18.11 Improvement of Crumb Softness and Shelf-Life

The freshness of stored wrapped baked goods is judged by appearance, taste, crust crispiness and primarily crumb softness. Since freshness correlates well with the compressibility of the crumb, it can be measured by rather simple mechanical devices. The lower the resistance to compression, the higher the freshness will be rated. Crumb softness depends on the volume of the baked product: The higher the volume, the less material is available to form the cell walls of the pores, so the resistance to compression decreases. Furthermore, structural changes taking place during storage result in an increased crumb firmness. Their main cause is retrogradation – or re-crystallization – of the starch, mainly of its amylopectin moiety, that was previously gelatinized in the baking process. Whereas the smaller amylose molecules migrate from the starch granule into the environment and re-crystallize soon after baking, the large amylopectin molecules remain in their amorphous form longer. In the amorphous (i.e. gelatinized) form, amylose and the linear parts of amylopectin form α-helices that are able to bind non-polar molecules. Complexes with iodine show the typical blue colour, proving that the environment – the inside of the helix – is non-polar (in polar solvents such as water, iodine is brown).
An involvement of protein in staling has been propounded by some authors, but so far it has not been confirmed by others.

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The role of water is not yet fully understood. According to one model, the water interferes with the formation of starch-gluten complexes which contribute to crumb hardening. This may happen in analogy to emulsifiers that attach to protein with their polar end, pointing their hydrophobic end to the outside, and thus prevent interaction with the polar starch molecules. Although the drying out of bread is not the cause of staling, bread containing a higher level of water stales more slowly (Bechtel *et al.*, 1953). Moisture migration plays an important role, as wrapped bread without a dry crust keeps fresh longer. Several authors have suggested that water is transported from starch to protein or vice versa, but there seems to be no final conclusion so far. A recent review on bread staling concludes that due to the entrapment of water in the re-crystallized amylopectin crystallites, the moisture migrates from the protein to the starch, which also results in modification of the gluten network (Gray and Bemiller, 2003).

The following phenomena can be perceived by the consumer as physical and sensory changes, and are referred to as the staling of baked goods:

- Shrinkage and formation of wrinkles
- Loss of crustiness
- Loss of crumb softness
- Increased opacity of the crumb
- Increased crumbling
- Reduced flavour
- Change in mouth-feel (firmer, dryer, shorter bite)

A comprehensive description of the changes that take place during the storage of baked goods is given by Zobel and Kulp (1996).

Flour additives are able to reduce the rate of staling by (a) degradation of or (b) interaction with the starch fractions involved, or (c) increasing availability of water.

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18.11.1 Enzymes

α-Amylase

One option for retarding the staling of baked goods is the application of enzymes that affect the starch fraction responsible for staling. If the crystalline regions of the amylopectin can be broken down or the amorphous amylopectin can be prevented in some other way from re-crystallization after baking, staling can be decelerated. α-amylase is able to attack amylose and amylopectin in the middle of the molecule, breaking them down first to smaller fractions and finally to short dextrins and branched limit-dextrin. The current theory of the anti-staling effects of amylases states

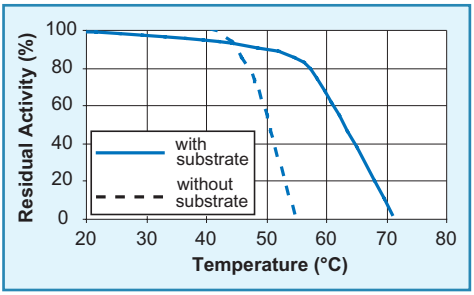

Fig. 149: Temperature-stability curve for fungal α-amylase from Aspergillus oryzae

that a) the smaller fractions of amylose and amylopectin have a lower tendency to crystallize and b) short dextrins interfere with other regions of the starch molecule in such a way that crystallization is prevented to some extent.

The native starch present in flour only hydrates to a limited extent during dough preparation since most of it is enclosed in compact starch granules. Only the starch in granules damaged by the milling process is accessible to water and can therefore swell in the relatively cold environment of a dough system.
Since most amylases can only act on hydrated starch, the effect of these enzymes during dough preparation and fermentation is rather limited, and so is any effect on staling.

Fungal α-Amylase

Gelatinization of wheat starch occurs between 62 and 75 °C (Pomeranz, 1984) provided that sufficient water is available. It is characterized by an opening of the dense starch granules followed by water uptake, which makes starch a good substrate for amylases. Unfortunately, standard fungal α-amylases do not survive the increasing temperature for long enough to be effective in hydrolyzing gelatinized starch: Their stability curve shows a steep decline above 55 °C (Fig. 149), even though starch as the substrate has a stabilizing function on the enzyme.

A new alternative is a novel amylase from a non-GMO fungus, a *Rhizopus oryzae* strain. In contrast to other fungal amylases, this enzyme (***Softase Six*** in Fig. 150) is able to partially hydrolyze even non-gelatinized starch and thus reduce the tendency towards re-crystallization. The effect on crumb softness is somewhere between that of one of the best monoglycerides for this purpose (glycerol monostearate) and a very efficient GMO amylase.

Cereal α-Amylase

Cereals contain two different amylases, α-amylase and β-amylase. The α-amylase contributes to anti-staling because it has a

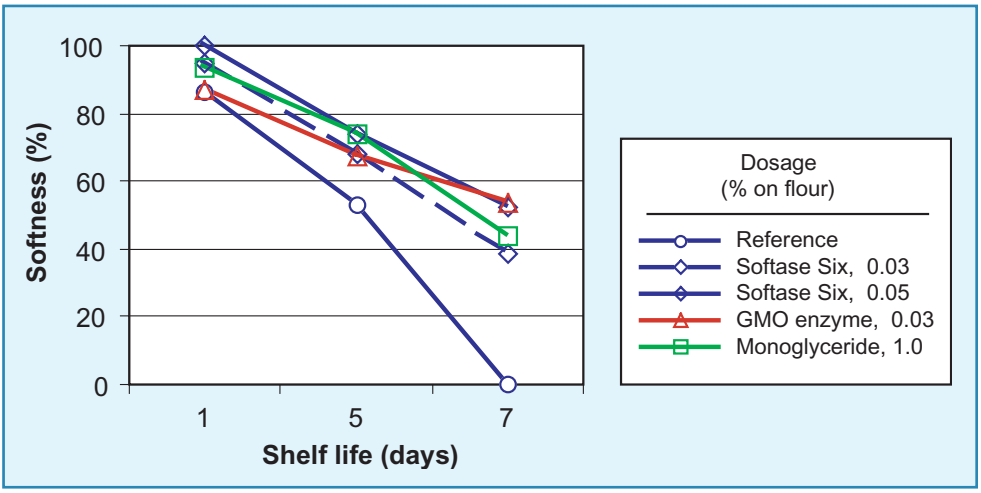

Fig. 150: Effect of enzymes and glycerol monostearate on the crumb softness of toast bread

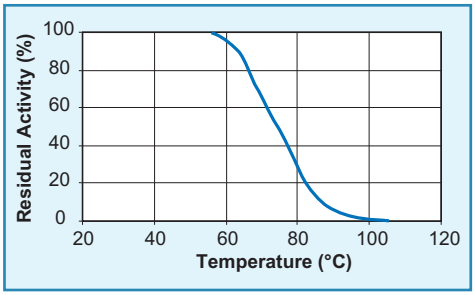

Fig. 151: Temperature-stability curve for cereal α-amylase

higher heat stability than fungal amylases (Fig. 151). It will therefore still be active when at least parts of the starch are already gelatinized. Nevertheless, the final baking temperature will be sufficient to completely inactivate the enzyme.

Cereal β-Amylase

According to Würsch and Gumy (1994), β-amylase has some power to inhibit amylopectin retrogradation. The enzyme reduces the length of external chains (also called A chains) within the molecule which are responsible for the formation of crystalline structures, together with parts of the B chains.

Bacterial α-Amylase

Bacillus species are not the only micro-organisms able to produce bacterial amylases with high heat resistance for the cereal industry, but they are the ones most commonly used for this purpose. One example of still relatively low heat resistance is shown in Fig. 152. Since some activity still remains even after 20 min at 95 °C, not only the desirable anti-staling effect through degradation of the starch molecules is obtained, but also a continuing liquefaction of the crumb upon storage. Nonetheless, bacterial α-amylases of similar types are still being used to improve crumb softness, but only combined with fungal α-amylases and at very low concentrations to avoid crumb liquefaction.

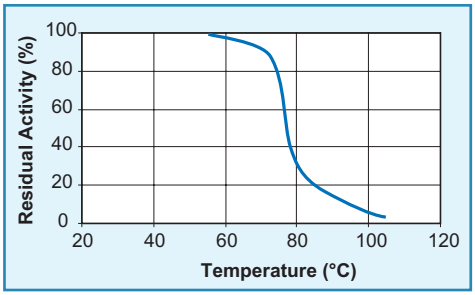

Fig. 152: Temperature-stability curve for bacterial α-amylase from Bacillus subtilis

Amylases from Genetically Modified Organisms

Amylases of intermediate heat stability are most suitable for prolonging crumb softness. They can be obtained by genetic modification of conventional micro-organisms (e.g. Diderichsen and Christiansen, 1986). The ability of some of these enzymes to create short-chain maltodextrins seems to be advantageous (Min *et al.*, 1998) and may be due to the same mechanism as for β-amylases. Kragh *et al.* (1999) claim that a non-maltogenic exo-amylase is also capable of retarding retrogradation, and this may also be due to the removal of A chains from the B chains.

Hemicellulases

Hemicellulases are able to break down the pentosans in flour. There are two pentosan fractions: one is water-soluble and the other is soluble only in a mildly alkaline environment. Conversion of the insoluble fraction results in soluble pentosan able to form gels with high water-binding capacity. Hydrolysis of the soluble fraction releases water. While increasing the water-binding capacity results in dryer dough, reducing it improves the formation of the gluten network because of the availability of water. Furthermore, pentosans are able to form covalent linkages to the gluten protein, thus increasing the strength of the gluten network. With pentosanases (which belong to the hemicellulase family) the rigidity of this network can be optimized for a maximum volume yield.

Hemicellulases are now probably the most important baking enzymes for obtaining a large volume yield. Originally only a scarcely noticed side-activity of certain enzyme preparations, they can now be obtained commercially in a

purified and standardized form. Generally speaking, bread with a large volume also has a softer crumb structure, so any staling process already starts at a higher softness level. Even if the staling rate (the slope of the softness curve) is not affected, the final softness will be superior to that of bread with lower volume.

Furthermore, some hemicellulases result in a finer crumb structure due to the optimization of the gluten structure. More pores per volume mean a large surface area. The cell walls are therefore thinner and result in a lower resistance to compression, i.e. better softness.

In 1992, Yin and Walker reported a significant decrease in the crumb firming rate during storage after the addition of pentosans obtained by commercial gluten separation. Later, in 1995, Van Eijk and Hille found in bread a correlation between pentosanase treatment and free pentosan, probably released from the pentosan-gluten network. An effect of pentosanases on crumb softness therefore appears likely.

So far there seem to be no reports on the contribution of the altered water absorption capacity of the pentosan to shelf-life, although in theory, at least, an increased availability of water due to the action of the enzymes should also decrease the staling rate.

Lipases

Lipase converts lipids already present in the flour, or added lipids, into free fatty acids and di- or monoglycerides. Fatty acids, especially long-chain saturated fatty acids, are able to interfere with the starch molecule and retard staling, as do mono- and diglycerides (see below). But most probably because of the close relationship between the flour proteins and lipids, the hydrolyzed lipids (di- and monoglycerides) interact not with the starch but primarily with the gluten. No improvement of the shelf-life can therefore be achieved by using lipases. (See also chapter 18.5.4, page 236 on lipolytic enzymes.)

Lipoxygenases

Improved crumb softness has also been reported for lipoxygenases (e.g. from soybeans). A complex model by Chung and Pomeranz (1977) suggests that free lipids are released from gluten-lipid complexes, which might then interfere with starch. In another approach the crumb softening is explained by the oxidation of lipids, as saturated lipids have improved anti-staling properties.

Other Enzymes

Many other enzymes have been tested for their effect on bread staling. Only recently, phospholipase has been claimed to reduce the staling tendency by forming lyso-phospholipids (see below) from endogenous or added phospholipids. Pullulanase, a debranching enzyme of the amylase family, attacks the α-1,6-branching sites in amylopectin (which α- and β-amylase cannot). This is also said to reduce the re-crystallization potential, although some authors have not found any positive effect (e.g. Si, 1995). On the other hand, a branching enzyme, i.e. an enzyme capable of building up larger, branched molecules by attaching glucose subunits to an existing fragment, may also be able to alter the structure of starch in such a way that organized re-crystallization becomes impossible.

As far as the author knows, none of these enzymes is actually being used in anti-staling enzyme compounds.

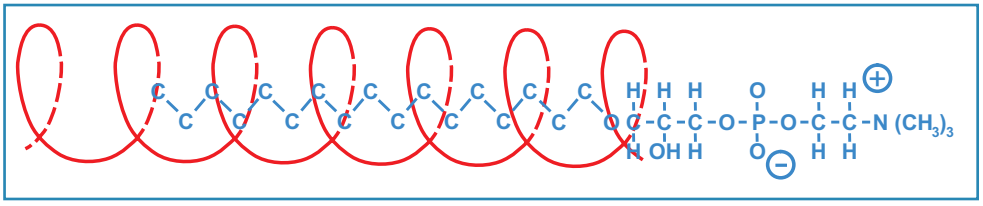

Fig. 153: Phospholipid-amylose inclusion complex as an example of the interaction between helical starch structures and emulsifiers

18.11.2 Emulsifiers

Emulsifiers modify the gelatinization and re-crystallization behaviour of starch due to their interaction with amylose and the helical regions of amylopectin (Fig. 153). They inhibit the re-crystallization of amylopectin and the formation of an inter-granular starch matrix by amylose. Their efficiency depends on the type of emulsifier. Molecules containing a long and straight non-polar tail are considered to be most suitable (Stauffer, 2000). For flour treatment, only powdered emulsifiers with good free-flowing properties can be used.

Lecithin

Two fatty acids and one phosphoric acid group are linked to a glycerol backbone. The phosphoric acid group can carry different functional residues, predominantly choline, serine, inositol, ethanolamine or hydrogen. The fatty acids constitute the non-polar end of the bipolar molecule, and the phosphatidyl group the polar end. Although the amylose-complexing capacity of native lecithin is inferior to that of some synthetic emulsifiers, including SSL, CSL and monoglycerides (in their alpha-form; see below), this natural emulsifier is recommended as an anti-staling agent because of its positive overall contribution to the baking process and its generally positive image of wellness and health.

Lysolecithin

Phospholipase A2 removes a fatty acid from the second carbon atom of the glycerol backbone of phospholipids, while phospholipase A1 takes away the fatty acid from the first carbon atom. The resulting hydrolysed phospholipids are more polar than the original molecule (improved water solubility) and also more effective in complexing starch (Fig. 154).

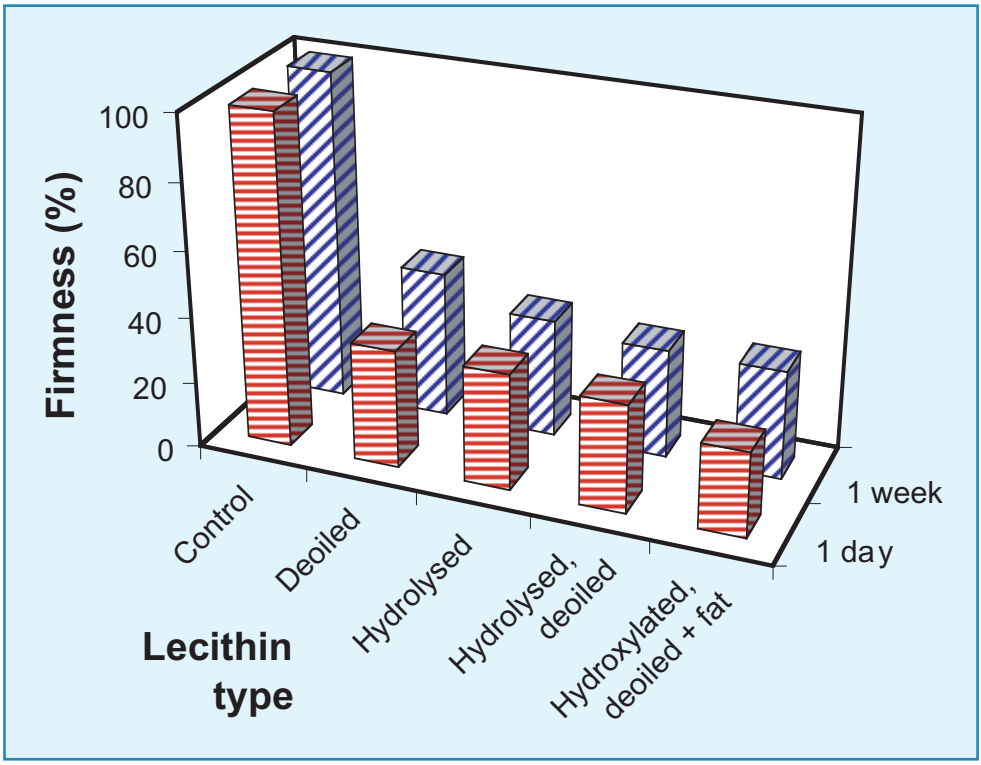

Fig. 154: Effect of hydrolysis on the anti-staling effect of lecithin (modified from Van Nieuwenhuyzen, 1998)

For sterical reasons, lysophospho-lipids with a fatty acid next to the phosphatidyl group are less effective than those with the hydrophobic end farther away from the hydrophilic end, so the latter have superior anti-staling properties. The anti-staling potency increases with the degree of hydrolysis (Van Nieuwenhuyzen, 1999). Sprayed on a carrier or in a de-oiled form, lysolecithin is also suitable for the milling industry.

Hydroxylated Lecithin

Chemical modification by hydroxylation also improves the complex-forming capability of lecithin. It is not clear whether the effect is due to the hydroxyl group added to the molecule or to partial hydrolysis under the applied alkaline conditions (Van Nieuwenhuyzen, 2001).

Mono- and Diglycerides

Fat molecules can be converted into emulsifiers by removing one or two fatty acids from the glycerol backbone. Distilled monoglycerides, especially, are widely used because of their anti-staling effect. Unfortunately, for full function they need to be in their hydrated alpha form. Only limited spontaneous formation of the alpha form occurs when monoglyceride powders are present during dough preparation. Pastes containing monoglycerides are therefore more effective than powders. However, combination with co-emulsifiers such as lecithin results in more complete production of the alpha form even from the powdered state. Spray-dried compounds of monoglycerides and lecithin have been used in the milling industry for several decades. The hydration speed of monoglycerides and thus their efficacy also improves with decreasing particle size of the powdered emulsifier.

SSL and CSL

Sodium stearoyl-2-lactylate (SSL) and calcium stearoyl-2-lactylate (CSL) are among the emulsifiers with the best amylose-complexing properties, only outperformed by some distilled, hydrogenated monoglycerides (e.g. Krog, 1971). They are widely used in bread improver compounds, especially for soft buns, since they impart a soft crust as well as a long-lasting crumb softness. Their use in the milling industry is not very common, partly because they are difficult to handle (tendency to form lumps).

DATEM

Diacetyl tartaric acid esters from mono- and diglycerides, mainly known for their effect of increasing volume and dough tolerance , are also able to complex amylose (Krog, 1971). Since they cause a large increase in volume, it is difficult to say how much of the improved softness is due to the larger volume and how much to inhibited retrogradation. For similar reasons to SSL and CSL, DATEM is used as a bread improver than as a flour treatment agent.

18.11.3 Oxidizing Agents

Oxidizing agents are hardly mentioned in connection with bread staling. The author does not know whether there has been any investigation into the effect of these substances on staling. Nevertheless, there are some simple facts that can be considered.

Potassium bromate, azodicarbonamide, ascorbic acid and the like do have the potential to increase the volume of a baked product if properly used. For reasons mentioned earlier, this improves the crumb softness at the beginning of storage. Even with the staling rate unchanged, the impression at the end of the storage period would be softer.

Furthermore, oxidative gelation of pentosans (Geissmann and Neukom, 1973) and oxidative formation of gluten-pentosan complexes (Hoseney and Faubion, 1981) have been demonstrated; both have the potential to increase the uptake of water, a prerequisite for a long shelf-life. On the other hand reducing agents such as cysteine or sulphite may shorten the shelf-life.

18.11.4 Hydrocolloids

Substances with a large water binding capacity are used in the milling industry to increase the amount of water that can be added to the flour without making the doughs sticky. They are also used in bread improver compounds

for specific applications such as deep frozen dough to absorb water released from cold damaged protein or starch. As we mentioned earlier, bread with a higher water content keeps its softness longer. But there are limits: in some countries the moisture content of bread is restricted, and according to Kulp (1979) there is also an optimum level of water for long-lasting freshness.

Guar gum, locust bean gum, pre-gelatinized waxy starches (rich in amylopectin) and cellulose derivatives such as carboxymethyl cellulose have already been in use for some decades. Pectin, agar agar and alginates, and also hydrocolloids from microbial sources such as xanthan, dextran, curdlan, or gellan have some promising properties too, but they are not used in flour mostly for cost reasons, since their performance/price ratios are lower than those of the thickening agents mentioned above.