Review Article

Food additives and processing aids used in bread-making

Abstract

Bread is the most consumed food in the world. Bread can be used as a delivery vehicle for nutrients and supplements to the general public. To have a good quality bread, the use of optimum ingredients is essential. This chapter gives the crucial role of food additives and their components in baking science from dough to final loaf. Delving into the fascinating world of dough enhancement, the chapter will explore various additives such as dough conditioners, enzymes, emulsifiers, preservatives, and flour improvers. Further, it covers how these substances contribute to the texture, shelf life, and overall quality of bread. The discussion will not only encompass the positive aspects but also touch upon potential controversies and health considerations associated with the use of these additives. A trend of gluten-free bread is gaining attention over traditional source ingredients like bread. In this chapter, we discussed how one can make gluten-free bread without altering bread quality characteristics. A comprehensive review of the effect of additives on bread quality and their acceptable daily intake (ADI) and generally recognised as safe (GRAS) status has been discussed.

Keywords: Dough conditioners, Enzymes, Emulsifiers, Flour improver, Preservatives.

1.0 **Introduction**

Bread and other fermented foods have consistently been essential components of our diets from ancient times in one way or another. Initially, people consumed bread for energy purposes. As bread became one of the main staple foods in Western countries and other regional parts, people began seeking nutritional advantages beyond just calories. This shift in perspective emphasizes the significant role these products play in not just supplying energy but also delivering various essential nutrients and cutting down on calories. This evolving understanding highlights the diverse and crucial contributions of these foods to our overall health and well-being. In today's era, there's a growing demand for a new generation of healthier food products, such as low-calorie, rich in fibres, rich in antioxidants, and other health-modulating products. Along with that, they must also boast excellent sensory quality. To achieve these goals, a common practice involves incorporating additives like oxidants, enzymes, emulsifiers, and hydrocolloids. These additives serve various purposes, enhancing the breadmaking process, aiding in ingredient processing, compensating for raw material variations, ensuring consistent quality, and preserving freshness and other desirable food properties.

Additives are the ones that are incorporated in less quantity to improve the flavour, texture, appearance or shelf life of a product [1]. The International Numbering System, established by the European Union, assigns E-numbers to all approved food additives. These E-numbers serve as a universal identification system adopted by many countries to facilitate easy recognition and categorization of these additives. The incorporation of additives in food products,

including bakery items, is subject to regulation by the Food and Drug Administration (FDA) in the United States. The FDA establishes specific limits on the quantity of each additive permissible in a product and provides guidelines for the labelling of products containing additives.

2.0 Hydrocolloids

Hydrocolloids have proven their effectiveness, versatility, and safety as valuable supplements in the crafting of wheat bread. These food additives serve as stabilizers, thickeners, and gelling agents, impacting the stabilization of emulsions, suspensions, and foams, while also influencing the process of starch gelatinization. Through specific interactions, particularly with gluten proteins, these additives can exert either positive or negative influences on the rheological properties of the dough. They have the potential to enhance bread volume, crumb porosity, moisture retention, water mobility, antistaling properties, and texture, resulting in products with improved technological quality [2]. Various studies have documented increased farinographic water absorption by incorporating different hydrocolloids across a range of concentrations (0.1% to 5% on a flour basis). Gums contribute to antistaling effects in part by retaining more moisture during storage, thus preserving the softness of the crumb.

Regulatory bodies such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Scientific Committee on Food (SCF) have extensively evaluated hydrocolloids, including carboxymethylcellulose (CMC), Hydroxypropyl methylcellulose (HPMC), xanthan gum, guar gum, carrageenan, etc. Their assessments have concluded an ADI for unmodified and modified celluloses as well as other hydrocolloids, with no specified numerical value due to low toxicity and the absence of genotoxic concerns. Chronic toxicity studies have shown no adverse effects at levels up to 9,000 mg/kg per day, and there is no evidence of carcinogenic or reproductive/developmental effects. Combined exposure to celluloses at the 95^{th} percentile of exposure assessment for the general population has been deemed safe. As a result, a numerical ADI isn't deemed necessary, and safety concerns remain minimal at reported use levels [3, 4].

2.1 Carboxyl methyl cellulose (E466)

Carboxymethylcellulose (CMC), derived from cellulose, undergoes chemical modification to introduce carboxymethyl groups onto the cellulose backbone, enhancing water solubility and thickening properties. Typically, in powdered form, CMC is dosed between 0.1 to 2.0% based on flour weight. It exhibits broader water solubility across various pH levels and its stronger thickening abilities may result in a denser crumb texture in the finished bread. Highly effective as a water binder, CMC enhances dough hydration and handling properties, leading to softer and more pliable dough during shaping and processing. Its addition also improves cell size and porosity [5]. CMC and xantham gums alter dough time development, with xanthan decreasing and CMC increasing it [6]. Additionally, they enhance mixing and extend shelf life by retaining moisture and forming barrier coatings during heating to reduce water loss and oil uptake. No ADI but safety concerns remain minimal at reported (12-15 g/day) use levels.

2.2 Hydroxypropylmethylcellulose (E464)

HPMC, a non-ionic modified cellulose, incorporates methyl (19-30%) and hydroxypropyl (4-12%) groups into the cellulose chain, enhancing its surface activity and responsiveness to hydration and temperature changes [7]. It effectively forms hydrophilic bonds with various flour components, such as rice, maize, and potato, enhancing water absorption in gluten-free

breads [8]. Acting as a gel-like network within the dough, HPMC strengthens its structure, aiding in gas retention from yeast fermentation for improved dough rising and uniform texture. Moreover, it imparts smoother, cohesive consistency to the dough, reducing stickiness and facilitating shaping into different forms like loaves or rolls. Typically used alone or combined with other hydrocolloids, HPMC concentrations range from 0.5 to 2% based on flour weight, depending on application and desired attributes. It serves as an effective anti-staling agent, delaying crumb hardening and amylopectin retrogradation. HPMC contributes to water retention while enhancing dough elasticity and extensibility, easing shaping without tearing [9]. Modified celluloses, including CMC and HPMC (0.1%–0.5% f.b.), decrease the hardening rate and final crumb firmness value [10]. It creates interfacial films around gas cells, enhancing cell stability against expansion and processing variations. No ADI but safety concerns remain minimal at reported (12-15 g/day) use levels.

2.3 Xanthan gum (E415)

Xanthum gum (XG) is a polysaccharide derived from the fermentation of simple sugars by the bacterium *Xanthomonas campestris*. It is a free-flowing powder with a white to cream color that dissolves both in hot and cold water but is insoluble in the majority of organic solvents. The two most important characteristics of XG are strong shear thinning and high low shear viscosity. XG provides stable suspension properties and stability to colloidal solution due to high viscosity at a low shear rate [11]. The addition of XG increases the water-absorbing and gas-retention capacity of the dough. XG contributes enzyme, shear, and freeze-thaw stability, lessens flour sedimentation, increases gas retention, and produces uniform coating and excellent cling in wet-prepared batters. Typical usage levels range from 0.5% to 1.5% of the total flour weight [12]. Higher levels may be required in gluten-free bread formulations to compensate for the absence of gluten. As compared to other cellulose derivative hydrocolloids like CMC and HPMC, it has less capacity to retain water as well as porosity is not so good enough but compared to locust bean, k-carrageenan it has better retention ability as well as gives good porosity [13, 14]. However, it has a good synergistic effect with the other hydrocolloids for gluten-free bread to improve the viscosity, softer crumbs and in improving water retention [15, 16]. ADI value is not defined for xanthan gum.

2.4 Carrageenans (E407)

Carrageenans (CA) are linear sulfated polysaccharides with a sulfate content of 18–40%, extracted from red seaweeds. It consists of galactose and 3,6-anhydro-galactose units, which can be sulfated or non-sulfated. These units are connected by alternating α-(1-3) and β-(1-4)-glycosidic links [17, 18]. CA derivatives include gelling κ-CA, iota-CA, and non-gelling λ-CA. The rheological properties of carrageenan are influenced by the number of sulfate esters present in the copolymers. Higher levels of ester sulfate result in lower gel strength. Consequently, κ-carrageenan produces rigid gels, ι-carrageenan forms flexible gels, and λ-carrageenan does not gel but yields viscous solutions. Among these options, κ-carrageenan is the most commonly used substitute [19]. Typical usage levels range from 0.5% to 3% of the total flour weight and it may vary with the ingredients. It is good for forming gels that have better mouthfeel and moistness. Carrageenan demonstrates superior freeze-thaw stability compared to CMC, HPMC, and xanthan gum. This attribute is crucial for maintaining the structural integrity and quality of bread during storage and distribution, especially in the case of frozen or partially baked goods [17]. As compared to other hydrocolloids it does not have

much potential to improve the bread properties like firmness and specific volume, especially in gluten-free breads [20]. ADI of carrageenan is 75 mg per kg of body weight.

2.5 Guar gum (E412)

Guar gum, derived from the seed endosperm of *Cyamopsis tetragonolobus,* is a polysaccharide composed of D-mannose and D-galactose in a molecular ratio of 1.6 to 1 and its molecular structure features a linear backbone of mannose units linked together in a β-(1-4) pattern [21]. This configuration makes guar gum highly viscous at low concentrations and found application as an effective thickening, stabilizing, and water-binding agent. Guar gum is insoluble in hydrocarbons, alcohols, esters, and organic solvents, in general. On the other side, in cold or hot water solutions, guar gum hydrates rapidly and forms high-viscosity colloidal solutions, but it does not form gels unless other polysaccharides are added. In bread manufacturing, guar gum plays a crucial role in improving mixing tolerance, prolonging shelf life by retaining moisture, and preventing syneresis in frozen food products [22]. Typically used at dosages ranging from 0.3% to 1% of the total flour weight, guar gum contributes to achieving optimal texture, moisture retention, and overall quality in bread products. The performance of guar gum is affected by factors such as pH, temperature, and the presence of certain ions. No ADI is defined for this gum.

2.6 Locust bean gum (E410)

Locust bean gum (LBG) is a naturally occurring neutral polysaccharide extracted from carob tree seeds (*Ceratonia silique*). It consists of D-galactose and D-mannose units, with a molecular ratio typically ranging between 73:27 and 86:14, so it possesses inherent thickening and stabilizing properties. Its dosage typically ranges from 0.1 to 3.0% based on flour weight, and it exhibits water solubility across a broad pH range [23]. LBG does not dissolve entirely in cold water; however, upon heating above 80 °C (176 °F), it fully dissolves and forms pseudoplastic solutions [17]. LBG demonstrates a significant ability to synergize with κ-carrageenan and xanthan, resulting in the formation of gels characterized by elasticity, high cohesion, and minimal syneresis. The addition of LBG to frozen dough improves the resistance to extension, specific bread volumes and also reduces the proof time [20]. No ADI defined.

3.0 Emulsifier

Emulsifiers, also referred to as surface active agents or surfactants, play a crucial role in bread making by reducing the surface tension between hydrophobic-hydrophilic interfaces. In bread production, emulsifiers must possess specific characteristics to effectively facilitate the formation and stabilization of air bubbles within the dough, essential for its expansion during fermentation and baking. This process results in a softer crumb structure that inhibits staling, improves moisture retention, and extends shelf life. Additionally, emulsifiers streamline dough handling and shaping processes, ensuring uniform mixing and forming. They also contribute to achieving a finer crumb texture and preventing premature staling, thereby preserving freshness over time. Common emulsifiers used in bread making include diacetyl tartaric esters of monoglycerides (DATEM), polysorbates, sodium stearoyl lactylate (SSL), mono and diglycerides and their derivatives, lecithin, and sucrose esters.

3.1 Lecithin (E322)

The first emulsifier employed in the baking industry was lecithin, a natural substance derived from maize or soy oil. Lecithin is a phospholipid that consists of glycerol with two fatty acid groups and a phosphate group to which choline is attached. The characteristic emulsifying qualities of lecithin are derived from the phosphate and fatty acid groups' interaction. Generally, lecithin is incorporated in making darker bread and mixed grain bread which shows higher water hydration, extended shelf life, improved emulsification of lipids, improved gluten stability, larger loaf volume and improved baking properties. Depending on the flour quality, the best result is achieved by incorporating 0.2% lecithin in flour. However, the higher quantity of lecithin reduces loaf volume, minor crumb structure and crumb softness [24]. In the case of gluten-free breads, it may be on the higher side of up to 9% [25]. In comparison to other emulsifiers, its natural origin resonates with consumers seeking clean-label ingredients, while its unique ability to balance emulsification and gluten stability enhances the overall quality of bread products. However, it will not have much effect on improving the dough strength of bread. EFSA does not declare any ADI for lecithin because of no evidence of genotoxicity/cytotoxicity from animal studies with higher doses.

3.2 DATEM (E472e)

DATEM is an ester of glycerol derivative with edible fatty acid and mono- and diacetyl tartaric acid which is generally used in yeast-leavened products such as white bread. The ideal dose of DATEM ranges from 0.25 to 0.50% (based on flour). DATEM is available in various forms, including a sticky viscous liquid like fats or yellow waxes, and in flakes or powder form. It exhibits higher hydrophilicity when compared to mono- and diglycerides [26]. The incorporation of 0.4% DATEM increases the loaf volume by approximately 10 %. DATEM is greatly effective in increasing loaf volume and dough stability so that it improves dough strength while less effective in improving crumb structure and loaf volume [27]. The crumb grain may develop a coarser texture, showing the tendency for the formation of holes during using weaker flours. This characteristic renders DATEM particularly effective as a bread enhancer for creating crusty bread varieties. Additionally, it can be applied in white bread recipes to achieve a more open crumb structure [24].

3.3 Stearoyl Lactylate (SL)

SL is an anionic emulsifier derived from stearic acid esterified with lactic acid in the presence of sodium or calcium, produced through the utilization of fatty acid, 88% lactic acid, and an alkaline catalyst referred to as sodium stearoyl lactylate (SSL-E481) or calcium stearoyl lactylate (CSL-E482). It typically contains sodium or calcium (3.5 to 5%), 34% lactic acid, and an ester value ranging from 150 to 190. SSL is water dispersible at neutral pH and solubility is limited if the pH value is 4 to 5 this is mainly because of 15 to 20% free fatty acids in it, whereas CSL is less water dispersible as compared to SSL but more soluble in oil [28]. Functioning primarily through interactions with flour proteins via non-polar regions, SL forms hydrophobic bonds, thereby enhancing the viscoelasticity of the dough. Additionally, the incorporation of the stearic acid component within the moderately non-polar helical structure of starch results in the formation of an insoluble complex, effectively resisting retrogradation. This complex contributes to both dough strengthening and crumb softening, potentially increasing mixing time while shortening proof times and promoting gassing power [28]. The utilization of SL correlates with an improvement in loaf volume, facilitating the formation of smaller and finer air cells within the crumb of the final product. Moreover, it enhances dough handling

properties, reduces crumb hardness and staling, ultimately leading to an increase in specific bread volume. Notably, SL demonstrates efficacy in reducing the staling of bread by decreasing water mobility and amylopectin crystallization. However, for peak heights with increasing the concentration of SL, it did not increase but instead increased the mixing time. The maximum usage level is 3 g/kg or 3 g/L in bread as per EFSA [29].

3.4 MDG (E471) (DMG AND EMG)

Approximately 70% of food emulsifiers produced worldwide are made of mono- and diglycerides and their derivatives. Around 60% of all monoglycerides are utilized in bakeries with 20% in cakes and sponge cakes and 40% in bread. Mono- and diglycerides are generally manufactured by esterification (glycerolysis) of triglycerides with glycerol, yielding a mixture of mono, di and triglycerides. Due to their lipophilic nature, monoglycerides dissolve in oil and stabilise water/oil emulsions, forming reversed micelles in the oil. The dispersibility characteristics of the emulsifiers during dough mixing determine the functioning of monoglycerides and other emulsifiers in bakeries [26]. Glyceryl monostearate exerts a pronounced impact on softness while displaying a comparatively less influence on loaf volume which gives a fine crumb with considerable elasticity. Glyceryl monostearate works by delaying the retrogradation of starch. The ideal dosage is 0.2% of the base flour [24].

3.5 Polysorbate (PS)

PS are non-ionic surfactants and are mixtures of partial esters of fatty acids with sorbitol and its anhydrides. PS 20, PS 40, PS 60 and PS 80, refer to PS derivatives where the main hydrocarbon tail is laurate, palmitate, stearate and oleate respectively (C12:0, C16:0, C18:0 and C18:1) [30]. PS 20 (E432), PS 80 (E433) and PS 60 (E435) are common emulsifiers used in the industry. These emulsifiers interact via hydrogen bonding, forming crosslinks among gluten, thus enhancing dough and bread properties such as loaf softening, dough strengthening and volume increase, however, they are not particularly effective for softening. These emulsifiers usually exert their effect during fermentation, dough handling, shaping and baking as with other dough strengtheners. Additionally, PS prolongs proof time and inhibits gassing power [31]. Reduces the dough development time and dough stickiness, increases the mixing tolerance index and thickens cell walls in the crumb. Notably, PS does not harden the crumb during storage and does not significantly contribute to the retrogradation of starch. Recommended doses range from 0.5% to 1% on a flour basis. The overdosage of polysorbate leads to gastrointestinal and metabolic syndrome has been reported. JECFA has given ADI for the polysorbate group is 25 mg/kg.

4.0 Enzymes

In recent years, the application of enzymes has increased because of more natural-sounding components. Enzymes in wheat flour are diverse and primarily found in the germ and aleurone layers. Enzymes are used as an alternative to chemical agents such as hydrocolloids, emulsifiers etc. Some enzymes are present in wheat flour while some are added. Proteases, oxidoreductases, and amylases are the three most significant types of enzymes. Enzymes increase the loaf volume and improve dough characteristics [26]. The factors affecting enzyme activity are temperature, pH, water activity and enzyme concentration.

4.1 Amylases

Bread staling is often the main problem in bread due to the retrogradation of starch specifically amylopectin undergoes molecular arrangement and transformation leads lower shelf life and causing huge losses to the industry. It not only causes economic loss but also downsizes the company's reputation. To cope with up above problem industry uses α-amylases (α-1,4-glucanohydrolase) enzymes to break down starch into low molecular weight dextrin and maltose. α-Amylase belongs glycosyl hydrolases family hydrolyze α-1,4-glycosidic bonds of the amylose or amylopectin chain and converts into oligosaccharides such as maltose and maltotriose, and dextrin. These α-amylases can be obtained from either fungal, cereal or through bacterial sources [32]. A thermal labile α-amylase enzyme can be produced from the *Bacillus licheniformis*. The enzyme is stable at 90°C. The use of α-Amylase lowers the crumb hardness, improves crumb structure and loaf volume, and hinders the staling in whole wheat and white bread [31].

β-amylase, is the exoenzymes that break down dextrin into maltoses, further maltase from yeast sources breaks down maltose into glucose. α- and β-Amylase only hydrolyzes the α-1,4-glycosidic linkages in starch. α- amylase randomly attacks the α-1,4 linkage, whereas β-Amylase attacks the α-1,4 linkage at the nonreducing sugar end. As a result, only 50–60% of the amylopectin is converted to maltose due to incomplete hydrolysis. Because amylose has a somewhat branched structure, the greatest degree of hydrolysis in this situation is 75–90% [32]. Amylose has a crucial role in the manufacturing of bread, as the specific loaf volume of gluten-free bread depends on the increasing level of amylose content in rice bread [33]. When a higher quantity of β-Amylase is added, the dough becomes non-machinable because of stickiness [26].

Glucoamylases are derived from the fungi *Aspergillus oryzae*, which can act on α-1,6 glycosidic linkage to produce a reducing sugar. Amylase from a fungal source exhibits lower temperature stability compared to its bacterial thermostable amylase from *Bacillus subtilis*. The use of bacterial amylase shows residual activity in the bread even after the baking process. To address this, an α-amylase with intermediate thermostability is used as an effective antistaling agent in baked goods [34].

As compared to fungal amylase, bacterial amylase breaks down starch more vigorously. It is because of its ability to effectively target the amorphous areas of starch granules that result in excessive dextrinization and a corresponding reduction in dough viscosity, giving open-textured crumb. Bacterial amylase effectively increases the shelf life of bread but overdosing may produce undesirable texture [24]. The European Union classifies amylase enzymes as processing aids because they do not exert a functional effect on the final product. The use of these enzymes in baked goods is regulated under Regulation (EC) No. 1332/2008 and Regulation (EC) No. 1169/2011

4.2 Protease

Protease enzymes are used commercially in the bakery industry. Protease reduces dough consistency, decreases mixing time, increases dough uniformity, and improves the flavour and texture of the bread. Proteases hydrolyze peptide bonds and reduce gluten elasticity which reduces the shrinkage of dough or paste after moulding and sheeting [32].

Malt proteases are mostly used in weak flours to hydrolyze gluten proteins and make the dough softer. *Aspergillus oryzae* is the source of fungal protease which is heat-labile so the activity is limited to the dough phase only. Bacterial proteases, derived primarily from *Bacillus subtilis*,

exhibit high activity and are exclusively employed in the United States to weaken rusk dough. In laminated dough (for crackers), bacterial proteases help prevent cracks, curl, and the development of gas bubbles [26].

An amino acid produced by an enzyme through proteolysis action reacts with reducing sugar at baking temperatures so-called Maillard reaction, producing the desired crumb colour and bread flavour. A treatment of rice flour with 50 μg of protease from *Aspergillus oryzae* resulted in high specific volume bread due to swelling of dough, more gas retention, change in dough viscosity and protein degradation [33]. Thermoase from *Bacillus stearothermophilus* was used to treat dough at 25℃ for 16 h. Glutelins and prolamines are digested by thermase enzyme, as a result, high volume, good crumb appearance and soft texture product obtained. During the storage period staling rate and crumb hardness of bread were considerably lower [35].

4.3 Lipases

Lipases also called triacyl glycerol acyl hydrolases hydrolyse triacylglycerol and produce monoacylglycerol (MAG), diacylglycerol (DAG), glycerol and free fatty acid. Even though cereal grains contain lipases but in less quantity which does not rancid the flour. Compared to proteases and α-amylases, the application of lipases has started recently [36-38]. Lipases enhance dough stability and gluten network which improve the qualities of dough handling and machinability. Lipases also increase loaf volume, harden the dough and improve the crumb texture [39].

In 1990, first-generation lipases came into existence and now third-generation lipases are used. Third-generation lipases are more effective in high-speed dough mixing and no-time dough process. Moreover, they produce less short-chain free fatty acid reducing the risk for off-flavour production during prolonged storage of the baked goods [40]. Some proportion of emulsifiers can be replaced by lipases which is why lipases are also used as antistaling agents [41].

4.4 Glucose oxidase

Glucose oxidase (GOX, EC 1.1.3.4) functions as an oxidoreductase, an alternative to oxidizing agents, effective fast oxidizing enzyme. The enzyme is produced mainly from Aspergillus spp. The enzyme catalyzes the β-D-glucose into gluconic-D-lactone in the presence of oxygen. Subsequently, it converts to gluconic acid and hydrogen peroxide. Hydrogen Peroxide serves as an alternative to calcium peroxide and indirectly oxidises the gluten sulfhydryl group to disulfide bonds [41]. It shows a drying effect in bread dough. GOX reduces resistance in whole wheat dough, similar to white dough.

Glucose oxidase acts as a chemical oxidant: reducing the extensibility of dough, increasing in resistance of dough and bread volume. GOX works by oxidising the water-soluble sulfhydryl group and oxidative gelation of pentosane [42]. GOX affect the water extractable fraction and does not affect the gluten protein extractability. The addition of GOX results in an increase in water absorption, dough elasticity and tenacity.

4.5 Hemicellulase

Hemicellulases are a broad class of hydrolyzing enzymes that catalyses hemicellulose such as arabinoxylan, xylan, xylobiose, and arabinogalactan etc. Arabinoxylen has significant importance in bread making, hence they are the primary focus for enzymatic activity. Among

hemicellulases, xylanase or endo-1,4-β-xylanase (4-β-D-xylan xylanohydrolase) is a widely used enzyme that hydrolyzes 1,4-β-D-xylosidic linkages in xylan and arabinoxylan [43].

Xylanase converts water-insoluble hemicellulose to water-soluble form that holds water in the dough which enhances bread volume, improves crumb uniformity, reduces dough firmness and reduces dough stickiness [44]. Utilising xylanase in combination with other enzymes yields superior outcomes than xylanase used alone. The use of different enzymes has a better effect on bread quality compared to a single enzyme. The combination of α-amylase and glucose oxidase enhances dough extensibility and bread volume. When commercial enzyme combinations including α-amylase and lipase activity are added to bread samples made by the straight dough method, the bread-keeping qualities will improve and a more thermostable amylose-lipid complex will be formed.

5.0 Oxidizing and Reducing Agent

Oxidizing agents or maturing agents oxidize the thiol group of gluten and form disulfide bonds. These additional disulfide bonds strengthen the gluten network shorten the mixing time and also increase the rate of natural aging or oxidizing of flour. Oxidizing agents also oxidize carotene and xanthophyll pigment which bleach the colour of flour [45]. Oxidants (for example, potassium bromate, ascorbic acid, azodicarbonamide, and ammonium persulfate) enhance the fermentation process, and elasticity, and improve the stability of the dough. As a result, the dough exhibited increased strength, yielded a larger oven rise, and featured a finer crumb grain. The application of oxidizing agents depends on flour quality, preparation method and legislation of that country [26].

5.1 Oxygen

The most unknowingly used oxidant is the molecular oxygen in dough mixing of bread making. This oxygen gets incorporated with the dough during the mixing stage and makes the dough stronger by gluten cross-linking. Mixing dough in the presence of oxygen than nitrogen atmosphere results in stronger dough with lower extensibility and higher resistance. Hydrated starch granules adsorb the oxygen during the mixing stage and it get desorbed during baking. Hence, contributing to loaf volume and helping in S-S bond breakage leads to higher extensibility and lower elasticity in the dough. Molecular oxygen is required for some chemicals to react, enzyme action and other biological reactions. Lack of oxygen during mixing and over-mixing leads to the desorption of oxygen at an early stage leading to lower bread quality. It's the first and universal oxidant used by most baking industries.

5.2 Ascorbic acid (E300)

Ascorbic acid is the most widely used improver in the baking industry. It is most popular in those countries where it is forbidden for use. Ascorbic acid is less effective compared to bromate in rising loaf volume. Typically used at the level of 20 to 150 ppm in bread making depending on cultivar and storage time. It is naturally found in fruits and vegetables and is also known as vitamin C. Most of the ascorbic acid used in industries is made from glucose using fermentation and chemical methods. L-threo-ascorbic acid is the most effective oxidant and inhibits protease enzymes in the dough. The SH/SS exchange reaction between neighbouring protein chains containing SH groups is suggested to be an essential process in the growth of the gluten network [46]. Ascorbic acid increases the dough strength, crumb structure & colour,

and reduces dough stickiness. It does not reduce the loaf volume at an overdosage level as in the case of other oxidants.

During the mixing of dough air incorporation takes place, and the oxygen gets entrapped inside the gluten network. In the presence of reducing agents like ascorbic acid reacts with oxygen later, the ascorbic acid oxidase enzyme which is present in wheat flour, converts ascorbic acid into dehydroascorbic acid. Further dehydroascorbic acid oxidizes reduced glutathione to oxidized form by an endoenzyme present in the wheat flour called glutathione dehydrogenase. Thereby breakage of S-S bonds and exchange of SH group takes place in bread dough. This action makes dough more elastic and higher dough volume, provides a more uniform and finer crumb texture and reduces extensibility [47]. Ascorbic acid will be active only when a sufficient amount of oxygen is incorporated and is available to convert ascorbic acid into oxidized form [48]. Ascorbic acid is considered as GRAS status by the FDA.

5.3 Azodicarbonamide (E927)

Azodicarbonamide (ADA) is used as a whitening agent in cereal flour and dough conditioner in bread making. It is a maturing agent utilized in flour premixes that promptly oxidizes available thiol groups (─ SH) within flour proteins, thereby enhancing dough strength. This process is notably efficient in altering the dough characteristics of low-quality flours by enhancing both the processing behaviour and gas retention properties [49]. The addition of ADA in dough alters the rheology of dough by increasing the storage modulus and the loss modulus. Dough consistency, extensibility ratio and strengthening of dough increase with the increase in the level of ADA [48]. It is reported that the optimum quantity of ADA enhances bread volume and fine crumb structure but overdose may reduce bread volume. The application of ADA is efficient in the Chorleywood bread process while in other methods it can be problematic due to the fast reaction rate. The use of ADA is banned in EU countries because of the presence of a reaction product, semicarbazide, which poses health risks but in other countries, it is still used [46].

5.4 Potassium bromate

Brazil, Argentina, China, India, Germany and Italy are the top 5 producers of potassium bromate. Potassium bromate, commonly referred to as bromate in the industry, has been extensively utilized in the baking industry as an oxidizing agent. It exists as an odourless, colourless white crystal or powder, exhibiting high solubility in water and lower solubility in acetone, ethanol, methanol, dimethyl sulfoxide and toluene. It reduces the ageing time, widely used for fermentation and proofing of a dough in a baking process. It interacts with the gluten-forming proteins in flour, enhancing dough strength, retention of carbon dioxide, improving gas cell structure and yielding bread with full loaf volume and a uniform fine-crumb structure. Bromate affects mainly the gelatinization temperature of flour, and bromate had nearly one-third of the birefringence value of that control. Bromate act as an improver by reacting with the gluten and releasing the free sulfhydryl group. These vital disulfide linkages are necessary for making bread with proper physical qualities. Bromate prevents the cross-linking formation between gliadin and glutenin, thereby ensuring the SH group is available for cross-link formation during the baking process. The formation of low molecular weight protein leads to a decrease in the elasticity thereby increasing the extensibility of dough.

Bromate is recognized as a slow-acting oxidant compared to others, manifesting minimal effect during the mixing phase. It is active at the last stages of proofing and baking. Its primary impact is observed during prolonged proving time and the early stages of baking. This is because bromate activity depends on temperature, as temperature increases, activity will also increase. Potassium bromate degrades with distilled water at 350 ℃ to yield oxygen and potassium bromide. Whereas it degrades in wheat flour at 150-200 ℃ due to the catalytic activity of metal ions present in wheat flour such as copper, iron, magnesium, aluminium and zinc ions. Bromate effectiveness and activity can be enhanced by combining with ascorbic acid [46]. The average level of bromate in bread making is 10 ppm.

Regardless of its numerous advantages, there have been reports of serious health issues associated with it, such as diarrhoea, abdominal pain, vomiting and irritation to the upper digestive tract. However, under a controlled level, it transforms into potassium bromide, which is considered to be safe for consumption. It is important to note that a complete reduction of bromate to bromide cannot be assumed as it depends on the baking temperature, time, form (powder or aqueous) and quantity of bromate used. Degradation of bromate can be achieved through the use of some reducing agents and ferrous sulphate. The usage level of bromate depends on the country's regulations. The European Union and JECFA, FAO and WHO panel banned the usage of potassium bromide. However, the FDA sets the acceptable limit as not more than 75 mg/kg of flour.

5.5 Iodate

These are much stronger oxidants than the bromates. Iodate oxidizes the free SH group and reduces to iodide form during the baking process. The use of high levels of iodate leads to the polymerization of high molecular weight glutenin as a result it prevents crosslink formation between them by oxidizing SH groups in higher sulfur oxidation states. Thereby it hinders crosslinking resulting in low-loaf volume bread. The extractability of glutenin and gliadin was reduced in iodate-treated flour during the mixing, fermentation and baking stages. Although iodide is generally recognized as safe, it presents a challenge in bread-making.

5.6 Chlorine dioxide and chlorine

Chlorine is a weak bread improver, mainly used for the bleaching of carotenoids in flour. It can be used up to 2,500 mg/kg. the other application of chlorine includes disinfectant of water, equipment and other food materials. The improving mechanism of chlorine is unclear and it may be due to the breakage of hydrogen and peptide bonds. Gliadin proteins were more prone to chlorine than glutenins. Chlorine oxidises both aromatic amino acids and the SH group whereas SS bonds were unaffected. The chlorination of flour improves the appealing quality, white crumb, fine and uniform gas cells, high loaf volume and good sensory properties of bread.

5.7 L-Cysteine (E920)

L-cysteine serves as a reductant or reducing agent utilized as an improving agent in bread-making. As a naturally occurring amino acid containing a free -SH group in its molecule, L-cysteine catalyzes disulfide bonds of gluten-forming proteins through SH/SS exchange reactions. This process leads to the formation of softer dough with decreased elasticity and increased extensibility. However, excessive use of reducing agents like cysteine can result in sticky and poorly handled dough. Cysteine is typically employed in hard flours and those with short gluten, where the gluten is resistant and possesses low extensibility, resulting in

diminished gas retention, volume, crumb, density, and porosity. The inclusion of cysteine weakens the dough, reducing its viscoelasticity, mixing time, and tolerance to mixing. Conversely, it enhances the adhesiveness, machinability, and extensibility of the dough.

In the activated dough development method, the reducing agent plays a crucial role in breaking down high molecular weight glutenins into lower molecules during the mixing phase. While high molecular weight glutenins contribute to dough elasticity, low molecular weight glutenins enhance dough extensibility. To restore the desired dough characteristics essential for breadmaking, additional oxidizing agents are introduced into the dough during proofing, promoting the formation of larger molecules. L-cysteine is typically used in concentrations ranging from 50 to 300 ppm (on a flour basis). However, adding only L-cysteine may not yield beneficial results, as it can decrease bread volume and result in a coarse crumb structure. Therefore, it is recommended to use L-cysteine in conjunction with oleo gels or thickening agents to achieve better quality bread. This dough conditioner has obtained GRAS status from the FDA for use in bakery applications and the ADI of cysteine is 2.2 g per day.

6. Leavening agents

A leavening agent expands the volume of dough and batter by foaming action to soften and lighten the food mixtures. In food items, leavening agents are utilised as the nucleation seed for gas production or to aid in the creation of structure and texture by gas expansion resulting from a chemical reaction [65]. The incorporation of air through mechanical action is an alternative to a leavening agent.

6.1 Yeast

The use of yeast in bread making started 6000 years ago for dough fermentation with ancient Egyptians [50]. The most widely used strain of yeast in bread making is baker's yeast *Saccharomyces cerevisiae*. Generally, yeast is used at the level of 1% of flour basis. The optimal temperature of 34-38 °C and pH of 4.5-5.5 is ideal for maximum yeast activity. Upon fermentation, yeast produces zymase enzyme to break down fermentable sugars yielding ethanol and CO_2. The yeast can withstand the 15% ethanol concentration during fermentation. Most of the alcohol will get volatized during baking and entrapped CO_2 provides leavening. This results in better crumb structure and increased bread loaf volume. Yeast not only imparts leavening action but also serves characteristics flavour, crust colour, nutritional supplement and a key functional component in bread and other baked goods [51].

The sugar exists in flour as a starch, added sucrose and maltose from enzyme activity act as a substrate for yeast fermentation. Sucrose, a substrate for yeast, can affect negatively if used in excess. High osmotic pressure exerted by sucrose leads to the dehydration of yeast cells which will interfere with the metabolic activity of yeast. The same effect can be observed with the addition of sodium chloride. The osmotic pressure of sodium chloride is 12 times higher than sucrose because of its low molecular weight and ability to form double ions. Therefore, sucrose and sodium chloride can be adjusted in formulation to regulate the fermentation rate, ensuring it is neither too fast nor too slow.

Baker's yeast is available in various forms but the main difference among them is moisture content. Compressed yeast is the most potent in terms of its leavening ability. It typically contains approximately 67-71% moisture content. It is unstable at higher temperatures due to high moisture content. However, when stored under refrigeration (2-7 °C), it remains relatively

stable for 3-4 weeks [52]. Bulk yeast is essentially identical to compressed yeast but available in granular form. It requires refrigeration like compressed yeast. Because of its particulate nature, it is more stable and offers rapid cooling. Active dry yeast contains a moisture content of 4-8 % and is prepared from a special form of yeast. It is stable during drying so has a shelf life of 2-12 months at room temperature and several months in refrigerated conditions. Before adding it to the dough it needs to dissolve in lukewarm water. Dry instant active yeast is similar to active dry yeast available in granular form. It has a moisture content of 4-6% and a shelf life of more than a year.

In sourdough bread making both yeast and bacteria (like Lactic acid bacteria) are used as leaving agents in which yeast produces CO_2 and bacteria produce acid (like lactic acid) to give a sour flavour to bread. The use of other leavening agents like Sodium bicarbonate, baking soda, ammonium bicarbonate and other double-acting leavening agents is limited in bread-making.

7. **Preservatives**

Preservatives are indispensable additives in bread making, vital for prolonging shelf life and safeguarding product quality by inhibiting microbial growth and preventing spoilage, effectively addressing bread storage problems. Bread typically exhibits a high water activity, approximately around 0.96. This high water activity makes bread susceptible to microbial growth, including yeast, bacteria, and mold. The growths of microbes contribute to the reduction of the shelf life, ultimately resulting in detectable mold growth and the generation of mycotoxins that are not identifiable. Preservatives like propionate, sorbate, acetate and ascorbic acid are strategically incorporated into bread formulations. These act by altering pH levels, disrupting cellular processes, or interfering with microbial metabolism, these compounds ensure bread remains fresh, flavourful, and safe for consumption.

7.1 Sorbates

Sorbic acids and their potassium salts are generally recognized as safe for their use in bakery goods and these are effective in inhibiting mold growth. In commercial use of sorbates fungi static are twice as effective as propionates in bread and yeast-raised products. However, incorporation affects yeast and produces sticky dough which is difficult to handle and also baked products may have irregular structures and reduced loaf volume. These potassium sorbates have a great effect against Penicillium and Aspergillus spp. The usage levels range from 0.001 to 0.3% in bakery products. The challenge can be addressed by applying sorbate onto the product post-baking or blending anhydrates of sorbic acid with fatty acids like palmitic or the use of encapsulated sorbic acid. Moreover, the utilization of sorboyl palmitate has proven effective in managing mold growth without disrupting the fermentation process. During baking, the heat causes sorboyl palmitate to hydrolyze, releasing sorbic acid that hinders mold development during storage.

7.2 Propionates

Propionic acid, a naturally occurring organic acid, and its salts, including sodium, potassium, and calcium, are white, free-flowing powders with a cheese-like flavour. These preservatives serve dual purposes: firstly, to slow down mold development, and secondly, to prevent bacterial spoilage of bread, commonly referred to as "rope," caused by specific Bacillus spp., notably *B. subtilis* and *B. licheniformis*. Despite their effectiveness, the utilization of calcium propionate

comes with practical drawbacks, such as its adverse impact on loaf volume. This affects yeast activity and alters the dough characteristics leading to a reduction in the loaf volume. Furthermore, concerning propionic acid, excessive dietary intake has been linked to propionic acidemia in children. Complications associated with this condition encompass learning disabilities, seizures, arrhythmia, gastrointestinal symptoms, and recurrent infections, among others.

7.3 Acetates

Acetic acid exists as a colourless liquid or crystalline solid, while acetates are typically white crystalline powders or granules. These acetates can be used in different forms, such as free acetic acid or sodium, potassium, and calcium acetates (known as E261, E262, and E263, respectively). In baking, acetic acid, a key component of vinegar, has long been used to fight against bacterial spoilage, notably addressing the common "rope" issue and extending the mold-free shelf-life of bread. Its application not only gives products a natural appeal but also shows effectiveness against "rope" at concentrations equivalent to 0.1–0.2% of acetic acid on a flour basis. However, its ability to fight molds decreases at such levels. Moreover, higher concentrations can give the bread an undesirable vinegar smell. Furthermore, in the typical preparation of sourdough bread, lactic acid bacteria (LAB) are commonly utilized, resulting in the production of various acetates, including ethyl acetate and isoamyl acetate. These acetates serve as natural preservatives, helping to extend the shelf life of the bread.

8.0 Advanced Food additives in bread-making

8.1 Bacterial nanocellulose (BNC)

BNC is a microbial cellulose made from a low molecular weight carbon source called D-glucose. BNC is a biopolymer produced by aerobic bacteria mainly *Gluconacetobacter* spp., which forms a 3-D morphological network structure [53]. An exopolysaccharide excreted to air as a ribbon shaped of 2--4 nm nanofiber less than 100 nm wide. The USA Food and Drug Administration has classified BNC under GRAS status [54]. Similar to other kind of hydrocolloids, it can be used as thickening, gelling, stabilizing and suspending agents in various product development. Further cohort workers used BNC as a fat mimic and replacing agent. The fine structure of BNC helps in better water holding. BNC was added at 0.14% level in bread making, the resultant bread had higher specific volume and crumb moisture content. Firmness of the bread was reduced by 25%, and both cohesiveness and resilience were lower compared to the control [55]. This improvement enhances gas retention, resulting in a more porous crumb and a softer bread texture. The addition of BNC reinforced the elastic property of a dough by strengthening the cross-linking network.

8.2 *Cistus incanus*

Cistus incanus L. (CI) is a medicinal plant belonging to the shrub family, it exhibits antioxidant, antimicrobial, anti-inflammatory, anti-diabetic, anti-ulcerogenic and cytotoxic properties [56]. CI is a rich source of antioxidant compounds like flavonoids, and terpenoids. ellagic and gallic acids and terpenoids [57]. The Cistus spp. is used as a herbal infusion, food additive and nutraceutical product. CI leaves have been used in the preparation of food supplements, herbal tea, pasta, Aronia juice and bread making to enhance the functional properties of food.

Cohort workers used both CI leaves and CI extract at 1-5% level in the preparation of dough. The addition of CI to bread increased the bread yield due to more absorption of water during the dough development stage. Whereas, the specific volume and loaf volume are slightly less at higher concentrations. However other properties like porosity, baking loss, acidity, and moisture of bread were improved with CI level. The addition of CI leaves increased the hardness of the bread and reduced the springiness, chewiness and cohesiveness [57]. Whereas CI extract incorporation was reduced 50% hardness than a control, bread springiness, gumminess, cohesiveness and resilience were decreased considerably [58]. Moreover, CI can be used for improving the nutraceutical properties of bread as the addition increases the total phenolic and antioxidant activity of bread. But the overall acceptability of bread considerably decreased with the CI level, so up to 3% CI level addition gives the promising quality bread without lowering other quality attributes. As the CI is rich in tannins, it may interfere with the protein and some mineral absorption.

8.3 Propolis

Propolis is a resinous mixture compound prepared by honeybees during the collection of nectar from various parts of plants, exudates and buds. This resinous compound is mixed with wax and saliva, and is used to repair hives and also as a protection barrier against invaders. The chemical composition of the propolis varies with the type of plant, geographical region, temperature, etc. It is a rich source of polyphenols, terpenoids, sesquiterpenoids, cinnamic acid, essential oils, aromatic acids, benzaldehyde resins, sterols, minerals and other organic compounds [59]. Due to the presence of these compounds aqueous and alcoholic extract of propolis shows remarkable antibacterial, antimicrobial, antiangiogenic, cardioprotective, hepatoprotective and antioxidant properties [60].

Propolis-supplemented bread does not affect the nutritional composition of bread, only increases the mineral content of bread. The use of low concentrations of propolis does not affect the specific volume and gas retentional properties of gluten. Propolis affects the textual properties of bread such as hardness, chewiness, cohesiveness and fracturability [61]. Propolis intensifies the formation of amylopectin crystals after baking leading to an increase in the crystalline to amorphous ratio. Among alcoholic and aqueous extracts, alcoholic extracts cause intense colour changes in bread due to better solubility of pigments in alcohol compared to water. The addition of propolis to bread makes the nutraceutical food by improving antioxidant properties [66]. The water activity of bread is usually around 0.95 and is more susceptible to mould growth. To overcome these bakers generally add chemical preservatives i.e., calcium propionate. But nowadays consumers prefer chemical-free bread, Propolis-enriched bread is a good option for bakers for better shelf life and to claim health benefits.

8.4 Transglutaminase

The enzyme EC 2.2.13 belongs to a class of transferase enzymes. An extracellular enzyme produced by Streptomyces mobaraensis. A transglutaminase (TGase) is a glutamine g-glutamyltransferase protein that catalyzes an acyl transfer reaction between a γ-carboxyamide of protein-bound glutamine or peptide by introducing a covalent cross-link between amines, peptides, proteins and other deaminated products of glutamine. The catalysis of the glutamine-lysine crosslink by TGase leads to the formation of high molecular weight polymers, thereby altering the functional properties of proteins and enhancing the rheological and textural characteristics of food products. In the absence of lysine residue or other primary amines, water

serves as an acyl acceptor, causing the carboxyamide groups of glutamine residue to deamidate, forming glutamic acid and ammonia residues. This biochemical reaction results in a change in the surface charge of proteins, consequently affecting their solubility and function. It is noteworthy that the crosslinking reaction catalyzed by TGase in food systems precedes the deamidation reactions. Considering that covalently linked peptides function as endogenous amino acids, the crosslink formation catalyzed by TGase has the potential to improve the nutritional value of proteins. By forming cross-linkage, the enzyme influences the functional textural properties of a food product. Indeed, this cross-linkage affects protein gelling ability, water-holding capacity, elasticity and thermal stability [62]. As consumer preference is changing over time from wheat bread to gluten-free and vegan bread. However, it's important to note that gluten plays a crucial role in the formation of a 3-D network during breadmaking and this network helps in entrapping gas during the yeast fermentation process, which in turn contributes to the volume and porosity of the bread. Prevalence of celiac disease restricted the consumption of wheat bread. To overcome this and to have a similar viscoelastic property of gluten, cohort workers searching for alternatives to date.

TGase helps in moisture retention thereby reducing the amount of yeast and hardness of bread crumbs. The yeast quantity was reduced from 2.4% to 1.2% in gluten-free bread without losing its structure and other textural properties [63]. The use of TGase reduces the use of thickening and other gelling agents and proofing time in bread making. The addition of TGase along with whey protein shows higher specific volume and crumb porosity. The use of TGase improves the mixing stability, maximum resistance of dough, strengthens gluten network, complex modulus, pore structure, loaf volume, thermal stability of dough, bread yield and specific volume. Whereas other properties like crumb hardness, storage modulus and extensibility of dough, and weight loss of bread decreased. The efficiency of the enzyme depends on the protein source and level of the enzyme. Cohort workers used enzymes up to 2% level of flour in bread preparation. TGase is mainly found in applications in gluten-free and composite flour bread. However, TGase can be used in the case of insect-damaged wheat grains and low-gluten-quality wheat grains. Since all the enzymes are protein in nature, they can be easily inactivated with baking. FDA gave GRAS status in 1998 for microbial TGase.

8.4 Laccase

Laccase (LAC, EC 1.10.3.2) is an enzyme containing copper ions that catalyzes the oxidation of various phenolic compounds by removing a single electron, thereby generating reactive phenolic radicals. Its substrates include tyrosine residues in proteins and ferulic acid esters linked to arabinoxylan. LAC facilitates the oxidation of tyrosine and tyrosine-containing peptides, leading to the polymerization of these compounds. Moreover, LAC possesses the ability to displace phenoxyl radicals to SH groups, producing SH radicals, thus potentially acting via SH/SS interchanges [64].

Treatment with LAC generally results in increased dough firmness and decreased extensibility. The firmness of LAC-treated dough decreases over time, possibly due to radical-catalyzed depolymerization of the arabinoxylan network initiated by radicals produced by LAC. Thermal inactivation of LAC after gel formation of water-extractable arabinoxylan stops free radical production and stabilizes gel hardness, leading to bread with increased specific volume.

The effect of LAC is primarily attributed to the crosslinking of ferulic acid residues esterified to the arabinoxylan fraction of dough, resulting in a robust arabinoxylan network. The

difference in firming between flour-based doughs and doughs from hydrated gluten suggests that arabinoxylan, more abundant in flour dough than in gluten dough, is the preferred substrate for LAC. However, despite LAC primarily acting on arabinoxylans as an oxidoreductase, it can also impact gluten quality

9. Conclusion

Physical staling, chemical changes, and microbiological spoilage lead to the quality degradation of freshly baked bread that impacts product shelf life. To combat these challenges, bakers need advancement in the ingredients they use. Bread additives and processing aids play a significant role in modern breadmaking. The utilization of enzymes, hydrocolloids, reducing agents, emulsifiers and dough conditioners offers a range of benefits such as improving texture, extending shelf life, and reducing allergenicity. From enzymes like amylases, laccase, transglutaminase and xylanases to reducing agents like L-cysteine, these additives help optimize dough characteristics and enhance the overall quality of bread products. Furthermore, advancements in additive technology continue to drive innovation in the industry, allowing for the development of gluten-free and vegan bread options that cater to evolving consumer preferences. As research in this field progresses, it is essential to prioritize the safety and health implications of these additives while striving to meet the ever-changing demands of consumers for high-quality, nutritious, and flavorful bread.

Conflict of Interest

The authors declare no conflict of interest.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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Details of the AI usage are given below:

1. ChatGPT

2.

3.

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