

# Efficient application

A NEW GENERATION OF ENZYMES IS EMERGING. "PROTEIN ENGINEERING" IS THE BUZZ WORD FOR ENHANCING THE APPLICATION CAPACITY OF ENZYMES



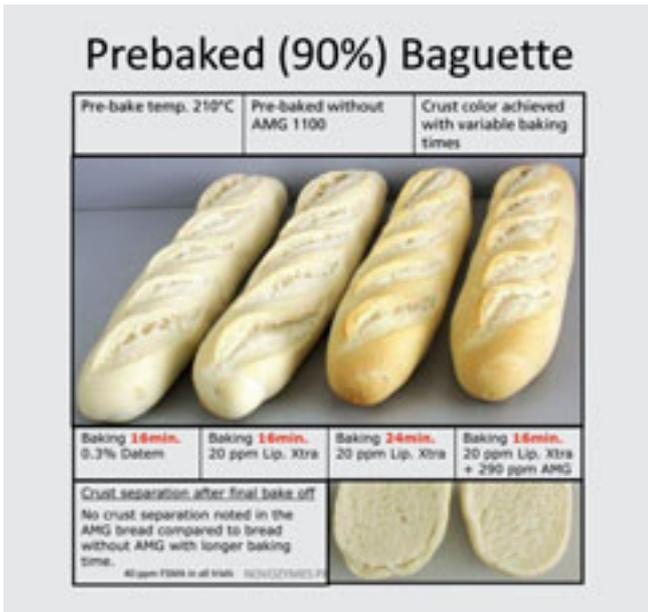
**+** Enzymes are proteins composed of 20 different amino acids. They act as a catalyst and accelerate chemical reactions. Differences in their three dimensional structure determine their activity. The German physiologist, Wilhelm Friedrich Kühne was the first to use the term "enzyme", in 1878. Up Enzymes are indispensable for metabolic function, for healing wounds and the production of hormones in the body.

The enzyme's capability to act as a bio catalyst has already aroused the interest of the industry. Washing agents with enzymes can even clean the laundry at low temperatures. In the food industry, enzymes trigger targeted and quick reactions and results, which is also true of the baking industry. Maltogenic amylases, for example, degrade starch in such a way that the crumb of the bread remains soft after baking and during storage. This capability is predominately used in the production of sliced bread. Lipases and similar enzymes can modify fats and other lipids by increasing the polar characteristics of free lipids. This way, the use of emulsifiers that have to be labeled can be minimized in many applications. According to European labeling legislation, enzymes are considered to be processing aids which have no effect on the final product and therefore they do not have to be labeled.

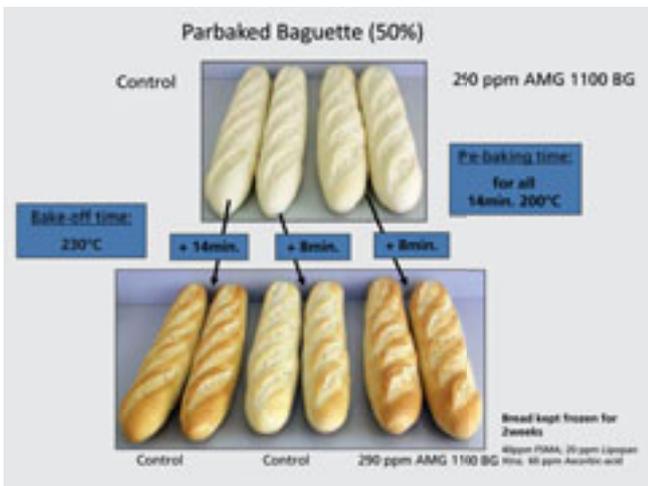
Enzymes are produced by microorganisms. However, naturally occurring bacteria, yeasts and moulds display activities undesired for industrial application. They produce not only the desired enzyme but also some enzymatic side activities which require elaborate cleaning and separation processes. The production could be improved by selective processes,

## Key enzyme suppliers to the food industry

- + AB Enzyme, United Kingdom
- + Ajinomoto, Japan
- + Amano, Japan
- + Beldem SA/NV (subsidiary of Puratos), Belgium
- + Biovet, Bulgaria
- + Chr. Hansen, Denmark
- + DSM, Netherlands
- + Genencor (division of Danisco), Denmark
- + Kerry Group, Ireland
- + Lyven, France
- + Novozymes (part of Novo Nordisk Foundation), Denmark
- + SternEnzym, Germany +++



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The use of GMO for the production of enzymes does not have to be labeled in Europe. For one thing, the enzyme is the product of a genetically engineered microorganism and it is not genetically modified itself. According to the latest European regulation, in the future the use of enzymes needs only to be labeled in the b2b sector but not for the final consumer. Currently, respective implementing regulations are not yet available.

In the future, the European Food Safety Authority (EFSA) will conduct a uniform safety review for all enzymes approved for use within the EU. It remains to be seen whether such a review of procedure will increase the safety or if it is just being used to raise the market entry barriers for smaller suppliers.

The concentration on the market is large enough as it is. Worldwide there are about one dozen enzyme suppliers representing about 90% of the entire market. Most of them are members of the Association of Manufacturers and Formulators of Enzyme Products (AMFEP).

The large companies are the market leaders: Novozymes, DSM, Danisco Grenencor and AB Enzymes. The family of enzyme suppliers is rather eager to cooperate as everyone buys from everyone. Currently, there is no production facility for enzymes in Germany, although there is one supplier who has expertise and patents, mainly for specialized enzymes, which are then produced by contractors, namely SternEnzym, a subsidiary of the Wywiol Group, located in Ahrensburg near Hamburg. According to company information, it seems that there will soon be food enzyme production once again in Germany.

The next technological advance in the enzyme industry is the so-called “protein engineering” (PE). This term describes the capability of a modern biotechnologist to modified enzymes in such a way that certain parts of a molecule or the atomic bonds change in order to achieve certain modes of action. It is possible for example, to strengthen the intra-molecular bonds in certain areas of the enzyme molecule with the effect of improved thermal stability. At the ▶

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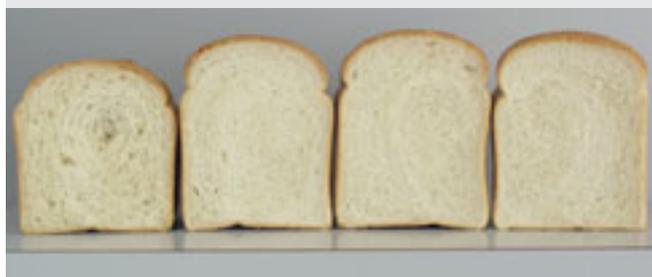


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but genetic modification has proved to be more efficient. There are two different types of genetic modification: a) the amplification or concentration of already present activities and b) the implementation of certain activities from other microorganisms, plants or animals. In both ways, the aim is to set up a safe and reliable production of enzymes. It is estimated that more than half of the enzyme quantity used is produced by GMOs.

**Lipopan Xtra BG**

Good dough strengthening performance and significant cost savings via emulsifier replacement with Lipopan Xtra BG. In a typical Chorleywood process, approximately 20 ppm of Lipopan Xtra give same volume and crumb appearance compared to 0.25% SSL oder 0.2% DATEM.



Control	0.25% SSL	0.2% DATEM	20 ppm Lipopan® Xtra
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Novozymes

same time, it may be possible to control the enzyme's activity via pH and temperature resulting in targeted browning of the dough pieces within a certain time period. Other modifications change the enzyme so that inhibitors cannot dock to the molecule. Inhibitors are substances naturally present in raw materials. They prevent or at least aggravate certain processes by docking to the originator of the process and in this case, the enzyme.

Protein engineering can be done either by applying genetic engineering or by using chemicals which require additional processing steps with resulting higher costs. Up until now, genetically modified microorganisms produced more enzymes and less undesired by-products. Protein engineering is used to modify the microorganisms in such a way that they produce a modified enzyme. Whether this enzyme has to be called genetically modified or not is still an open question. In enzyme research and development, there are currently several developments.

Amyloglucosidases is one example. They influence the browning behavior by releasing glucose which compensates for the sugar used up by the yeast in processes with proofing retardation. At the same time, they reduce the risk of the crust splitting off in frozen baked goods. Today, amyloglucosidases are mainly produced without the use of GMOs and PE. In the very near future, it is expected that these enzymes will also be enhanced in terms of production, targeted precision and efficiency by genetic engineering and protein engineering. Another application where enzymes will be increasingly used in the future is salt-reduced baked goods. Enzymes can help to partly replace the gluten strengthening effect of the salt. Added to that, they can support the formation of short chained sugars and proteins as aroma precursors.

Enzymes that reduce the formation of acrylamide during baking are already available on the market. The so called asparaginases degrade the amino acid asparagine, the precursor for acrylamide, into harmless components.

Lipolytic enzymes, often also called lipases, have a leading position in enzyme research. These enzymes split off fatty acids from the fats and fatty substances that are present in the flour or that are added to the dough. With this, they



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modify the interaction between fats and gluten. The result is increased volume, proofing tolerance and dough stability. In this field, GMO and PE may ensure, in the future, that the interaction chains can be controlled as precisely as possible and targeted to the fats contained in the flour. Added to that, the lipolytic enzymes available on the market today have a beneficial mode of action in dough with long resting times. If the dough, at the same time, is exposed to a lot of oxygen, as for example during lamination, this group of enzymes can also be applied to brighten up the crumb color. Lipopan Xtra BG is a lipase produced using GMO and PE. It is available from Novozymes.

The enzyme producers are also looking into the matter of the water binding capacity of the dough in relation to the baking time and the energy consumption of the baking process. Currently, this issue cannot be linked to a certain group of proteins. For wafer dough and the production of breadings, a technological effect has already been achieved with hemicellulases.

And last but not least, the increasing market for liquid improvers has caused the enzyme researchers to develop enzymes that also maintain their stability and efficacy in aqueous solutions. +++