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# Food Additives and Processing Aids used in Breadmaking

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## Abstract

The main classes of additives used in breadmaking are: (i) oxidants/reductants; (ii) emulsifiers; (iii) hydrocolloids; and (iv) preservatives. The main processing aids used are enzymes. Historically, market trends have developed from the use of ingredients in greater quantities - to obtain specific effects in bread (such as fat for crumb softness) - to the use of additives at much lower levels (max. 1%) and, more recently, to enzymes which are used in parts per million (ppm). According to many regulations, enzymes do not need to be declared on the label of the final product, attending the “clean label” trend. We will describe the food additives used under each class, individually describing their mode of action and effects on dough rheology, during the breadmaking process, and on product quality. We will also describe the main enzymes currently used, dividing them according to the substrate they act on (gluten, starch, lipids, non-starch polysaccharides or NSPS), individually describing their mode of action and effects on dough rheology, during the breadmaking process, and on product quality. Legal aspects will also be addressed. We will conclude with future trends in the use of additives and processing aids in breadmaking.

**Keywords:** bread, oxidants, reductants, emulsifiers, hydrocolloids, preservatives, enzymes

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## 1. Additives in breadmaking

The main classes of additives used in breadmaking are: (i) oxidants/reductants; (ii) emulsifiers; (iii) hydrocolloids; and (iv) preservatives. Maximum dosages permitted may vary according to the application and from country to country; so local legislation must always be consulted. Usually, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) of

the Codex Alimentarius, the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) are taken as guides. The International Numbering System, created in the European Union, assigns E-numbers to all approved food additives, and these are used in many countries to facilitate identification.

### 1.1. Oxidants and reductants

Oxidants and reductants are normally included to assist with gluten network development [1]. Oxidants improve stability and elasticity of the dough, which becomes stronger, increasing oven rise, and making crumb grain finer. They act on the gluten proteins of flour, i.e. oxidizable thiol ( $-SH$ ) groups, creating additional disulfide bonds ( $S-S$ ) [2]. Oxidative enzymes such as glucose-oxidase and hexose-oxidase are now used to replace or support the action of traditional redox materials [3]. Reductants have the opposite effect, but may help to optimize gluten network formation.

#### 1.1.1. Azodicarbonamide (ADA) (E927)

Azodicarbonamide (ADA) is a fast-acting oxidizing agent. Its action is to oxidize free thiol groups ( $-SH$ ) in flour proteins and to strengthen the dough. This action is particularly effective in modifying the dough properties of poor-quality flours, for instance by improving the processing behavior and gas retention properties. ADA used at the correct level increases bread volume and improves crumb properties, but overdosing depresses loaf volume [4].

Azodicarbonamide is a maturing agent used in flour premixes, providing immediate oxidation when water is added. It is consumed in the mixer, in the early stages of the baking process. Azodicarbonamide is added at dosages of 10–40 ppm (flour basis) [4].

The use of ADA is banned in EU countries, but is still used in others. The key reason for the ban is the presence of a reaction product, semicarbazide, which is present in bread crumb and crust, posing a health risk. The use of oxidizing agents depends on legislation, flour quality and production process. In European countries, only ascorbic acid is permitted [4].

#### 1.1.2. Ascorbic acid (E300)

Ascorbic acid is commonly used as an improver in the baking industry. In some countries, it is the only oxidation improver allowed. It has an intermediate speed of reaction and its effect is greatly noticed in the proofing chamber. Its key mechanism of action is the sulfhydryl/disulfide reaction, which plays an important role in the rheological properties of bakery systems [3].

Ascorbic acid itself is a reducing agent. However, in the presence of oxygen and an enzyme, ascorbic acid-oxidase, which is naturally found in wheat flour, it is converted to its dehydro form, that participates in oxidation reactions, stabilizing the gluten network [4]. Its effect on gluten and dough is to reduce extensibility and increase elasticity, giving better volume, shape, and finer and more uniform texture to the finished breads [5]. It is applied in pan bread from 50 to 200 ppm (flour basis) levels.

Some plants and fruits have high levels of ascorbic acid and this presents an opportunity to use them to provide the ascorbic acid requirement in bakery products. This has an advantage in that the chemically synthesized version has an E-number and must be declared on the label as ascorbic acid, vitamin C or E300, while plant or fruit products are declared as ingredients [4].

### 1.1.3. *L-Cysteine (E920)*

*L-Cysteine* is a reductant or reducing agent, with an inverse effect to oxidants. It is an amino acid that contains a free –SH group in its molecule, which breaks disulfide bonds between gluten-forming proteins, reducing the number of cross-links. The resulting dough is softer, lower in elasticity and greater in extensibility. *L-Cysteine* used alone would not be beneficial to a dough system, as it would result in bread with low volume and coarse crumb structure [4].

The advantages of using *L-cysteine* are improved machinability, shorter mixing time and reduced proofing time [4], a process called activated dough development (ADD). In ADD, reducing agents convert high molecular weight glutenins into smaller molecules during mixing. Extra oxidizing agents added to the dough form larger molecules again during proofing, re-establishing desired dough characteristics for breakmaking. *L-Cysteine* opens the disulfide bonds during mixing (less energy) while ascorbic acid closes the remaining bonds. The added oxidant must not be strong, for otherwise *L-cysteine* will be oxidized to cystine (dough strengthener) [2].

As *L-cysteine* relaxes the gluten structure during the mixing process and enhances dough development, when the dough temperature is an issue, *L-cysteine* may be used to reduce the work input requirement thus assisting to control the final dough temperature [5]. Its application dosage varies from 50 to 300 ppm (flour basis).

'Natural' alternatives to synthetic *L-cysteine* are available, which are based on inactivated yeast. In this case, the reducing effect is based on a mixture of glutathione and proteolytic enzymes released from the disrupted yeast cells [5].

## 1.2. Emulsifiers

Emulsifiers are common additives used in breadmaking and can be classified according to two main functions: (i) crumb softeners; and (ii) dough conditioners or gluten strengtheners. Mono- and diglycerides are the main examples of the first group, while diacetyl tartaric acid (DATA) esters of mono- and diglycerides (DATEM) and polysorbate are two prominent examples of the second. Lactylates can be classified as having both functions.

Emulsifiers are often evaluated according to their physicochemical properties. The hydrophilic/lipophilic balance concept (HLB) is the most widely used concept, although not very common in the bakery industry [6].

### 1.2.1. *Mono- and diglycerides (E471)*

Mono- and diglycerides and their derivatives account for about 70% of the production of food emulsifiers in the world. Overall, bakery is by far the field of greatest application.

Approximately, 60% of all monoglycerides are used in bakery – 40% in bread and 20% in sponge cakes and cakes [6].

Mono- and diglycerides are generally manufactured by esterification (glycerolysis) of triglycerides with glycerol, yielding a mixture of mono, di and triglycerides. The hardness of a monoglyceride is mainly determined by the hardness of the edible fat from which the monoglyceride has been produced [6]. As the monoglycerides are the functional part, molecular distillation can be carried out to increase their concentration.

The content of monoglycerides in commercially distilled monoglycerides is usually 90–95% [6]. Two crystalline forms are generally present: alpha and beta. The alpha form is the most functional type of monoglycerides in bakery products. The monoglycerides marketed for bakery applications include plastic, hydrated, powdered and distilled monoglycerides [7].

Monoglycerides possess a lipophilic character and are therefore assigned with a low HLB number (3–6). They dissolve in oil and in stabilized water-in-oil (w/o) emulsions to form reversed micelles in oil. Any functionality of monoglycerides and other emulsifiers in bakery depends on the dispersibility properties of the emulsifiers during mixing of the dough. The factors that influence dispersibility properties during dough mixing are a balance between particle size and hardness or melting point of the monoglyceride [6].

Distilled monoglycerides are considered anti-staling agents in breads, as they soften the crumb of the product after baking and retain this softness during the beginning of shelf-life. They act by binding to the amylose fraction of wheat starch at the high temperatures typical of baking. In doing so, they slow down retrogradation of the starch during cooling and subsequent storage [5].

Distilled monoglycerides have the greatest effect on softness compared to other types of emulsifiers, and less effect on loaf volume. The result is a fine crumb with considerable elasticity. The optimal dosage is 0.2% (flour basis) [2].

#### *1.2.2. Diacetyl tartaric acid esters of mono- and diglycerides (DATEM) (E472)*

DATEM include glycerol derivatives esterified with edible fatty acids and mono- and diacetyl tartaric acid [8], generally permitted for the use in foodstuffs and as dough conditioners for all baked products, particularly yeast-leavened products, such as white bread. Their HLB value is 8–10. The optimal dosage is between 0.25 and 0.50% (flour basis) [2].

DATEM comes as a sticky viscous liquid, or with a consistency like fats, or yellow waxes, or in flakes or powder form. DATEM is more hydrophilic compared to the mono- and diglycerides, and its starting materials [8].

When the flour used for breadmaking contains an inadequate amount, or less than ideal quality, of protein, the inclusion of DATEM assists in dough performance during manufacturing (tolerance toward raw material quality, mechanical resistance, sticking to manufacturing equipment, mixing and fermentation tolerance) and provides dough with reasonable oven spring [5].

Ionic emulsifiers, such as DATEM, offer a huge ability toward the formation of hydrogen bridges with amidic groups of the gluten proteins [8]. Diacetyl tartaric acid (DATA) esters

bind rapidly to the hydrated gluten proteins and, as a result, the gluten network formed becomes stronger, more extensible and more resilient, producing a uniform and stable gas cell structure [5].

DATA esters enhance gas retention when incorporated into most yeast-raised wheat flour-based doughs. They have a strong improving effect on loaf volume and dough stability, which generates a more symmetrical appearance for the baked bread. Internally, breads have a finer gas cell structure with thinner cell walls, resulting in whiter crumbs, and a finer, more even texture, that is softer and more resilient [5].

For whole meal and grain breads, the major difficulty is the disruption of the gas cell network by larger particles, such as bran and seeds. This can be solved by adding extra wheat gluten, by using DATEM (or DATA esters), or by using a combination of both [5].

### 1.2.3. Lactylates: calcium stearoyl-lactylate (CSL) (E482) and sodium stearoyl-lactylate (SSL) (E481)

Lactylate esters are synthesized from food-grade fatty acids and lactic acid. For lactylates as emulsifiers, the fatty acid represents the non-polar portion and the ionic lactic acid polymer represents the polar portion [9].

Calcium stearoyl-lactylate (CSL) and sodium stearoyl-lactylate (SSL) are typical dough conditioners with HLB values of 8–10 and 10–12, respectively. Both are commonly used in the manufacturing of white bread and are employed as dough strengtheners. Also, they act as anti-staling agents, aeration aids and starch/protein complexing agents. Their optimal dosage is 0.25–0.50% (flour basis) [2].

Because of their high degree of hydrophilicity, lactylate salts hydrate readily in water at room temperature. The sodium salts hydrate more rapidly than the calcium salts, giving SSL and CSL different functionalities in short baking processes [9].

The strengthening effect of lactylates relates to their ability to aggregate proteins, which helps in the formation of the gluten matrix. It is believed that they interact with proteins through: (i) hydrophobic bonds between the non-polar regions of proteins and the stearic acid moiety of lactylates; and (ii) ionic interactions between the charged amino acid residues of proteins and the carboxylic portion of lactylates. In the case of bread dough, these effects result in increased dough viscosity, better gas retention and, ultimately, greater bread volume [9].

The effects of lactylates on dough handling properties and proofed dough volume are also related to protein complexing. As proofed dough is heated in the early baking phase, the lactylates are transferred from the protein to the starch. The coating on the starch significantly delays starch gelatinization, which keeps the viscosity low and allows additional expansion of the dough in the oven. As the resultant dough is softer than the unemulsified dough, it allows more abusive mechanical working without causing irreversible damage to the protein structure. Both CSL and SSL provide very good yeast-raised dough strengthening effects [9].

SSL enhances gas retention in the dough, but is less efficient than other dough strengthening emulsifiers, such as DATEM. It also has effects on crumb softening, extending shelf-life, through binding to amylose, showing similar action to distilled monoglycerides. However,

bakers tend to prefer DATEM as a dough conditioner for maximum gas retention, and add distilled monoglycerides at the desired level when extra softness is needed [5].

SSL may be replaced by CSL at similar levels, with similar effects in breadmaking. The need to reduce sodium in bakery products, for health reasons, has led to an increased interest in CSL as an SSL replacer [5].

#### 1.2.4. Polysorbates (E491–E496)

Polysorbates are sorbitol derivatives and they form part of a group of emulsifiers known as sorbitan esters, which can be further modified to polysorbates [10].

The polysorbate family of products is among the most hydrophilic or water soluble emulsifiers allowed in foods, due to the long polyoxyethylene chain, so the addition of small amounts of polysorbate emulsifiers to water results initially in a dramatic decrease in interfacial tension [10].

The unique qualities of each polysorbate are attributed to the different fatty acids used in each product. The ethylene oxide chain length is controlled at an average of 20 moles and it does not change between products. The short-chain fatty acid polysorbate 20 has the highest HLB at 16.7, followed by the others with longer-chains, such as polysorbates 40, 60, 65, 80 and 85 [10].

Sorbitan esters and polysorbates are emulsifiers regulated by governing bodies. For instance, in North America, the market where they are most popular, the specific applications for these compounds in foods are defined and the use level is controlled. Most polysorbates are used in bakery goods. In most bakery applications, polysorbates are used below 0.3% (flour basis) [10].

Polysorbates are added as dough strengtheners to improve baking performance. They stabilize the dough during late proofing and early stages of baking, when there are great stresses on the inflating cells. Their use results in loaves with greater volume and a fine and uniform crumb structure [10].

Regardless of its good effects in breadmaking, and the fact that the polymerized forms of ethylene oxide used in polysorbates have been shown to be safe, the unreacted free-ethylene oxide has been classified as “carcinogenic to humans (Category 1)” by the International Agency for Research on Cancer, and thus, the European Commission Scientific Committee on Food is concerned with these impurities. So, even if the potential risk of impurities in polysorbates is low, a responsible food manufacturer should be aware of these concerns. Food producers would be prudent to source their polysorbates from a reputable supplier [10].

### 1.3. Hydrocolloids

Hydrocolloids are widely used in the food industry, because they modify the rheology and texture of aqueous systems. These additives play a very important role in foods, as they act as stabilizers, thickeners and gelling agents, affecting the stabilization of emulsions, suspensions, and foams, and modifying starch gelatinization [2].

During baking, starch gelatinization and protein coagulation take place and the aerated structure obtained during leavening is fixed, forming the bread crumb. It has been stated that granule swelling can be reduced by the presence of hydrocolloids (particularly at high concentrations), which can interact with the molecules leached out from starch granules, leading to a stiffening effect. Thus, due to these interactions, crumb structure and texture are positively influenced by the presence of gums [11].

In the baking industry, hydrocolloids are very important as breadmaking improvers, because they enhance dough-handling properties, improve the quality of fresh bread, and extend the shelf-life of stored bread. They must be used in small quantities (<1% flour basis) and are expected to increase water retention and loaf volume, while decreasing firmness and starch retrogradation [2].

Polysaccharides such as carboxymethyl cellulose, guar gum and xanthan gum are employed as stabilizers in bakery products in particular.

### 1.3.1. Xanthan gum (E415)

Xanthan gum is an anionic polysaccharide employed to modify rheological properties of food products [1]. It is produced industrially from carbon sources through fermentation by the Gram-negative bacterium *Xanthomonas campestris* [12]. Structure-wise, it is a polymer with a D-glucose backbone. Trisaccharide side-chains formed by glucuronic acid sandwiched between two mannose units are linked to every second glucose of the main polymer chain. The carboxyl groups in xanthan gum may ionize creating negative charges, increasing the viscosity of the solution in water [1].

Xanthan gum easily disperses in cold and hot water, quickly producing viscous solutions. These solutions are stable to acid, salt, and high temperature processing conditions, and show good efficiency at low concentrations, around 0.1% (flour basis). Also, products that contain this gum have fluidity, good mouthfeel, and adhesion. These advantages make xanthan gum a suitable thickener, stabilizer, and suspending agent in many foods [12]. In bakery products, it improves wheat dough stability during proofing. Also, it has the ability to increase dough stability during freeze-thaw cycles in frozen dough [2].

### 1.3.2. Guar gum (E412)

Guar gum is made of the powdered endosperm of the seeds of *Cyamopsis tetragonolobus*, a leguminous crop. The endosperm contains a complex polysaccharide, a galactomannan, which is a polymer of D-galactose and D-mannose. This hydroxyl group-rich polymer forms hydrogen bonds with water, imparting significant viscosity and thickening to the solution. Due to its thickening, emulsifying, binding and gelling properties, quick solubility in cold water, wide pH stability, film forming ability and biodegradability, guar gum finds applications in a large number of industries, including the bakery industry. At the level of 0.5% (flour basis) in bread, it improves both softness and loaf volume. It is also used for increasing dough yield in baked goods [13].

### 1.3.3. Carboxymethylcellulose (CMC) (E466)

Carboxymethylcellulose (CMC) is a cellulose derivative, and it is also called cellulose gum. It finds applications in the food industry as a food stabilizer and thickener. It contains carboxymethyl groups ( $-\text{CH}_2\text{COOH}$ ) attached to  $-\text{OH}$  groups within the glucopyranose monomers forming a carboxymethyl gum backbone. This anionic polysaccharide is often used as a food additive in its sodium salt form (sodium carboxymethylcellulose). In sodium carboxymethylcellulose, some of the carboxyl groups have been replaced by sodium carboxylate groups. The degree of substitution by sodium ions, chain length of the cellulose backbone and clustering of the carboxymethyl substituents determine CMC functionality [1].

CMC has a combined effect with enzymes and emulsifiers on textural properties of both dough and fresh bread. For example, CMC contributes to yielding high volume and retarding staling. Both CMC and guar gum have proven to be beneficial in the formulation of gluten-free breads [2].

## 1.4. Preservatives

Preservatives are intended to inhibit the growth of molds and thermophilic bacteria. The preservatives permitted for use in bread are commonly limited by legislation [5]. Propionic, sorbic and benzoic acids (E280, E202 and E210, respectively) are among the most commonly used food preservatives. Propionic acid inhibits molds and *Bacillus* spores, but not yeasts to the same extent, and has, therefore, been the traditional choice for bread preservation [14].

Preservatives are often added in their salt form, which is more soluble in aqueous solutions. Their effectiveness depends on the pH of the system to which they are added, as the dissociated acid alters the antimicrobial effect. The pKa values (pH at which dissociation occurs) of propionic acid and sorbic acid are 4.88 and 4.76, respectively. Maximum pH for their activity is around 6.0–6.5 and 5.0–5.5 for sorbate and propionate, respectively. At pH 6, only 7% of the propionic acid will be undissociated, compared to 71% at pH 4.5 [14].

### 1.4.1. Propionates

The sodium, potassium and calcium salts of propionic acid are used as bread preservatives in many countries. These preservatives have two functions: (i) to retard the rate of mold development; and (ii) to prevent the bacterial spoilage of bread known as “rope” caused by certain *Bacillus* spp., notably *B. subtilis* and *B. licheniformis*. Calcium propionate (E282) is more widely used than propionic acid, because it is easier to handle the solid salt than the corrosive liquid acid [15]. Its regular dosage is around 0.3% (flour basis).

Although effective at retarding molds and preventing “rope” spoilage, there are some practical disadvantages associated with the use of calcium propionate, among which is the effect on loaf volume. A decrease in loaf volume is caused by the combination of reduced yeast activity and altered dough rheology [15].

Regarding propionic acid, high levels of dietary intake have been associated with propionic acidemia in children. Complications of this disease can include learning disabilities, seizures, arrhythmia, gastrointestinal symptoms, recurrent infections and many others [16].

#### 1.4.2. Sorbates

Sorbates are more effective at inhibiting mold growth than propionates by weight [16]. However, sorbic acid and its salts are of less value in bread and yeast-raised goods, because of their detrimental effects on dough and bread characteristics. They can produce sticky doughs which are difficult to handle; and the baked products may have reduced volume and an irregular cell structure. The use of encapsulated sorbic acid is an alternative to overcome these negative effects. Also, sorbic acid or its salts may be sprayed on the surface of breads after baking [14]. In the dough, its dosage is around 0.1% (flour basis).

#### 1.4.3. Acetates

Acetic acid in the form of vinegar has been used by bakers for many years to prevent the bacterial spoilage of bread known as “rope” and to increase mold-free shelf-life. It gives products a more “natural” appeal and is effective against “rope” at concentrations equivalent to 0.1–0.2% of acetic acid (flour basis). However, at such concentrations, its effect against molds is limited. Significantly higher concentrations lead to an unacceptable odor of vinegar in the bread [15].

#### 1.4.4. Fermentates

An increasing number of natural preservatives are being marketed as “clean label” or “label friendly” shelf-life extension solutions for the bakery industry. Among these are fermentates, which are food ingredients produced by the fermentation of a variety of raw materials by food grade microorganisms. Such microorganisms include lactic acid bacteria or propionic acid bacteria that produce weak organic acids with a preservative effect. However, weak organic acid preservatives have actually been reported to have no effect on the shelf-life of bakery products with pH values close to 7 [16].

Preservatives inhibit microbial spoilage, but do not destroy microorganisms. Therefore, it is important to process baked goods following good manufacturing practices (GMP), including the use of good quality raw-materials and appropriate hygiene systems that are correctly monitored [5].

## 2. Enzymes in breadmaking

Enzymes, also called biocatalysts, are proteins with special properties. They are able to catalyze chemical reactions at low energy requirements without being consumed by these reactions; and the resultant effects modify the structure and/or the physicochemical properties of the environment. Each kind of enzyme has its own specific substrate on which it acts, which provides excellent process control for the use in breadmaking. As the enzymes used are not active in the final products, once they are denatured in the oven, they are classified as “processing aids”, and do not need to be included in the list of ingredients in product labels, according to the legislation requirements in many countries. The Enzyme Commission (EC) number for each enzyme mentioned is shown in this chapter. This is an international numerical classification

for enzymes, where classifying criteria are the chemical reactions each enzyme catalyzes [17]. For a logical comprehension, we have classified food enzymes used in baking by the substrate each one acts on, as follows.

## 2.1. Substrate: polysaccharides

The main polysaccharide present in wheat flour is starch, which is present in the form of granules composed of two fractions. One fraction is amylose (25–28%), the linear fraction, composed by glucose molecules linked by  $\alpha$ -1,4 bonds; and the other fraction is amylopectin (72–75%) which is a branched fraction. Amylopectin is also a glucose polymer formed by  $\alpha$ -1,4 bonds and branches are linked to the linear backbone by  $\alpha$ -1,6 bonds. In the milling process, some starch granules become damaged and it is necessary to have between 7 and 11% of this damaged starch in wheat flour, once it is the substrate for  $\alpha$ -amylase action [18–20].

### 2.1.1. Fungal $\alpha$ -amylase (EC 3.2.1.1)

This kind of endoamylase randomly hydrolyzes  $\alpha$ -1,4 bonds of damaged starch granules from wheat flour, generating low molecular weight dextrans and oligosaccharides (maltose, maltotriose, etc.). Each generated dextrin has its own non-reducing end. Subsequently, the endogenous wheat flour  $\beta$ -amylase hydrolyzes generated dextrans to maltoses [19], which will be hydrolyzed to glucose by maltase enzyme produced by the yeast [18, 20].

The maximum activity pH range of fungal  $\alpha$ -amylase varies from 5 to 6, and fits with the pH of most bread doughs [20]. Fungal  $\alpha$ -amylases are mostly denatured by heat before starch gelatinization temperature range is reached. This fact explains why it is necessary to have damaged starch to be hydrolyzed by this enzyme: it is a more easily degradable substrate than native starch granules. There is a smaller risk of over-action of fungal  $\alpha$ -amylase due to its lower thermostability [18].

The combined use of fungal  $\alpha$ -amylase with endogenous  $\beta$ -amylase produces higher levels of maltose, stimulating yeast fermentation. Consequently, higher gas production enhancing bread volume occurs [20].

Endogenous  $\alpha$ -amylase is present in ungerminated wheat, but its activity varies and can be indirectly measured by the Falling Number (FN). Its activity is low in ungerminated wheat, providing high FN results. On the contrary, in germinated wheat, its activity is high, causing low FN results, and this situation can be a disaster for baking. So, it is necessary to standardize flour with fungal  $\alpha$ -amylase to guarantee the same good results in baking in terms of bread volume, crust, color and general loaf quality [18].

$\alpha$ -Amylase also contributes to a better crumb texture. Once it degrades damaged starch, the dough consistency decreases and machinability is enhanced [18, 20].

Another important contribution of fungal  $\alpha$ -amylase for baking is that reducing sugars generated during mixing and fermentation will participate in the Maillard reaction (combination of

low molecular weight reducing sugars with proteins under high temperature). Maillard reaction is responsible for the non-enzymatic browning of bread crust and generation of bread characteristics including aroma and flavor [18, 20].

Amylases also permit oven spring to occur for a prolonged period. The bread volume is increased once they avoid quick viscosity rising during starch gelatinization [18].

#### 2.1.2. $\beta$ -Amylase (EC 3.2.1.2)

This endogenous enzyme is present in mature ungerminated wheat, and hydrolyzes only damaged starch granules [18]. In breadmaking, this exo-amylase acts sequentially from the non-reducing ends of starch fractions (amylose and amylopectin) or dextrans, hydrolyzes  $\alpha$ -1,4 bonds and releases maltoses and  $\beta$ -limit dextrans. The generated maltoses will be substrate for yeast fermentation after maltase action, enhancing the gassing power of the dough [19].  $\beta$ -Amylase action ceases one glucose molecule before an  $\alpha$ -1,6 bond of amylopectin. The  $\alpha$ -1,6 bond is the branching point of amylopectin [20]. This effect also contributes to reduce bread firmness [18]. The maltoses generated that are not consumed by the yeast contribute to crust color [19].

#### 2.1.3. Bacterial amylase

This enzyme hydrolyzes starch more aggressively than fungal  $\alpha$ -amylase. This effect is due to its efficiency to act on amorphous regions of starch granules, generating excessive dextrinization, with excessive decrease in dough viscosity, producing an open texture crumb [20].

Bacterial amylase provides a softer crumb, despite greater recrystallized starch content in comparison with a control. However, stickiness and gumminess were verified in crumb treated with this enzyme. Such effect occurs by greater thermostability of bacterial amylase, which keeps its capacity to hydrolyze gelatinized starch inside the oven, when fungal  $\alpha$ -amylase is already denatured, and its action may continue during storage [18, 20].

It was proven that bacterial amylase was efficient to extend bread shelf-life. However, small overdosing provokes great and undesirable texture modification [20].

#### 2.1.4. Bacterial maltogenic $\alpha$ -amylase (EC 3.2.1.133)

Bacterial maltogenic  $\alpha$ -amylase is obtained from genetically modified *Bacillus stearothermophilus*. This enzyme hydrolyzes  $\alpha$ -1,4 linkages of easily accessible outer gelatinized starch molecules, in both amylose and amylopectin fractions, producing  $\alpha$ -maltose and other malto-oligosaccharides [21], decreasing bread staling speed. The hydrolyzed amylopectin branches project themselves to the intergranular spaces hampering their reorganization, avoiding crystallization and/or amylose-amylopectin interactions, providing a weaker and less firm starch structure, yielding softer bread [18].

This exo-enzyme is unable to hydrolyze  $\alpha$ -1,6 linkages, so it stops its action one glucose molecule before starch branching. Also, there are some evidences of endo-activity, shown by

amylose and  $\beta$ -limit dextrin hydrolysis. The lower molecular weight branched oligosaccharides resulting from maltogenic  $\alpha$ -amylase action on amylopectin, maltotriose and/or maltotetraose, act as anti-firming agents in baked goods [18, 22].

According to Gerrard et al. [23], the use of maltogenic  $\alpha$ -amylase did not affect rheological properties of bread dough due to its low activity at mixing temperatures (lower than 35°C). Its higher activity is observed at starch gelatinization temperatures during the baking stage, which is enough for the hydrolysis of glycosidic bonds in gelatinized starch by this enzyme. The inactivation of this enzyme by high temperatures occurs during baking time, and starch hydrolysis produces a limited amount of soluble dextrans.

The produced maltodextrins inhibit starch-starch and starch-protein interactions causing a delay in amylopectin reassociation and retrogradation, resulting in a slower crumb firming process. This effect is known as anti-staling [18].

#### 2.1.5. *Amyloglucosidase or glucoamylase (EC 3.2.1.3)*

This exo-amylase directly releases  $\alpha$ -glucose molecules from native or damaged starch granules, increasing the production rate of fermentable sugars in the dough, enhancing yeast fermentation rate [18]. The level of added sugars can be reduced by using amyloglucosidase, and crust color can be improved, as enzyme activity remains after yeast inactivation. As glucose continues to be generated and is no longer consumed by the yeast, glucose remaining in the dough during baking contributes to crust browning and also to an increase in bread sweetness [18].

This enzyme has limited action on  $\alpha$ -1,6 linkages, overriding side chains. However, some theories state that amyloglucosidase completely converts starch molecules to glucose [18].

## 2.2. Substrate: proteins

Proteins are composed of sequences of amino acids linked by peptide bonds. The main proteins of wheat flour are gliadin (a prolamine) and glutenin (a glutelin), which form, in the presence of water and mechanical energy, a cohesive protein network called gluten. This structure is very important for breadmaking. It has special viscoelastic properties (extensibility and elasticity) that allow the dough to flow. At the same time, it is able to retain CO<sub>2</sub> generated by the yeast during the fermentation step [18].

#### 2.2.1. *Glucose-oxidase (EC 1.1.3.4)*

Glucose-oxidase converts glucose (from the hydrolysis of starch) and oxygen (present inside the dough) into gluconolactone and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The gluconolactone is natural and spontaneously converted to gluconic acid. H<sub>2</sub>O<sub>2</sub> readily oxidizes the free thiol (–SH) groups of wheat flour dough proteins, promoting the formation of disulfide bonds (S–S) between gliadin and/or glutenin, that strengthen the gluten network. Thus, this enzyme is very important for breadmaking [18].

The cross-linking effect of proteins is responsible for the gluten network strengthening, that contributes for better crumb structure and bread volume improvement [18]. Nevertheless, high dosages of glucose-oxidase produce excessive stiffness of the dough reducing machinability, and must be avoided [19].

#### 2.2.2. *Hexose-oxidase (EC 1.1.3.5)*

This kind of oxidoreductase has similar effects to those of glucose-oxidase. However, most widely, its substrates are mono and oligosaccharides, other than glucose. The corresponding lactones are obtained, and the generated  $H_2O_2$  acts exactly the same way as in the case of glucose-oxidase as described under Section 2.2.1, producing similar effects in breadmaking [18].

#### 2.2.3. *Transglutaminase (EC 2.3.2.13)*

This kind of acyl transferase promotes the reaction between amines, such as those presented by the  $\gamma$ -carboxamide from L-glutamine with the  $\epsilon$ -amino group from L-lysine. This enzyme catalyzes the formation of covalent cross-linkages between proteins having these amino acid residues. It gives an additional strengthening effect to the gluten network comprising disulfide bonds. The result is the formation of larger and insoluble gluten polymers that affect not only the biochemical characteristics of the dough, but also its rheological properties [24]. Such an effect permits to replace the use of oxidants and even chemical emulsifiers in bakery formulations. Thus, transglutaminase is sometimes recommended in high-fiber and rye bread production. Gluten-free baked goods are also a promising field of action, as the utilization of transglutaminase enhances the protein network formation in breadmaking [18].

This enzyme increases water absorption of wheat flour doughs, provokes dough strengthening, enhances dough stability, reduces dough extensibility, improving crumb texture and bread volume [18].

Transglutaminase is recommended for reinforcing weak protein networks, and also for enhancing freeze-thaw stability of frozen doughs, like frozen croissants and puff pastry, as it decreases their deterioration during frozen storage [18].

#### 2.2.4. *Protease*

Proteins present in baking doughs are substrates for proteases, which hydrolyze peptide bonds irreversibly, in order to reduce mixing time of bread doughs, or to reduce the strength of biscuit doughs, improving their machinability [19]. The disulfide cross-linkages of gluten are not affected by proteases and thus remain intact. The extension of protease effects depends on the amount of enzyme added and on the period of time that it is allowed to work before its inactivation by oven temperatures or pH changes. The main results of protease action are: (i) increase in protein water solubility; (ii) decrease in dough viscosity; (iii) decrease in the average molecular weight of protein fractions; and, consequently, (iv) decrease in gluten complex elasticity [18, 20].

Neutral or sulfhydryl proteases have been used more effectively due to their active pH range (from 5 to 8), that fits the pH of the majority of breads and biscuit doughs. Almost all the

fungal proteases from *Aspergillus oryzae* are neutral type, while vegetable proteases, like papain and bromelain, are sulfhydryl type [20].

For long fermentation times, like in saltine cracker production, the dough can reach pH 4 or lower, and in this case, acidic protease is better used. Otherwise, in soda cracker production, the dough rises up to the alkaline region after soda addition, making serine protease (trypsin) more effective for gluten breakdown. This kind of protease is extracted mainly from bacterial sources like *Bacillus subtilis* [20].

High levels of protease cause such gluten network weakening that produces the coarse texture desired for English muffins, or favors cookie dough flow in the oven. However, care must be taken to avoid excessive proteolysis in bread doughs, because weak gluten networks generate undesirable coarse texture and low bread volume [20].

In the sponge process, it is usual to add small amounts of protease at the beginning of mixing, allowing its action on the gluten network during the sponge fermentation. When fresh flour is incorporated to the sponge, the newly added flour is poorly hydrolyzed during dough mixing. This blend of hydrolyzed and almost non-hydrolyzed gluten generates good smooth dough in the mixer that permits a decrease in mixing time [20].

It is useful to add small amounts of protease in the straight dough process for pan bread, to avoid tight doughs that give incomplete pan filling, or to avoid undesirable breaking along the loaf side. Similarly, in the production of hamburger and hot-dog breads, the dough must flow to fill in the molds during the short fermentation time. The addition of small amounts of protease in the mixer improves dough flow and enhances bread shape and symmetry [20].

In pizza dough production, the make-up work to spread and round the dough into a thin layer becomes easier as a result of adding small amounts of protease during mixing. In this case, the enzyme is able to work during proofing time, adequately reducing the strength of the gluten network, avoiding dough contraction during sheeting and preserving the desired oven spring [20].

The amino acids released by the proteolytic action react with the reducing sugars at high temperatures in the so-called Maillard reaction, enhancing color and flavor of breads and biscuits [18].

### **2.3. Substrate: lipids**

Wheat flour lipids are composed of high levels of linoleic acid (C18:2), and lower levels of palmitic (C16:0) and oleic (C18:1) acids. These fatty acids may occur in the free form, or bound to starch and proteins. Starch lipids, mainly lysophospholipids, form complexes with amylose during gelatinization and have little importance for breadmaking [18].

Non-starch lipids (NSLs) (75% of total wheat flour lipids) are divided 1:1 into polar and non-polar lipids. Most of bound NSLs are composed by triacylglycerols (non-polar). Free NSLs are mainly composed of glycolipids and phospholipids; both are polar molecules that positively contribute to dough handling properties. They have a great effect on loaf volume, due to their

effect on the stability of the gas cells, as they can form thin lipid monolayers inside gas cells that enhance CO<sub>2</sub> retention by the dough [18].

#### 2.3.1. Phospholipase (EC 3.1.4.3)

Phospholipases are a particular kind of lipase with higher specificity toward phospholipids (polar fraction), that converts them *in situ* into lipids with even higher polarity and surface activity [25]. These act as dough strengthening emulsifiers, with dough stabilizing properties [18]. With the use of phospholipases, traditional emulsifiers like DATEM, CSL and SSL can be completely or partially substituted in breadmaking with similar results [25]. Phospholipases also improve dough machinability, as the stickiness is reduced, and the bread volume ultimately increases [18].

#### 2.3.2. Glycolipase (E.C. 3.1.1.26)

Glycolipases are a particular kind of lipase with higher specificity toward glycolipids (polar fraction), that, similarly to phospholipase, converts them *in situ* into emulsifiers. Having similar effects in breadmaking as those from phospholipases, these enzymes increase dough stability. This effect is possible once the generated surface-active lipids maintain stable gas cell structures, due to the interaction of polar lipids with proteins at the liquid lamellae that surround gas cells [25].

#### 2.3.3. Lipase (EC 3.1.1.3)

This kind of enzyme is classified as a glycerol ester hydrolase due to its capacity to hydrolyze acylglycerol ester linkages, releasing preferably fatty acids at positions -1 and -3 from the glycerol structure. The products formed include mono- and diacylglycerol residues, which act as crumb softening emulsifiers in breadmaking. This effect is due to the acylglycerols capacity to penetrate amylose helicoidal structure forming amylose-lipid complexes, retarding amylose retrogradation, increasing bread volume and providing better crumb structure and texture [18].

#### 2.3.4. Lipoyxygenase (EC 1.13.11.12)

The substrates of lipoyxygenase are polyunsaturated fatty acids, such as linoleic (C18:2) and linolenic (C18:3) acids, and  $\beta$ -carotene and chlorophylls from wheat flour [18, 19].

This enzyme, present in enzyme-active soy flour, oxidizes endogenous wheat flour pigments, providing a bleaching effect, resulting in a whiter crumb. Also, dough strengthening occurs during breadmaking [20]. The accessible thiol (-SH) groups from wheat flour proteins are oxidized by the hydroxyperoxides generated by lipoyxygenase action on fatty acids. This oxidation provokes intermolecular disulfide bond formation among gluten proteins, increasing mixing tolerance, improving dough machinability, enhancing rheological properties for breadmaking, increasing bread volume and improving internal texture. Nevertheless, high dosages of lipoyxygenase produce undesirable flavors in breads, due to the decomposition of the hydroxyperoxides of fatty acids generated by lipoyxygenase action, and must be avoided [18].

## 2.4. Substrate: non-starch polysaccharides (NSPS)

There are several non-starch polysaccharides (NSPS) in wheat flour: pentosans,  $\beta$ -glucans and cellulose, all classified as dietary fiber constituents [18]. Pentosans are the most important NSPS due to their great water absorption capacity, despite their low content (2–3%) in wheat flour.

Around 50% of pentosans are water soluble, and 50% insoluble. About 75% of pentosans are xylans, and almost 25% are galactans. Due to their strong hydrophilicity, pentosans affect dough viscosity and, consequently, loaf volume [20].

Xylans are xylose polymers linked by  $\beta$ -1,4 bonds. They can have arabinose molecules linked to the xylan backbone by  $\beta$ -1,3 bonds; then, they are called arabinoxylans (AXs). Some linkages can be  $\beta$ -1,2, mainly in the insoluble or water unextractable arabinoxylans (WU-AXs). Soluble or water extractable arabinoxylans (WE-AXs) present a 3:1 xylose:arabinose ratio, while WU-AXs have a greater proportion of arabinose [20].

AXs are the main NSPS that constitute wheat endosperm cell walls, and, in solution, provide high viscosities, which depend on AXs molecule length. Both WE-AXs and WU-AXs have great water-binding capacity, which, in breadmaking, increases dough consistency, stiffness and resistance to extension, while decreasing mixing time and dough extensibility [18].

The WE-AXs are weakly linked to wheat endosperm cell walls and have gelling properties in the presence of oxidants [25]. The main components responsible for the increase in viscosity of flour suspensions are the WE-AXs, and this ability stabilizes protein films during temperature elevation [18]. According to Wang et al. [27], WE-AXs are considered beneficial to bread quality, enhancing gas retention.

The WU-AXs are structural components of wheat cell walls that link AXs, proteins, cellulose and lignin, through covalent and non-covalent bonds [26]. Experiments have shown better loaf volume and bread quality when WU-AX content decreases, and this effect is due to: (i) physical barriers to gluten development represented by the WU-AX, which impair gliadin and glutenin approximation; (ii) high water absorption capacity, making water unavailable for gluten network development; and (iii) gas cell perforation by these structures, provoking their coalescence [18, 27].

If the AXs do not receive appropriate enzymatic treatment during dough processing, the water added to the wheat flour becomes constrained in these hydrophilic structures, causing a water scarcity for gluten network development, enzyme action, yeast activity and starch granule gelatinization, impairing bread final quality.

### 2.4.1. Fungal xylanase (EC 3.2.1.8)

This enzyme is used to release water from xylans. It has great influence on dough viscosity. Thus, it improves dough tolerance to the breadmaking processes, as dough elasticity is reduced [19]; and contributes to increase bread volume up to 20% when compared with a control, mainly in high-fiber doughs, such as breads made with whole wheat flour and other whole cereals [20]. Xylanases enhance gas retention capacity of dough, contributing to a softer

and finer crumb [19]. This kind of endo-xylanase is extracted from *Aspergillus* spp. and this enzyme preferentially hydrolyzes WE-AX, promoting gluten protein aggregation [26], due to its water releasing capacity which is beneficial for gluten network formation [19].

Excessive dosage levels must be avoided, because, in this case, slack and sticky wheat flour doughs are produced. This effect is caused by the excessive hydrolysis of AX, provoking excessive loss in water binding capacity [19]. The resultant breads present in appropriate crumb structure, with ragged gas cell distribution, besides inappropriate crust color [18].

#### 2.4.2. Bacterial xylanase (EC 3.2.1.55)

This kind of endo-xylanase is extracted from *B. subtilis*. It preferentially hydrolyzes WU-AX, enhancing dough stability. Due to this effect, the dough is able to keep maximum volume for a longer period during the fermentation step, and it maintains a great resistance to mechanical stress during the breadmaking process [18]. Oven spring is prolonged and bread volume is enhanced due to dough relaxation and better gas retention [19, 26], which produces finer grains that provide a softer and more homogeneous bread crumb [18].

For the same reason as for fungal xylanase, excessive dosage levels of bacterial xylanase must also be avoided [18].

#### 2.4.3. Cellulase (EC 3.2.1.4)

This enzyme hydrolyzes cellulose (linear homopolysaccharide composed by a glucose polymer backbone linked by  $\beta$ -1,4 bonds) from wheat cell walls, mainly from the wheat grain outer layers. Cellulose chains are organized in crystalline and amorphous regions. In cellulose crystalline structure, the molecules are highly ordered and chain arrangement blocks water and enzyme penetration into the microfibrils. In the non-crystalline (amorphous) regions, water and enzymes have greater access, and these sites are more easily hydrolyzed than the crystalline ones. Thus, the amorphous regions are firstly attacked and degraded by the cellulases [28]. This produces lower molecular weight fragments that can bind more water.

Cellulase action on cellulose has numerous benefits in the breadmaking process: (i) water absorption increases; (ii) dough viscosity increases; (iii) high-fiber dough stickiness decreases; (iv) machinability is enhanced; (v) the release of glucose increases, and; (vi) the cut opening for French rolls increases [28].

### 3. Future trends

There is currently huge pressure on the food industry to produce healthier products. “Clean” or “friendly” labels, with shorter and simpler ingredient lists are a strong trend. These include the search for more natural and healthier alternatives for chemical additives which have a negative impact on consumer acceptance. The bakery industry is trying to eliminate E-number ingredients from its formulations using, for example, (i) enzymes and vital wheat gluten (an ingredient) to eliminate emulsifiers and chemical oxidants; (ii) hydrocolloids as a

more “friendly” choice than other additives; and (iii) natural preservatives such as fermentates, for mold control. However, in some cases, these alternatives are expensive and not as effective as chemical additives.

Enzymes do not need to be declared as processing aids on the labels of food products in many countries, so they are an interesting strategy for “clean labels”. Some enzymes are under development and will probably soon become commercially available for use in breadmaking. An example is laccase (EC 1.10.3.2), an oxidative enzyme that oxidizes different kinds of phenolic compounds, increasing dough stability and strength, promoting quicker dough formation and reducing dough stickiness [18]. Another example is  $\beta$ -glucanase (EC 3.2.1.6), which hydrolyzes the  $\beta$ -glucans present in barley, rye and oat flours, enhancing microstructure, volume, texture, shelf-life and taste in breads made with these composite flours [29].

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