX can partially replace vital wheat gluten in some applications, thus providing another opportunity to reduce the cost of ingredients.



5 Dough conditioning

Novozymes' Pentopan®, Panzea® and Celluclast® can be used for all types of bread and baked products to improve dough machinability, stability, extensibility and gas retention, all of which contribute to the finished product's appearance, volume, bloom and crumb structure.

Novozymes' Pentopan® is a fungal 1,4 β -Xylanase which randomly hydrolyzes β -1,4-glycosidic linkages within xylan. Panzea® is a bacterial xylanase and Celluclast® is a cellulase which breaks down cellulose into glucose, cellobiose and longer glucose polymers. Blends of these enzymes are a great option for whole wheat and multigrain products as these baked goods contain high amounts of non-starch polysaccharides, such as cellulose, pentosans (arabinoxylans) and β -glucans.

Xylanases hydrolyse arabinoxylans (AXs). The breakdown of WU-AXs (detrimental form) into medium-to-high molecular weight WE-AXs (beneficial form), improves dough handling properties and leads to a greater uniformity in quality characteristics. When xylanases are used for the degradation of WU-AXs, they provide dough strengthening and confer a stabilizing effect on gas bubble structure.

The following are a few mechanisms of how xylanases perform their function:

As AXs are cleaved by xylanase, water which was previously held by the polysaccharide, is freed and redistributed to sites where it is most needed (e.g. gluten-forming proteins). The release of water is responsible for the extra viscous behaviour and mobility of the dough.

In the presence of hydrogen peroxide, ferulic acid residues link adjacent AX chains by forming diferulic acid bridges. This cross-linking induces oxidative gelation and creates larger and more stable molecules known as AX gels, which upon hydration can hold > 30 g of water per gram of polysaccharide.

WE-AX gels function as natural gums or hydrocolloids. They hold large amounts of water and increase the viscosity of the liquid phase of dough, thus providing extra foam stability and gas retention. Higher foam stability makes it possible to have a finer and more homogeneous crumb structure and higher bread specific volume.

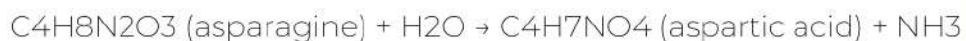
They promote the intimate interaction between WE-AXs and gluten proteins to reinforce the dough and enhance oven spring.

Enzymatic solutions such as Novozymes' Panzea®, Pentopan® and Celluclast® help produce high-quality variety breads consistently.

6 Acrylamide reduction

An effective way to reduce acrylamide is to add enzyme asparaginase (L-asparagine amidohydrolase). Asparaginase converts the amide amino acid asparagine into aspartic acid, which bypasses the acrylamide formation, reducing its levels in the baked goods. Some bakery products tend to have higher acrylamide levels per unit weight than others.


Conversion of asparagine to aspartic acid via asparaginase biocatalysis takes place as follows:⁵



New tougher EU requirements to reduce acrylamide levels, as well as scrutiny from other regulatory authorities around the globe, are driving companies to take measures to reassure consumers that they care about their health and safety.

An effective, easy-to-implement solution is Novozymes Acrylaway®, which can reduce acrylamide levels by up to 95% without affecting the taste, texture or appearance of the final products.





Chapter 4:

Novozymes & enzymes

BETTER BUSINESS WITH BIOLOGY

Novozymes is an industrial biotechnology company and the world's largest provider of enzyme and microbial technologies.

We believe in creating better business with biology, and together with customers, partners and the global community, we improve industrial performance while preserving the planet's resources and helping build better lives.

Our bioinnovation enables fresher tasting bread during shelf life, higher agricultural yields, low-temperature washing, energy-efficient production, renewable fuel and many other benefits that we rely on today and in the future. We call it **Rethink Tomorrow**.

A reliable partner in the baking industry

Staying ahead of the curve in this dynamic industry requires the best technology and expertise to help you become more sustainable, more flexible, and even more profitable.

Novozymes is the trusted pathway to new opportunities and growth based on a comprehensive portfolio of baking enzymes and services. We pride ourselves on the consistency of our high-quality products, which are all made with the help of renewable energy.

And, our production strains, using assessed and approved raw materials, are produced under strict, controlled processes that have full traceability. We can help you meet your targets, whether related to sustainability or the bottom line. Let's transform your business, together.

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