Functional oxidation in bread making

This paper explores the effect of glucose oxidase on dough properties for industrial bread making. By the action of glucose oxidase on glucose present in the dough, hydrogen peroxide is produced that induces protein cross-links via disulfide bridges and arabinoxylan cross-links via ferulic acid bridges. The extent of cross-linking in dough depends on the production rate and concentration of hydrogen peroxide. A new glucose oxidase from *Penicillium chrysogenum* shows a self-regulating mechanism that prevents undesirably high levels of hydrogen peroxide being produced. This will create new opportunities for the use of glucose oxidase as a tool for replacing chemical oxidizers (such as ADA or Bromate) or in applications such as frozen dough.

The increased need for functional oxidation

Consumers’ are demanding more from their foods. Desiring a healthier lifestyle remains paramount and we demand greater diversity in our food, but also for it to be natural and authentic. The impact of climate change and population growth also leads us to source our foods in more sustainable ways and distribute it efficiently to ever growing cities and towns. In the bakery industry, this has led to an increased industrialization and scale of operations, usually coupled to a decline of the smaller artisanal or craft establishments. However, we still demand the same standards of freshness and authenticity from the large plant bakeries.

To harness the natural variability of wheat flour, bakers started to use chemical oxidizing agents to strengthen gluten proteins. High speed bakeries, lean formulations and changes to wheat flour quality all led to increased use of chemicals such as Azodicarbonamide, Potassium Bromate and Ascorbic Acid. These chemical oxidants have been highly effective in maximizing the potential from the wheat protein available.

Due to consumer demands for the reduction of chemical additives, and legislative restrictions on their use, glucose oxidase was introduced later on in the 1980s and enzymatic oxidation became commonplace. This offered new benefits and fitted well with consumer demand for healthy products with natural and easy-to-understand ingredient listings.

Challenges to the baker have continued up to the present day, particularly with global urbanization on the rise. We buy our groceries at increasingly consolidated retail channels. Also, we travel more than ever before and buy our baked goods on the way to work and expect to be able to buy them at our travel destinations; we desire familiar, healthy, tasty and high quality bakery products wherever we go. The large industrial bakeries need to produce the same quality, if not better, than the local craft store used to make.

Bakers continue their quest to increase consistency, freshness and naturalness regardless of the quality and availability of wheat. This requires ever more flexible and adaptable ways to strengthen wheat, to modify it to maximize the potential of the flour, whilst offering the process tolerance demanded by the plant bakery. There is consequently an increasing demand for functional oxidation - adaptable oxidative improver systems that can be tailored to meet the challenges of variable wheat quality and standardize product quality, independent of geographical location.

The Gluten Network as key to dough performance

It is generally recognized that the baking quality of wheat flour is related to the amount and properties of gluten proteins. The gluten proteins can be divided into two groups: the monomeric gliadins and the polymeric glutenins, of which there are roughly equal amounts. Gliadins can only form intramolecular cysteine bonds, the glutenins contain cysteine residues that can also form intermolecular bonds, creating a polymeric glutenin structure (Lindsay & Skerritt 1999).

The interactions between the different glutenins and between the gliadins are critical to dough behavior, but they are still not clearly understood at a molecular level. What is clear, however, is the...
important role played by disulfide linkages formed between the gluten proteins, especially the glutenin subunits. The presence and properties of very large glutenin aggregates, known as the glutenin macropolymer (GMP), is important for baking properties and the quality of flour (Don et al. 2003).

In addition to gluten, arabinoxylans also influence dough and bread quality. The water-extractable arabinoxylan molecules increase the viscosity of the dough liquid phase, stabilizing the liquid films surrounding the gas cells. In addition, cross-linking of arabinoxylans may strengthen the gluten network (Decamps et al. 2013). Some authors have described the formation of cross-links between gluten protein tyrosine residues and arabinoxylan ferulic acid residues - albeit at low levels (Wang et al. 2002) - whereas others have not observed this cross-linking, and suggest that gluten proteins and arabinoxylans form separate networks (Labat et al. 2001).

**Different oxidation systems**

Disulfide bridges between gluten proteins are essential for the formation of the gluten network and for the viscoelastic properties of dough. Having an appropriate level of sulfhydryl oxidation is crucial for optimal dough properties (Bonet et al. 2006; Lagrain et al. 2006).

The functionality of the gluten proteins during bread making can be altered by the incorporation of redox agents. These redox agents can be divided into chemical additives and enzymes (Joye et al. 2009). A weak dough can be strengthened by the addition of oxidizing agents, whereas a dough made from for example Italian or Australian flour can be made more extensible by the addition of reducing agents, such as glutathione. Examples of chemical oxidants include, among others, ascorbic acid, potassium bromate and azodicarbonamide (ADA). These differ in how quickly they act; ADA is a fast-acting oxidant, bromate is a slow-acting oxidant and ascorbic acid has an intermediate speed of oxidation.

Another aspect differentiating the oxidants is the effect they have on dough handling. For example, ascorbic acid and to a greater extend calcium peroxide will allow the dough to absorb additional water and will impart a dry surface to the dough. Iodate or ADA might create a slightly opposite effect and leave the dough more pliable and soft. Each of these oxidants have their own right for existence and are not easily inter-changeable.

The use of enzymes as oxidizing agents is an attractive alternative because of regulatory/legislative restrictions and also in view of the current trend towards more natural and easy to understand ingredients on labels. Examples of such enzymes in breadmaking include laccase, tyrosinase, hexose oxidase and glucose oxidase of which glucose oxidase is the most commonly used. It is generally recognized in the bakery industry that glucose oxidase improves dough handling properties and dough stability during bread making. The glucose oxidases currently available in the market are part of a toolbox to replace chemical oxidizers such as ascorbic acid, due to their ability to act fast, allowing the dough to absorb water and create a dry dough surface.

**Glucose oxidase: the working mechanism**

In the presence of molecular oxygen, glucose oxidase catalyzes the oxidation of β-D-glucose, yielding hydrogen peroxide and D-glucono-δ-lactone, which in turn spontaneously hydrolyzes to gluconic acid when water is available. The effect of glucose oxidase on dough properties can be attributed to the hydrogen peroxide formed. The hydrogen peroxide causes the oxidation of free sulfhydryl groups in gluten proteins, leading to the formation of disulfide linkages.

Apart from the effect of glucose oxidase on the formation of disulfide bridges between gluten proteins, the hydrogen peroxide may also effect the arabinoxylans; a phenomenon also known as oxidative gelation (Vemulapalli et al. 1998; Miller & Hoseney 1999). Oxidative gelation is the coupling of two ferulic acid residues of neighbouring arabinoxylans initiated by the production of hydrogen peroxide from glucose by glucose oxidase. The hydrogen peroxide and peroxidase present in the wheat flour produce a radical from ferulic acid. Two of these ferulic acid radicals can be linked. Consequently arabinoxylan chains are linked, leading to increased water-binding capacity. The result is dryer dough.

Since hydrogen peroxide is believed to be responsible for the effect on dough rheology, the amount and rate of hydrogen peroxide production determines the extent of cross-linking. High levels of hydrogen peroxide, especially during the mixing phase, might lead to a decrease in the size of the gluten aggregates formed rather than the formation of an extended gluten network. Mechanical shear during mixing of the dough will break
some of the newly-formed disulfide bridges, leaving fewer SH groups to form linkages at a later stage building a less extensive gluten network. These smaller aggregates will be less effective in stabilizing the dough structure than the gluten aggregates formed when lower levels of hydrogen peroxide are present (Decamps et al. 2014).

Introducing a new glucose oxidase

The use of a glucose oxidase during the dough making process is well known within the bakery industry. Most common glucose oxidases on the market are originating from Aspergillus sp. Recently, DSM developed a new glucose oxidase, BakeZyme® Go Pure, originating from Penicillium chrysogenum. Biochemical analyses of BakeZyme® Go Pure have shown that this enzyme exhibits a self-regulating mechanism, unlike glucose oxidase originating from Aspergillus niger. The production of hydrogen peroxide takes place in a controlled manner avoiding over-oxidizing of the gluten network. A possibly larger and more extensive gluten network is formed, improving the overall strength of the gluten network. Moreover, it allows for the dough to become elastic, maintaining its ability to stretch.

Figure 3 shows the results of the biochemical analyses. During dough mixing and later during fermentation, samples were analysed for gluconic acid content. The amount of gluconic acid measured is a direct indication of the amount of hydrogen peroxide formed induced by the enzymatic reaction of glucose oxidase. At the onset of the dough making process, glucose oxidase ex Penicillium (PenGox) and ex Aspergillus (AspGox) show a similar profile of hydrogen peroxide formation. Differences are noticeable at the end of the mixing phase where the dough containing PenGox shows lower levels of hydrogen peroxide production compared to dough containing AspGox. This will avoid over-oxidation of the gluten network that might result in the formation of smaller aggregates rather than the formation of an extended gluten network.

The formation of a larger gluten network will also allow for an increase in dough extensibility. The dough can be stretched further before breakage occurs. Figure 4 shows results of the dough extensibility measured on the Extensograph (Brabender), a rheology method often used in the bakery industry. In this test BakeZyme® GO 10.000 (glucose oxidase ex. Aspergillus) is compared to BakeZyme® Go Pure (glucose oxidase ex. Penicillium). The addition of small dosages of BakeZyme® GO 10.000 immediately decreases the extensibility while dough containing BakeZyme® Go Pure remains extensible. Only further increase of dosage will decrease the dough extensibility gradually.

Due to the difference in hydrogen peroxide formation, providing regulated oxidative power during the mixing process, Bakezyme® Go Pure is suitable for bread making processes such as Chorleywood Bread Processes. Typical characteristics of this process such as short time and high shear mixing, over oxidizing and damage to the gluten structure easily occurs when using glucose oxidase originating from Aspergillus. This results in bread with an open and coarse crumb structure. When using Bakezyme® Go Pure in the dough, it will remain soft and pliable improving the stability during fermentation. More importantly, the final bread will show a nice and fine crumb structure. Results of these tests are shown in figure 5.
Conclusions: closing the gap
The effect of glucose oxidase on dough properties is due to the production of hydrogen peroxide that induces protein cross-linking via disulfide bridges as well as arabinoxylan cross-linking via ferulic acid bridges. The extent of cross-linking in dough depends on the production rate and concentration of hydrogen peroxide. A new glucose oxidase allows for a different working mechanism, in particular it shows a self-regulating mechanism avoiding high levels of hydrogen peroxide being produced. This will create opportunities for the use of glucose oxidase as a tool for replacing chemical oxidizers (such as ADA or Bromate) or in applications such as frozen dough.

Meet our baking experts at IFT15, Chicago to learn more about BakeZyme® Go Pure.

References

For more information: info.food@dsm.com | www.dsm.com/food

DSM – Bright Science. Brighter Living.™

Although diligent care has been used to ensure that the information provided herein is accurate, nothing contained herein can be construed to imply any representation or warranty for which we assume legal responsibility, including without limitation any warranties as to the accuracy, currency or completeness of this information or of non-infringement of third party intellectual property rights. The content of this document is subject to change without further notice. Please contact us for the latest version of this document or for further information. Since the user’s product formulations, specific use applications and conditions of use are beyond our control, we make no warranty or representation regarding the results which may be obtained by the user. It shall be the responsibility of the user to determine the suitability of our products for the user’s specific purposes and the legal status for the user’s intended use of our products.

The General Terms of Conditions of Sale of DSM Food Specialties B.V. apply to and are part of all our offers, agreements, sales, deliveries and all other dealings. The applicability of any other terms and conditions is explicitly rejected and superseded by our General Terms and Conditions of Sale. The current version of our General Terms and Conditions of Sale can be found at www.dsm.com, a hard copy will be forwarded upon your request.