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More Knowledge About How Much Does Preservatives Affect the Food Production and Preservation Society

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Abstract

The main classes of food additives used in foodmaking 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 food (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 food-making 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 food-making process, and on product quality. Legal aspects will also be addressed. We will conclude with future trends in the use of food additives and processing aids in food making.

Keywords: food additives, food making, quality.

Introduction

The food improver is a blend of ingredients that activate the gluten and help produce gas which assists and improves the processes of dough kneading and the fermentation. The result is a lighter loaf with better texture and keeping qualities. The Food improvers simplify the work of foodmaking, allowing them to show off their full expertise. They can be used with any technology, under the most widely varying production conditions. As needed, they can Improve texture and volume, Enhance machinability, Boost tolerance in control proofing, especially for raw frozen and pre-cooked specialty foods, Texture & volume, Increase shelf life, Increase yields, and cost reduction All our Improvers are 100% Bromate Free. Although the use of food additives is negatively viewed as a result of incorrect and inadequate information to the public today, the use of food additives is needed due to the process between the production and consumption of food in today's world. Food additives, which are used to minimize the problems that may occur during this process, enable us to consume healthier and more reliable foods **[1,2,3,4,5,6,7]**. Vitamin C, another food additive ingredient, increases the quality of the proteins in the flour and provides a better volume of food. It increases the nutritional value of food. Cysteine, DATEM, and Soy Flour are not used in food improvers because they are not needed and are not in compliance with the food communiqué. The Soy Flour; Today, it is not preferred in food improvers due to the dominant flavor and smell of soybeans. It whitens the dough color. Food used to be like white, but not anymore**[8,9,10,11,12,13,14]**. The **DATEM**: Diacetyl tartaric acid esters of mono- and diglycerides were used to ensure the smooth inner texture of the food. It has now been replaced by natural enzymes as it changes the flavor of food and is expensive. **Cysteine**: It is an essential amino acid. It causes the dough to be more fluid and weak. There is a weakness in the dough due to the low gluten quality of Turkish wheat, high bran content, and low salt content in the dough. For this reason, it is necessary to strengthen the dough with vitamin C instead of weakening it with cysteine. The cysteine amino acid was used to be produced by a manufacturing technique that was not very pleasant for all religious circles (Muslims, Jews, Christians, and Buddhists) and all peoples. However, cysteine, which is an essential amino acid today, can be produced much cheaper by fermentation. In order to increase the fluidity of the dough when needed, deactivated yeast and enzymes are preferred today due to their easy supply. The food improvers should preserve the flavor and natural smell of the food while improving all the elements you expect in food quality. Although the use of additives is negatively viewed as a result of incorrect and inadequate information to the public today, the use of food additives is needed due to the process between the production and consumption of food in today's world. Food additives, which are used to minimize the problems that may occur during this process, enable us to consume healthier and more reliable foods. Using food additives (enzyme mixtures for bakery according to the new communiqué) or processing aids created with legally permitted ingredients has no health negative effects and it allows you to produce healthier foods. The Processing aids products (enzyme and vitamin C) should be added to foods. A few grams of vitamin C with a few grams of enzyme is useful, but it has no known harms

[8,9,10,11,12,13,14]. The vitamin C and the enzymes be used in bakeries rather than mills, Vitamin C and enzymes (processing aids) cannot be mixed well enough due to the technology of flour mills. The Processing aids products that are placed in an environment with 15% moisture such as flour lose 30% in a month. The effects of substances that undergo specific reactions such as enzymes can be determined by controlled food studies. Specialized personnel are required for this type of R&D unit to be established in the mills. The food improver is one of the food production ingredients used with flour, water, salt, and yeast all over the world, especially in developed countries such as Germany and the USA. The food improver is a mixture of vitamin C and enzymes used in intensive and rapid food production processes to increase food production quality **[15,16,17,18,19,20,21,22]**.

The alpha-amylase enzyme found in food improvers naturally exists in the flour. The Alpha-Amylase enzyme converts some of the damaged starch in flour into sugar and shortens the leavening time of the dough. The sugar formed is consumed by the yeast and carbon dioxide gas is released in the meantime. The released gas expands at the oven temperature and helps the food to turn into a more voluminous, smooth, easily digestible, higher-quality food item. Since the original sugar converted from starch by alpha-amylase enzyme is easily and quickly caramelized even at low temperatures during baking, the food takes color easily. Thus, while baking the food for a shorter period of time would be sufficient for the food to take color, it does not cause the formation of carcinogenic substances known as acrylamide by baking the food more than necessary for its coloring. In this way, alpha-amylase enables us to produce and consume much healthier foods **[23,24,25,26,27,28,29,30]**.

The Processing aids and vitamin C used in mills are used according to the minimum requirement in flour. It is problematic to put in a fixed amount without taking into account the final product needs and changing process conditions. a. Many products such as baked food, Trabzon food, pita, flatbread, bagel, cake, food crumbs, black food, and so on are obtained from the food flours produced in the mills. However, if pita is to be produced from these flours, less vitamin C should be added, and if food is to be produced, more vitamin C should be added. Another example is cake production. One of the most important reasons why many large producers are still unable to produce products at European standards in cake production is the enzymes used in mills for food flour. While special flour cannot be produced in the mills for "sponge cake", technological use of additives continues unconsciously. The amount of vitamin C used in mills must be constant and minimal due to an economical process. However, the need for vitamin C in food production varies depending on the amount of water added during dough forming, kneading time, dough temperature, mechanical process length and type, food production time, flour strength, whether the dough is reprocessed, the amount of yeast, the characteristics of the flour in the blend, the processing technique, the volume of food and what kind of product to be produced. The need for vitamin C in food flour also includes regional differences. For example, high vitamin C is required in Kayseri and Adana, low vitamin C in the Eastern Black Sea region, medium level vitamin C in Erzurum, and very high vitamin C in Istanbul, while it may not be needed at all in some regions. In the countries, that export flour to all corners of the world, it would be a more correct approach to leave the need for additives to the decision of the end consumer, as customer demands and needs differ **[31,32,33,34,35,36]**.

The Food Additives in Foodmaking

The main classes of food additives used in foodmaking are the oxidants/reductants; the emulsifiers; the hydrocolloids; and the preservatives. The Maximum dosages permitted may vary according to the application and from country to country; so the 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 **[37,38,39,40,41,42,43,44]**.

The Oxidants and the Reductants

The Oxidants and reductants are normally included to assist with gluten network development. Oxidants improve the 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). The Oxidative enzymes such as glucose-oxidase and hexose-oxidase are now used to replace or support the action of traditional redox materials. The Reductants have the opposite effect but may help to optimize gluten network formation **[45,46,47,48,49,50,51,52]**.

The Azodicarbonamide

The Azodicarbonamide (ADA) is a fast-acting oxidizing agent. Its action is to oxidize free thiol groups (─ SH) in the 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. The ADA used at the correct level increases food volume and improves crumb properties, but overdosing depresses loaf volume. 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. The Azodicarbonamide is added at dosages of 10–40 ppm (flour basis). The use of the 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 food crumbs and crust, posing a health risk. The use of the oxidizing agents depends on legislation, flour quality, and production process. In European countries, only ascorbic acid is permitted **[53,54,55,56,57,58,59,60]**.

The Ascorbic Acid

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. 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 dihydro form, which participates in oxidation reactions, stabilizing the gluten network. 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 foods. It is applied in pan food 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 **[61,62,63,64,65,66,67,68]**.

The Cysteine

The 1-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 food with low volume and coarse crumb structure. The advantages of using l-cysteine are improved machinability, shorter mixing time, and reduced proofing time, a process called activated dough development (ADD). In addition, 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 breadmaking. 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 (the dough strengthener **[69,70,71,72,73,74,75,76]**. 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 in controlling the final dough temperature. 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 the glutathione and the proteolytic enzymes released from the disrupted yeast cells **[77,78,79,80,81,82,83,84]**.

The Emulsifiers

The Emulsifiers are common food additives used in foodmaking and can be classified according to two main functions: the crumb softeners; and the dough conditioners or gluten strengtheners. The 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. The 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 food industry **[85,86,87,88,89,90,91,92]**.

The Mono- and Diglycerides

The Mono- and diglycerides and their derivatives account for about 70% of the production of food emulsifiers in the world. Overall, the bakery is by far the field of greatest application. Approximately, 60% of all monoglycerides are used in bakery − 40% in food and 20% in sponge cakes and cakes. The 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. 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%. 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 **[93,94,95,96,97,98,99]**. The 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 the bakery depends on the dispersibility properties of the emulsifiers during the 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. The Distilled monoglycerides are considered anti-staling agents in foods, 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 the retrogradation of the starch during cooling and subsequent storage. The 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) **[100,101,102,103,104,105,106]**.

The Diacetyl Tartaric Acid Esters of Mono- and Diglycerides (DATEM)

The DATEM includes glycerol derivatives esterified with edible fatty acids and mono- and diacetyl tartaric acid, generally permitted for use in foodstuffs and as dough conditioners for all baked products, particularly yeast-leavened products, such as white food. Their HLB value is 8–10. The optimal dosage is between 0.25 and 0.50% (flour basis). The DATEM comes as a sticky viscous liquid, 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. When the flour used for food making 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. The 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. The 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 food. Internally, foods 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. For the whole meal and the grain foods, 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 **[107,108,109,110,111,112]**.

The Lactylates: Calcium Stearoyl-Lactylate (CSL) (E482) and Sodium Stearoyl-Lactylate (SSL)

The 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. The 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 food 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). 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. 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: the hydrophobic bonds between the non-polar regions of proteins and the stearic acid moiety of lactylates; and the ionic interactions between the charged amino acid residues of proteins and the carboxylic portion of lactylates. In the case of food dough, these effects result in increased dough viscosity, better gas retention, and, ultimately, greater food volume. The effects of lactylates on dough handling properties and proofed dough volume are also related to protein complexing. As the proofed dough is heated in the early baking phase, the lactylates are transferred from the protein to the starch **[113,114,115,116,117,118]**. 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. The 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, food-making tends to prefer DATEM as a dough conditioner for maximum gas retention and add distilled monoglycerides at the desired level when extra softness is needed. The SSL may be replaced by CSL at similar levels, with similar effects in food making. The need to reduce sodium in bakery products, for health reasons, has led to an increased interest in CSL as an SSL replacer **[119,120,121,122,123,124,125]**.

The Polysorbates

The Polysorbates are sorbitol derivatives and they form part of a group of emulsifiers known as sorbitan esters, which can be further modified to the polysorbates. 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. 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. The 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). The 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. Regardless of its good effects in food making, 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. The Food producers would be prudent to source their polysorbates from a reputable supplier **[126,127,128,129,130,131,132]**.

The Hydrocolloids

Hydrocolloids are widely used in the food industry because they modify the rheology and texture of aqueous systems. These food 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. During baking, starch gelatinization and protein coagulation take place and the aerated structure obtained during leavening is fixed, forming the food 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. In the baking industry, hydrocolloids are very important as foodmaking improvers, because they enhance dough-handling properties, improve the quality of fresh food, and extend the shelf-life of stored food. 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. Polysaccharides such as carboxymethyl cellulose, guar gum, and xanthan gum are employed as stabilizers in bakery products in particular **[133,134,135,136,137,138]**.

The Xanthan Gum

Xanthan gum is an anionic polysaccharide employed to modify the rheological properties of food products. It is produced industrially from carbon sources through fermentation by the Gram-negative bacterium *Xanthomonas campestris*. Structure-wise, it is a polymer with a d-glucose backbone. The 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. The 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. 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 **[139,140,141,142,143,144]**.

The Guar Gum

The 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 food, it improves both softness and loaf volume. It is also used for increasing dough yield in baked goods **[144,145,146,147,148]**.

The Carboxymethylcellulose

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 ($-CH_2COOH$) attached to $-OH$ 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. The CMC has a combined effect with enzymes and emulsifiers on the textural properties of both dough and fresh food. 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 foods **[130,131,132,133,134,135]**.

The Preservatives

The Preservatives are intended to inhibit the growth of molds and thermophilic bacteria. The preservatives permitted for use in food are commonly limited by legislation. Propionic, sorbic, and benzoic acids 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 food preservation. The 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 p*K*a values (pH at which dissociation occurs) of propionic acid and sorbic acid are 4.88 and 4.76, respectively. The 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 **[111,112,113,114,115,116]**.

The Propionates

The sodium, potassium, and calcium salts of propionic acid are used as food 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 food known as “rope” caused by certain *Bacillus* spp., notably *B. subtilis* and *B. licheniformis*. Calcium propionate is more widely used than propionic acid because it is easier to handle solid salt than corrosive liquid acid. 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. 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 **[100,101,102,103,104,105]**.

The Sorbates

The Sorbates are more effective at inhibiting mold growth than propionates by weight. However, sorbic acid and its salts are of less value in food and yeast-raised goods, because of their detrimental effects on dough and food characteristics. They can produce sticky doughs that 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 foods. In the dough, its dosage is around 0.1% (flour basis) **[55,56,57,58,59,60]**.

The Acetates

Acetic acid in the form of vinegar has been used by foodmaking for many years to prevent the bacterial spoilage of food 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 food **[88,89,90,91,92,93,94]**.

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The 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 been reported to not affect the shelf-life of bakery products with pH values close to 7. The 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 **[17,18,19,20,21,22,23]**.

The Enzymes in Food Making

Enzymes, also called biocatalysts, are proteins with special properties. They can 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 specific substrate on which it acts, which provides excellent process control for use in food making. 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. For logical comprehension, we have classified food enzymes used in baking by the substrate each one acts on, as follows **[33,34,35,36,37,38,39]**.

The Substrate: The 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 of glucose molecules linked by α-1,4 bonds; and the other fraction is amylopectin (72–75%) which is a branched fraction. Amylopectin is also a glucose polymer formed by α-1,4 bonds and branches are linked to the linear backbone by α-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 α-amylase action **[28,29,30,31,32,33,34]**.

The Fungal α-Amylase

This kind of endo amylase randomly hydrolyzes α-1,4 bonds of damaged starch granules from wheat flour, generating low molecular weight dextrins and oligosaccharides (maltose, maltotriose, etc.). Each generated dextrin has its non-reducing end. Subsequently, the endogenous wheat flour β-amylase hydrolyzes generated dextrins to maltoses, which will be hydrolyzed to glucose by the maltase enzyme produced by the yeast. The maximum activity pH range of fungal α-amylase varies from 5 to 6 and fits with the pH of most food doughs. Fungal α-amylases are mostly denatured by heat before the 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 α-amylase due to its lower thermostability. The combined use of fungal α-amylase with endogenous β-amylase produces higher levels of maltose, stimulating yeast fermentation. Consequently, higher gas production enhancing food volume occurs. The Endogenous α-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 α-amylase to guarantee the same good results in baking in terms of food volume, crust, color, and general loaf quality. α-Amylase also contributes to a better crumb texture. Once it degrades damaged starch, the dough consistency decreases and machinability is enhanced. Another important contribution of fungal α-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). The Maillard reaction is responsible for the non-enzymatic browning of food crust and the generation of food characteristics including aroma and flavor. The Amylases also permit oven springs to occur for a prolonged period. The food volume is increased once they avoid quick viscosity rising during starch gelatinization **[45,46,47,48,49,50,51]**.

The β-Amylase

This endogenous enzyme is present in mature ungerminated wheat and hydrolyzes only damaged starch granules. In food making, this exo-amylase acts sequentially from the non-reducing ends of starch fractions (amylose and amylopectin) or dextrins, hydrolyzes α-1,4 bonds and releases maltoses and β-limit dextrins. The generated maltoses will be a substrate for yeast fermentation after maltase action, enhancing the gassing power of the dough. β-Amylase action ceases one glucose molecule before an α-1,6 bond of amylopectin. The α-1,6 bond is the branching point of amylopectin. This effect also contributes to reducing food firmness. The maltoses generated that are not consumed by the yeast contribute to crust color **[82,83,84,85,86,87,88]**.

The Bacterial Amylase

This enzyme hydrolyzes starch more aggressively than fungal α-amylase. This effect is due to its efficiency in acting on amorphous regions of starch granules, generating excessive dextrinization, with an excessive decrease in dough viscosity, producing an open texture crumb. The Bacterial amylase provides a softer crumb, despite greater recrystallized starch content in comparison with a control. However, stickiness and gumminess were verified in crumbs treated with this enzyme. Such an effect occurs by greater thermostability of bacterial amylase, which keeps its capacity to hydrolyze gelatinized starch inside the oven, when fungal α-amylase is already denatured, and its action may continue during storage. It was proven that bacterial amylase was efficient in extending food shelf-life. However, small

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overdosing provokes great and undesirable texture modification **[77,78,79,80,81,82,83,84]**.

The Bacterial Maltogenic α-Amylase

The Bacterial maltogenic α-amylase is obtained from genetically modified *Bacillus stearothermophilus*. This enzyme hydrolyzes α-1,4 linkages of easily accessible outer gelatinized starch molecules, in both amylose and amylopectin fractions, producing α-maltose and other malto-oligosaccharides, decreasing food staling speed. The hydrolyzed amylopectin branches project themselves to the intergranular spaces hampering their reorganization, avoiding crystallization and/or amylose-amylopectin interactions, and providing a weaker and less firm starch structure, yielding softer food. This exo-enzyme is unable to hydrolyze α-1,6 linkages, so it stops its action one glucose molecule before starch branching. Also, there is some evidence of endo-activity, shown by amylose and β-limit dextrin hydrolysis. The lower molecular weight branched oligosaccharides resulting from maltogenic α-amylase action on amylopectin, maltotriose, and/or maltotetralose, act as anti-firming agents in baked goods. The use of maltogenic α-amylase did not affect the rheological properties of food 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 dextrins. 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 **[38,39,40,41,42,43,44]**.

The Amyloglucosidase or Glucoamylase

This exo-amylase directly releases α-glucose molecules from native or damaged starch granules, increasing the production rate of fermentable sugars in the dough, and enhancing yeast fermentation rate. 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 food sweetness. This enzyme has limited action on α-1,6 linkages, overriding side chains. However, some theories state that amyloglucosidase completely converts starch molecules to glucose **[11,12,13,14,15,16,17]**.

The Substrate: The Proteins

The 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 foodmaking. It has special viscoelastic properties (extensibility and elasticity) that allow the dough to flow. At the same time, it is able to retain CO_2 generated by the yeast during the fermentation step **[61,62,63,64,65,66]**.

The Glucose-Oxidase

The Glucose-oxidase converts glucose (from the hydrolysis of starch) and oxygen (present inside the dough) into gluconolactone and hydrogen peroxide (H_2O_2). The gluconolactone is natural and spontaneously converted to gluconic acid. H_2O_2 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 foodmaking. The cross-linking effect of proteins is responsible for the gluten network strengthening, which contributes to better crumb structure and food volume improvement. Nevertheless, high dosages of glucose-oxidase produce excessive stiffness of the dough reducing machinability, and must be avoided **[71,72,73,74,75,76,77,78]**.

The Hexose-Oxidase

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 **[25,26,27,28,29,30,31,32]**.

The Transglutaminase

This kind of acyl transferase promotes the reaction between amines, such as those presented by the γ-carboxamide from l-glutamine with the ε-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. Such an effect permits to replacement of the use of oxidants and even chemical emulsifiers in bakery formulations. Thus, transglutaminase is sometimes recommended in high-fiber and rye food production. Gluten-free baked goods are also a promising field of action, as the utilization of transglutaminase enhances protein network formation in food making. This enzyme increases water absorption of wheat flour doughs, provokes dough strengthening, enhances dough stability, reduces dough extensibility, improving crumb texture and food volume. Transglutaminase is recommended for reinforcing weak protein networks, and also for enhancing the freeze-thaw stability of frozen doughs, like frozen croissants and puff pastry, as it decreases their deterioration during frozen storage **[144,145,146,147,148]**.

The Protease

The Proteins present in baking doughs are substrates for proteases, which hydrolyze peptide bonds irreversibly, in order to reduce the mixing time of food doughs, or to reduce the strength of biscuit doughs, improving their machinability. 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 an increase in protein water solubility;

a decrease in dough viscosity; a decrease in the average molecular weight of protein fractions; and, consequently, a decrease in gluten complex elasticity. Neutral or sulfhydryl proteases have been used more effectively due to their active pH range (from 5 to 8), which fits the pH of the majority of foods and biscuit doughs. Almost all the fungal proteases from *Aspergillus oryzae* are neutral type, while vegetable proteases, like papain and bromelain, are sulfhydryl type. For the 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*. The 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 food doughs, because weak gluten networks generate undesirable coarse texture and low food volume. 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 into 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. It is useful to add small amounts of protease in the straight dough process for pan food, 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 foods, 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 food shape and symmetry. In pizza dough production, the makeup 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. The amino acids released by the proteolytic action react with the reducing sugars at high temperatures in the so-called Maillard reaction, enhancing the color and flavor of foods and biscuits **[141,142,143,144,145,146]**.

The Substrate: The 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 foodmaking. The Non-starch lipids (NSLs) (75% of total wheat flour lipids) are divided 1:1 into polar and non-polar lipids. Most bound NSLs are composed of 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_2 retention by the dough **[10,11,12,13,14,15,16]**.

The Phospholipase

The 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. These act as dough-strengthening emulsifiers, with dough-stabilizing properties. With the use of phospholipases, traditional emulsifiers like DATEM, CSL, and SSL can be completely or partially substituted in food making with similar results. Phospholipases also improve dough machinability, as the stickiness is reduced, and the food volume ultimately increases **[71,72,73,74,75,76,77]**.

The Glycolipase

The Glycolipases are a particular kind of lipase with higher specificity toward glycolipids (polar fraction), that, similarly to phospholipase, convert them *in situ* into emulsifiers. Having similar effects in food making 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 **[101,102,103,104,105,106]**.

The Lipase

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 food making. This effect is due to the acylglycerol's capacity to penetrate amylose helicoidal structure forming amylose-lipid complexes, retarding amylose retrogradation, increasing food volume, and providing better crumb structure and texture **[135,136,137,138,139,140]**.

The Lipoxygenase

The substrates of lipoxygenase are polyunsaturated fatty acids, such as linoleic (C18:2) and linolenic (C18:3) acids, and β-carotene and chlorophylls from wheat flour. This enzyme, present in enzyme-active soy flour, oxidizes endogenous wheat flour pigments, providing a bleaching effect, and resulting in a whiter crumb. Also, dough strengthening occurs during foodmaking. The accessible thiol (─SH) groups from wheat flour proteins are oxidized by the hydroxyperoxides generated by lipoxygenase action on fatty acids. This oxidation provokes intermolecular disulfide bond formation among gluten proteins, increasing mixing tolerance, improving dough machinability, enhancing rheological properties for food making, increasing food volume, and improving internal texture. Nevertheless, high dosages of lipoxygenase produce undesirable flavors in foods, due to the decomposition of the hydroxyperoxides of fatty acids generated by lipoxygenase action, and must be avoided **[120,121,122,123,124,125]**.

The Substrate: Non-Starch Polysaccharides (NSPS)

There are several non-starch polysaccharides (NSPS) in wheat flour: pentosans, β-glucans, and cellulose, all classified as dietary fiber constituents. 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. The Xylans are xylose polymers linked by β-1,4 bonds. They can have arabinose molecules linked to the xylan backbone by β-1,3 bonds; then, they are called arabinoxylans (AXs). Some linkages can be β-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. The 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 foodmaking, increases dough consistency, stiffness, and resistance to extension, while decreasing mixing time and dough extensibility. The WE-AXs are weakly linked to wheat endosperm cell walls and have gelling properties in the presence of oxidants. 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. WE-AXs are considered beneficial to food 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. Experiments have shown better loaf volume and food quality when WU-AX content decreases, and this effect is due to physical barriers to gluten development represented by the WU-AX, which impair gliadin and glutenin approximation; high water absorption capacity, making water unavailable for gluten network development; and gas cell perforation by these structures, provoking their coalescence. 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 food final quality **[48,49,50,51,52,53,54]**.

The Fungal Xylanase

This enzyme is used to release water from xylans. It has a great influence on dough viscosity. Thus, it improves dough tolerance to the foodmaking processes, as dough elasticity is reduced; and contributes to increasing food volume by up to 20% when compared with a control, mainly in high-fiber doughs, such as foods made with whole wheat flour and other whole cereals. Xylanases enhance the gas retention capacity of dough, contributing to a softer and finer crumb. This kind of endo-xylanase is extracted from *Aspergillus* spp. and this enzyme preferentially hydrolyzes WE-AX, promoting gluten protein aggregation, due to its water-releasing capacity which is beneficial for gluten network formation. The 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. The resultant foods present an appropriate crumb structure, with ragged gas cell distribution, besides inappropriate crust color **[128,129,130,131,132,134]**.

The Bacterial Xylanase

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 the maximum volume for a longer period during the fermentation step, and it maintains a great resistance to mechanical stress during the food-making process. Oven spring is prolonged and food volume is enhanced due to dough relaxation and better gas retention, which produces finer grains that provide a softer and more homogeneous food crumb. For the same reason as for fungal xylanase, excessive dosage levels of bacterial xylanase must also be avoided **[135,136,137,138,139,140]**.

The Cellulase

This enzyme hydrolyzes cellulose (linear homopolysaccharide composed of a glucose polymer backbone linked by β-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 first attacked and degraded by the cellulases. This produces lower molecular weight fragments that can bind more water. The Cellulase action on cellulose has numerous benefits in the foodmaking process water absorption increases; dough viscosity increases; high-fiber dough stickiness decreases; machinability is enhanced; the release of glucose increases, and the cut opening for French rolls increases **[141,142,143,144,145,146,147,148]**.

Conclusion

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, enzymes and vital wheat gluten (an ingredient) to eliminate emulsifiers and chemical oxidants; hydrocolloids as a more “friendly” choice than other additives; and 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 foodmaking. An example is laccase, an oxidative enzyme that oxidizes different kinds of phenolic compounds, increasing dough stability and strength, promoting quicker dough formation, and reducing dough stickiness. Another example is β-glucanase, which hydrolyzes the β-glucans present in barley, rye, and oat flours, enhancing microstructure, volume, texture, shelf-life, and taste in foods made with these composite flours.

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