Enzymes - Best Friends of Flours

The Miller's Little Helpers

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For a long time, α- and ß-amylase were thought to be the only enzymes that could be used in the milling industry. This view has changed dramatically since the introduction of hemicellulases two decades ago, and has now received another blow through the success of lipolytic enzymes. There are many more enzymes (Tab. 1) that still play niche roles for certain applications, but which may turn out one day to be as versatile as the aforementioned types. This presentation focuses on lesser-known properties of common enzymes and some niche applications of well-known enzymes.

Tab. 1: Enzymes suggested for bread and flour improvement (not exclusive)

Enzyme	**Claimed Effect**
α-amylase, fungal	Energy supply for yeast
α-amylase, bacterial	Liquefaction
α-amylase, intermediate heat stable	Anti-staling
Amyloglucosidase (glucoamylase)	Energy supply, colour, flavour
Branching enzyme (glucotransferase)	Water binding
Cellulase	Water binding
Furanosidase, arabinofuranosidase	Dough structure, water binding
Ferulic & cumaric acid esterase	Dough structure, water binding
Glutathion oxidase	Protein strengthening
Glycolipase, galactolipase	Dough stability & volume yield
ß-glucanase	Structure, liquefaction
Glucose oxidase, galactose oxidase, hexose oxidase	Protein strengthening
Hemicellulase, xylanase, pentosanase	Dough structure, water binding, volume yield
Laccase, polyphenol oxidase	Dough strengthening
Lipase	Flavour, *in-situ* emulsification, dough stability & volume yield
Lipoxygenase, lipoxidase	Dough structure, decolorization
exo-Peptidase	Colour, flavour
Peroxidase	Protein strengthening
Phospholipase	Pore structure & volume yield
Protease, proteinase	Protein relaxation, liquefaction
Pullulanase	Structure, water binding
Sulphhydryl oxidase	Protein strengthening
Sulphydryl transferase	Protein strengthening
Transglutaminase	Protein cross-linking, gluten stabilization

The term hemicellulase designates a family of enzymes. All the members shown in Fig. 1 are able to break down the pentosans, but their impacts on dough and baking properties vary widely.

It is assumed that pentosans form a network with gluten; the more pentosans are involved, the firmer the network. That is why darker wheat flours and mixtures containing rye flour have a lower volume yield than white flours. The volume yield of all flours can be increased considerably by adding hemicellulases.

Most of these enzymes are derived from *Aspergillus* strains selected for or specializing in the production of hemicellulases.

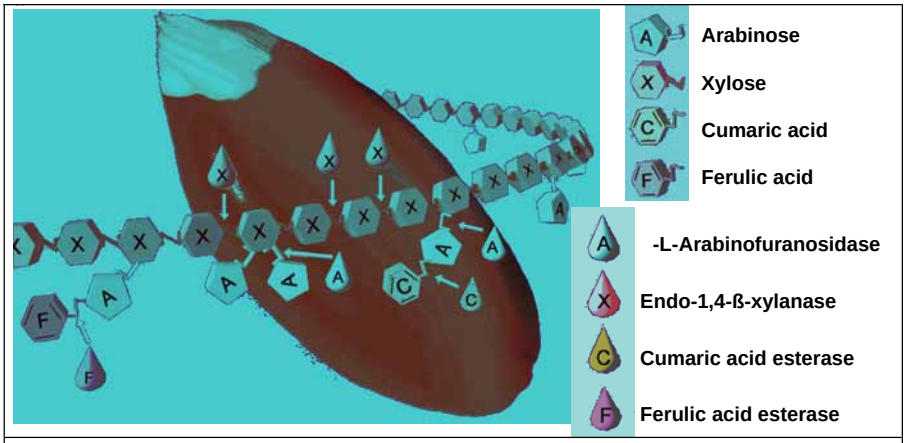

Fig. 1: Hemicellulolytic enzymes

Hemicellulases are mostly sold in compounds with amylase. It is not possible to give a general dosage recommendation as there is no standard method of determining hemicellulase activity. The available methods are usually based on determining the release of reducing sugars, the reduction of viscosity or the breakdown of synthetic or coloured molecules and are very difficult to relate to each other. Moreover, even the use of a standard method for different hemicellulases does not necessarily permit conclusions in respect of baking properties, presumably because the points at which hemicellulases of different origin attack the pentosan molecules are too various.

Protease

Proteases (also known as proteinases or peptidases) split the protein strands of the gluten molecule (Fig. 2) and thus lead first to a softening and then to a complete collapse of the structure. A purified single and very specific protease would only be able to break down a few of the peptide bonds, resulting in only limited softening.

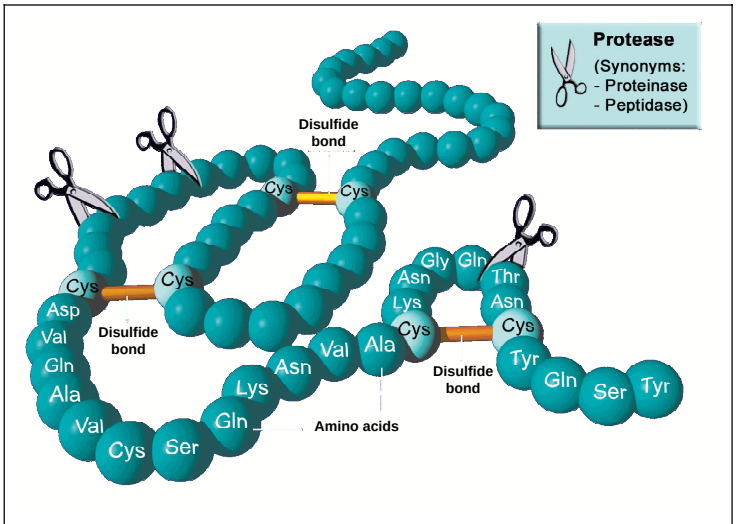

Fig. 2: Action of protease on gluten

With short gluten structures a slight softening may well be desirable; in this case it has a similar significance to the use of cysteine. The proteolytic action is more time-dependant than the function of cysteine. As a result, it increases with the fermentation time of the dough.

That is why there is a considerable demand for enzyme preparations that do not contain even traces of protease.
The use of protease is less crucial with flours that are rich in gluten. It is even very common in the production of pan (toast) bread, where a soft dough that precisely fills the tin is required. Proteases are also very useful in the production of cracker, biscuit or wafer flours where elasticity of the gluten is not desirable.

Enzymes for biscuits, crackers and wafers

Whereas a high protein content and a strong gluten are desired properties in many bread processes, flours with little and weak gluten are preferable for durable baked goods. The tendency of dough to spring back after rolling and the undesired formation of gluten lumps in wafer batter are the reasons for this requirement. Whether a flour with low and weak protein is available or not, the use of elasticity-reducing agents will have benefits in all stages of the process: The lamination will be more uniform; reduction of the thickness of the dough sheet can be performed faster and more reproducibly; relaxing periods for the dough sheet can be shortened or even omitted; the dough pieces will keep the shape given by the cutting; shrinkage and bending in the oven as well as the formation of hairline cracks (checking) are avoided. With suitable amylases, expensive recipe components such as milk solids otherwise necessary for sufficient browning can be omitted. Furthermore, the whole process will be less dependent on flour quality.

Biscuit and cracker applications

Tab. 2 shows the recipes for simple hard biscuits made without and with protease (Alphamalt BK 5020). The last row compares the dimensions of the biscuits. As the length:width ratio shows (average of 25 biscuits), there is almost no difference between the length and width of biscuits with enzyme addition, while those without enzyme showed shrinkage in one direction.

Tab. 2: Biscuits baked with and without bacterial protease

Component (kg)	Reference	With Enzyme
Flour	100	100
Fat	50	50
Sugar	50	50
Salt	0.2	0.2
Water	10	10
Alphamalt BK 5020	-	0.05
Length / width (mm)	62.3 / 59.6	63.6 / 63.3

Since the protease takes away most of the internal tension, the products are less inclined to bend during baking: The first row of Fig. 3 shows the bottom of biscuits without protease; coloration occurred mainly at the margins, which were still touching the oven stone when the cookies became convex due to asymmetric protein shrinkage upon thermal denaturation. Biscuits made with protease remained flat and showed uniform browning (bottom row). This, too, is a common problem that can be observed with many commercially produced hard biscuits.

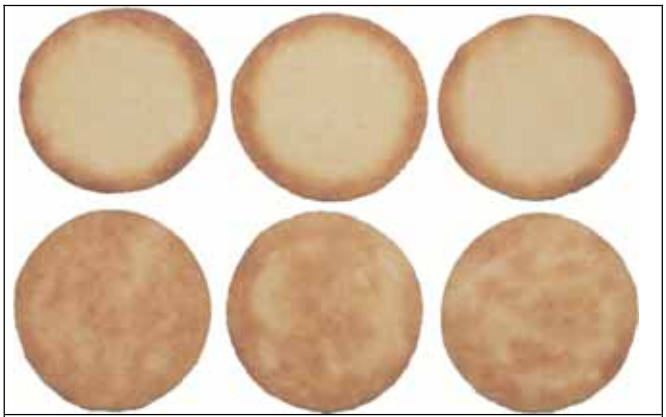

Fig. 3: Underside of hard biscuits baked without (top) and with Alphamalt BK 5020 (bottom)

Wafer applications

Batters for wafer production contain a large amount of water. A low viscosity and a uniform dispersion of all ingredients is essential for even wafers with a homogeneous structure. Since the formation of gluten lumps during mixing can result in standstill of the machinery due to blocked tubes and sieves, or in non-uniform browning and reduced stability of the baked goods, the use of low protein flour is desirable, but may not be sufficient. Liquefying hydrolytic enzyme complexes are able to decompose any gluten present in a liquid batter, resulting in a uniform mixture with optimum flow properties. Due to the viscosity reduction, the amount of water used in the recipe can be lowered, which results in a lower energy consumption for baking and a higher oven throughput. Such enzymes are most suitable for semi-continuous processes with batch times of at least 10 min, because the enzyme reaction needs some minutes to take effect.

We used the Brabender Amylograph at a constant temperature for a simple test to demonstrate the effect of a ‘wafer enzyme’ on the rheological properties of a liquid dough system (Fig. 4). Standard wheat flour for bread making was used in all the tests; 250 g flour was premixed with 330 mL of water in a Braun mixer for 1 min 45 s and then put into the reaction jar. The wafer enzyme Alphamalt LQ 4020 was added to one sample at 20 g per 100 kg flour before starting mixing.

Whereas the reference sample remained at almost the same viscosity for about 40 min, the enzyme caused an immediate viscosity drop. Furthermore, all the gluten strands were destroyed, which is evident from the definite shape of the curve. By contrast, the reference curve shows large fluctuations due to gluten lumps or strands adhering to the mixing tool of the Amylograph.

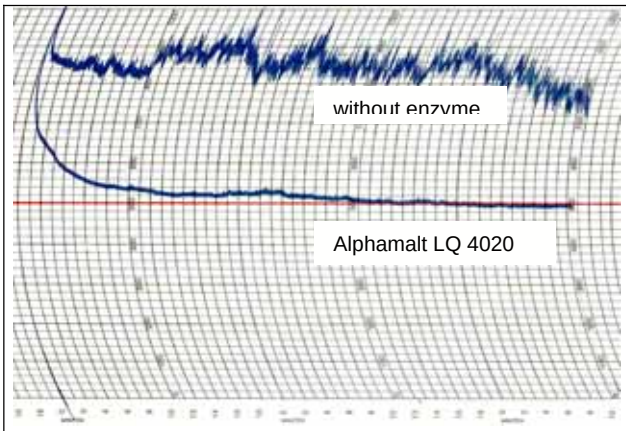

Fig. 4: Effect of Alphamalt LQ 4020 on the viscometric behaviour of wheat flour batter

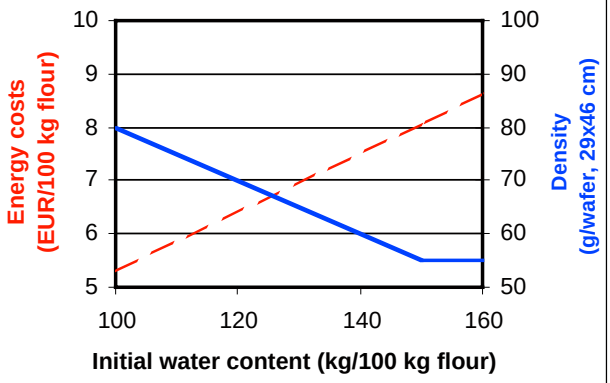

Fig. 5: Effect of water addition on evaporation costs

In baking trials with a pilot-scale plant it was possible to control the water addition and thus the weight and density of the wafers with the help of the enzyme compound. This offers great economic advantages (reduced energy demand, higher throughput) and more freedom for product development (Fig. 5). Wafers of higher density are crisper and remain crisp longer because of reduced water absorption.

Replacement of sodium metabisulphite (SMB) in cracker and wafer production

This strong reducing agent splits the inter-chain and intra-chain disulphide bonds of the gluten, causing an immediate fall in dough resistance or batter viscosity. SMB is very cheap and easy to use.

In many countries, therefore, SMB is still used in wafer and cracker production. Unfortunately, SMB destroys vitamin B1, and can cause health problems in sensitive persons. Furthermore, it inhibits the browning reaction and causes a sulphureous off-taste. Not only do enzymes offer a healthy alternative to SMB; they also have decisive technical advantages, namely constant dough properties once the reaction is accomplished, including a comparable texture of return dough and fresh dough, the reduction of water addition to wafer batters and control of wafer density and stability.

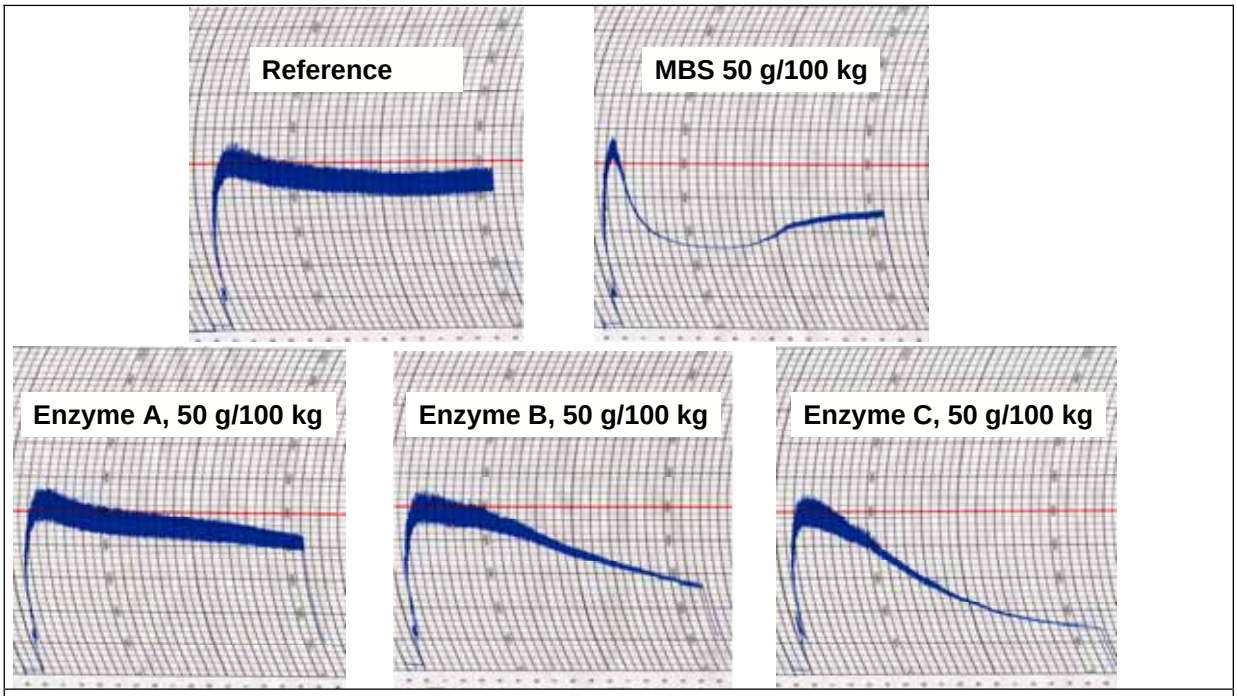

Fig. 6: Farinographs with sodium metabisulphite (SMB) or enzymes. A: proteolytic enzyme for liquid wafer batters; B: proteolytic biscuit and cracker enzyme; C: proteolytic, amylolytic and hemicellulolytic enzyme complex for fast breakdown of gluten.

When tested in the Farinograph, both SMB and enzymes show a decline in kneading resistance (Fig. 6). The reaction of SMB occurs much faster, but probably due to the presence of atmospheric oxygen, some of the resistance is restored upon continued mixing, when disulphide bonds broken by SMB recover (upper right). The slower but persistent reaction of the enzymes results in a minimum resistance, when all the substrate of the enzymes has been degraded.

Glucose oxidase

The enzyme glucose oxidase (GOD) is usually derived from the mould *Aspergillus*, sometimes from *Penecillium* species. Honey is also a rich source of GOD. The enzyme stems from the pharyngeal glands of the bees. However, its suitability is rather restricted by the taste of its carrier.

One effect of GOD in the dough is to oxidize glucose to form gluconic acid with the aid of atmospheric oxygen, but the slight souring that occurs in the process is negligible; its other effect is to transform water into hydrogen peroxide (Fig. 7). This oxidizing agent acts on the thiol groups of the gluten, either directly or via several pathways, inducing formation of disulphide bonds and thus tightening of the protein. The limiting factor in this process is the availability of oxygen. Besides other chemical reactions that consume oxygen, yeast needs oxygen before starting the actual fermentation, as it initially breathes instead of fermenting. This means that the conditions for GOD are only good on the surface of the dough where plenty of oxygen is always available. This limitation can be solved by technical measures during dough preparation, for example overpressure or the supply of extra oxygen through the mixing tool.

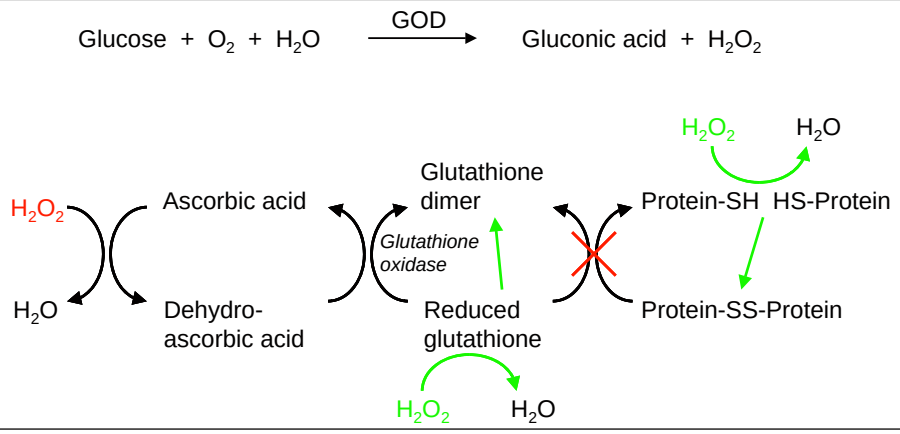

Fig. 7: Reaction of glucose oxidase and probable effects on dough components

Lipolytic enzymes

Lipase is yet another miracle enzyme, under-estimated for a long time. The enzyme converts non-polar lipids into diglycerides and monoglycerides, i.e. emulsifiers (Fig. 8). There are also polar lipids in wheat flour, namely phospholipids and glycolipids (Fig. 9), which can be converted into more hydrophilic lyso-forms by some special lipases or phospholipases.
The *in situ* formation of emulsifiers results in dough strengthening and larger volume yield, but not improved shelf-life. This is in contrast to the effect of mono- and diglycerides which are added to a bread formula. Due to interaction with starch they are able to reducing the staling rate. On the other hand, their effect on volume yield is very limited. Most probably, the action of enzymatically formed emulsifiers on volume yield is pronounced because they are already located at the right sites of the dough for improving the protein properties; but for anti-staling effects, not enough emulsifier is formed to interfere with starch retrogradation. Interestingly, it is being disputed whether the doughs have to contain additional fat, and if so, what kind of fat, for the lipase to work satisfactorily. According to our findings, fat reduces the efficacy of lipase, probably by 'distracting' the lipase from the 'right target', i.e. the flour lipids.

There is also the problem of a possible impairment of taste due to the release of flavour-active fatty acids, particularly if butter is involved. In any case, there are certain applications where lipases are of considerable use.

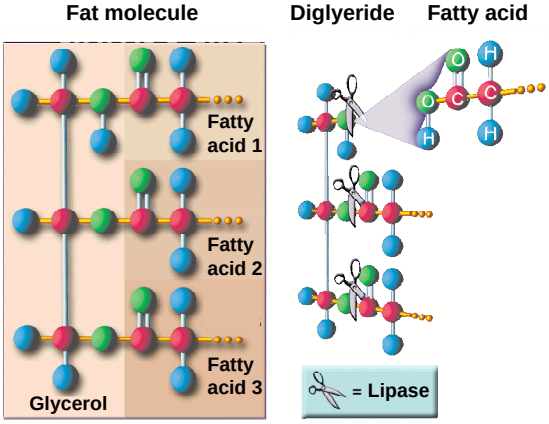

Fig. 8: Effect of lipase on fat molecules

Total lipids	1,280
Non-polar lipids	457
Polar lipids	823
Phosphatides	250
Phosphatidyl acid	30
Phosphatidylglycerol	51
Phosphatidylcholine	27
Phosphatidylethanolamine	traces
Phosphatidylserine	15
Lyso-phosphatidylcholine	117
Lyso-phosphatidylethanolamine	10
Total galactolipids	249
Other polar lipids	320

Fig. 9: Average lipid composition (mg/100 g) of wheat flour (0.405 % ash)

Steamed bread

Chinese steamed bread is often made from a wheat flour of low or medium protein, depending on the type of steamed bread. The preparation process is sometimes quite similar to western style pan bread, but the final product is cured in a steam chamber or basket, not baked in an oven. Therefore, there are some differences in appearance. Steamed bread is white in colour and has a soft and shiny surface. The common types of steamed bread weigh about 30 - 120 g, with shapes either pillow-like or round (Fig. 10 and Fig. 11).

Fig. 10: Pillow-shaped steamed bread

Fig. 11: Round steamed bread

Enzymes such as amylases or hemicellulases improve the overall appearance of steamed bread. Some kinds of steamed bread seem to be ideal playing grounds for lipases. Particularly after extensive kneading or long fermentation processes, a dramatic effect on dough stability and volume yield can be seen. In the example shown in Fig. 12, the volume increase was a net 70 %. Due to the strong dependence on processing conditions, this effect cannot be reproduced with all dough preparation methods.

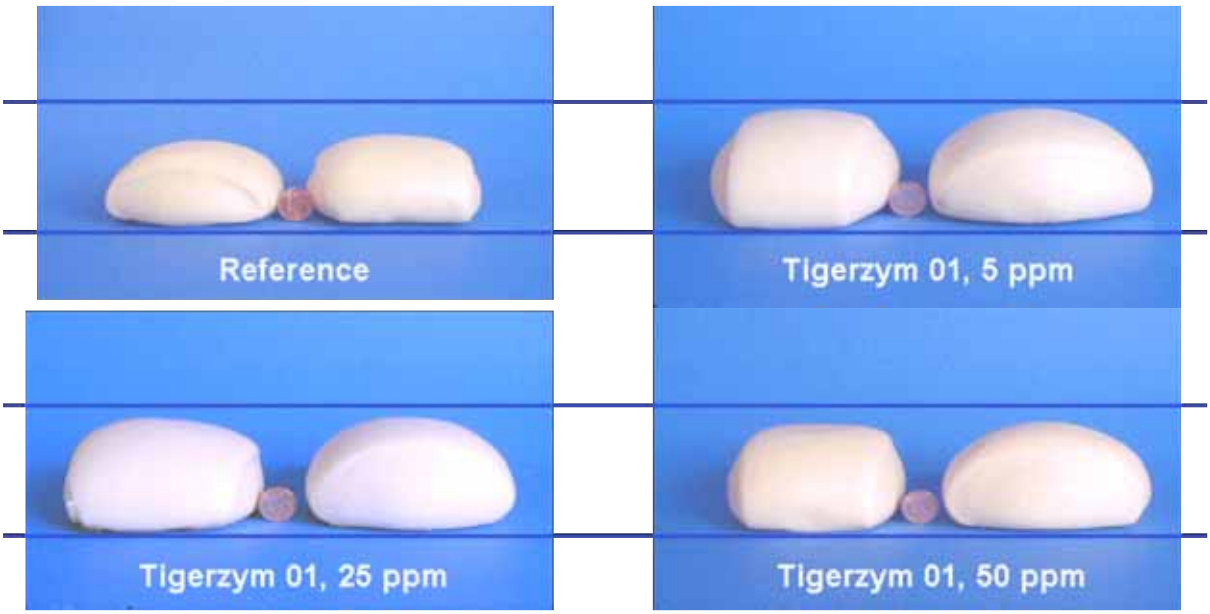

Fig. 12: Effect of an enzyme compound containing lipase (Tigerzym 01) on the size of steamed bread. The volume yield per 100 g of flour was 300, 447, 477 and 512 mL, resp. (from upper left to lower right)

Development of the dough by sheeting favours the beneficial effect of lipase. This is probably due to a more extensive exposure to atmospheric oxygen: lipolyse exposes the fatty acids to the action of wheat lipoxygenase, which – in the presence of sufficient oxygen – is converted into hydroperoxides; these in turn will react with flour components. In addition to dough strengthening, a bleaching effect occurs due to the oxidation of flour carotenoids. Since lipases are specific for the type of fatty acid present in the triglyceride, not all lipases are suitable for the improvement of steamed bread.

Noodles

The effect of lipase is also visible with noodles. Pastazym is a compound based on lipase, but containing a selection of other enzymes as well. The lipase is responsible for the brightening effect shown in Fig. 13 and for most of the firming effect shown in Fig. 14. Both effects are not only detectable in the laboratory, with sophisticated instruments, but also by the consumer (Fig. 15). There are certain limitations, of course. Pasta made from durum wheat only cannot be improved, and the use of eggs also masks the effect of enzymes. The greatest efficacy is achieved in noodles made from hard or soft wheat only. The bleaching effect shown in Fig. 13 and Fig. 15 is not always required, as some consumers prefer yellowish noodles. Nevertheless, it can be useful in this case too, if for instance a speckled or greyish flour is used: the enzyme reduces both the speckles and the dark colour, and therefore provides a bright background for permissible yellow food colorants (Fig. 16).

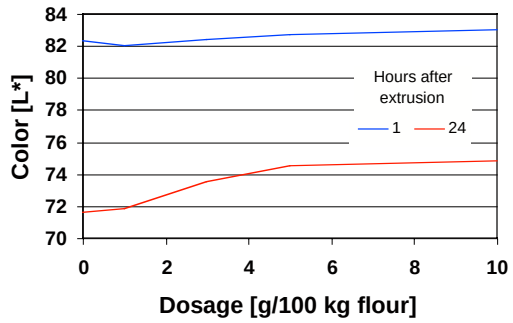

Fig. 13: Effect of Pastazym on the colour of fresh, uncooked noodles (determined with the Minolta colour measuring system)

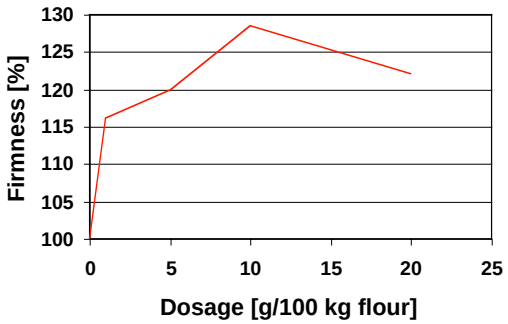

Fig. 14: Firmness of fresh, uncooked noodles as affected by Pastazym (determined with Texture Analyser TA XT2)

Fig. 15: Effect of Pastazym on the colour of fresh, uncooked noodles

Fig. 16: Uncooked noodles, untreated (left) and treated with Pastazym + EMCEcolor BC (ß-carotin)

Replacement of potassium bromate

Less spectacular but probably even more important from a global point of view is the replacement of potassium bromate in bread making processes. This very efficient and cheap bread improver is being banned in more and more countries for health reasons.

Apart from alternative oxidizing agents, using oxidative enzymes was a very early approach to tackling this challenge. Surprisingly, these enzymes turned out to be of limited use. Amylase, xylanases and nowadays lipases proved to be far more efficient if combined with safe oxidizing agents such as ascorbic acid. Oxidases can nevertheless support their function. Fig. 17 shows an early test for replacing bromate in 'no-time' bread making processes. Fig. 18 represents the state of the art, the effect of the market-leading bromate-replacing enzyme compound Alphamalt BX, which can be used for short processes as well, and is most suitable for long fermentation (3 – 24 h).

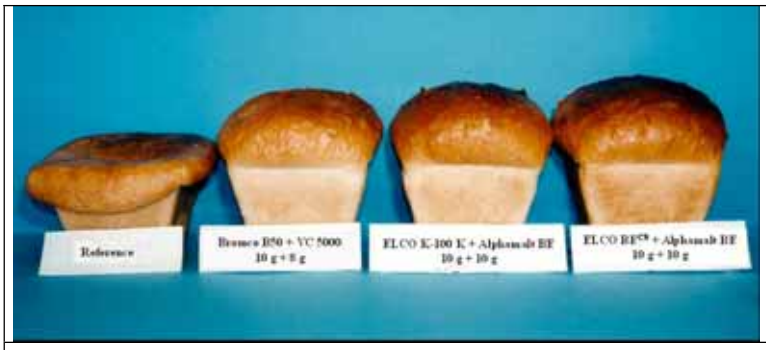

Fig. 17: Replacing potassium bromate in no-time dough. Bromco B50 = 50 % potassium bromate; Alphamalt VC 5000 = fungal α-amylase with 5,000 SKB/g; Alphamalt BE = enzyme compound; ELCO K-100 K = ascorbic acid, 100 %; ELCO BE CS = coated ascorbic acid, 70 % ascorbic acid.

Fig. 18: Replacing potassium bromate in long fermentation. Alphamalt VC 5000 = fungal α-amylase with 5,000 SKB/g; Alphamalt BX = enzyme compound with oxidizing elements.