



Microbial Preservation and Contamination Control 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 methods. Besides lactic acid bacteria and yeast, other microorganisms with unique properties, such as enzymes, new aromas and flavors, exopolysaccharides, and vitamins, among other compounds with beneficial properties, could be added to bread manufacture, improving bread quality and health effects for the consumers. The preservation of microbial cultures and starters is crucial in bread-making. New encapsulation methods, cryoprotectants, spray-drying, fluidized bed drying, and vacuum drying are employed for microorganism cultures that will be used as starters or biological additives in fermentation. A development is observed in the antimicrobial methods used as bread preservatives, 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 that incorporates innovative methods, biological additives, new methods, and processes focusing on microbiological protection.

Keywords: fermentation; yeast; LAB; bread; microbial contamination; inoculum preservation

1. Introduction

Bread is one of the oldest foods produced by humans, and its consumption dates back to findings more than 14,400 years ago in Northeastern Jordan [1]. Since then, refining this process has resulted in the industrial production of the initial specialized pressed yeasts for baking in the Netherlands in 1780 [2]. This innovation completely transformed the bread production process, particularly when compared to natural fermentation. Bread is a source of essential nutrients, including carbohydrates, fiber, vitamins, and minerals. It has diverse shapes and forms, and it is one of the most consumed food products worldwide, with an average consumption of 70 kg (41–303 kg)/year/capita [3]. Europe consumes less bread, with an average annual consumption of 59 kg [4,5].

Fermentation by yeast and lactic acid bacteria (LAB) in typical bread or sourdough is the critical manufacturing process. In this context, microbiology has increased participation in the baking industry, acting in several steps, from preparing the bread dough, including inoculum and starters, to the preservation process and spoilage control. We can tell that baking is currently one field of microbiology application, plus being a source of innovation, improving all bread production processes and giving baking products a better texture, flavor, and better health properties [6]. 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 used in sourdough and yeast cultures drives the quality of the bread. Besides yeast and LAB, adding other microorganisms, probiotics, postbiotics, or microbial enzymes during the bread preparation



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). makes it possible to introduce unique characteristics such as decreased gluten content or increased mineral bio availability [7–9].

The preservation techniques of microorganisms for baking or fermenting inoculum are critical and are in progressive development. In this context, freeze-drying, spraydrying, fluidized bed drying, microencapsulation, and other technologies can preserve the microorganisms [10–12].

During baking, the high temperatures effectively kill most bacteria in the dough, rendering the bread free of microorganisms 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 [13].

Nowadays, one approach to avoid bread spoilage is the application of biological methods such as microbial fermentation using LAB strains and yeast because of their antifungal activity and shelf life-extending capacity. Some examples include *Lactiplantibacillus plantarum* LB1 (formerly *Lactobacillus plantarum*) and *Furfurilactobacillus rossiae* LB5 (formerly *Lactobacillus rossiae*), which have been shown to inhibit fungal development for up to 21 days with the lowest contamination score [5,14]. This antifungal property is used for the biopreservation of quinoa and rice bread [15]. Innovative chemical approaches include essential oils and plant extracts [5]; however, preservatives such as potassium sorbate, benzoic acid, and sodium benzoate, among others, are commonly used [16]. Another way to preserve is through physical methods, such as radio frequency heating [17].

This comprehensive review discusses the preservation and storage techniques used for starters and microorganisms in baking. In addition, the major contamination points in the baking process are analyzed, looking into the traditional and contemporary landscape of emerging and alternative technologies for microbial contamination control.

2. Bread Fermentation Process

Two methods of bread dough fermentation can be employed: the first is straight dough using the industrial baking yeast Saccharomyces cerevisiae, and the second is the natural fermentation known as sourdough. It is important to note that natural fermentation can also involve industrial baking yeast [18]. Currently, bread production is predominately undertaken using the first method, which consists of adding all ingredients together in a vessel using the following sequential process: mixing, resting, modeling, proofing, and baking. Straight dough is efficient in leavening dough and reducing fermentation time [19], in contrast to the natural fermentation process, which is slower and more sensitive to environmental and process conditions [20]. Based on the production technology used, there are four types of sourdoughs: type I (traditional sourdough), type II (starter cultureinitiated sourdough), type III (dried sourdough), and type IV (mixed dried sourdough) [21]. Sourdough is a source of LAB and wild yeasts and their enzymes, which are involved in a complex interaction with the raw material and the baking process's physical-chemical conditions, determining the properties of the bread [18,20,22]. The genus Lactobacillus is the most predominant in sourdough, especially L. plantarum, which has stood out as predominant in 142 out of 312 studies in a meta-analysis of the microbiota of sourdough bread in 15 worldwide sourdoughs [23]. Enzymatic activity from L. plantarum involving esterases, decarboxylases, reductases, and glycosyl hydrolases is essential in the dough properties [20]. *Fructilactobacillus sanfranciscensis* has also been described as predominant [24]. Other genera found in sourdough include Leuconostoc, Weissella, Pediococcus, Enterococcus, and Lactococ*cus* [25,26]. It is possible to obtain mature sourdough after 5–10 days of the back-slopping process in type I and at least 24 h in sourdough type II when a starter is added in the first step [24].

Among the yeasts, *S. cerevisiae* is the most predominant. However, other genera are also observed, such as *Kazachstania*, *Kluyveromyces*, *Pichia*, and *Torulaspora* [24]. The adaptability of *S. cerevisiae* to the fermentation environment in baking and years of use have brought a performance challenge that surpasses others [8]. Other genera of bacteria

less studied in sourdough are *Acetobacter*, *Gluconobacter*, *Komagataeibacter*, *Bacillus*, *Pantoea*, *Kosakonia*, *Pseudomonas*, and *Paraburkholderia* [8,27–31].

There has been a growing trend in incorporating probiotics into baking processes. This trend is driven by the desire to enhance baked goods with additional health benefits, moving beyond traditional nutrition. Probiotics offer a unique opportunity to transform baked products into functional foods with therapeutic potential. By adding probiotics to bread fermentation, for example, bakers can create products with improved digestive health support and potential immune system benefits. This approach aligns with the evolving consumer demand for healthier food options [32]. 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 [33]. In the new planet scenario with pollution problems and climate change, a more natural production, without or with fewer chemical additives, aligns with consumer preferences [34].

3. Bread Native Microbiology, Ingredients, and Additives

The ingredients used in baking can bring microorganisms. They consist primarily of cereal flour with a focus on wheat flour. Wheat flour plays a crucial role by forming the essential gluten network upon hydration, a critical factor in shaping the structure of the bread. This ingredient also serves as a potential reservoir for various microorganisms, including bacteria, fungi, and yeast, which can be present in bread, impacting fermentation and bread quality. Table 1 summarizes the microorganisms found in wheat flour [35].

The type of flour used in bread-making can have a significant impact on the composition of native microorganisms that are involved in the fermentation process. Several studies have investigated how different types of flour, such as wheat flour sourced from different regions and whole wheat flour with varying extraction rates, can affect the microbial communities in sourdough. Enterobacteriaceae constitute the main component of the refined soft and durum wheat flour microbiome, while wholemeal durum wheat flour, mainly Xanthomonadaceae, can also be found [36]. Studies have shown higher levels of microorganisms in whole rye than in whole wheat flour [37].

Water is another vital ingredient, ranking as the second-largest component in bread dough alongside the microorganisms that drive the fermentation process. Water plays a pivotal role in the microbiota of bread dough. A study conducted with ten samples of potable water sourced from various regions in Italy revealed discernible differences in chemical and microbial compositions [38]. Water activity (a_w) is essential to microorganisms' growth. It influences the bread's quality and shelf life, and water activity control can help prevent or minimize microbiological spoilage. Molds, for instance, can grow within an intermediate range of water activity between 0.6 and 0.84 [39,40].

It is noteworthy that the presence of LAB and other microorganisms in sourdough can be related to endophytic wheat flour [25,41], water quality [38], the local environment [42], and insects [20].

Chemical leavening agents are widely used in baking applications and consist of mixtures of acids and bases. They produce gas (CO₂) by a chemical reaction instead of yeast fermentation. They can be baking soda (sodium bicarbonate) or baking powder (a mixture of baking soda and powdered acids, all creating carbon dioxide bubbles and causing the bread to rise) [43]. Chemical leavening used in baking is not inherently prone to carrying microorganisms.

Table 1. Wheat flour microbiota.

Microorganism	Name	Reference
Bacteria	Bacillus cereus Escherichia coli Salmonella spp.	[37]

Microorganism	Name	Reference
	Actinobacteria phylum	
	Delftia	
	Deljim Enterphactoriaceae and Oralphactoriaceae familion	
	Enterococcus faecium	
	Fruinia	
	Lacticaseibacillus naracasei	
	Lactinlantihacillus nentosus	
	Lactobacillus brevis	
	Lusinobacillus	[25 41 44]
	Paenihacillus	
	Pediococcus	
	Pseudomonas	
	Serratia	
	Sphingomonas	
	Stenotronhomonas	
	Enterococcus	
	Lactobacillus	
	Lactococcus	
	Streptococcus	
	Aureobasidium pullulans	
	Candida phangngaensis	
	Filobasidum magnum	
	Kazachstania	
	Naganishia albida	
	Papiliotrema rajasthanensis	
Yeasts	Pichia	[24,44]
	Rhodotorula graminis	
	Rhodotorula mucilaginosa	
	Saccharomyces	
	Sporidiobolus metaroseus	
	Vishniacozyma victoriae	
	Alternaria sp.	_
	Aspergillus	[37]
Eilen en terre franzi	Penincillus	
rnamentous fungi	Cladosporium sp.	
	Talaromyces rugulosum	[45]
	Wallemia sebi	

4. Microorganisms' Entry in Bread-Making

Bread ingredients promote the growth and proliferation of microbes during various phases of bread preparation, processing, packing, and storage. It is highlighted that after baking, the final microbial load of the bread is reduced, surviving only spore-forming microorganisms, such as some bacteria and molds, which have endured the intense heat treatment. Upon exiting the oven, the bread will be exposed to environmental microbial contamination (air, packaging, baking tools, food manipulators, and insects, for instance) [45]. Figure 1 illustrates microorganisms' major entry points in the baking process.

The bakery environment influences the microbiota of the bread before and after baking. A study on four bakeries in Italy microbiologically analyzed the walls of the room where the fermented doughs were handled, the storage boxes, and the clean mixer bucket. The results demonstrated that the dominant sourdough LAB species and yeasts dominated the house microbiota [42].



Microorganisms in baking

Figure 1. Origin of microorganisms in bread baking. Bacteria, yeast, and molds come from packing manipulators, air, and insects. Molds and bacteria with endospores are baking-resistant and could be present in bread after baking.

The genus *Lactobacillus*, the most representative of LAB in fermented dough, can have different origins. According to their metabolic flexibility, they could originate from a free life or nomadic style or be present in insects or different human niches, including the oral cavity and intestinal tract [20,46,47].

On the other hand, the air in bakery rooms can be a reservoir of undesirable microorganisms that can populate surfaces, the dough, and the final bread. A multigrain wholemeal bread processing factory study revealed filamentous fungi in the air, mainly in areas after oven-baking, such as cooling, slicing, and packaging. These same fungi (Penicil*lium paneum* and *Penicillium polonicum*) were observed in the flour and bread, indicating cross-contamination in the production environment [48]. Some pathogenic microorganisms such as Salmonella spp. (ecologically present in eggs), Vibrio spp. (aquatic habitat), Klebsiella spp., Pseudomonas spp., and Staphylococcus spp. were present at high levels in bread from bakeries in Dhaka, Bangladesh, indicating the poor hygienic control of workers, facilities, and processes [49]. A study was conducted in Aliero, Kebbi State, to assess the hygienic conditions of local bakeries. The study collected data on socio-demographics and sanitary conditions. It was found that there were problems with water supply, garbage storage, and other hygienic issues in the bakeries. Bread samples from the bakeries were found to be contaminated with both pathogenic and non-pathogenic microorganisms. Specifically, E. coli, Pseudomonas spp., Proteus spp., Bacillus spp., Penicillium spp., Aspergillus spp., Rhizo*pus* spp., and *Fusarium* spp. were identified as contaminants [50].

A study carried out in Brazil demonstrated that cross-contamination of bread production areas caused contamination after baking since the mold present in the raw material (wheat flour) was present in the air sampling in the cooling, slicing, and packaging areas, leading to contamination of the bread [45].

The results obtained with research done in Alexandria, Egypt, showed that handling bread without gloves, lack of coverage, and packaging are associated with a higher number of microorganisms. Specifically, these practices are linked to higher total plate count, yeasts and molds, and coliform counts. Additionally, not wearing gloves and displaying bread outside the shop are significantly associated with *Staphylococcus aureus*. To ensure proper handling of bakery products to avoid contamination, it is recommended that health education be provided to workers, guidelines for microbiological quality should be established, and standards and rules on bread safety should be defined and clarified [51].

It is essential to implement good manufacturing practices to reduce the possibility of undesirable microorganisms entering the baking process.

5. Preserving and Storing Microbial Cultures for Baking

In bread-making, the choice of using baker's yeast in either wet tablet or dry form has evolved. The transition to a dried format has greatly facilitated storage, eliminating the need for refrigeration and extending shelf life. In contrast, wet baker's yeast typically requires refrigeration, has a reduced shelf life, and is prone to spoilage by mold. The bread industry has demonstrated that commercial preparations of yeasts (compressed, dry, or liquid) can contain microorganism contaminants. In a study conducted in Italy, vegetative forms of *Bacillus* sp. were found in compressed yeast. *Bacillus* spp. spores (in dry yeast), *Enterobacteriaceae*, total and fecal coliforms (in compressed yeast), and *Enterococci* (in compressed and dry yeast) were also isolated. Additionally, fungi and lactic acid bacteria were detected, including *L. plantarum*, *Leuconostoc mesenteroides*, *Pediococcus pentosaceus*, and *Pediococcus acidilactici* [52].

In terms of microorganisms, *S. cerevisiae*, globally used in bakeries, can be preserved on a large scale in two different forms from the production batch: yeast cells in the wort are centrifuged and filtered to obtain wet yeast with 60–75% moisture, or further dried to obtain dry yeast with humidity as low as 4–6%. The yeast can be further dried to obtain dry yeast with moisture as low as 4–6%. Maintaining yeast cell viability (preference up to 76%) and high CO₂ production is essential. [53,54]. In the past, baker's yeast concentrates were the first starters to be commercialized. Since then, there has been a technological evolution in producing active dry yeast and other microorganisms. The dryer's temperature must be adequate for cell preservation, and the loading rate of the dryer is a critical parameter related to the quality of the baker's yeast [55].

Both wet and dry yeast are available on the market with different advantages and shelf lives. The activity of wet yeast is usually higher than that of dry yeast. However, the simplicity in transportation, storage, and the long shelf life of dry yeast makes it incredibly popular worldwide [55]. Similarly, to obtain sourdough on an industrial scale, the method chosen needs to be efficient to the point of achieving a reduction in moisture (<7%) and, mainly, in water activity (below 0.3) sufficient to prevent reactions from occurring in the biochemical and microbiological processes in the dried powder, thus ensuring stability and extended shelf life [56].

Significant advancements have enhanced the quality of commercial baking products, notably with S. cerevisiae [7]. However, drying new microorganisms, including LAB and generally recognized as safe (GRAS) microorganism strains, for their utilization as starters in bread fermentation processes, poses a continuing challenge that necessitates further progress [57]. Researchers have evaluated drying techniques, such as spray-drying, freezedrying, or vacuum drying, to preserve the high fermentation capacity and cell viability of microorganisms [57]. Other methods can be used to produce large amounts of dried bacteria and yeast. Some of the most commonly known are fluidized bed drying and microencapsulation [58]. Gelinas studied dry methods for yeast, including patent docs, and applied scientific publications about methods for drying brewer's yeast. The author concluded that the long-term survival of dehydrated yeast cells progressively improved with specific strains, growth conditions, and, to a lesser extent, drying conditions [59]. De Marco's study found that preserving nutrients and sourdough type III microorganisms' viability is best achieved through low temperatures and vacuuming during freeze-drying and a short residency time during spray-drying [10]. The dry process can be used for sourdough starters, yeasts, Lactobacillus, and other bacteria with unique properties.

These drying and protective techniques are commonly utilized currently. The differences in use depend on the target microorganisms. For example, vacuum and freeze-drying are appropriate for heat-sensitive microorganisms, but spray-drying is a viable alternative for operational costs and continuous production [10]. It is crucial to rehydrate the starter culture powder before using it and after drying it. Various methods have been suggested in the literature for rehydration. Some of these methods include mixing the dried starter with wheat flour and sucrose in a physiological solution [56], while others suggest using unbleached wheat flour and water [60]. Some authors also recommend activating microorganisms such as bacteria and yeast before drying by adding, for instance, sugar, corn starch, baker's wheat flour, and water into fresh sourdough and incubating it for 2 h at 30 °C and 60% relative humidity in a climate chamber [61].

This section discusses the differences in cost and drying time of these methods.

5.1. Microencapsulation

Microorganisms face challenges maintaining viability during baking due to extreme conditions, such as temperatures around 180 °C for 40 min [62]. Microencapsulation can enhance viability, minimize losses during baking, extend shelf life, and safeguard against gastrointestinal conditions [63]. The survival of probiotics and sensitive microorganisms in extreme conditions, like altered temperature, pH, and salinity, can be a problem, and several micro- and nanoencapsulation techniques can improve viability [64].

Microencapsulation involves entrapping active compounds within inert materials through physicochemical processes. Various techniques exist for this procedure, with different protective agents available on the market, differing in application, feasibility, and cost [65].

In one study, the probiotic *Bifidobacterium animalis* subsp. *lactis* was added to bread inside a three-layered microcapsule structure containing lactose, stearic acid, Na alginate, and polyethylene. This study showed that the microencapsulation effectively stabilizes the bacteria against elevated temperatures to allow their incorporation into bread. The high viability rate of *B. animalis* subsp. *lactis* found after bread baking at 180 °C for 40 min and after 60 min exposure to a simulated gastric fluid with a pH of 1.2 is evidence of the method's feasibility. Several microencapsulation methods are under study, including chitosan-coated alginate/gellan gum [62] and milk protein [66], among others.

5.2. Freeze-Drying

Freeze-drying, or lyophilization, can encapsulate microorganisms, 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 °C). 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 stage, 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 [58,67,68].

A cryoprotectant may enhance the survivability of bacteria during the freeze-drying process. Glucose, lactose, sorbitol, sucrose, glycerol, sugar, mannose, and trehalose can be used in proportions of 5–15%. 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 [58,69,70]. The sourdough powder has several advantages over fresh sourdoughs, such as longer shelf life, constant product quality, ease of formulation and mixing, and lower transportation costs [61]. In one study, highland barley sourdough powder with inulin was prepared using the freeze-dried method. The bread quality and dough gluten network were investigated. The FT-IR spectra of gluten proteins in bread dough with inulin were analyzed, and the proportion of α -helix and β -sheet from gluten in the dough was higher than that in the dough without inulin, indicating that sourdough powder containing inulin possessed a denser gluten network structure. The results show

that inulin was essential in preserving lactic acid bacteria and acid-producing ability compared to sourdough powder without inulin [71]. It has also been demonstrated that the pure freeze-dried strain retained the aroma characteristics of sourdough and could give bread proper acidity, improving viscoelasticity and the gluten network structure, enhancing the bread quality. S. cerevisiae XZFM15F1, *L*. plantarum (L.P) 2979, L.P3355, and *Lactococcus lactis* were tested [72]. Several authors have reported the influence of different cryoprotectants in the freeze-drying process and the survival rate of some microbial starters used in sourdough. The sourdough type I starter has a survival rate of 66.20% after freeze-drying [56], while *Lactobacillus brevis* ED25 and sourdough type II have survival rates of 94.07% and 83%, respectively [61,73]. Table 2 presents an overview of the survival rate for bread-making starters after the freeze-drying technique.

Starter	Мо	Before Freeze-Drying (log CFU/g)	After Freeze-Drying (log CFU/g)	Survival Rate (%)	Reference
Sourdough type I	LAB	9.50	8.93	94.00	[73]
Sourdough type I	LAB Yeast	$\begin{array}{c} 9.17 \pm 0.17 \\ 7.53 \pm 0.12 \end{array}$	6.07 5.03	66.19 66.80	[56]
Yeast starter	S. cerevisiae 88-4	7.00	6.65	95	[74]
Sourdough type I	LAB Yeast	$8.7 \pm 0.0 \\ 8.6 \pm 0.0$	$8.0 \pm 0.6 \\ 8.0 \pm 0.0$	91.95 93.02	[61]

Table 2. Overview of freeze-drying technique. Mo = microorganisms.

The freeze-drying process has several advantages. It causes minimum damage to the product and is excellent for sensitive materials. Additionally, it provides a large surface area for encapsulation, resulting in a porous structured powder. On the other hand, cryoprotectants are necessary, and drying takes a long time (24–35 h). The equipment is complex, and the investment and maintenance costs are considered disadvantages [58].

5.3. Spray-Drying

Spray-drying has been one technique for encapsulating biocomponents since 1920. It is widely used in the industry due to its robustness, rapid drying, flowable powders, and ability to manipulate particle size [75]. It is based on a two-phase system: liquid and air. In this technique, the active material is dissolved, prepared, and homogenized. 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 [67,76]. The whole process consists of three main stages: (i) atomization, (ii) mixing, and (iii) separation. Atomization is the first and most important process during 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 [77]. There are few studies on drying this specific type III sourdough, with freeze-drying being the preferred method due to its better cell preservation. However, this process is expensive and time-consuming. In contrast, spray-drying is a cheaper method, with continuous production, and operates on a large scale.

Among the systematic review of drying methods (based on 23 studies) presented by Marco et al. [10], 65% (n = 15) evaluated cell viability before and after the drying process, of which 33% (n = 5) were type I sourdough. When comparing cell viability between drying techniques, freeze-drying was the most efficient, with small reductions in LAB and yeast counts. However, the authors reported exceptions where the freeze-drying process did not guarantee the expected viability. For example, in type I sourdough with initial viability of

9.17 and 7.53 log CFU/g for LAB and yeast, respectively, after the freeze-drying process, a drastic reduction of 3 log CFU/g occurred for both groups of microorganisms.

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 [67].

Table 3 briefly presents an overview of the survival rate of bread-making starters after spray-drying.

Starter	Мо	Before Spray-Drying (Log CFU/g)	After Spray-Drying (Log CFU/g)	Survival Rate (%)	Reference
Kombucha sourdough type I	LAB Yeast	$\begin{array}{c} 11.00 \pm 0.05 \\ 10.50 \pm 0.46 \end{array}$	$\begin{array}{c} 9.93 \pm 0.10 \\ 9.40 \pm 0.15 \end{array}$	90.27 89.52	[60]
Sourdough type I	LAB Yeast	$8.7 \pm 0.0 \\ 8.6 \pm 0.0$	$\begin{array}{c} 5.0\pm0.0\\ 4.9\pm0.1\end{array}$	57.47 56.97	[61]
Sourdough type I	LAB Yeast	$\begin{array}{c} 9.17 \pm 0.17 \\ 7.53 \pm 0.12 \end{array}$	$7.9 \pm 0.1 \\ 5.7$	86.15 75.69	[56]

Table 3. Overview of the spray-drying techniques. Mo = microorganisms.

5.4. 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 [78].

This technology is a commercially effective method to produce instant active dry baker's yeast. Akbari et al. [55] showed the optimum operating parameters of an industrial continuous fluidized bed dryer for making instant active dry baker's yeast. Working conditions, such as temperature, the loading rate of compressed yeast granules, and hot air humidity, directly affect yeast viability. As a result, the most critical factors that affected the quality of the product in the study were the loading rate and the operational temperature in each zone on the bed. The data analysis resulted in an optimal operating point at a loading rate of 350 kg/h and temperatures between 29 and 33 $^{\circ}$ C as well as high yeast cell viability of up to 76%, which was 27% higher than the viability of the yeast in the normal operating conditions of the plant. According to Vorländer et al. [79], fluidized bed application enables faster drying than lyophilization, on the one hand, and lower temperatures than spraydrying, on the other hand, the two predominantly used techniques for life-sustaining drying of microorganisms. In this study, the authors showed different protectants for S. cerevisiae, such as mono-, di-, oligo- and polysaccharides, but also skimmed milk powder and one alditol, as they, or chemically similar molecules, are known from other drying technologies to stabilize biological structures such as cell membranes, and thus, improve survival during dehydration. As a result, with the combined use of trehalose and skimmed milk powder, survival rates were 300 times higher than without protective additives.

Concerning the microorganisms in the fluidized bed, dried pre-encapsulated cells 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 microorganisms forms a homogenous layer, which may be completed in multi-layers [80].

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 [81,82].

Coating baker's yeast with a fluidized bed system was successfully tested to prevent dried yeast's moisture absorption from the flour mixture (moisture content of 13% wb) by Altay et al. [83]. This problem could lead to the loss of yeast activity. Amounts of 50 g of yeast, 0.25 MPa of nozzle pressure, 13.09% of coating material to yeast ratio, and the palm–Na caseinate–maltodextrin coating material were in the best condition. The coated yeasts demonstrated protective properties against moisture transfer until the end of the second month. Due to the exceptional use of fluidized beds for baker's yeast, studies have evaluated its usefulness for other microorganisms that are interesting for application as starters in bread fermentation, such as probiotics.

According to Wirunpan et al. [84], the survival rate of *L. 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 [79]. Wu et al. [85] evaluated the optimization of the process parameters using a fluidized bed to dry and encapsulate *L. brevis* RK03; the authors achieved a survival rate of 95% using casein and whey protein as carriers.

5.5. Vacuum Drying

Vacuum drying resembles freeze-drying; however, the samples are dried by evaporation, not sublimation. Removing water from heat-sensitive microorganisms such as probiotics without damaging or keeping them viable is challenging.

Therefore, vacuum drying is an alternative for more sensitive microorganisms. It works at a higher temperature, around 25–30 °C, and a higher pressure (10 mbar), compared to generally below 10 mbar for freeze-drying. Low-temperature vacuum drying is gentler, limiting the loss of viable heat-sensitive microorganisms even though the cell wall and cell membrane can be damaged when this technique is used. The drying parameters can be altered to diminish the damage by adding protecting agents or by pre-treatment of the cells. Table 4 presents some examples of vacuum drying of probiotics [86,87].

The operation parameters of a vacuum dryer allow it to have an energy consumption that is about 40% lower than freeze-drying [88,89]. However, there are disadvantages, such as the long processing time, the dried product shrinkage, and the formation of a denser structure. The reduced drying temperatures, higher drying rate, and reduced oxygen concentration are considered advantages [87,89,90].

Strain	Protectant	Temperature	Pressure	Time	Survival Rate	Reference
Lc. paracasei F19	Trehalose 25% (w/w)	15 °C	15 mbar	22 h	70%	[91]
Lactobacillus helveticus	Sorbitol (1% w/w)	43 °C	100 mbar	12 h	18%	[92]
L. acidophilus	Trehalose (20% w/w)	Room temperature	0.11 mbar	96 h	37.9%	[93]

Table 4. Vacuum drying of probiotics. Parameters and survival rates.

6. Microbial Contamination of Baking: Bread Spoilage

Bread spoilage can be caused by chemical and microbiological factors. Chemical spoilage, such as rancidity, is the most common type after baking high-fat bread products. It is known as lipid degradation, resulting in off-odors and flavors. There are two types of rancidity: oxidative and hydrolytic. The former induces the degradation of unsaturated fatty acids by oxygen, forming aldehydes, ketones, and short-chain fatty acids. The hydrolytic form is due to triglyceride hydrolysis producing malodorous fatty acids and glycerol. Moisture and endogenous enzymes such as lipases and lipoxygenases enhance the problem. Our focus is microbiological spoilage caused by molds, yeasts, and bacteria and influenced by several factors, including bread ingredients and external contamination due to lack of hygiene, insects, and other factors discussed in Section 4 [5,94]. The major types of microbiological contamination are discussed below.

6.1. Molds and Toxins

It is important to note that microbial spoilage of bread after baking is responsible for huge waste generation and public health concerns [95]. Moreover, filamentous fungi during bread-making can produce mycotoxins that can be stable at high temperatures, such as aflatoxin and ochratoxin, which are highly toxic and associated with cancer [96]. There is no intake limit reference, although some countries have limited their concentration in grain, and there is evidence that 1 ng/Kg body weight per day contributes to the risk of liver cancer [97]. In Brazil, the cereal concentration limit of aflatoxin is 5 μ g/kg. A study performed in Brazil showed that in 180 samples of wheat grain, wheat bran, whole wheat flour, and wheat flour, only one wheat grain had aflatoxin above the permitted level, according to legislation [98]. The principal genera associated with bread mold are *Penicillium* (*Penicillium* roqueforti, Penicillium brevicompactum, and Penicillium chrysogenum), Wallemia, Aspergillus (formerly Eurotium), and other common molds, including Chrysonilia sitophila, Rhizopus, and Mucor [45]. The P. roqueforti is associated with spoilage by a mycotoxin hazard in bread, contrasting with species widely used in the dairy industry. However, studies have proved that *P. roqueforti* is a case of species domestication, identifying five populations with traits specific for cheese, non-cheese, wood colonizers, silage, and food spoilers [99–101].

In a study with Portuguese wheat flour regarding mycotoxin, no wheat flour exceeded the legislation limit, which in cereal is 4 μ g/kg for total aflatoxin and 3 μ g/kg for ochratoxin, in the European Union [37]. However, a study showed that the thermal stability of aflatoxin and ochratoxin depended on the food matrix involved. Aflatoxin was degraded in temperatures up to 160 °C, and ochratoxin was stable in temperatures up to 180 °C [96]. A recent study demonstrated that bread contaminated with mycotoxins, aflatoxin B1, and ochratoxin A decreased the contamination percentage with a combination of LAB starter and yeast in the fermentation dough for 24 h [102]. It is noteworthy that mold can affect the sensory characteristics of bread and bring safety insecurity with mycotoxin production [95].

6.2. Yeasts: Chalk Molds

Chalk molds are a type of bread spoilage having the appearance of white powder, caused by yeasts, especially *Saccharomycopsis fibuligera*, *Hyphopichia burtonii*, *Zygosaccharomyces bailli*, *S. cerevisiae*, and *Wickerhamomyces anomalus* (formerly known as *Pichia anomala*). They are common in sliced and rye bread, and some of them, such as *H. burtonii*, present biocontrol properties against *Aspergillus niger* and *P. paneum* [103].

A recent study demonstrated the effectiveness of highly sensitive, quantitative polymerase chain reaction (qPCR) and digital droplet polymerase chain reaction (ddPCR) for the early detection and quantification of *S. fibuligera* and *W. anomalus* cells directly in bread for the first time. These analyses represent a promising strategy for applying high-throughput approaches to monitor bread quality [104].

6.3. Bacillus sp.: Ropiness

The microbial bread spoilage caused mainly by the *Bacillus* genus is called ropiness. Ropiness is characterized by an unpleasant odor due to volatile compounds like diacetyl, acetoin, acetaldehyde, and isovaleraldehyde. Moreover, the slimy crumb is due to bacterial polysaccharides and protein extracellular production, as well as degradation of the breadcrumb because of extracellular hydrolase production (peptidase and amylase). The breadcrumb can be almost liquefied in advanced stages, forming long, silky strands when pulled apart. However, the mechanism of rope spoilage is not entirely elucidated [105–108]. The *Bacillus mesentericus* group is one of the first *Bacillus* which was attributed to rope formation [107,109].

Currently, *B. subtilis*, *B. clausii*, *B. cereus*, *B. licheniformis*, and *Bacillus* species that are not identified have been associated with ropiness [110,111]. In Portuguese sourdough bread, besides the best-known species identified, *B. brevis*, *B. circulans*, *B. laterosporus*, *B. macerans*, *B. mycoides*, *Bacillus pumilus*, and *Bacillus stearothermophilus* have been found [112]—other *Bacillus* such as *Cytobacillus firmus* (formerly *Bacillus firmus*), *Niallia circulans* (ordem *Bacil-*

lales), Paenibacillus polymyxa (formerly *Bacillus polymyxa*), and *Priestia megaterium* (formerly *Bacillus megaterium*) and *L. plantarum* subsp. *plantarum* (formerly *L. plantarum*) have been reported to cause ropiness in bread [107,113]. *Bacillus mesentericus* was isolated as a bread contaminant related to rope formation [111], but they are currently included in the *B. subtilis* group [107,109].

The primary reservoir of *Bacillus* endospores comes from naturally contaminated soil and crops, which will contaminate the flour. Baking eliminates the vegetative forms, but after, the endospores will germinate, causing rope spoilage [107,114].

Rumeus [111] demonstrated that visible contamination by *Bacillus* was observed only 40 h after bread baking, indicating the possibility of the consumer not perceiving the risk of consuming toxin-contaminated bread, which could lead to poisoning or infection. Several other fermented foods can present lipopeptides with antimicrobial activity produced by *Bacillus* or other favorable properties, and some strains can be probiotics [115].

7. Control of Microbial Contamination in the Bread Chain

The control of microbial spoilage in bread is initiated by the effective quality control of wheat grain and wheat flour. New methods are being studied to improve the quality of contaminated cereal grain, including thermal processes, cold plasma, and organic acids, among others, since actual methods could damage the grain and have some concerns regarding consumers, such as pesticides, dying grain, debranding, chlorine, and hypochlorite [116]. Maintaining appropriate moisture levels, especially in milling equipment, is essential to thwart mold proliferation and subsequent wheat flour contamination. During storage, temperature management and suitable packaging selection are crucial in mitigating oxygen and humidity exposure [117,118]. In the process of bread-making, maintaining a hygienic environment is essential to minimize the presence of microbial cells and spores in the air, on surfaces, and in the hands of those handling the dough. Such contaminants risk affecting the bread after baking, as evidenced by a study conducted in a bakery factory, which observed potential cross-contamination by the air and packaging gloves [45]. Moreover, after baking, bread must be preserved far from humidity and high temperatures [107]. Some other strategies have been studied to contribute to or make up for the deficiency at the beginning of the bread chain, such as modified atmosphere packaging, active packaging, chemical preservatives, essential oils, and plant extracts (Figure 2).



Figure 2. Microbial control points in the bread chain.

Bread spoilage control is essential for consumers' health and food 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 both the industry and consumers. Preservation refers to techniques and methods used to extend the shelf life of baking products, maintaining quality, freshness, and safety for extended periods. Moreover, different preservation categories, including chemical, biological, and physical methods, are used for this purpose [5]. Traditional and innovative technologies used for preserving bread, as well as their perspectives for application in future years, are described in what follows.

7.1. Chemical Methods: Organic Acids

Using weak organic acids in bread or even other food is considered a conventional strategy to extend the shelf life of products. However, the baking industry must follow local legislation that regulates the maximum concentration of these acids in baked products [119,120]. The mechanism of action of these chemicals consists of destabilizing plasmatic membrane components (H⁺-ATPase) and inhibiting intracellular vital enzymes (phosphofructokinase in glycolysis) 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 bread baking. Natural preservatives, like honey, sugar, and salts (potassium, sodium, calcium), can also help extend shelf life.

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 two days, preventing early spoilage [120]. However, some fungi can be insensitive to these acids. For example, a study using bread and cake found resistance to propionic and sorbic acids at maximum legal limits for usage in *P. brevicompactum* and *P. roqueforti* [121]. In another study, management of pH and temperature was insufficient to control yeast spoilage in bread, and the efficiency of propionic acid was yeast-dependent [103].

A Brazilian study with sliced bread produced in Brazil used calcium propionate or potassium sorbate preservatives and isolated *P. roqueforti, H. burtonii* (HB17), and *Paecilomyces variotii* (PV11). This fact indicated a difference in sensibility between mold and yeast for these preservatives [122].

Although 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 they effectively inhibit microbial growth without affecting the bread 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 [16,120].

A combination of different acids or strategies can also be performed for preservation. Quattrini et al. [123] 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 after adding sugar (4%) to sourdough fermentation with *L. brevis* for six days, the bread was free of *A. niger* growth. Ricinoleic acid (up to 0.15%) and *Lactobacillus hammesii* presented the same preserving effect.

Organic acids have been studied and used as preservative agents in food and beverages for a long time, proving their efficiency. The baking industry uses these compounds individually or combined, or even as products of natural bacteria fermentation in the dough matrix [124]. Thus, the application of these compounds as preservatives in bread will still be in high demand due to their antimicrobial properties and flavoring aspect [125].

7.2. Biological Preservatives

7.2.1. Essential Oils

Chemicals are primarily used to sanitize food product rooms. However, nowadays, there are concerns regarding the residues of these chemicals on the surfaces in contact with

food. Because of this, green technologies have been studied to promote a better hygienic environment for bread-making that diminishes microbial spoilage.

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 [126–128]. They are known for their bioactive properties, including inhibition of the growth of bacteria, yeasts and molds, viruses, protozoa, and insects, and their antioxidant properties [127,129–131].

These molecules are promising for application in the food industry as natural preservatives. They can be used for product preservation in the bakery industry. Essential oils can be added to the headspace of packaged bakery products, directly in bread, or in combination with other strategies to increase shelf life. Essential oils are also an alternative for controlling toxigenic fungi in cereal grains. Fungi contamination affects grain development and seed germination, reducing grain quality and nutritional value. In addition, some fungi can produce mycotoxins, toxic secondary metabolites. The antifungal and antimycotoxigenic properties and the action mechanisms of various essential oils have been studied. Vapor and nanoencapsulation can apply essential oils directly in the grains [132]. However, there are limitations in the process, and studies are in progress to answer several questions relating to toxicity, the concentration of use, and the cost of production, among other points [133]. The literature shows promising results. Black cumin seed (*Bunium persicum*) essential oil was evaluated for acute and subacute toxicity in male Wistar rats. After 14 days, the results showed that black cumin essential oil did not affect the immune system, tested enzymes, or organs. No mortality was observed at the doses tested [134].

It is important to note that applying essential oils to baking products may alter the sensory properties of bread, such as taste, texture, and color, being considered a negative aspect of a biopreservative [5,128,135].

The antimicrobial mechanism disrupts membrane cells and ergosterol reduction, inhibits enzymes, inhibits mycelial growth, inhibits spore germination, and alters proteins due to the cleavage of disulfide bonds, among other effects [133,135–138].

Table 5 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.

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	[139–142]
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, E. rubrum, Fusarium oxysporum, P. corylophilum, Penicillium italicum, P. paneum	Bread shelf life	[121,133,139, 143–145]

Table 5. Preservation of bread by plant essential oils.

Essential Oils	Major Compounds	Targeted Molds	Action	Reference
Lemongrass (Cymbopogan citratus)	Citral, geraniol, limonen, neral, nerol, myrcene, citronellal	A. flavus, A. niger, E. amstelodami, E. Herbariorum, E. repens, E. rubrum, P. corylophilum, Penicillium expansum	Mold growth inhibited	[139,146]
Rosemary (Rosemary officinalis)	Carnosic acid, carnosol, rosmarinic acid, hesperidin	Penicillium sp. Aspergillus sp.	Fungal generation reduced	[133,147]
Oregano (Origanum vulgare L.)	Carvacrol, thymol, rosmarinic acid, p-Cymene, terpinene, linalool, naringin, β-caryophyllene	A. flavus, A. niger, Aspergillus fumigatus, Aspergillus ochraceus, A. parasiticus, A. terreus, Eurotium fibuliger, P. commune, P. roqueforti	Mold growth inhibited	[140]
Cinnamon (Cinnamomum jersenianum HandMazz)	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	[139,140]

Table 5. Cont.

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. A study developed a nanofiber by fish gelatin, with dual encapsulation of essential oils in β -cyclodextrins, which presented efficient activity against microbial spoilage in wheat bread at ambient temperature for ten days. The nanofiber encapsulated with eugenol, a major component of clove essential oil, showed the best result [148]. Thus, even if potentially considered for usage in bread conservation, more studies must be conducted to meet both preservation and quality standards in baking products.

7.2.2. 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. They have been described as having exciting properties, such as antimicrobial activity [149,150] and medical application [151,152]. 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 in the food industry one of the receptors of these bioproducts [153]. Recent works investigating the benefits of plant-based extracts in breads have also been observed [154,155]. Studies also concern the interaction of plant extracts with other components present in the food matrix and the regulations about food safety [156].

Regarding food application, plant-derived extracts can efficiently inhibit microbial pathogens and molds. Negi [157] has described an extensive review regarding phytochemicals with antimicrobial activity, such as *Cinnamomun* and *Garcinia* species, as well as *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 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 [158].

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. [159] observed that star anise may cause lipid peroxidation in the cell membrane

and interaction with 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. [160], 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 the bread ingredients. No adverse effects were observed for its texture, and the extended shelf life of the bread was achieved due to fungistatic activity.

To conclude, Figure 3 summarizes how plant extracts and essential oils interact with target microorganisms and thus act as preserving agents in baking products. Similar to essential oils, perspectives on using plant extracts in bread and other products for conservation purposes may depend on their ability to avoid early spoilage without altering the sensory aspects of the product.



Figure 3. Mechanism of action of plant extracts and essential oils.

7.2.3. Lactic Acid Bacteria (LAB)

Besides their role in fermentation, lactic acid bacteria also act as a preservative agent, enhancing the shelf life, flavor, and quality of baking goods. Spontaneous acidification due to the fermentation of local microbiota occurs in sourdough. 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. LAB presents considerable importance for preservative purposes in the baking industry [9,161]. Table 6 describes LABs with potential use to increase the shelf life of bread.

Table 6. Lactic acid bacteria in bakery products can potentially increase bread's shelf life.

Antifungal Lactic Acid Bacteria	Microorganisms	Reference
L. plantarum FST 1.7	Fusarium culmorum and Fusarium graminearum	[162]
L. plantarum CRL 778, Lactobacillus reuteri CRL 1100, L. brevis CRL 772 and CRL 796	Aspergillus, Fusarium, and Penicillium species	[163]
Lactobacillus amylovorus DSM 19280	A. niger FST4.21, P. expansum FST 4.22, P. roqueforti FST 4.11, F. culmorum FST 4.05	[164]

L. amylovorus DSM19280

L. plantarum L244 with Schleiferilactobacillus

harbinensis L172 (formerly Lactobacillus harbinensis)

L. plantarum CH1, L. paracasei B20, L. mesenteroides L1

L. plantarum UMCC 2996, F. rossiae UMCC 3002, P.

pentosaceus UMCC 3010

Table 6. Cont.		
Antifungal Lactic Acid Bacteria	Microorganisms	Reference
L. plantarum	A. niger FST4.21, F. culmorum TMW 4.0754, P. expansum LTH S46	[165]
L. plantarum LB1, F. rossiae LB5	P. roqueforti DPPMAF1	[166]
F. rossiae LD108, Companilactobacillus paralimentarius PB12 (formerly Lactobacillus paralimentarius)	Aspergillus japonicus, E. repens and Penicillium roseopurpureum	[167]
Latilactobacillus sakei (formerly Lactobacillus sakei) KTU05-6, P. acidilactici KTU05-7, P. pentosaceus KTU05-8, P. pentosaceus KTU05-9, P. pentosaceus KTU05-10	Molds	[168]

Molds

P. commune, Mucor racemosus, and R. mucilaginosa

M. racemosus UBOCC-A-109155, P. commune UBOCC-A-116003, Yarrowia lipolytica

UBOCC-A-216006, Aspergillus tubingensis AN, A. flavus T5, Paecilomyces formosus AT

A. flavus ITEM 7828, P. paneum ITEM 1381, A. niger

ITEM 7090

Furthermore, microbial growth inhibition can result from antimicrobials secreted by LAB, preserving these baked goods. Rizzello et al. [172] 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 [173]. Preserving baked goods with LAB can also occur due to unsaturated or saturated fatty acids such as 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 [174,175].

In an experiment by Illueca et al. [176], commercial yeast bread and sourdough bread were prepared as controls. The system of study was sourdough bread supplemented with L. plantarum 5L1 lyophilized. The results showed significant differences. A higher total phenolic, lactic acid, alcohol, and ester content in bread with higher amounts of L. plantarum 5L1 was observed. L. plantarum 5L1 additionally delayed fungal growth and reduced the content of the aflatoxins (AFB1 and AFB2) compared to the control.

7.3. Physical Methods

7.3.1. Pasteurization and Radio Frequency Heating

Major bread components such as wheat flour and cereals display low water activity at 25 °C ($a_{w,25^{\circ}C} \leq 0.85$). Besides being less susceptible to microbial spoilage in bread, this property disappears due to the water content of bread. Bread is an intermediatemoisture food with 35–42% moisture and water activity (a_w) above 0.95. This fact makes bread susceptible to microbial spoilage, with the main effect coming from the growth of various molds [177]. An approach to control contamination and spoilage is to reduce the microorganisms, mainly molds, by treating the flour with thermal methods such as radio frequency and pasteurization. Pasteurization influences physical and technological properties precisely due to changes in gluten structure and starch configuration. Heat treatment of flour decreases gluten extensibility, and partial gelatinization of the starch granules occurs [178,179]. Studies with radio frequency (RF) in bread flour

[119]

[169]

[170]

[171]

(10 kW—27.12 MHz) have indicated that the RF process did not cause a significant change in the physicochemical and rheological properties of the flours treated. In contrast, the RF process applied in the 75–85 °C temperature range decreased bread volume and specific volume. Also, the disrupted gluten matrix reduces the dough's gas-holding capacity [180].

7.3.2. Cold Atmospheric Plasma Treatment

Cold atmospheric plasma (CAP) is an emerging green and safe technology resulting from non-thermal gas ionization into free electrons, ions, reactive atomic and molecular forms, and ultraviolet (UV) radiation. CAP treatment of bread inhibits yeasts, molds, and mesophilic bacteria. However, the texture becomes damaged, indicating that more studies must be implemented [181].

7.3.3. Electrolyzed Water

Electrolyzed water decreases the growth of spoiled bread by *P. roqueforti* and *H. burtonii* [182]. A study in the literature showed using four different types of electrolyzed water (Anolyte NaCl, Catholyte NaCl, Anolyte Na₂CO₃, and Catholyte Na₂CO₃) improves the bread quality. It was observed that each one brings different properties, such as increased antioxidant activity and the water-holding capacity of the dough, as well as a higher loaf volume [183].

7.4. Packaging Strategies

7.4.1. Modified Atmosphere Packaging (MAP), Active Packaging, and Intelligent Packaging

A promising strategy focused on different packaging for baking products has contributed to bread preservation methods.

Atmospheric air is recognized as a leading cause of contamination in bread products, particularly post-baking. This is attributed to fungal spores present during slicing and packaging, significantly elevating the risk of contamination. Modified atmosphere packaging (MAP) is an inexpensive and easy method to replace atmospheric air with a mixture of carbon dioxide (CO₂) and inert gas nitrogen in packaging, extending bread's shelf life. The objective is to decrease the oxygen (O₂) content to less than 1%. Oxygen, besides microbial respiration, produces lipid oxidation reactions. The antimicrobial properties of CO₂ inhibit mold and bacteria growth [12,184]. A combination of techniques has also been studied to improve preservation effects. One work observed that combining high temperature (200 °C) and time with a modified packaging atmosphere technique could improve the quality and shelf life of par-baked bread [124]. The modified atmosphere packaging technique has shown promising results for preservation in bakery products and other types of food, illustrating that it is a well-established technique that can be widely used in the baking and food market [185–187].

As discussed previously, spoilage bread intensifies after baking because of crosscontamination. Therefore, controlling the hygienic conditions of bread-making is crucial. Chalk yeast reduced the incidence to values of 0.3–0.98% in sliced bread in modified atmosphere packaging (MAP). Previously, with the use of conventional sanitizer, the incidence of deterioration ranged from 6.03% to 11.59%. This intervention allowed bread to be free of chemical preservatives in the MAP [188]. Fik and colleagues [189] observed that a packed atmosphere composed of 60% CO₂ and 40% N₂ showed good protection against microbial degradation for wholemeal bread for up to 27 days. In another study, a modified atmosphere packaging of 50% CO₂–50% N₂ or 20% CO₂–80% N₂ presented satisfying results for extending soy bread shelf life without using calcium propionate as a chemical preservative [190].

However, studies have shown that MAP in bread packaging only delays mold growth but cannot prevent growth [191], and the use of MAP must be evaluated before use. To attend to this demand, the European Food Safety Authority (EFSA) developed innovative concepts for packing foods, such as active packaging (AP) and intelligent packaging (IP). These methods allow effective interaction between the packaging material and the packing components, forming a protective layer around the food, increasing hygiene, safety, and shelf life and decreasing sensory losses. Active packaging uses components with scavenging and releasing agents such as oxygen scavengers, moisture absorbers, antimicrobial agents, essential oils, sachets/films containing ethanol emitters, and antioxidants, among others, to improve the package system quality and enhance the shelf life. With these properties, active packaging preserves the food from extrinsic factors such as heat, UV rays, oxygen, water vapor, and pressure through effective packing techniques. There are several types and combinations. AP uses essential oils such as thyme, cinnamon, oregano, and lemongrass as antimicrobials, which have been used for hot dogs and sliced bread [192]. Moisture absorbers with essential oils are another example, and this method has been tested for sliced and gluten-free bread [193]. Ethanol emitters are effective in slowing down mold growth in rye bread. Sachets are prepared with antifungal, antioxidant, and antimicrobial effects. Active packaging is considered an effective and cheap method for industrial use [194].

In recent years, innovations such as nanomaterial packaging have emerged, incorporating metallic nanoparticles, biodegradable packaging, and edible coatings or films containing essential oils [195]. Intelligent packaging is a smart packaging system and is the most innovative technology. The method monitors changes in the interior and exterior of packed food and transmits the status of the packaging system to support decision-making. Biosensors, temperature regulators, ripeness monitors, and time-temperature monitors are responsible for monitoring the quality and safety of the product [196].

7.4.2. Coating and Biodegradable Packaging

Another strategy involving packaging techniques is described as implementing properties to the coating layer where food will be stored [197]. Viscusi and colleagues [198] observed that a polypropylene package with sorbate as an active molecule efficiently controlled molds and inhibited the growth of several pathogenic bacteria for 12 days. In a similar work, a packaging film of polyhydroxybutyrate and clove essential oil as an antimicrobial agent was developed and presented antibacterial and antifungal activity, improving the shelf life of brown bread for up to 10 days [199].

In a different context, sustainability and the environment have created a high demand for biodegradable packing materials. They are 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 [200–202]. A study focused on bread products observed that a biopackage composed of lignin nanoparticles, cinnamaldehyde, and polybutylene succinate presented antifungal activity against *A. niger* and *Penicillium* sp. and created an efficient barrier against moisture and oxygen deterioration for up to 14 days at 25 °C [200].

Natural waxes and other lipids have been used to produce films and coatings for food due to their capability to interact with biopolymer matrices, altering crystallinity and plasticity. They can be recycled with little change in properties and are abundant environmental sources [203]. For instance, it is a current practice to cover horticulture with a waxy coat, which maintains the exchange respiration and prevents wet loss. Moreover, the edible coating can be produced from sugarcane wax, carnauba wax, honey beeswax, and others like palm oil, which preserve the freshness of mangoes for 16 days [204].

A study has shown that using an edible beeswax and diacetyl tartaric ester monoglycerides film as a coating for hamburger bread is a viable option. The study found that this coating type can extend the bread's shelf life and improve consumer acceptability [205].

Whole wheat bread packed in beeswax–chitosan-coated paper had good consumer acceptance. It was microbiologically stable for eight days and 12 days for wheat bread stored at ambient temperature and refrigerated, respectively [206]. Therefore, considering the diversity in crust textures, continued evaluation of the effectiveness of these wax wraps

for various types of bread is crucial. Despite the preliminary nature of these studies, they present a promising avenue to address the current reliance on plastic foil in food packaging.

8. Conclusions

Baking processes are constantly developing to obtain the best sensory and health properties at each step of production. From the inoculum choice and preparation to the final product and preservation, all steps receive special attention and research. As reflected in the literature, the recent advancements demonstrate a dynamic field where research continually propels the baking industry forward. The age-old art of bread-making has integrated global scientific research results. Current results with treatments of flour and bread concerning production and commercialization demonstrate the advances in this theme. Several factors influence the selection of methods. These include the processing facility's unique characteristics (bread chain), the production stages, and the complexity inherent in bread manufacturing, even in artisanal settings. This complexity underscores the knowledge required in this field. It is strongly advised against using just one method, highlighting the importance of appropriate legislation for producing bread, where the methods and essential control points must be clearly outlined. The hygiene of the handlers, the cleaning conditions of the production premises, the origin of the water, the quality of the raw materials used, and other factors of the process can all potentially be sources of contamination. This highlights the role each individual plays in preventing contamination. The manipulators must have basic knowledge of the microbiology of the process. Vigilance and care of these possible entry points for microorganisms are crucial when considering physical, chemical, and biological control methods.

Incorporating sustainable, biodegradable, ecologically friendly additives and new packages in baking aligns with environmental concerns and highlights a conscious effort to enhance human health. In particular, the emphasis on natural preservatives, innovative dry procedures, and unique strains with inhibitory properties introduces a new dimension to bread production. Preserving microbial cultures and starters constitutes a crucial point in bread production, more relevant when new biological technologies are being incorporated into baking, such as adding microorganisms with special properties concerning metabolites and enzymes.

Controlling and mitigating contamination is crucial in bread production. Regulatory initiatives play an essential role in setting guidelines and requirements and ensuring protocol compliance. It is also recommended that relevant stakeholders receive comprehensive training and specialized education.

These approaches contribute to extending shelf life and preventing spoilage and play a crucial role in elevating the overall quality of bread. The intersection of science and baking is a testament to the ongoing quest for better, healthier, and more sustainable food production. It is remarkable how the age-old art of bread-making has integrated global scientific research results. Tradition and innovation reflect a promising future for the baking industry: sustainable and health-conscious.

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