TECHNICAL PAPER

How adding BakeZyme® AAA to bread reduces ascorbic acid dosage

while maintaining ideal texture and volume

July 2018

HEALTH · NUTRITION · MATERIALS

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Introduction

Ascorbic acid is the preferred oxidizing agent to improve the gluten network needed to bake bread with the taste, texture and volume appreciated by consumers. In response to the current price volatility of ascorbic acid, DSM has developed the enzyme blend BakeZyme® AAA. It provides a cost-effective way to reduce ascorbic acid dosage in bread by up to 50%, while maintaining the high-quality bread that consumers expect.

This technical paper explains the science behind the formation of the gluten network by the gliadin and glutenin proteins, the effect of glutathione on dough characteristics, the mechanism of ascorbic acid as oxidizing agent and how BakeZyme® AAA reduces the amount of ascorbic acid.

Consistent bread quality depends on good gluten quality

Gluten is a water-insoluble protein that is formed when wheat flour is mixed with water. Gluten quality is one of the major parameters for good and consistent bread quality. Many factors affect the development and strength of gluten. These include the variety of wheat; the amount, hardness and pH of water; and the presence of enzymes, salt, fat, oil, emulsifiers and sugars. Wheat comes in many varieties (genotypes) and is grown in many areas around the globe, which leads to fluctuations in its composition and quality, and hence also of gluten quality. The milling industry thus faces a daily challenge to produce flours with good and consistent baking quality.
To improve the quality of bread, bakers add bread improvers to further modify dough properties and improve bread quality. These contain, among other components, oxidizing agents, to create the perfect gluten network that bakers need to bake bread with the taste, texture and volume appreciated by consumers. Bakers have been using oxidizing agents for over one hundred years.

The important role today of ascorbic acid

The three oxidizing agents traditionally used are potassium bromate, ADA (azodicarbonamide) and ascorbic acid. Potassium bromate is a very cheap and – due to its slow action – effective oxidizing agent. It has an excellent effect on dough tolerance, loaf volume crumb structure and bread softness. However, concerns about its safety to public health[1] have led to it being banned in many countries. It is still used in parts of the USA where its use needs to be declared on the consumer label. In countries where potassium bromate is not permitted, ascorbic acid is the oxidizing agent of choice, sometimes combined with ADA, where allowed. However, a few years ago ADA also came under pressure and many bread improver formulations have been altered since.

> “Gluten quality is one of the major parameters for good and consistent bread quality. Many factors affect the development and strength of gluten.

Ascorbic acid is thus currently the most important and most widely used chemically synthesized oxidizing agent in the world for the baking industry. Typical levels of ascorbic acid vary between 20-150 ppm or 2-15 grams per 100 kg flour, depending on the type and quality of flour, the application, other dough components, the type of process, the mixing time and intensity, and the required loaf volume.

Over 95% of the world's ascorbic acid is produced in China. Regulatory measures introduced by the Chinese government to reduce carbon emissions from its factories have impacted the production of ascorbic acid and led to its price skyrocketing during 2017[2]. Consequently, bakers are seeking ways to be less reliant on ascorbic acid.

Gluten-forming proteins are crucial for formation of the desired protein network

The gluten network is a continuous three-dimensional protein network formed when two of the naturally occurring proteins in flour – the monomeric gliadins and the polymeric glutenins – stick together through the formation of crosslinks. Disulfide (SS) bonds[3] between two adjacent cysteine residues are the most important crosslinks. The number and distribution of SS bonds significantly affects the properties of the dough[4].

Gliadins are much smaller than glutenins, which can vary in size from 500,000 amino acids to several millions, and are thus among the largest proteins found in nature. Both fractions play an important role in the rheological properties and thus the quality of the flour, but their functionality differs.

Gliadins contain cysteine residues that exclusively form intra-molecular SS bonds and are therefore not involved in reactions with glutenins. Consequently, gliadins have an effect on dough viscosity and extensibility, and act as plasticizers. The cysteine residues in the glutenins on the other hand can form inter-molecular SS bonds and form the continuous phase of the dough. Glutenins therefore have an effect on dough cohesiveness and elasticity. Table 1 displays the main differences between gliadins and glutenins.

Gliadins	Glutenins
Dissolve in 70% aqueous alcohol	Do not dissolve in 70% aqueous alcohol
Intra-molecular SS bonds via cysteine residues	Inter-molecular SS bonds via cysteine residues
Small sized	Belong to the largest proteins found in nature
Affect dough viscosity and extensibility	**Affect dough cohesiveness and elasticity**

Table 1. The differences between the two gluten-forming proteins and their effects on flour quality and bread making.

Any given flour is a combination of several protein fractions where the ratio between gliadins and glutenins, the molecular weights of the glutenin sub-units and the ratio between high- and low-molecular weight glutenin sub-units determine the quality and the properties of the flour[5].

Glutathione negatively interferes with the gluten network and weakens the dough

Also present in flour are several low molecular weight thiols (free SH groups), mainly glutathione, which is a tripeptide (γ-glutamylcysteinglycine); its structural formula is shown in Figure 1. Glutathione is present in its reduced (GSH) and oxidized (GSSG) forms and as a glutathionylated protein (PSSG)[6]. Glutathione has a strong dough weakening effect as the free SH groups react with glutenin to create PSSG and therefore negatively interferes with the intermolecular SS bonds between the large glutenin protein molecules in the gluten network. Fewer intermolecular disulfide bridges will be formed, resulting in a weaker dough.

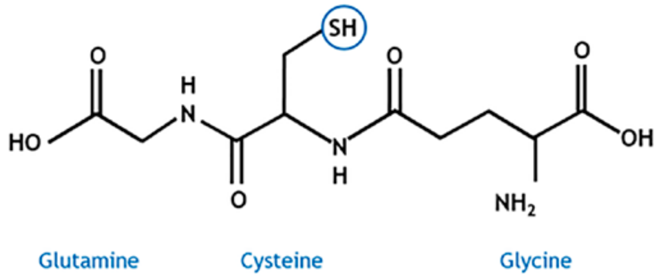

Figure 1. Structural formula of reduced glutathione. The SH group in the central cysteine molecule is the free SH group responsible for the weakening of the gluten network.

Glutathione is mainly present in the germ of the kernel and under the aleurone layer; thus the outside of the wheat kernel. Higher levels of GSH are found in flours with a higher extraction rate, such as whole wheat flours.

Ascorbic acid reacts with glutathione

Glutathione weakens the dough and less reduced glutathione means a stronger dough, as discussed earlier. Ascorbic acid acts by removing free SH groups present in flour, thus preventing the glutathione from interfering with the intermolecular SS bonds between the large proteins in the gluten network.

In contrast to potassium bromate and ADA, ascorbic acid is a reducing agent. It acts as an effective dough strengthener because it is rapidly oxidized to dehydro-ascorbic acid (DHA) in the presence of atmospheric oxygen[7] (see figure 2). DHA is the actual functional component. Oxidation is accelerated by the endogenous enzyme ascorbic acid oxidase (AAO) and is limited by the availability of oxygen. This is illustrated in laboratory tests which show increasing ascorbic acid oxidation at increasing mixer speed due to increased incorporation of atmospheric oxygen into the dough.
This explains the need for higher ascorbic acid dosages when shorter duration and more intensive mixing systems are applied, especially under partial vacuum where less oxygen is present.

DHA oxidizes free reduced glutathione (GSH) to ascorbic acid and oxidized glutathione (GSSG)[8]. This reaction is catalyzed by an enzyme, a reductase named glutathione dehydrogenase (GSH-DH). The redox reaction is illustrated in figure 2.

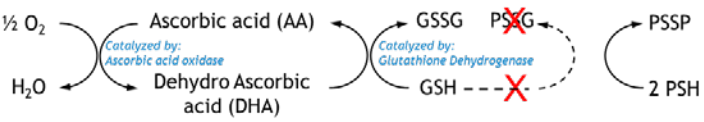

Figure 2. Proposed mechanism of ascorbic acid. Ascorbic acid oxidizes into dehydro-ascorbic acid (DHA), which oxidizes GSH into the oxidized form GSSG, preventing GSH from reacting with gluten which would weaken the dough. This allows the gluten to form disulfide bridges and hence a stronger dough.

Figure 3 shows the effect of ascorbic acid in bread. It illustrates the result of a shock test with ascorbic acid (1) and without ascorbic acid (2). Removing ascorbic acid (2A) reduces loaf volume. Without ascorbic acid and with a shock test (2B) the volume is even less and the structure is no longer smooth and uniform.

With 40 ppm ascorbic acid	Without ascorbic acid

SHOCK SHOCK

Figure 3. Removing ascorbic acid from a simple application leads to loss of volume and stability. 1: With 40 ppm ascorbic acid. 2: Without ascorbic acid. A: Without shock. B: With shock.

Figure 4 is another example of the effect of reducing ascorbic acid on the dough volume. The lower the amount of ascorbic acid, the lower the loaf volume.

	1	2	3
AA dosage	40 ppm	20 ppm	0 ppm
Volume at 90 min proofing	100%	96%	88%
Volume at 120 min proofing	100%	97%	88%

All loafs contain 6 ppm BakeZyme® P500, 20 ppm BakeZyme® HSP 6000 and 5 ppm Panamore® Golden

Figure 4. Dose response of ascorbic acid in white tin bread (Straight dough process). When ascorbic acid is reduced, oven spring and dough stability and thus robustness decrease.

As mentioned, wholemeal flours have higher amounts of glutathione. Therefore, wholemeal flours require a much higher amount of added ascorbic acid than white flour to reach the same volume.

With the increasing consumer trend for more natural ingredients, in some countries chemically synthesized ascorbic acid is replaced by Acerola, a natural but costly source of vitamin C. Its mechanism is the same as above.

> “ Ascorbic acid acts as an effective dough strengthener and DHA is the functional component.

BakeZyme® AAA optimizes the gluten network formation and reduces ascorbic acid dosage

Ascorbic acid leads to dough with proper strength and is a strong and stable foundation for further improvement with other components such as amylase, xylanases, glucose oxidases and lipases. In response to the current price volatility of ascorbic acid, DSM has developed the enzyme blend BakeZyme® AAA, a well-balanced and thoroughly tested blend of baking enzymes.
It offers a cost-effective way to reduce ascorbic acid in bread by 20-50%, while maintaining normal bread quality.

> BakeZyme® AAA adds extra functionalities on top of the GOX functionality to optimally stimulate the gluten network formation.

As explained earlier, decreasing the amount of ascorbic acid influences the dough volume. This partly can be compensated by using glucose oxidases (GOX). The use of GOX to support the oxidation effect of ascorbic acid in bread doughs is well known. However, the working mechanism of a glucose oxidase is different than that of ascorbic acid.

In the presence of oxygen, GOX catalyzes the oxidation of glucose into hydrogen peroxide and gluconic acid (Figure 5). Hydrogen peroxide is a strong and effective oxidizer and increases the formation of SS bonds and/or dityrosine bonds. But GOX does not always show the same volume increase as ascorbic acid. Increasing GOX dosage can even lead to over oxidation, and a too strong and rigid protein network due to an excess of disulfide bonds. This results in rather inflexible and stiff dough, and a loaf with a loss of volume.

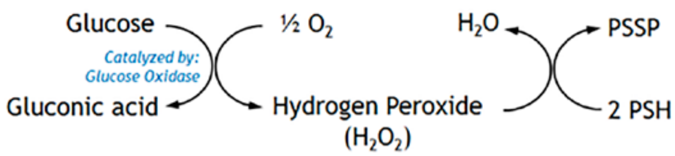

Figure 5. The action of glucose oxidase on glucose.

To solve this issue, DSM application experts developed the enzyme blend BakeZyme® AAA, the latest addition to the proven BakeZyme® range.
By using BakeZyme® AAA, the performance of ascorbic acid will be mimicked, allowing the amount of ascorbic acid to be reduced.

With BakeZyme® AAA, DSM created on top of the GOX functionality extra functionalities to optimally stimulate the gluten network formation and the required volume increase while maintaining an acceptable cost-effectiveness.

BakeZyme® AAA supports the oxidizing properties of the gluten network during dough mixing and solves the negative effects of high glucose oxidase levels that can make doughs too rigid. Due to optimization of the composition, a synergistic effect between the enzymes in the blend is achieved that supports dough rheology, stability, loaf volume and uniformity of the baked products.

BakeZyme® AAA allows 25-50% ascorbic acid reduction without compromising on dough rheology (figure 6), loaf volume and other bread quality attributes. Every 1 ppm BakeZyme® AAA allows a reduction of 10 ppm ascorbic acid up to an ascorbic acid usage rate of around 100 ppm or 10 grams per 100 kg flour.

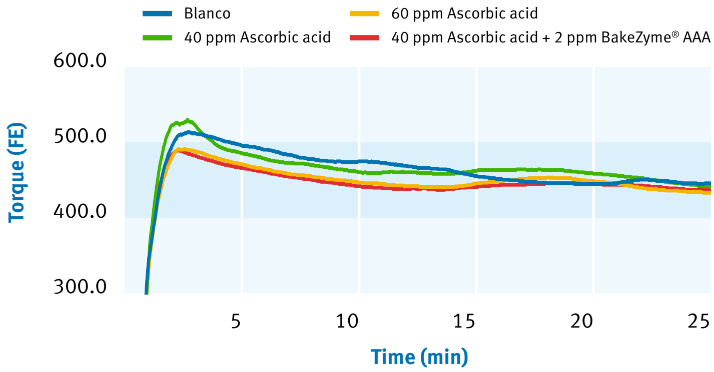

Figure 6: Farinogram data of the reference, 60 ppm ascorbic acid, 40 ppm ascorbic acid and 40 ppm ascorbic acid plus 2 ppm BakeZyme® AAA. The results show that 40 ppm ascorbic plus 2 ppm BakeZyme® AAA has the same mixing behavior as 60 ppm ascorbic acid.

In batard bread, a reduction of one third of ascorbic acid can be reached by adding BakeZyme® AAA, as shown in figure 7. This figure shows the result of a baking trial in which one third of 60 ppm ascorbic acid was replaced by BakeZyme® AAA. The same volume is reached by using 40 ppm ascorbic and 2 ppm BakeZyme® AAA as by using 60 ppm ascorbic acid; a reduction of ascorbic acid of one third.

	1	2	3
Ascorbic acid:	60 ppm	40 ppm	40 ppm
BakeZyme® AAA:	0 ppm	0 ppm	2 ppm
Loaf volume:	100%	94%	100%

Figure 7. Results of baking trial with BakeZyme® AAA in batard bread.

In a white tin bread, 50% reduction of ascorbic acid can be reached. Figure 8 shows the results of a baking trial with BakeZyme® AAA in white tin bread. By adding 30 ppm ascorbic acid and 3 ppm BakeZyme® AAA the same volume can be reached as by adding 60 ppm ascorbic acid.

	1	2	3
Ascorbic acid	60 ppm	40 ppm	30 ppm
BakeZyme® AAA	0 ppm	2 ppm	3 ppm
Loaf Volume (SV: ml/g)	(6.6) 100%	100%	99%
SV after shock test	(6.4) 100%	102%	100%

Figure 8. Results of baking trial with BakeZyme® AAA in white tin bread.

Conclusion

In most countries, ascorbic acid is the only approved chemically synthesized oxidizing agent. It is used in most breadmaking systems for volume breads, typically varying in dosage from 20 to 150 ppm. Adding ascorbic acid to the dough results in an enforced gluten network and improved dough strength.

However, due to the price volatility of ascorbic acid, bakers are greatly interested in ways to reduce ascorbic acid dosages in bread. In response to this challenge, DSM has developed the enzyme blend BakeZyme® AAA. This well-balanced and thoroughly tested blend of baking enzymes offers a cost-effective way to reduce ascorbic acid dosages in bread improvers by up to 50%, while maintaining the high-quality bread that consumers expect.

In contrast to adding only glucose oxidase, BakeZyme® AAA supports the dough strengthening effect of ascorbic acid and the volume improving functionality of ascorbic acid. The enzyme is an add-on to existing bread improvers and flour correctors which reduces the development time for reformulation and smooth transition. Existing glucose oxidase levels in improvers can generally be maintained. BakeZyme® AAA has been extensively tested in various flours, applications and processes.

Acronyms

AAO:	Ascorbic acid oxidase
ADA:	Azodicarbonamide
DHA:	Dehydro-ascorbic acid
GOX:	Glucose oxidase
GRAS:	Generally Recognized As Safe
GSH:	Glutathione in the reduced form
GSH-DH:	Glutathione dehydrogenase
GSSG:	Glutathione in the oxidized form
PSSG:	Protein with oxidized glutathione
SS:	Disulfide bond

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