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Review

Microorganisms, Drying, and Preservation Processes in the Baking Industry

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Abstract: The required processes and steps for making bread include technological and innovative concepts. The current trend is the use of less toxic compounds and green processes. Besides lactic acid bacteria and yeast, other microorganisms with special properties, such as enzymes, new aroma and flavor, exopolysaccharides, and vitamins, among other compounds with beneficial properties, could be added to bread manufacture, improving bread quality and health effects for the consumers. Freeze drying, new encapsulation methods, cryoprotectants, spray drying, fluidized bed drying, and vacuum drying are used for probiotics and microorganism cultures that will be used as starters or biological additives in the fermentation. The same development is observed in the preservation methods, and studies with plant extracts and essential oils have been proposed and introduced, replacing chemical agents, such as propionate, within the clean-label bread formulations concept. Baking science is a growing research line incorporating innovative methods and biological additives.

Keywords: baking; yeast; LAB; sourdough; essential oils; plant extracts; probiotics; drying preservation

1. Introduction

Bread is one of the oldest foods produced by humans, with its consumption dating back to findings more than 14,400 years ago in northeastern Jordan [1]. Since then, this process has been perfected, leading to the industrial production of the first specific pressed yeasts for baking 1780 in the Netherlands [2], completely altering the bread production process, especially in comparison to natural fermentation. Bread is a source of essential nutrients, including carbohydrates, fiber, vitamins, and minerals. It is one of the most consumed food products worldwide, with an average consumption of 70 kg (41–303 kg)/year/capita worldwide [3]. The fermentation by yeast and lactic acid bacteria (LAB) in the typical bread or sourdough is the critical manufacturing process. In this context, microbiology has an increasing participation in the baking industry, acting on several steps, from the preparation of the bread dough to the preservation process and spoilage control. We can tell that baking is currently one field of microbiology application, a source of innovation, improving the fermentation process and giving the bread a better texture, flavor, and health properties [4]. Microbial enzymes act as biological catalysts in baking, helping break down complex molecules into simpler ones, transforming raw ingredients into finished products more efficiently. The starter culture drives the quality of the bread. Besides yeast and LAB, adding other microorganisms, postbiotics, or microbial enzymes to the bread preparation could introduce special characteristics such as lower gluten content or more mineral bioavailability through their enzymes [5–7]. Freeze-drying, spray drying, fluidized bed drying, microencapsulation, and other technologies can preserve the microorganisms [8].

During baking, the high temperatures effectively kill most bacteria in the dough, rendering the bread sterile when it comes out of the oven. However, contamination can occur at various production steps, including cooling, slicing, transportation, and packaging. In addition, recent studies have shown that bakers may be a source of yeast and bacteria in breads [9].

Preservatives control microbial spoilage and ensure their quality and safety. Nowadays, one approach to avoid bread spoilage is microbial fermentation using LAB strains and yeast because of their antifungal activity and shelf-life extending capacity. *Lactiplantibacillus plantarum* LB1 (formerly *Lactobacillus plantarum*) and *Furfurilactobacillus rossiae*. LB5 (formerly *Lactobacillus rossiae*) inhibited fungal development for up to 21 days with the lowest contamination score [10]. This antifungal activity of LAB was used in the biopreservation of quinoa and rice bread [11]. Innovative approaches include essential oils and plant extracts [10]. However, chemical preservatives such as Potassium sorbate, benzoic acid, and sodium benzoate, among others, are commonly used. Another way to preserve is through physical methods such as violet (UV) light, Infrared (IR), microwave (MW) heating, and ultra-high pressure (UHP) [12]. Freezing, modified atmosphere packaging, and other methods are used [13,14].

This review describes the importance of microorganisms, probiotics, and microbiological knowledge in baking. It covers the status of the drying and preservative technologies currently used.

2. Microorganisms and probiotics in baking

In 1970, the first bacterium was identified in sourdough bread, the *Fructilactobacillus sanfranciscensis* (formerly *Lactobacillus sanfranciscensis*), which predominated in the microbiota of the sourdough of San Francisco bread, a traditional sourdough bread in the United States [15]. Bread produced with industrial baking yeast, namely *Saccharomyces cerevisiae*, is efficient in leavening dough, reducing fermentation time, and standardizing the process. It has a widely appreciated aroma and flavor, significantly less acidic and less intense scent than sourdough, precisely due to the fermentative alcoholic metabolism characteristic of yeast [16]. Zhang et al. [17], during the fermentation by *S. cerevisiae* in traditional Chinese bread, identified 27 aromatic compounds such as aldehydes, alcohols, ketones, organic acids, esters, and aromatic amino acids.

Starting from the 1990s, driven mainly by political-economic factors, particularly in France, a movement toward returning to the tradition of producing bread through natural fermentation began [18]. The properties of more natural production, without or less chemical additives, align with consumer preferences [19]. In this context, the science of baking was driven. The microbiota of fermented dough for bread was characterized, generating studies that concluded that mature sourdough is a source of LAB and wild yeasts. These microorganisms and their enzymes are in a complex interaction with the raw material (wheat flour) and together with the physical-chemical conditions of the baking process [20–22].

The term "probiotic" is defined as live microorganisms that positively affect the host when administered in appropriate quantities [23]. LABs are historically recognized as probiotics for their association with health properties for humans and animals [24]. However, more recent studies highlight the need to analyze other microorganisms found in smaller quantities or with less evidence in sourdough microbiome but which have been observed as persistent in natural fermentation. An example is the *Bacillus* genus due to its ability to survive in a low pH environment [25,26]. Some *Bacillus* strains are recognized for their probiotic properties [27]. Others may be associated with bread spoilage and public health issues, such as *Bacillus subtilis, Bacillus cereus, Bacillus licheniformis*, and *Bacillus pumilus*, which, due to their spore-forming characteristic, can sometimes survive the high baking temperatures [28]. Acetic acid bacteria have also been persistent in sourdough environments [29]. Additionally, advances in omics technologies have facilitated the study of the microbiome of natural fermentation to access uncultivable microorganisms, thereby increasing knowledge of other microorganisms present in the system.

A recent metagenomic analysis of the microbiome of *jalebi*, a traditional wheat flour dough fermented, sweetened, and fried in oil, demonstrated the presence of 12 phyles, 80 families, 179 genera, and 664 species of bacteria. Bacteria represented 91.91% abundance, with 8.024% eukaryotes, 0.063% viruses, and 0.003% archaea. In this research, the authors detected genes with putative probiotics, antioxidants, vitamins, and stress tolerance functions were detected, too. Despite comparisons with sourdough from Canada, the USA, New Zealand, and Belgium, *jalebi* fermentation exhibited a pH between 4.81 ± 0.016 to 5.06 ± 0.037 [30], which differs from the pH of mature natural fermentation, known to be below 4.0. Moreover, the metagenomic analysis showed changes in the microbiota of natural fermentation from the propagation until the maturity of the sourdough [31].

Among the microorganisms recognized as probiotics in sourdough, the genus *Lactobacillus* is the most predominant, especially *L. plantarum*, which has stood out as predominant in 142 out of 312 studies in a meta-analysis on the microbiota of sourdough bread [32]. This capacity of *L. plantarum* is attributed to its enzymatic activity involving esterases, decarboxylases, reductases, and glycosyl hydrolases [22]. *F. sanfranciscensis* has also been described as considerably predominant [33]. Other genera found in sourdough include *Leuconostoc, Weissella, Pediococcus, Enterococcus*, and *Lactococcus* [34,35].

Among the yeasts, *S. cerevisiae* is the most predominant. However, other genera are also observed, such as *Kazachstania, Kluyveromyces, Pichia,* and *Torulaspora* [33]. The adaptability of *S. cerevisiae* to the fermentation environment in baking and years of use have brought a performance challenge to surpass [6]. However, other yeasts have exciting properties that are currently being explored. For example, *Kluyveromyces marxianus* can reduce FODMAPs through inulinase production, significantly reducing the fructan content in whole wheat flour bread [36]. *Kazachstania, Lachancea, Pichia, Torulaspora*, and *Wickerhamomyces* also reduce FODMAPs and have excellent performance in resistance to stress, high salt concentration, and low pH [37]. *Kazachstania unispora*, in conjunction with LAB, can improve the sensory and preservation characteristics of bread produced with spelled flour, a cereal of low technological quality but recognized for its nutritional value and sustainability [38].

It is essential to note that natural fermentation has evolved beyond traditional methods, where wheat flour and water are allowed to ferment naturally over several days with back-slopping. It now includes the use of inoculums of microorganisms, isolated or not from sourdough, that can confer specific desired properties to bread based on their enzymatic capabilities, termed natural fermentation type II [39]. From this perspective, the selection of native yeasts in sourdough for use in the baking industry has increased due to their adaptability to the sourdough environment [40], which ensures their stability [41]. Similarly, bacteria, especially LAB, are extensively studied in industrial bread production [42]. Consequently, there is a growing number of consortiums with specific goals in baking, such as the use of *F. sanfranciscensis* B450, *Leuconostoc citreum* B345, and *Candida milleri* L 999, which improved the antioxidant and texture properties of bread [43] and many others inoculum studied [7].

An important concept in baking is the postbiotics, which are byproducts of the microorganism's metabolism produced during fermentation and bread production, such as short-chain fatty acids, peptides, and vitamins. The baking temperature of bread leads to the death of these microorganisms, but their metabolites cause modifications during fermentation with technological, nutritional, or well-being consequences through the enzymes produced, besides active biomolecules and other postbiotics that remain in the bread even after baking [19,44–46]. Due to the growing interest in consuming bread with these sensory and health characteristics, the number of patents published related probiotics for use as starters in fermented dough production has increased.

Among the metabolites produced by natural fermentation, organic acids, aldehydes, esters, alcohols, and ketones are prominent [47], as are exopolysaccharides [48], enzymes [6,7], vitamins B12, folate, and riboflavin [49], free amino acids [50,51], and an increase in the bioavailability of minerals [52–56]. Furthermore, bacteriocins, proteins, or peptides that do not pose problems for consumption do not induce resistance [57] and serve as natural preservatives. The organic acids produced reduce the pH to safe levels concerning pathogenic microorganisms [51,58]. In addition to these molecules, γ-aminobutyric acid (GABA), a neurotransmitter in mammals with properties of blood pressure regulation, antidepressant, diuretic, and antioxidant, synthesized from the amino acid L-glutamate by the action of the enzyme glutamate decarboxylase [59] has been widely associated with LAB production in natural fermentation [45,60–63]. As described, the microorganisms produce several bioproducts during baking. This theme is an open area of research, development, and innovation for this sector.

3. Drying and encapsulation processes of probiotics and microorganisms

On a laboratory scale, the preserving and storing of live cultures can be done by cryopreservation. However, regarding an industrial scale, this method has significant disadvantages, such as high energy consumption and the need to maintain and transport samples below zero temperature. However, choosing a drying method must also consider the cell viability after the process; intrinsic and extrinsic factors can influence cell death [8,64].

For producing large amounts of dried probiotics, it is preferred to use the most suitable process for each case. Some of the most known are freeze drying, spray drying, fluidized bed drying, microencapsulation, and extrusion [65].

3.1. Freeze drying

Freeze-drying, or lyophilization, can encapsulate probiotics, keeping them protected and stabilized. This technique involves three major steps: (i) freezing, (ii) primary drying, and (iii) secondary drying. During freezing, the solvent crystallizes under atmospheric conditions and initiates the separation of water molecules from the solution by ice crystals; this stage is usually conducted outside the dryer. In the primary drying process, the frozen crystals are removed by sublimation under vacuum conditions at a controlled temperature below the triple-point (p = 6.104 mbar; T = 0.0099 ºC). At the triple point, the aggregate coexists in three forms (solid, liquid, and gaseous). Thus, below the triple point, the water goes directly from the solid to the gaseous state. Finally, at the secondary drying, a considerable amount of unfrozen water is retained with the product (15 – 20 %) and removed by desorption. This process is mainly governed by diffusion. Compared to sublimation, desorption is slow, depending on the desired residual water content [65–67].

A cryoprotectant may enhance the survivability of bacteria probiotics during the freeze-drying process. Glucose, lactose, sorbitol, sucrose, glycerol, sugar, mannose, and trehalose, in proportions of 5 to 15 %. can be used. The mechanism of action of the cryoprotectant can be described as an improvement of cold tolerance by increasing the unfrozen fraction, thus providing more space to cells and preventing cellular damage, mechanical damage, and osmotic cells [65,68,69].

Several authors have reported the influence of different cryoprotectants in the freeze-drying process and the survival rate of some probiotics. **Table 1** summarizes some of the results.

Table 1. Overview of survival rate of freeze-dried encapsulated probiotics.

Strain	Cryoprotectant (w/v)	Survival rate after freeze-drying	References
Limosilactobacillus reuteri (formerly *Lactobacillus reuteri*) (CICC6226)	Sucrose (15%), skimmed milk (10%)	95.98% ± 6.69	[70]
L. plantarum (LP105)	Trehalose (5%), whey protein concentrate (10%), pullulan (4%)	94.36% ± 1.06	[71]
Pediococcus pentosaceus (GS4)	Skimmed milk powder (13%)	81.76% ± 4.05	[72]
Enterococcus faecalis (PK1202)	Skimmed milk powder (8%)	96 %	[73]

Despite the survival rate presented in **Table 1**, the freeze-drying process still presents some disadvantages, and they must be considered when the drying process is selected. These disadvantages are listed in **Table 2**.

Table 2. Advantages and disadvantages of the freeze-drying process.

Advantage	Disadvantages	References
Minimum damage to the product	Need of additional cryoprotectants	[74]
It provides a large surface area for the encapsulation	Lengthy drying time [24 – 36 h]	[75]
The most widely used method for sensitivity materials	Complex equipment and difficult to change the process	[76]
Porous structured powder due to sublimation of water	High capital and maintenance cost	[66]

3.2. Spray drying

Spray drying has been one technique for encapsulating biocomponents since 1920. It is widely used in industry due to its robustness, rapid drying, flowable powders, and ability to manipulate particle size [77].

It is based on a two-phase system: liquid and air. The active material is dissolved, prepared, and homogenized in this technique. The process occurs continuously, and the product to be atomized is sprinkled in a chamber in which there is a circulation of heated air, thus forming droplets and making them solid. The procedure enables the evaporation of the bonded solvent and the transfer of the solid encapsulated material to the cyclone for recovery [66,76].

The whole process consists of three main stages: (i) atomization, (ii) mixing, (iii) separation. The atomization is the first and most important process during the spray-drying. In this phase, the liquid is disintegrated into micro-sized droplets, which leads to a vast surface area that enables the rapid evaporation of the solvent. The residence time of the droplets is determined by their size distribution and velocity, depending on the nozzle type [78].

Evaporation water rate at spray-drying processes is governed by a series of parameters, including droplet temperature (T), droplet moisture content (X), the rate of temperature change (dT/dt), and drying rate ($-dX/dt$). Depending on the parameters, the transformation from droplet to particle may occur too rapidly, imposing environmental changes on probiotics. Thus, it is necessary to use some protectant during the operation [8,65,79].

The benefits of spray-drying are a rapid drying process, direct conversion of the dried powder from the liquid feed, easy-to-change parameter values to improve quality indicators, high production efficiency, and less operator requirement [66]. **Table 3.** briefly presents an overview of the process parameters of the spray-drying technique, the protectant, and the survival rate for some strains.

Table 3. Overview of the spray-drying techniques: strain, parameters, and survival rate.

Strain	Inlet/outlet air temperature	Protectant	Survival rate	Reference s
Enterococcus rivorum (S22C)	140 ºC± 2/60 ºC± 2	Maltodextrin (12%), glucose (4%), whey protein (4%)	92%	[80]
Lactobacillus acidophilus (ATCC4356)	200 ºC/90 ºC	Whey protein (5%), maltodextrin (10%)	84.87%± 0.02	[81]
Saccharomyces cerevisiae var. *boulardii*	125 ºC/61 ºC	maltodextrin (40.39%)	92.84%	[82]
Lacticaseibacillusr hamnosus (formerly	98 ºC±4/65±3 ºC	Lactose (10%), trehalose (10%)	80% – 100%	[83]

Lactobacillus rhamnosus) GG						

3.3. Fluidized bed drying

Fluidization occurs when a gas flows through solid particles with a velocity more significant than the settling velocity. The particles tend to suspend with the gas; after reaching the top of the equipment, the gravitational pull causes them to fall, and the process starts continuously. This technique provides excellent gas-solid contact, high thermal efficiency, and a low cost of operation [84].

Concerning the probiotics in the fluidized bed, dried pre-encapsulated probiotics are suspended in the hot air. Subsequently, they are encapsulated with the desired biopolymer. Due to the high airflow rate and the rapid drying, the biopolymer coating over the probiotics forms a homogenous layer, which may be done in multi-layers [85].

Fluidized bed drying requires relatively low temperatures without causing thermal stress. In addition, the microbial biomass is dried not on its own but with other materials that act like a protective matrix. Hence, it can preserve heat-sensitive probiotics. The protectant matrixes may be wheat flour, skimmed milk powder, casein, maltodextrin, starch, microcrystalline cellulose, inulin, and NaCl [86,87].

According to Wirunpan, Savedboworn, and Wanchaitanawong [88], the survival rate of *Lactobacillus lactis* 1464 in shrimp feed pellets ranges from 89.54 % to 96.87 % at 80 and 50 °C, respectively. The survival rate in the fluidized bed drying process is intrinsically related to process temperature, drying time, and cell concentration [89]. Wu et al. [90] evaluated the optimization of the process parameters using a fluidized bed to dry and encapsulate *Lactobacillus brevis* RK03; the authors achieved a survival rate of 95% using casein and whey protein as carriers.

3.4. Vacuum drying

Vacuum drying resembles freeze-drying; however, the samples are dried by evaporation, not sublimation. Probiotics are usually heat-sensitive living cells, and because of that, removing water is challenging without damaging them or keeping them viable. Therefore, vacuum drying is an alternative. It works at a higher temperature, around 25–30 ºC, and a higher pressure (10 mbar), compared to generally below 10 mbar for freeze dryers. The operation parameters of a vacuum dryer allow it to have an energy consumption of about 40 % lower than freeze drying [91,92].

Regarding temperature, vacuum drying is gentler, limiting the loss of viable heat-sensitive probiotics, even though the cell wall and cell membrane can be damaged when this technique is used. To diminish the damage the drying parameters can be altered by adding protecting agents or pre-treatment of cells to diminish the damage. **Table 4** presents some examples of vacuum drying of probiotics [93,94].

Table 4. Vacuum drying of probiotics process parameters and survival rate.

Strain	Protectant	Temperature	Pressure	Time	Survival rate	Reference
Lacticaseibacillus paracasei (formely *Lactobacillus paracasei*) F19	Trehalose 25% (w/w)	15 ºC	15 mbar	22h	70%	[95]
Lactobacillus helveticus	Sorbitol (1% w/w)	43 ºC	100 mbar	12h	18%	[96]

L. acidophilus	Trehalose (20% w/w)	Room temperature	0.11 mbar	96h	37.9%	[97]

Despite the advantage of less energy consumption of vacuum drying, there are some disadvantages, as seen in **Table 5**.

Table 5. Some advantages and disadvantages of vacuum drying.

Advantages	Disadvantages	References
Reduced drying temperatures	long processing time	[94]
Higher drying rate	the dried product might have shrinkage	[92]
Reduced oxygen concentration	Denser structure	[98]

4. Preservation methods

Once the bread is baked, it could spoil. Physical, chemical, biological, and natural methods could be used for preservation [10]. The deterioration can alter bread's flavor and texture, influencing its nutritional properties and quality. So, to avoid these effects on product properties and to extend their shelf life, preservatives can act in different ways, preventing spoiling and economic losses for industry and consumers. Preservation refers to techniques and methods used to extend the shelf life of baking products.

Regarding bread spoilage, fungi of the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor*, and *Rhizopus* are noteworthy, as they can produce highly harmful mycotoxins. Their control has found alternatives using sourdough, which has natural preservatives by LAB [99]. These agents and preservation methods function as antimicrobials, antioxidants, and anti-browning agents [12]. These agents can be grouped into physical, chemical, and biological preservatives, as described below.

4.1. Physical agents

Various physical preservation methods ensure that baked products maintain quality, freshness, and safety for extended periods. Bakeries use different strategies to avoid spoilage and eventual economic losses. Baking practices and technology have evolved, and new methods for preserving baked goods may emerge.

Controlling the temperature of baked goods storage is a simple technique that prevents early spoilage. Using low temperatures in storage can help preserve bread. Cao et al. [13] observed that quinoa bread was better preserved in frozen storage mode. Also, they observed that the texture of the bread under these conditions was even softer and that repeated freezing and thawing damaged the texture of the bread. Thus, it must be evaluated as a preservative agent. A work observed that combining high temperature (200 ºC) and time with a modified packaging atmosphere technique could improve the quality and shelf-life of par-baked bread [100]. Studying the package for baking products is aimed at baking industries as an example of preservation methods. A strategy consists of using modified atmosphere packaging where CO_2 or N_2 concentration is aimed to increase.

In contrast, oxygen concentration (required for mold growth) is decreased, thus delaying spoilage in baked products [14]. A high demand for biodegradable packing materials has arisen because of the sustainability exigencies and being studied for usage in the food market. Biodegradable packaging can provide a shelf-life extension to bread and other products due to efficient protection against spoilage once it acts as an antimicrobial, antioxidant, and UV-blocking agent, besides barrier function [101–103]. A study focused on breadmaking observed that a biopackage composed of lignin nanoparticles, cinnamaldehyde, and polybutylene succinate presented antifungal activity against *Aspergillus niger* and *Penicillium* sp. and created an efficient barrier against moisture and oxygen deterioration for up to 14 days at 25 ºC [101].

4.2. Chemical agents

Using weak organic acids in food, specifically in baked goods, is considered a conventional strategy to extend the shelf life of products. The mechanism of action of these chemicals consists of destabilizing plasmatic membrane components (H+-ATP-ase) and inhibiting intracellular vital enzymes (phosphofructokinase in glycolysis via) of microorganisms. Thus, certain organic acids, such as acetic, citric, propionic, and sorbate, can be used as preservatives to inhibit mold and bacterial growth in baked goods. Natural preservatives like honey, sugar, and salts (potassium, sodium, calcium) can also help extend shelf life. However, the baking industry must follow local legislation that regulates the maximum concentration of these acids in baked products [104,105].

Propionic acid and its salts (calcium propionate and sodium propionate) are commonly used preservatives in the baking industry, usually employed at concentrations up to 0.2%, being directly added to the dough or applied to the surface of the bread. Molds can be inhibited at these levels for a few hours or 2 days, preventing early spoilage [105]. However, some fungi can be insensitive to these acids. For example, resistance to propionic and sorbic acids at maximum legal limits for usage was found in *Penicillium brevicompactum* and *Penicillium roqueforti* in a study using bread and cake [106].

Sorbic acid controls mold growth in bakery products at 0.001 to 0.3%. Incorporating sorbic acid and sorbates into bakery products requires careful consideration of dosage levels to ensure that they effectively inhibit microbial growth without affecting the baked goods' taste, texture, or overall quality. Sorbates can be sprayed on the surface of the bread after it is baked, or sorbic acid anhydrates can be combined with fatty acids to diminish adverse effects [12,105].

A combination of different acids or even different strategies can be performed to preserve baked goods. Quattrini et al. [107] observed that combining acetic acid with propionate and sorbate caused an additive effect against *P. roqueforti* and *A. niger*. Moreover, in the same work, they studied using both chemical and biological strategies for preserving bread products. They observed that by adding sugar (4%) to sourdough fermentation with *L. brevis* for 6 days, the bread was free of *A. niger* growth. Ricinoleic acid (up to 0.15%) and *Lactobacillus hammesii* presented the same preserving effect. Fermentation of dough with lactobacilli is an ancient and very effective method for preserving baked goods, and it will be further discussed in this review.

4.3. Natural and biological agents: essential oil, plant extracts, LAB, yeasts, and enzymes

Natural and biological agents offer a sustainable alternative to synthetic preservatives. In addition to common natural preservatives, such as honey, sugar, and salt, which are widely used to extend the shelf life of baking products, other natural agents can be an option for preserving these products. Natural products that can be used as preservatives are essential oils, plant extracts, lactic acid bacteria, yeasts, and enzymes. These agents act as preservatives and add a healthier value to the final product. Microorganisms used are classified as Generally Recognized as Safe (GRAS), and can be incorporated in food [12,104,108].

4.4. Essential oils

Essential oils are complex mixtures of volatile chemical compounds extracted from different plant parts (such as leaves, bark, seeds, and flowers) by distillation and pressing. These compounds are a variety of secondary metabolites synthesized by aromatic plants in small quantities with a hydrophobic liquid nature, being poorly soluble in water and primarily dissolved in organic solvents [109–111]. They are known due to their bioactive properties, including inhibition of the growth of bacteria, yeasts and molds, viruses, protozoa, and insects, besides antioxidant properties [110,112–114]. These molecules are promising for application in the food industry as natural conservants. They can be used differently for product preservation in the bakery industry. They can be added to the headspace of packaged bakery products, directly in breads, or in combination with other strategies. Spraying essential oil on the surface of baked products has also been studied as an option [111]. However, there are limitations in the process, and studies are in progress to answer several questions relating to toxicity, concentration of use, and cost of production, among other points [115]. Another limitation is the possibility that essential oils interfere with flavoring and final taste quality in food [111,116].

The antimicrobial mechanism is a disruption of membrane cells and ergosterol reduction, inhibiting enzymes and altering proteins due to the cleavage of disulfide bonds [115–117]. **Table 6** overviews the most relevant essential oils, their targets, and their effects on baking. The antifungal activity has already been tested for use in the baking industry. Due to the potential application of new essential oils as preservative agents in the food industry, a consistent search for new compounds is expected to continue with these promising findings.

Table 6. Preservation of bread by plant essential oils.

Essential Oils	Major compounds	Targeted Molds	Action	Reference
Clove (*Syzygium aromaticum L.)*	Eugenol, Acetyleugenol, Caryophyllene, Gallic Acid, Kaempferol, Quercetin, Tannins	*Aspergillus flavus, A. niger, Aspergillus parasiticus, Eurotium amstelodami, Eurotium herbariorum, Eurotium repens, Eurotium rubrum, Penicillium corylophilum, Penicillium commune, P. roqueforti, Penicillium citrinum, Endomyces fibuliger, Rhizopus nigricans, Penicillium* sp.	Reduced yeast and mold growth	[118–123]
Thyme (*Thymus vugaris L.*)	Thymol, Carvacrol, Linalool, P-Cymene, Camphene, Myrcene, Caryophyllene, Rosmarinic Acid	*A. flavus, A. niger, Aspergillus terreus, Alternaria alternata, E. amstelodami, E. herbariorum, E. repens, Eurotium rubrum, Fusarium oxysporum, P. corylophilum, Penicillium italicum, Penicillium paneum*	Bread shelf-life	[106,115,119, 124–126]
Lemongrass (*Cymbopogan citratus)*	Citral, Geraniol, Limonen, Neral and Nerol, Myrcene and Citronellal	*A. flavus, A. niger, E. amstelodami, E. Herbariorum, E. repens, E. rubrum, P. corylophilum, Penicillium expansum*	Mold growth inhibited	[119,127]
Rosemary (*Rosemary officinalis)*	carnosic acid, carnosol, rosmarinic acid and hesperidin	*Penicillium* sp. *Aspergillus* sp.	Fungal generatio n reduced	[115,128,129]
Oregano (*Origanum vulgare L.*)	Carvacrol, Thymol, Rosmarinic Acid,	*A. flavus, A. niger, Aspergillus fumigatus, Aspergillus ochraceus,*	Mold growth inhibited	[118]

	P-Cymene, Terpinene, Linalool, Naringin, β-Caryophyllene	*A. parasiticus, A. terreus, Eurotium fibuliger, P. commune, P. roqueforti*		
Marjoram *(Origanum majorana L.)*	Terpinen-4-ol, α-Terpinene, γ-Terpinene, Linalool, Sabinene, P-Cymene, Myrcene, Thymol	*A. flavus Penicillium chrysogenum, Rhizopus* spp.	Protection of seeds during incubation Shelf life	[130]
Mandarin (*Citrus reticulate L.*)	Limonene, Myrcene, Linalool, γ-Terpinene, Nobiletin, Hesperidin, Rutin, Ascorbic Acid	*A. flavus, A. niger, P. chrysogenum, Penicillium verrucosum*	Mold growth inhibited	[131]
Cinnamon (*Cinnamomum jersenianum* Hand.-Mazz)	Cinnamaldehyde, Eugenol, Cinnamyl Acetate, Coumarin, Proanthocyanidins	*A. flavus, A. niger, A. ochraceus, A. terreus, E. fibuliger, E. amstelodami, E. Herbariorum, E. repens, E. rubrum, P. corylophilum, P. citrinum; P. commune, Penicillium viridicatum, P. roqueforti*	Reduction of the targeted mold growth	[118,119,132]

4.5. Plant extracts

Plant extracts derived from different parts of the plant have also been investigated for various applications due to the knowledge of the bioactive properties they can have. It has been described as having interesting properties, such as antimicrobial activity [133,134] and medical application [135,136]. Plant extracts, herbs, and phytochemicals are highly sought for application in the treatment of cancer, diabetes, and cardiovascular diseases, as well as components in functional foods, nutraceuticals, and health care, being thus the food industry one of the receptors of these bioproducts [137]. Recent works investigating the benefits of plant-based extracts in baking products are also observed [138,139]. Studies also concern the interaction of plant extracts with other components present in the food matrix and the regulations about food safety [140].

Regarding food application, plant-derived extracts can efficiently inhibit microbial pathogens and molds. Negi [141] has described an extensive review regarding phytochemicals with antimicrobial activity, such as *Cinnamomun* and *Garcinia* species, *Punica granatum L.*, and their potential in food application. Cedarwood, sweet tobacco, and frankincense exhibited marked antimicrobial activity and inhibited mold growth, extending the shelf life of the bread until the end of the trial period. The results of this study suggest that packing bread with certain sachets of tree and leaf essential oils can inhibit and delay food spoilage, presenting an effective alternative to conventional synthetic preservation practices [142].

Focusing on baking industry applications, star anise (*Illicium verum*) is known for its natural compounds, including essential oils, which may possess antifungal properties. Bao et al. [143] observed that star anise may cause lipid peroxidation in the cell membrane and interaction with

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membrane proteins, altering their conformation, thus resulting in cell membrane dysfunction. Because of these mechanisms of action, they observed that the extract extended the shelf life of bread by up to 6 days. Torgbo et al. [144], using an electrothermal technique to extract bioactive compounds from rambutan peel fruit, showed the promising potential of this strategy and the benefits of these compounds for the bread. In the mentioned work, gallic acid, corilagin, geraniin, and ellagic acid were identified after ohmic heating extraction and incorporated into bread ingredients. No adverse effects were observed for its texture, and the extended shelf life of bread was achieved due to antioxidant and fungistatic activity. To conclude, **Figure 1** summarizes how plant extracts and essential oils interact with target microorganisms and thus act as preserving agents in baked products.

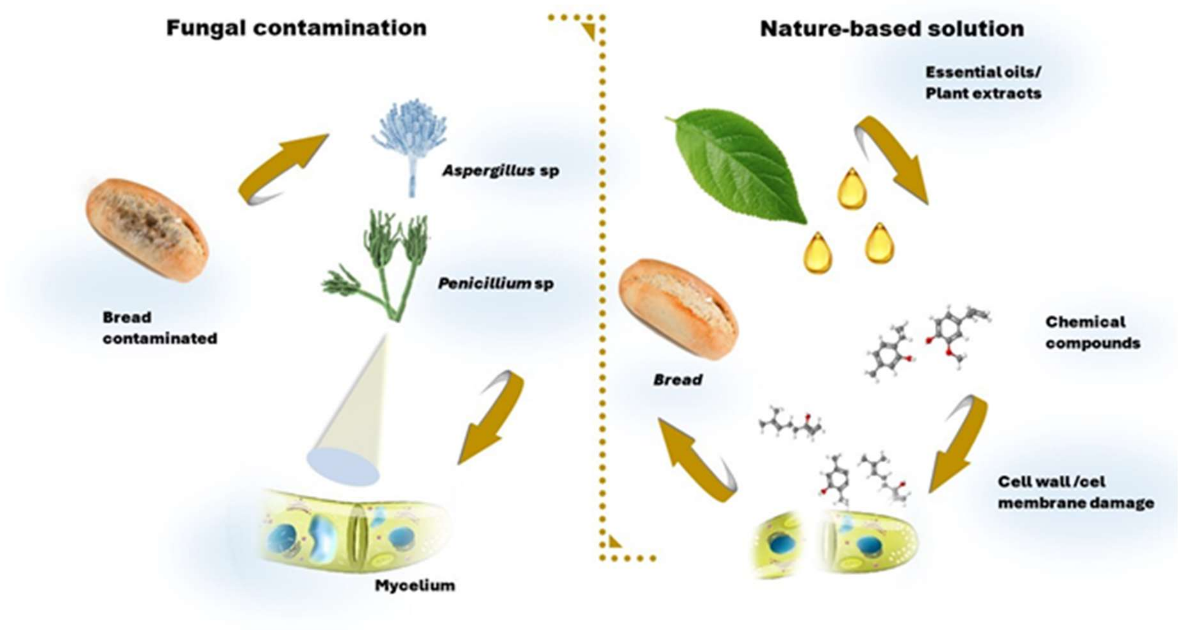

Figure 1. - mechanism of action of plant extracts and essential oils.

4.6. Lactic acid bacteria (LAB) and yeasts

Microbial fermentation can also be employed as a biological agent acting as a preservative in the baking industry to enhance the shelf life and quality of baked goods. The primary goal of microbial fermentation in baking is related to leavening and flavor development. However, it can also act as a preservative effect. Spontaneous acidification due to the fermentation of local microbiota occurs in sourdough, which uses several types of flour and water. This strategy is a valuable tool in the baking industry, enhancing the shelf life, flavor, and texture of baked products while reducing reliance on synthetic preservatives [7,145].

Besides the healthy and sensorial properties for consumers, sourdough fermentation also preserves the baked products. LAB presents considerable importance for preservative purposes in the baking industry. They secrete different organic acids, such as acetic, propionic, and lactic acid, into the sourdough matrix, creating a low-pH environment that inhibits the growth of some spoilage microorganisms due to the synergism activity between the acids [20,99]. Examples of reports of LAB with the potential to increase the shelf life of bread can be better observed in **Table 7**.

Table 7. Lactic acid bacteria in bakery products can potentially increase bread's shelf life.

Antifungal lactic acid bacteria	Organism	Reference
L. plantarum FST 1.7	*Fusarium culmorum* and *Fusarium graminearum*	[146]

L. plantarum CRL 778, *L. reuteri* CRL 1100, *L. brevis* CRL 772 and CRL 796	*Aspergillus*, *Fusarium*, and *Penicillium* species	[147]
Lactobacillus amylovorus DSM 19280	*A. niger* FST4.21, *P. expansum* FST 4.22, and *P. roqueforti* FST 4.11, *F. culmorum* FST 4.05	[148]
L. plantarum	*A. niger* FST4.21, *F. culmorum* TMW 4.0754, *p. expansum* LTH S46	[149]
L. plantarum LB1 *F. rossiae* LB5	*P. roqueforti* DPPMAF1	[150]
F. rossiae LD108 *Companilactobacillus paralimentarius* PB12 (formerly *Lactobacillus paralimentarius*)	*Aspergillus japonicus*, *E. repens* and *Penicillium roseopurpureum*	[151]
Latilactobacillus sakei (formerly *Lactobacillus sakei*) KTU05-6, *Pediococcus acidilactici KTU05-7*, *P. pentosaceus KTU05-8*, *P. pentosaceus KTU05-9 and*, *P. pentosaceus KTU05-10*	Molds	[152]
L. amylovorus DSM19280	Molds	[104]
L. plantarum L244 with *Schleiferilactobacillus harbinensis* L172 (formerly *Lactobacillus harbinensis*)	*P. commune*, *Mucor racemosus* and *Rhodotorula mucilaginosa*	[153]
L. plantarum CH1, *Lc. paracasei* B20 and *Leuconostoc mesenteroides* L1	*M. racemosus* UBOCC-A-109155, *P. commune* UBOCC-A-116003, *Yarrowia lipolytica* UBOCC-A-216006, *Aspergillus tubingensis* AN, *A. flavus* T5 and *Paecilomyces formosus* AT	[154]
L. plantarum UMCC 2996, *F. rossiae* UMCC 3002, *P. pentosaceus* UMCC 3010	*A. flavus* ITEM 7828, *P. paneum* ITEM 1381, *A. niger* ITEM 7090	[155]

Furthermore, microbial growth inhibition can result from antimicrobials secreted by LAB and yeasts, preserving these baked goods. Rizzello et al. [156] observed that in a sourdough prepared with pea flour hydrolysate, *L. plantarum* 1A7 released antifungal peptides during fermentation and extended the shelf life of the bread until 21 days. Another work describing antifungal compounds secreted by LAB showed that *L. reuteri* R29 could extend the shelf life of a bread system against *F. culmorum*, producing euterin molecules and other metabolites [157]. Preserving baked goods with LAB can also occur due to unsaturated fatty acids, the caproic acid. These compounds have antifungal activity and work synergistically with organic acids, acting as detergents while disrupting cell membranes. They also can inhibit enzyme activity and protein synthesis in target microorganisms [99,158].

Yeasts also contribute to natural fermentation in the sourdough as well as in the extending sensorial and physic-chemical properties of the bread. Natural yeast reduces pathogenic microbial activities, as it has antifungal activity, favoring the reduction of preservatives. Certain yeast strains, such as *S. cerevisiae*, *Pichia anomala*, and *P. roqueforti*, have been used as natural preservatives in food and beverage production. Yeasts can compete with spoilage organisms for nutrients and produce ethanol and other inhibitory compounds during fermentation. Many studies have shown that they maintain essential qualities that make them more palatable to be used as biopreservatives [159,160]. Jin et al. [161] described sourdough bread with *P. pentosaceus* and *S. cerevisiae* as starter cultures that inhibited *A. flavus* growth and enhanced sensory aspects of the bread. Due to the importance of yeasts in the baking industry, other areas of the food industry have also investigated potential applications of these microorganisms as preservative agents.

Some yeasts can also produce killer toxins, which have bioprotective qualities against food-degrading bacteria and diseases. Several genera of yeast produce extracellular and intracellular antibacterial compounds. The effectiveness of yeast as biopreservatives is also a result of the large amounts of ethanol and organic acids produced, which causes a change in the pH of the solution [10]. Yeasts such as *Wickerhamomyces anomalus* and *Meyerozyma guilliermondii* have already been reported as producers of organic compounds that cause inhibition of molds [100]. Due to their minimal water and nutrient needs, many can live on dry surfaces. In the fermentation process, several yeast species are used as starter cultures.

4.7. Enzymes

The usage of enzymes in the baking industry, or in food and beverage in general, is widely known and important for developing these products. Enzymes are biological catalysts produced by all living cells for specific chemical reactions, being classified in Enzyme Commission Number (EC Number) as oxidoreductases (EC1), transferases (EC 2), hydrolases (EC3), lyases (EC4), isomerases (EC5) and ligases (EC6). These enzyme classes can be used in food processing in different supply chains to alter raw food and materials and the final product to consumers, especially the ones produced from microorganisms since they are a great source of industrial enzymes. For example, in bread-making and sourdough processes, hydrolases (amylases, xylanases, lipases, peptidases, for instance) comprise an important role in biotransforming the flour and starch content and producing new compounds such as organic acids and nutrients and improving sensory and even healthy characteristics of the bread [7,162–164].

In addition, enzymes can be used as natural preservatives to inhibit spoilage and enzymatic browning once enzymes like catalase break down hydrogen peroxide, which can help extend the shelf life of specific products by preventing oxidative damage. Recent work demonstrated that the incorporation in bread of hydrolysates of gluten protein obtained after treatment with latex peptidases could delay fungal growth on the product [165].

With a different strategy, pitaya fruit was fermented with LAB strains. Its fermented product was used in bread composition where enhanced antioxidant activity and delay of fungal growth were observed due to its incorporation [166].

5. Conclusion

Baking processes are constantly developing to obtain the best sensory and health properties—each step of production, from the inoculum choice and preparation to the final product and preservation, receives special attention and research. Using sustainable, biodegradable, non-toxic, ecologically friendly green additives and processes is necessary in the current industrial scenario focused on environmental issues and human health. Natural preservatives, new dry procedures, and strains with unique inhibitory properties, among others, are innovative approaches to avoiding bread spoilage and improving the quality. The art of making bread now includes the results obtained from scientific research carried out around the world.

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