18.5 Enzymes

Enzymes have been in common use in the food industry for years. In contrast to most other applications in which enzymes find their way into foods, the enzymes in this case do not react at the place where they are added, namely in the mill; they do not take effect until the baker adds water.
This difference in time and place is a great challenge to the flour treatment sector in general, but in the case of enzymes it is an especially complex matter. On the other hand enzymes are highly specific; that is, if they are pure enough they act on selected targets and only have to be added in small quantities. Moreover, they are entirely natural as they can only be obtained from micro-organisms by way of fermentation or from vegetable or animal tissue and fluids by means of extraction (Fig. 116).

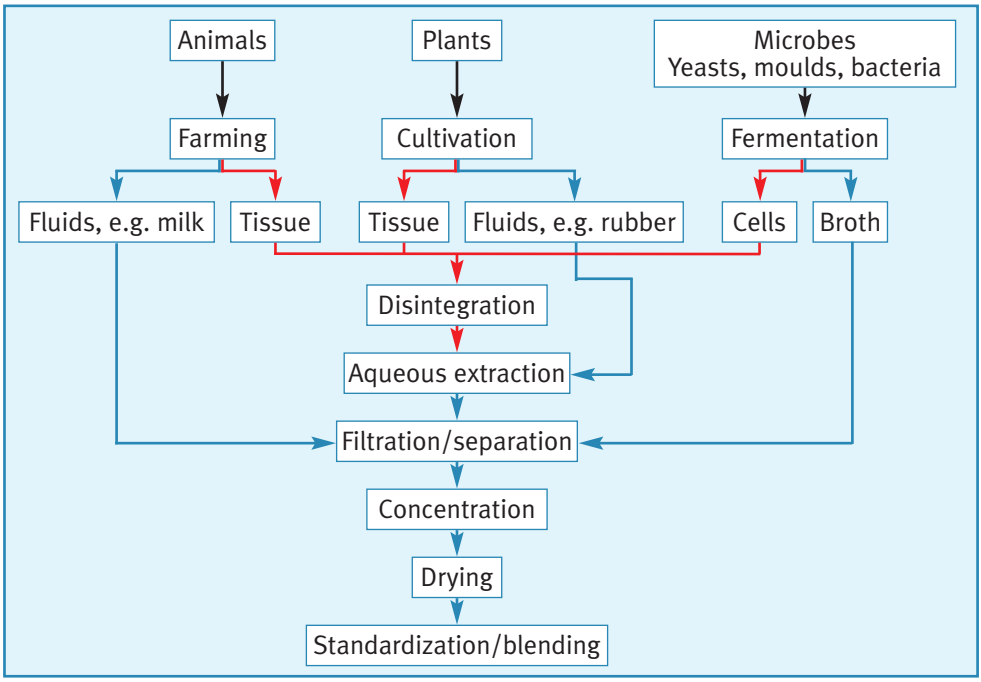

Fig. 116: Diagram showing the principle of enzyme production

Like all highly concentrated natural substances, enzymes have a potential for causing allergies when inhaled by workers; cases have been well documented. For this reason care must be taken during processing to reduce dust formation and exposure to dust. The use of protective masks and gloves is recommended for handling concentrated enzymes at the flour mill.
The baker only handles enzymes in a very diluted form, either in the flour or in bread improvers. About 10 ppm pure enzymes are added to flour. Bread improvers typically contain 1,000 to 10,000 ppm enzyme protein, which makes them more risky for the baker.
Enzyme producers are aware of the problem and offer enzyme preparations with reduced dust formation.
The flour itself still accounts for most of the bakers' allergies. It is also interesting to note that many enzyme allergies come about through the baker's contact with moulds, e.g. on contaminated tools or bakery walls.

Tab. 89: The amylase family

IUBMB[a] no.	Common name
EC 3.2.1.1	α-amylase
EC 3.2.1.2	β-amylase
EC 3.2.1.3	glucan 1,4-α-glucosidase
EC 3.2.1.10	oligo-1,6-glucosidase (isomaltase)
EC 3.2.1.41	pullulanase (inlcuding amylopectin 6-glucanohydrolase)
EC 3.2.1.54	cyclomaltodextrinase
EC 3.2.1.57	isopullulanase
EC 3.2.1.60	glucan 1,4-α-maltotetraohydrolase
EC 3.2.1.68	isoamylase
EC 3.2.1.94	glucan 1,6-α-isomaltosidase
EC 3.2.1.98	glucan 1,4-α-maltohexaosidase
EC 3.2.1.116	glucan 1,4-α-maltotriohydrolase
EC 3.2.1.133	glucan 1,4-α-maltohydrolase
EC 3.2.1.135	neopullulanase
EC 3.2.1.142	limit dextrinase
EC 2.4.1.19	cyclomaltodextrin glucanotransferase

[a] *Union of Biochemistry and Molecular Biology*

18.5.1 Amylases

The amylase family has many members (Tab. 89). In baking, the amylolytic enzymes most often used are α-amylase, β-amylase and amyloglucosidase (glucan 1,4-α-glucosidase).

α-Amylase splits linear, unbranched sections of the starch molecule into smaller components (Fig. 117). Like most enzymes, amylase only acts on dissolved substrate, i.e. swollen, damaged starch in the dough. The short-chain dextrins formed by the action of α-amylase serve as a substrate for β-amylase or amyloglucosidase; these in turn split off sugar (maltose, glucose) that can be used by the yeast. This chain of reactions reduces the dough viscosity, increases the fermenting power and thus the volume yield, enhances flavour and browning and prolongs the shelf-life (of the crumb softness).

Enzyme-Active Malt Flour

Like all other living material, grain needs enzymes for its vital functions. As it does not come back to life, so to speak, until germination, this is the phase when enzymes are produced in large quantities. Bakers have long put this characteristic to use by germinating cereals before processing them further.
Malt flour is the dried product made from germinated barley, wheat or rye. The functions of the three of them are largely identical.

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Malt flour contains primarily α- and β-amylase, but it also contains protease, glucanase and many other enzymes. Some of these may have a positive effect on the baking process (amylases and glucanases), but some can also cause damage (proteases). Like the flour's own amylases, the amylase of the malt flour has a pronounced effect on the Falling Number. If this is very high (i.e., the flour's own enzymatic activity is very low), anything up to 150 g or more of malt flour to 100 kg of flour may be needed to bring the Falling Number into the range of 250 - 300 s. With Falling Numbers around 300 s, no more than 50 g should be added, or the doughs will become too sticky. The activity of malt flours is often expressed in DP for diastatic power (or DU for diastatic units)

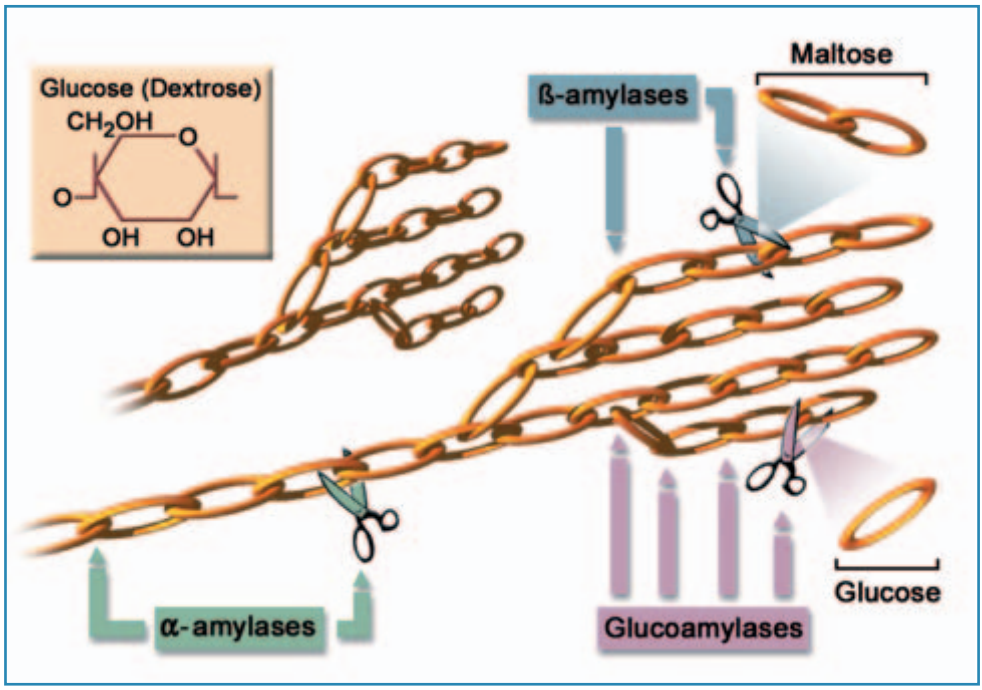

Fig. 117: Simplified representation of the action of amylases on amylopectin

and is usually about 400 DP. Occasionally it is stated in SKB/g (see below) and is in the range of 80 - 120.

Fungal Amylase

Moulds of the genus *Aspergillus* are often used in the production of enzyme preparations for applications in food as this genus includes numerous well-described strains that do not produce any toxins as by-products which might find their way into the finished product. In large fermentation equipment the moulds are made to produce amylase and give it off into their environment (the culture medium) as far as possible. A multi-stage purification and separation process (including centrifugation, precipitation, filtration and ultrafiltration; Fig. 118) then results in a crude enzyme concentrate that is usually spray-dried to form a powder with a good shelf-life. Various carriers – mainly maltodextrin, starch or flour – are added to make the substance more convenient to use at the mill with regard to dosing and flow properties.

Fungal amylase is usually α-amylase. Most side activities can be eliminated by strain selection and in the production process. In contrast to the cereal amylase in malt flour, it has only a very slight effect on the Falling Number since it reacts more sensitively than cereal amylase to the high temperatures at which the Falling Number is determined and is inactivated before it can break down the starch gel. Fungal amylase can be detected with a modified Falling Number method at a lower maximum temperature.

The dosage of α-amylase depends on its concentration, or more precisely its activity. The usual international unit is SKB per gram, named after Sandstedt, Kneen and Blish who developed the determination method (Sandstedt *et al.*, 1939). Many manufacturers do in fact use the units from their own assays, but they can usually express them in terms of SKB/g if wished. A typical dose for a wheat flour that is neither sprout-damaged nor treated with malt flour is 500 SKB per kg flour (i.e. 10 g

of an amylase with 5,000 SKB/g to 100 kg of flour). But even in the case of flours with a very low Falling Number it is sometimes useful to add small amounts of fungal amylase (1 - 2 g at 5,000 SKB/g) as this slightly improves the properties of the dough and results of the baking process without affecting the Falling Numbers. Values above 400 s indicate a low endogenous enzyme content of the flour requiring a higher dosage of fungal amylase, e.g. 30 g of a 5,000 SKB preparation or even more.

Amyloglucosidase

Amyloglucosidase (AMG; also called gluco-amylase, sometimes referred to as γ-amylase in the past) is a natural side activity of many amylase preparations, but it can also be obtained in a purer form from specialised *Aspergillus* strains. AMG breaks down starch into its smallest sub-units, namely glucose, and in contrast to α-amylase it does not stop at the branching points of amylopectin.
However, it would take a very long time to reduce viscosity through the effect of AMG alone, as the enzyme only acts on the starch from one end (chemically called the reducing end) and only splits off a single glucose molecule at a time. This means that the main significance of AMG lies in browning and in maintaining the fermentation process over an extended period (controlled fermentation). As it always occurs in conjunction with α-amylase, AMG is usually dosed in very small amounts (less than 0.1 g to 100 kg), unless the purpose of its use is to replace sugar.

18.5.2 Hemicellulase, Pentosanase and Xylanase

Wheat flour with an ash content of about 0.5% contains about 2.5% pentosans (typical rye flour about 7%) that can bind up to ten times their weight of water. These pentosans belong to the category of the hemicelluloses, "relatives" of cellulose (Fig. 119), and are made up of different sugar molecules (including glucose, xylose and arabinose). The prevailing polymer consists of a xylose backbone with arabinose side chains (Fig. 120) and is therefore called xylan or arabinoxylan, and the enzymes accordingly xylanases or arabinoxylanases, or – less specifically – pentosanases.

Approximately one third of these pentosans are soluble in water, while two thirds are larger molecules that are water-insoluble.

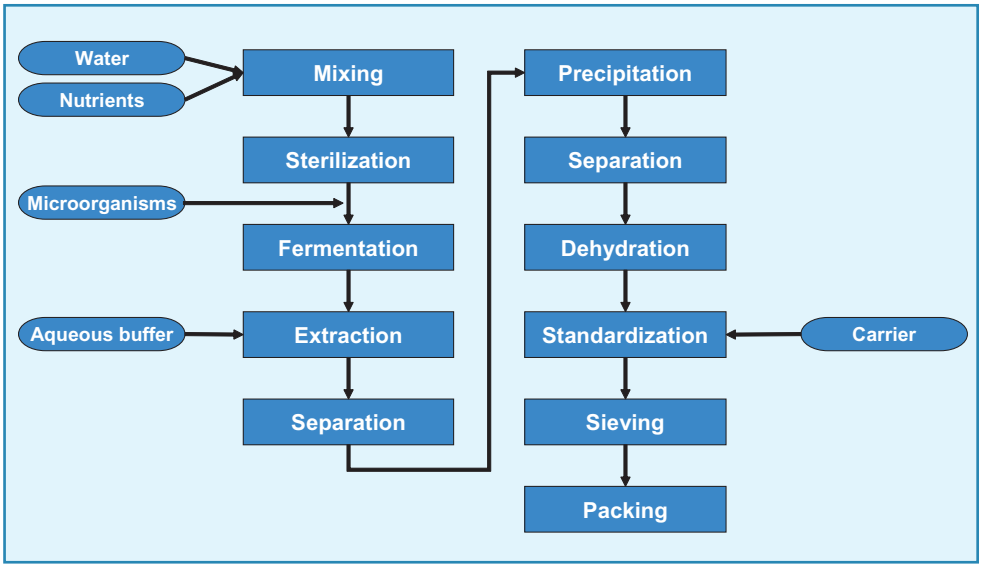

Fig. 118: Flow diagram of microbial enzyme production

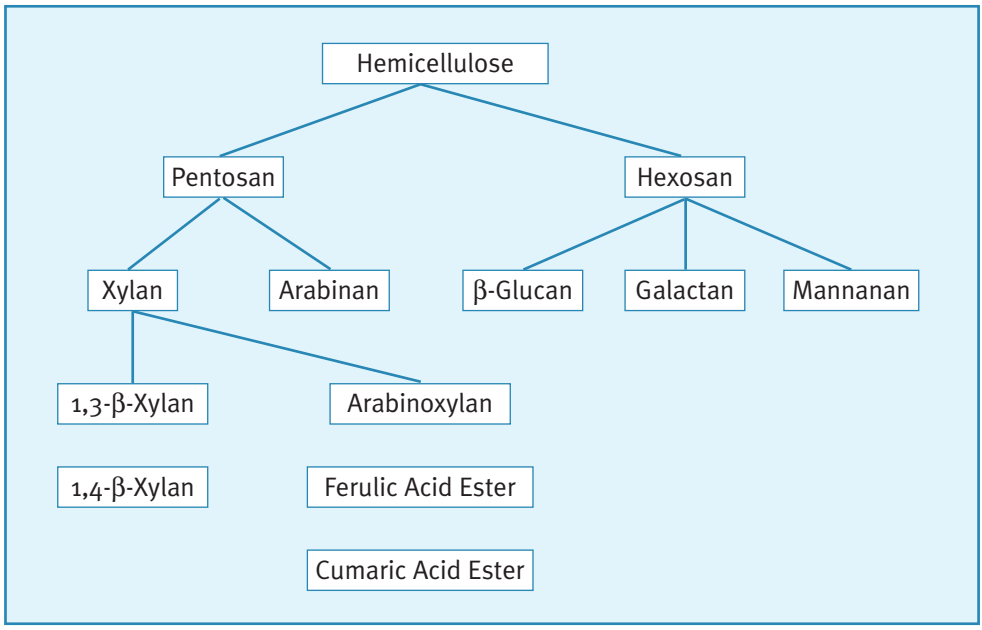

Fig. 119: Hemicelluloses

Xylanases break these substances down. This process initially leads to the formation of more soluble molecules from the water-insoluble pentosan, and this increases the binding of water and thus viscosity (Fig. 140, page 248). These molecules are broken down still further as the process continues; water is released and the viscosity reduced.

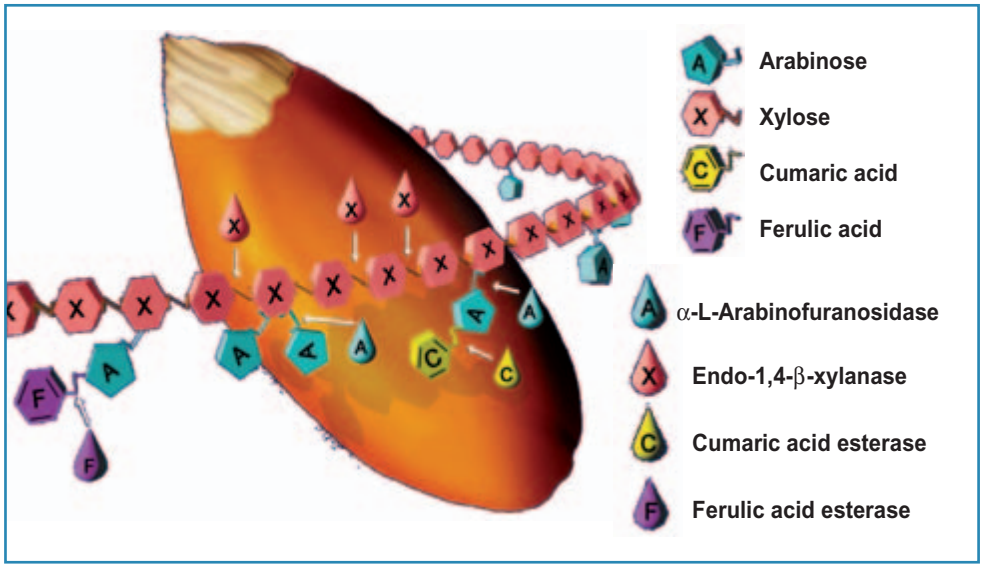

Fig. 120: Enzymatic hydrolysis sites in wheat xylan

It is assumed that pentosans form a network with gluten (e.g., Hoseney and Faubion, 1981), for instance by crosslinking via ferulic acid residues on the xylan molecule (Fig. 121); the more pentosans are involved, the firmer is the network. This is one reason why darker wheat flours and mixtures containing rye flour have a lower volume yield.

The volume yield can be increased considerably by adding hemicellulases which are only present in minor amounts in flour. The beneficial effect of hemicellulases (an enzyme family comprising pentosanases, xylanases and other enzymes acting on hemicelluloses) on dough properties and the volume yield of baked bread was discovered about two decades ago. Xylanases are now probably the most important "volume enzymes" for baked goods. Xylanases differ largely in respect of their specificity towards the arabinoxylan molecule. Proper selection will result in dryer or stickier dough surfaces, less or more water absorption, softer or stiffer dough, finer or coarser crumb structure, and – last but not least – a larger volume yield.

These have only a limited effect on the Falling Number, but their activity can sometimes be recognised very clearly in the Amylogram (lower gelatinization temperature and maximum viscosity) and also in the Alveogram where some hemicellulases cause a change in the curve similar to that produced by cysteine but without any breakdown of the protein (Fig. 122). It should be mentioned that the softening effect is the sum of the loosening of the pentosan-protein network and the release of water from the pentosan gel, which makes the water available for further hydration of the gluten and hence softening.

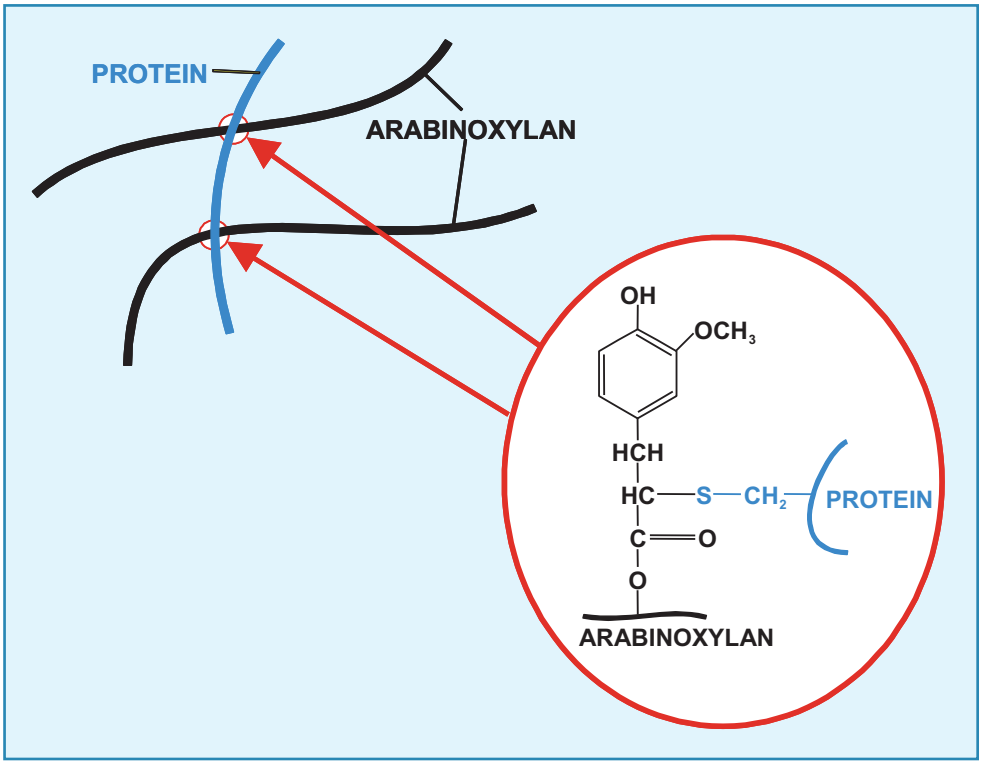

Fig. 121: Cross-linking of gluten and hemicellulose (modified from Hoseney and Faubion, 1981)

Many of these enzymes are also derived from *Aspergillus* species, just like fungal amylases, but these are strains that have been selected for or specialised in the production of hemicellulases. More recently, bacterial strains, either conventional or GMM, have been developed to produce very specific xylanases with excellent baking properties. A gene of a new type of xylanase has been obtained from algae and transferred into a bacterial production strain. The xylanase from this organism has a very low affinity to cereal xylanase inhibitors, e.g. TAXI (*Triticum aestivum* xylanase inhibitor). Hence, its effect should not be influenced by natural fluctuation of the inhibitor level, and results will be more uniform in different wheat lots (Gebruers *et al*., 2002; Frisbæk, 2003).

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 the points at which hemicellulases of different origin attack the pentosan molecules are too various.

18.5.3 Protease

Protease (also known as proteinase or peptidase) splits the protein strands of the gluten molecule (Fig. 123) and thus leads first to a softening and then to a complete collapse of the structure. Sometimes a rather surprising initial increase in viscosity or dough stability is observed. Although the causes are not clear, this may be due to improved water solubility at an early stage of hydrolysis when the main structure is still intact.

With short gluten structures a slight softening may well be desirable; in this case it has a similar significance to the use of cysteine. But unlike the amino acid, protease does not stop acting when the additive is used up. As a result, its effects increase 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. This fear may be exaggerated, at least if purified, single proteases are available: a single protease only acts on a few specific amino acid pairs (peptide bonds). Since there are 20 different amino acids, there are many combinations that cannot be hydrolysed by one particular protease. So the reaction will stop once all the suitable peptide bonds have been cut.

The use of proteases is less of a problem with flours that are rich in gluten. They are therefore used in bread improvers for burger buns or

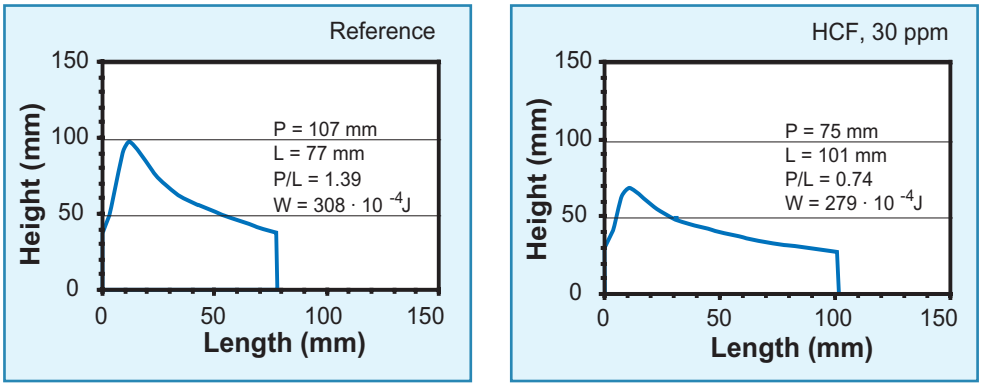

Fig. 122: Effect of a xylanase from Trichoderma *ssp. on the Alveogram. Left: no enzyme added; right: with 30 ppm xylanase on flour*

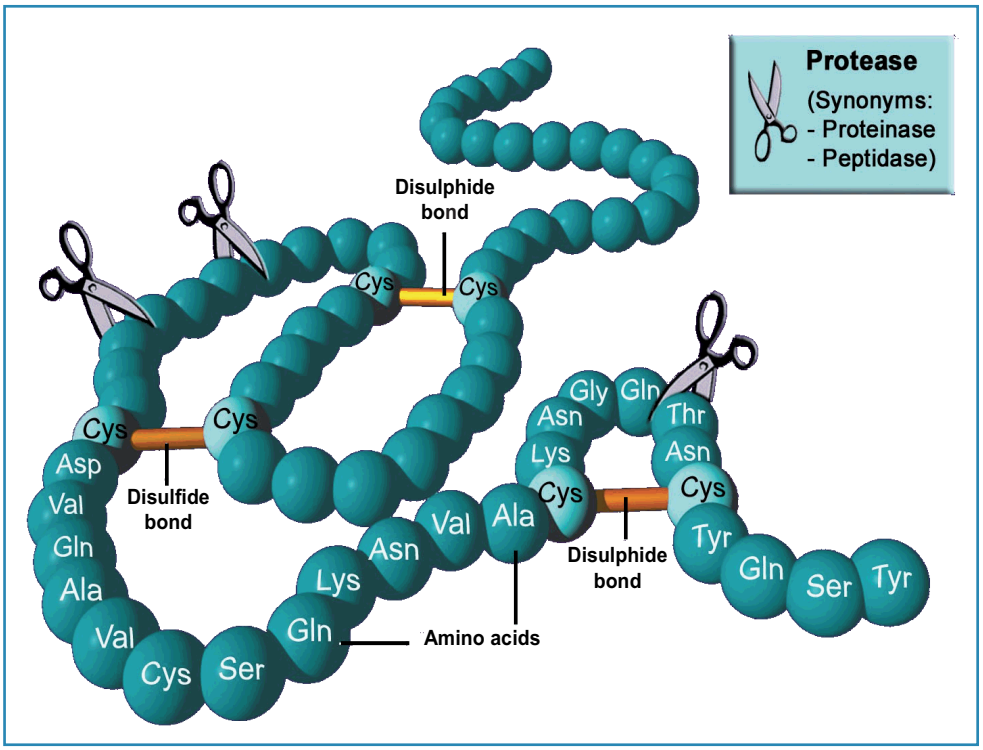

Fig. 123: Simplified representation of proteolytic enzymes attacking a protein molecule

toast bread made from strong flour to achieve a smooth dough structure. Furthermore, proteases are very useful in the production of cracker, biscuit or wafer flours where elasticity of the gluten is undesirable but extensibility a prerequisite for proper processing.

18.5.4 Lipolytic Enzymes

55 There are also frequent references to lipase, phospholipases and galactolipases. These enzymes split non-polar triglycerides present in flour (Tab. 90) into mono- and diglycerides and polar phospho- and galactolipids into more hydrophilic, i.e. more water-soluble lyso-forms (Fig. 124 - Fig. 126).

Theoretically, this should lead to the *in situ* formation of emulsifiers with well-known effects. Interestingly, the amount of emulsifier formed *in situ* does not explain the positive effects on dough properties and baking behaviour. Presumably, the lipids affected by

Tab. 90: Lipids in wheat flour [a]

Lipid	Concentration [b] mg/100 g
Total lipids	1,280
Non-polar lipids	457
Polar lipids	823
Phosphatides (lecithin)	250
Phosphatidyl acid	30
Phosphatidyl glycerol	51
Phosphatidyl choline	27
Phosphatidyl ethanolamine	traces
Phosphatidyl serine	15
Lyso-phosphatidyl choline	117
Lyso-phosphatidyl ethanolamine	10
Galactolipids	249
Other polar lipids	320

[a] *0.405% ash* [b] *14% moisture basis*

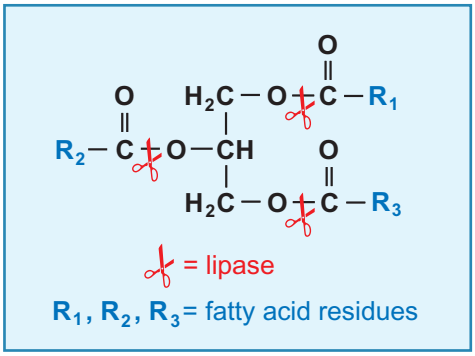

Fig. 124: Action of lipase on fat (triglyceride) molecules

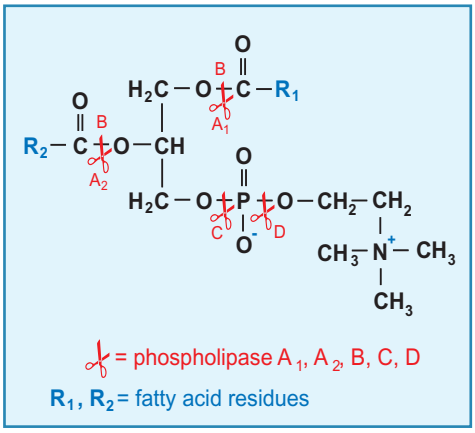

Fig. 125: Action of phospholipases on lecithin (phosphatidyl choline)

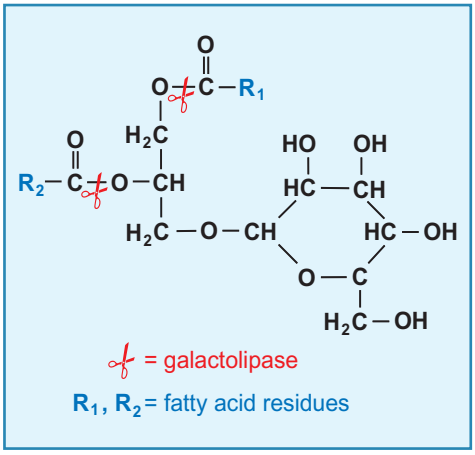

Fig. 126: Action of galactolipase on monogalactosyl-diglyceride

lipase are already located "in the right positions" (close to protein), at least once the dough has been prepared, where they are more effective than emulsifiers added to the flour. Surprisingly, no effect on crumb staling has be detected so far. In contrast to the above, the new molecules are possibly "in the wrong place" to prevent starch retrogradation, or the concentration is simply insufficient. The efficacy of monoglycerides in retarding staling is well-documented, but the typical dosage is 0.1 to 1% on flour.

Unfortunately, there is the problem of a possible impairment of taste due to the release of fatty acids. The author has occasionally noticed a rather cheesy smell in his baking laboratory, particularly when lipases were being tested in frozen butter croissants. For flour mills this is an obstacle because they do not have full control over the recipes used by their customers, the bakers.

18.5.5 Transglutaminase

Transglutaminase (TG) is able to crosslink protein chains either with adjacent loops of the same protein or with other proteins of the same or a different type. It requires lysine and glutamine groups, as shown in Fig. 143 (page 251), as a target. Although lysine is a limited amino acid in wheat flour, there is sufficient lysine for the action of TG to take place. The result is a strengthening effect on the dough, similar to that of AA, as shown in the Extensogram (Fig. 144, page 251).
Since it is much more expensive than AA, TG is little-used. Its main use is in prolonged or retarded fermentation, when the enzyme has enough time to achieve its function even at a low dosage.
Bauer *et al.* (2003) provide more detailed information on the function of TG.

18.5.6 Other Enzymes

Cellulase and β-glucanase are present as natural side activities in many enzyme preparations, e.g. xylanases. They do have some effect on the water-binding behaviour of dough, particularly from whole meal or rye flour containing larger amounts of fibrous

components than standard bread flour (see also chapter 18.13.2, Rye and High-Fibre Flour). But their potential for improving the baking properties of white wheat flour, e.g. dough stability or volume yield, is only limited and negligible in comparison with other enzymes.

Glucose oxidase is often mentioned and has already been described above under oxidation (chapter 18.3.3, page 223). Glucose oxidase was the hope of many who wanted to omit potassium bromate or other oxidizing agents. A similar enzyme has recently been launched: hexose oxidase. The enzyme may be regarded as a glucose oxidase with less specificity, as it not only oxidizes glucose – which is a hexose, i.e. a sugar molecule with 6 (greek: *hexa*) carbon atoms – but also other hexoses such as galactose which can be found in flour in smaller amounts. So the effect does not differ significantly from glucose oxidase.

Arabinofuranosidase and sulfhydryl oxidase (Fig. 127) have also been tested for their possible suitability as flour improvers. So far they have not found wide distribution because of high costs or the lack of obvious benefits as compared to more common enzymes or ascorbic acid.

The development of microbial lipoxygenase as an alternative to the enzyme in soy and bean flour is a further highly interesting topic. Initial approaches failed because of the unsuitable pH optimum of the microbial enzyme and presumably the fact that it is not type II or III lipoxygenase. Only those are capable of oxidizing lipid-bound fatty acids which subsequently bleach lutein, the main carotenoid in the flour (see also chapter 18.3.2, Enzyme-Active Soy Flour).

Polymer-producing enzymes such as alternan sucrase or dextran sucrase produce hydrocolloids during the fermentation process. This results in increased water absorption and dough stability (Tab. 91) *(Popper, 2002)*.

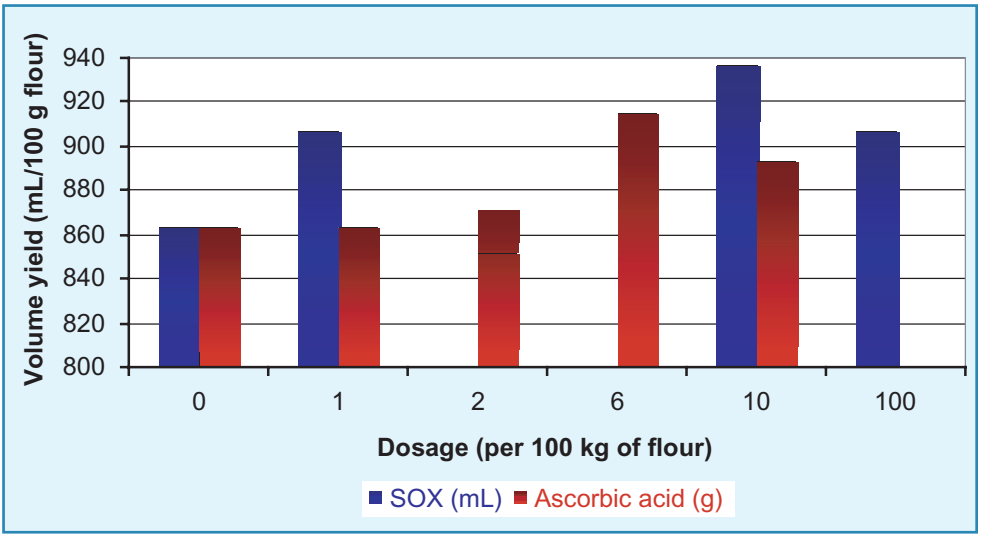

Fig. 127: Steamed bread baking trials with sulfhydryl oxidase (SOX) in comparison with ascorbic acid