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Review

Biotechnological Approach of Technological Advancements for Sustainable Probiotic Bread Production

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Abstract: The pursuit of probiotic-enriched bread, driven by the dual objectives of enhancing nutritional value and promoting health while ensuring sustainability, has spurred significant research and technological advancements. However, a persistent challenge lies in preserving the viability of microorganisms throughout the rigorous processes of production, storage, and exposure to the stomach's acidic environment. This study investigates biotechnological innovations for sustainable probiotic bread production, conducting a thorough review of probiotic encapsulation methods and analyzing prior research on the viability of encapsulated probiotics in bread across different baking conditions and storage periods. Encapsulation emerges as a promising strategy, involving the protection of microorganisms with specialized layers, notably multilayered alginate-chitosan coatings, to shield them from degradation. Studies suggest that encapsulated probiotics, particularly the *L. casei* 431 strain within smaller-sized products subjected to shorter baking times, exhibit minimal viability reduction. Moreover, incorporating microcapsules into the dough, rather than post-baking surface application, further mitigates bacterial losses during storage. Despite these advancements, further investigations are necessary to identify strains resilient to processing, storage, and consumption while prioritizing sensory attributes to meet consumer preferences. Ultimately, research in probiotic bread production aims for a sustainable approach, placing significant emphasis on health considerations and disease prevention. Implementing encapsulation technology aligns with consumer demands for healthy, environmentally friendly products, highlighting the urgent need for innovation in this field with a focus on sustainability.

Keywords: probiotic bread; microencapsulation; nutritional value; sustainability; technological advancements; health benefits

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1. Introduction

The increasing global focus on sustainability, coupled with the need for more health-conscious food options, has led to significant advancements in the biotechnological production of food. This approach integrates various technological and biological innovations to enhance the nutritional content of food while minimizing its environmental footprint [1]. By harnessing probiotic strains derived from agricultural and food industry waste, such as by-products from dairy and plant-based food processing, this approach addresses two key issues: the reduction in food waste and the provision of functional foods that promote gut health. The use of such waste not only reduces the environmental impact of food production but also presents a valuable opportunity to recycle nutrients back into the food chain [2]. By utilizing clean technologies, such as bioreactors and controlled fermentation processes, probiotic bread production can achieve greater consistency and efficiency, while minimizing the use of harmful chemicals and minimizing energy consumption. This ensures that the final product is not only nutritionally enhanced but also environmentally friendly, making it a sustainable choice for health-conscious consumers [3].

The term bread encompasses a wide range of bread and bakery products, predominantly buns. It has been a dietary staple across cultures since ancient times, providing

essential carbohydrates, dietary fiber, proteins, vitamins (primarily from the B group of vitamins), and minerals [4]. Dietary fiber, being a plant-based fraction resistant to digestive enzymes, is especially valuable for its beneficial effects, including the enhancement of fermentation processes in the large intestine, a reduction in serum cholesterol levels, and improvements in postprandial glucose levels. Notably, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) recommend a daily intake of dietary fiber of 25 g/day.

Worldwide, bread and composite flour consumption varies significantly based on socioeconomic factors such as income levels, family size, and cultural preferences [5]. In recent years, there has been a notable decline in per capita bread consumption. For instance, in Poland, from 2000 to 2020, the average monthly consumption of bread per person decreased from 6.61 kg to 2.75 kg [6]. This decline can be attributed to various factors, including changes in product quality, price, and availability, shifts in consumer attitudes, psychology, and lifestyle, as well as economic and sociocultural influences [7]. One prominent reason for the reduced consumption of bread is a growing awareness of the impact of dietary choices on health, leading individuals to adopt more balanced and rational diets. A balanced diet is crucial for providing the necessary energy, regulatory, structural, and fluid components, taking into account individual factors such as age, sex, physical activity level, and health status [8]. This trend has led to an increased preference for minimally processed and whole-grain products over light wheat bread, driven by misconceptions regarding the fattening properties of bread and a misunderstanding of its nutritional value [9]. Moreover, the inclination towards gluten-free diets has also contributed to the reduction in bread consumption. While such diets are often perceived as healthier and associated with weight loss, scientific research does not support these claims, emphasizing the importance of a balanced and varied diet. The indiscriminate adoption of gluten-free diets may result in a deficiency of essential nutrients found in bread, including dietary fiber, B vitamins, and minerals like calcium and iron [10].

Recent scientific research has also begun to delve into the potential health benefits of these alternative bread production techniques. Studies have shown that sourdough fermentation can lead to the production of bioactive compounds with antioxidant and anti-inflammatory properties, which may contribute to overall health and well-being [11]. Additionally, the sprouting process has been found to increase the bioavailability of certain nutrients, such as vitamins and minerals, in bread, potentially offering greater nutritional value to consumers [12].

Parallel to these dietary changes, socioeconomic factors have also played a significant role in altering consumption patterns. An improvement in economic conditions has lessened the financial burden of food expenses, allowing individuals to explore alternative meal options, including eating out. This trend has led to a growing preference for fresh and custom-prepared bread from catering establishments, as opposed to packaged or mass-produced varieties [13]. Technological advancements and broader access to information have also influenced consumer choices, favoring products perceived as healthier or of higher quality. As a result, there has been an increased demand for probiotic-enriched bread, indicating a shift towards functional foods that offer additional health benefits [14]. In response to these changes in consumer preferences and the demand for more nutritious and functional food options, the bread industry has embraced innovation and sustainability. This involves the incorporation of high-quality, locally sourced ingredients, the reduction in food waste, the optimization of energy usage, water conservation, the use of eco-friendly packaging, and collaboration with local communities to understand their needs better [10].

The combination of biotechnological advancements not only leads to the production of healthier bread with added nutritional benefits but also contributes to a more sustainable food system overall. The increasing prevalence of lifestyle-related diseases, such as obesity, diabetes, and heart disease, has underscored the importance of preventative health measures. Probiotic bread, with its potential to support digestive health and immunity, represents a promising avenue for reducing the risk of these diseases. By providing consumers

with an accessible and convenient way to incorporate beneficial probiotics into their diets, probiotic bread aligns with the growing emphasis on proactive health management and disease prevention [15–17]. Among growing interest in probiotic-enriched bread production, a notable research gap persists in elucidating the optimal integration of emerging biotechnological methods, such as encapsulation techniques and probiotic strain selection, to ensure both the viability of probiotics and the sustainability of bread as a staple food.

The study aims to investigate the biotechnological advancements in sustainable probiotic bread production, integrating waste-derived probiotic strains, clean technologies, and health-promoting properties to enhance bread's nutritional content while minimizing its environmental footprint. It seeks to evaluate the potential benefits of probiotic bread in terms of sustainability, health, and overall food system resilience, thereby contributing to a more sustainable and health-conscious food system.

2. Methods

The methodology of the literature review was conducted systematically following established guidelines [18,19]. Keywords related to probiotic bread production, biotechnological advancements, sustainability, and health were used to search relevant literature in databases such as PubMed, Scopus, Web of Science, and Google Scholar. The literature was categorized based on relevance to the research objectives, and key themes were identified for analysis. The manuscript is divided into sections including an introduction, literature review methodology, findings, and conclusion. Each section will address specific aspects of probiotic bread production, focusing on practical considerations and current recommendations for probiotic use, ingredients and processes in baking technology, and enhancing probiotic viability through technological processes.

3. Practical Considerations and Current Recommendations for Probiotic Use

Microorganisms have played a significant role in food and beverage production since ancient times. The creation of acidic products such as fermented cabbage, yogurt, and kefir is impossible to achieve without proper microorganisms. Moreover, fermented products are often characterized by better nutritional and organoleptic value compared to non-fermented products. The digestibility of proteins, amounts of certain vitamins (mainly from the B group), and absorption of mineral components, e.g., calcium, increase in fermented products [15]. The fermentation process is mainly responsible for lactic acid bacteria (LAB), such as bacteria of the genera *Lactobacillus* and *Bifidobacterium* as well as some strains of *Enterococcus*, and yeasts of the genus *Saccharomyces*. These microorganisms are associated with beneficial health effects for the host organism [16].

Probiotics belong to functional foods, which, in appearance, resemble traditional foods consumed under normal conditions but have a proven beneficial effect on one or more body functions beyond the nutritional effect. The beneficial effect can be achieved only after consuming such an amount of a given product in which it is standardly supplied with the diet. Functional food cannot be consumed in capsules, tablets, or drops [17].

Probiotic organisms have high requirements. Strains should come from healthy human microbiota, i.e., the microflora of the small intestine. They must have the ability to colonize and inhabit the appropriate part of the large intestine and, consequently, must be resistant to the action of gastric acid, digestive enzymes, and bile salts. LAB also represent one of the microorganisms of the native microflora of the gastrointestinal tract. They occur in mucous membranes and milk. Probiotic microorganisms must be safe for the consumer's health and have a GRAS (generally recognized as safe) status. Factors guaranteeing safety include strain stability during processing, use, and storage; genetic stability; a lack of genes resistant to antibiotics and bacteriophages; and a lack of side effects and pathogenic or toxic properties. The probiotic strain must also demonstrate competitiveness against harmful microflora inhabiting the gastrointestinal tract and must be an obligatory or facultative anaerobe [18]. Physiological characteristics are also important for efficient production of lactic acid during the fermentation process. The above features ensure the microorganisms'

passage through the digestive system and colonization of the intestine. The scientific justification of the interaction between a specific strain and the host's gastrointestinal system provides the basis for classifying the microorganism as a probiotic. Without scientifically documented effects, the microorganism cannot be labeled as a probiotic. Therefore, not all lactic acid bacteria are probiotics [19].

The beneficial effect of probiotics involves increasing the amount of positive microflora, restoring homeostasis between strains, and, in the case of infections, reducing the number of pathogens. Regular consumption of probiotics results in the phenomenon of probiosis, consisting of a significant increase in the number of probiotic colonies that have multifaceted beneficial effects on the host's gastrointestinal tract and systemic effects by actively participating in the synthesis of compounds, including certain vitamins [20].

Ensuring the efficacy and safety of probiotic products necessitates rigorous quality control measures and standardized testing methods throughout the production process. Quality control ensures that probiotic formulations maintain viability and potency from production to consumption, safeguarding their beneficial effects [21]. Standardized testing methods assess factors such as bacterial viability, stability, and purity, providing crucial data for product evaluation and regulatory compliance. Moreover, understanding potential interactions between probiotics, medications, and underlying health conditions is paramount for both consumers and healthcare professionals. Such insights enable informed decision-making regarding probiotic usage, particularly for individuals with compromised immune systems or those taking medications that may interact with probiotics [22]. Continued research and regulation in the probiotics industry are essential to uphold consumer trust and safety standards, emphasizing the ongoing commitment to evidence-based practices and the promotion of public health.

The functioning of probiotic microorganisms consists of binding to the adhesion receptors of the gastrointestinal tract using fimbriae. Bacteria that do not have fimbriae in their structure are more quickly removed from the gastrointestinal tract and, therefore, must be supplied in larger quantities, including through food. Bacteria should rapidly multiply in the large intestine, forming a biofilm that serves as an immunological protective barrier. Probiotic bacteria have the ability to stimulate the production of immunoglobulins, thereby enhancing the immune system and humoral response in the host. In addition to seeking microbiological balance, the beneficial effect also includes beneficial effects on colonocytes—intestinal epithelial cells—by providing the energy necessary for the regeneration of intestinal cells. They also improve intestinal peristalsis, contributing to more effective removal of metabolites and toxins [16,19,20,23]. Table 1 provides examples of strains with documented probiotic characteristics, showcasing their diverse range of actions and potential benefits in various health conditions.

Table 1. Examples of strains with documented probiotic characteristics [15,16,19,20,23].

Action	Strain Name	Description
Stimulation of immune response	*Lactobacillus acidophilus* LC1	Adjuvant effect in oral vaccines, adherence to human intestinal epithelium, establishment of intestinal microflora balance
Prevention of adverse effects	*Lactobacillus acidophilus* NCFO 1748	Prevention of diarrhea and other adverse effects after radiotherapy and antibiotic treatment, treatment of constipation, and reduction in enzyme levels in stool
Treatment and prevention of diarrhea	*Lacticaseibacillus rhamnosus* GG	Treatment and prevention of diarrhea after rotavirus infections and recurrent diarrhea caused by *Clostridium difficile*, prevention of acute bacterial diarrhea, alleviation of Crohn's disease and pediatric rheumatoid arthritis, antagonist to bacteria associated with tooth decay, and prevention of recurrent vaginal inflammations

Table 1. *Cont.*

Action	Strain Name	Description
Inhibitory effect on cancer	*Lacticaseibacillus casei* Shirota	Inhibitory effect on the development of surface bladder and colon cancer, protection against intestinal disorders, treatment of rotavirus diarrhea, maintenance of intestinal microflora balance, positive effects in the treatment of bladder cancer, reduction in fecal enzyme activity, and protection against food mutagens
Stimulation of immune system	*Lactobacillus johnsonii* La1 (NCC533)	Adhesion to human intestinal cells and positive effects in the treatment of gastrointestinal disorders
Prevention and treatment of infections	*Lacticaseibacillus casei* DN 114 001	Good survivability in the stomach and duodenum, and reduction in the frequency and duration of acute diarrhea in children
Treatment of rotavirus diarrhea	*Bifidobacterium bifidum*	Restoration of intestinal flora balance, anti-ulcer properties, and elimination of *Helicobacter pylori*
Anti-ulcer properties	*Bifidobacterium breve* Yakult	Protection against mutagenic foods, maintenance of intestinal microflora balance, and protection against diarrhea
Reduction in fecal enzyme levels	*Limosilactobacillus reuteri*	Colonization of the gastrointestinal tract
Prevention and treatment of diarrhea	*Saccharomyces boulardii*	Prevention of traveler's diarrhea and prevention and treatment of diarrhea caused by *C. difficile*

Disruption of the native microflora of the intestine by pathogenic organisms is the cause of gastrointestinal diseases. Probiotics can neutralize and shorten the duration of some gastrointestinal diseases. Diarrheas are most often treated with probiotics, including traveler's diarrhea or those caused by antibiotic therapy, as well as ulcers. Diarrheas are caused, among others, by *Escherichia coli* or *Salmonella*, while ulcers are caused by *Helicobacter pylori* [24].

The action of probiotic organisms is a complex interplay of mechanisms that contribute to their beneficial effects on host health. They employ lactic acid production, which denatures intracellular proteins in pathogens, rendering them inactive. Probiotics engage in competition for both nutrients and receptors on the mucosa and intestinal epithelium. By outcompeting pathogenic microorganisms, probiotics limit their attachment and subsequent colonization, facilitating their excretion from the body via feces. Moreover, probiotics produce a diverse array of substances, including bacteriocins and organic acids, which inhibit the activity of disease-causing agents. Probiotics also play a crucial role in modulating the immune system response, bolstering host defenses and promoting overall health and well-being [20,25].

Scientific research best and most frequently demonstrates beneficial effects related to alleviating and treating bacterial and viral diarrhea, constipation, and intestinal inflammation. Probiotics can also indirectly be used in the prevention of atherosclerosis, which is caused by many civilization diseases and the accumulation of cholesterol in the lumen of blood vessels, which can lead to heart attack, stroke, and even death. Probiotic microorganisms, by producing metabolites, contribute to the esterification of cholesterol already in the mucous membrane of the small intestine, thanks to which its faster deposition is possible. The beneficial effects also include the inhibition of carcinogenesis by reducing pathogens, whose carcinogenic effect is based on the production of fecal pro-carcinogenic enzymes responsible for the growth of cancer cells. Probiotics are also used in prevention, combating compounds recognized as carcinogenic, such as acrylamide, aflatoxins, and nitrosamines [26].

Microorganisms are living organisms that need nutrients for life, growth, and multiplication. Oligosaccharides such as inulin, lactulose, fructooligosaccharides, galactose derivatives, and β-glucans are most often not digested by humans and are used as nutrients for probiotics. This type of nutrient is called a prebiotic. The most commonly used

prebiotics are fructans (inulin, oligofructose). These compounds reach the colon practically undisturbed by digestive enzymes, where they are broken down by saccharolytic bacteria in the fermentation process. Providing proper nutrition for probiotics increases their health benefits. In addition to providing energy and essential components, prebiotics, as undigested food components, show beneficial effects, including reducing the fraction of LDL cholesterol, which accumulates in the lumen of blood vessels; stimulating the immune system; balancing the pH of the intestines to optimal values; aiding in better calcium absorption; and having a low energy value. The introduction of prebiotics into food invites no objections. They remain stable during storage and at low temperatures. Additionally, these substances serve as substitutes—oligofructose acts as a sugar substitute, and inulin can be used as a fat substitute [27].

The combination of probiotics and prebiotics is called a synbiotic. The combination shows a synergistic effect. The synergistic effect can be enhanced by using several strains provided that they do not show antagonistic effects towards each other [20]. The most important characteristics of the complex include the development of desirable microflora in the intestines with simultaneous inhibition of pathogens, a decrease in the amount of toxic and carcinogenic substances, prevention of diarrhea and constipation, and a reduction in the level of LDL cholesterol [25].

Probiotic organisms are primarily found in fermented products such as fermented milk beverages, pickled vegetables and fruits, and acidophilic milk. The prevalence of probiotics in dairy products is due to the optimal living conditions they provide for microorganisms, including anaerobic conditions and low pH levels. Additionally, probiotics are classified as lactic acid fermentation bacteria, which naturally occur in fermented products. The reason is the maintenance of optimal living conditions for microorganisms (anaerobic conditions, low pH) and the fact that probiotics are classified as lactic acid fermentation bacteria, and these bacteria naturally occur in fermented products. Adding nutrients (prebiotics) to maintain viability is also not a problem and is even positively received by the consumer. Probiotics are also added to other products to increase their nutritional value. Such products can be cold cuts (not subjected to a thermal process), using modified milk with lyophilized cultures. Obtaining a food product containing an appropriate amount of a specific strain with good viability poses a challenge for manufacturers. Food is a dynamic substrate. Individual ingredients interact with each other, as well as with microorganisms. Additionally, it is necessary to carefully select the technological process, and then the time and storage conditions, to guarantee the presence of live colonies of bacteria in the final product during consumption [28]. This is a task that is possible to achieve, as evidenced by new probiotic products appearing on the market. In addition to food, selectively selected probiotic strains and whole synbiotic complexes can be found in the form of tablets, capsules, or in other forms as pharmaceutical preparations and dietary supplements. It is important to clearly distinguish functional food and dietary supplements from registered medicines, which show a therapeutic and preventive effect against a specific disease [15,28].

The minimum dose required to achieve a probiotic effect cannot be definitively determined, as it depends on the strain and the form in which it is consumed. The literature does not provide specific numerical data for this. Instead, dosing should be based on studies involving individuals where the lowest concentration for a beneficial probiotic effect was observed. Establishing a single minimum dose is impossible; however, it is suggested that the minimum daily dose to cause a physiological or therapeutic effect should be between 10^8 and 10^{10} CFUs [29] (colony-forming units). Different organizations also recommend varying minimum doses. For example, CODEX Alimentarius suggests a minimum of 10^6 CFUs/g for added microorganisms, excluding those added during the product's production process. The Fermented Milks and Lactic Acid Bacteria Beverages Association in Japan set a minimum dose at 10^7 per g or ml for fermented products. The Canadian Natural Health Products Directorate recommends a minimum dose of 5×10^9 CFUs/day for 5 consecutive days. Lastly, the U.S. Food and Drug Administration advises a minimum dose of 10^6 CFUs, although the desired effect, considering the food storage aspect,

can be achieved with a dose of 10^8–10^9 CFUs. Each strain with a proven probiotic effect must be named according to the International Code of Nomenclature. The name includes the following: the genus name, e.g., Lactobacillus; species name, e.g., acidophilus; and alphanumeric designation, e.g., LC1.

4. Ingredients and Processes in Baking Technology

Bread production involves processing flour through various methods using basic bakery ingredients such as water, yeast, salt, into semi-finished products (dough), and then finished product—bread. Additional auxiliary substances such as chemical leavening agents, sweeteners, fats, milk, eggs, etc., are also permitted [30]. The primary method of leavening bread is the biological method utilizing fermentation, made possible by the activity of yeast and bacteria. In breadmaking technology, flour is a crucial ingredient, obtained by grinding grains of cereals classified as bread grains (e.g., wheat and rye). From a technological standpoint, the most important component of flour is the endosperm, consisting mainly of starch and proteins. The type of flour is determined by specifying the grain used for milling, the ash content expressed in grams per 100 kg of flour (a higher ash content indicating a higher bran and germ content in the flour), and the commercial name. Water, the second most important ingredient after flour, acts as a solvent and enables the swelling of starch, proteins, gums, and mucilage, contributing to the formation of the dough structure. Water quality, particularly the hardness and pH, significantly affects dough development during fermentation processes [31]. Yeast, a fundamental ingredient in breadmaking, belongs to the *Saccharomyces cerevisiae* species and is capable of aerobic respiration and fermentation, leading to the production of carbon dioxide, which leavens the dough. Salt, added for flavor and structural properties, also affects fermentation dynamics, acting as a fermentation regulator [30–32]. Ingredients and processes play a crucial role in determining the quality of bread. Enzymes, which act as natural catalysts, are particularly important. For example, amylases break down starches into sugars, which are then fermented to create the characteristic texture and structure of bread. Proteases help develop gluten, improving dough elasticity and volume [33]. Balancing the activity of these enzymes is key to achieving the desired bread texture and taste. Advances in enzyme technology provide bakers with tailored blends to optimize dough handling and fermentation, ensuring consistent quality in bread production [34].

The dough is formed from the bakery ingredients mentioned above, with gluten proteins playing a crucial role in wheat dough formation by creating a network structure. Rye and mixed doughs have a different structure due to the absence of gluten proteins, leading to the formation of a granular dough structure. Fermentation of rye and mixed dough relies on spontaneous fermentation, with lactobacilli and yeasts playing significant roles. The resulting products exhibit distinct flavors and aromas due to by-products such as acetic acid [32]. Controlling the fermentation time and temperature is crucial for optimizing the flavor, texture, and nutritional properties of bread. This process allows enzymes and microorganisms to metabolize sugars, creating flavor compounds and enhancing the bread's digestibility. By regulating fermentation parameters, such as the duration and temperature, bakers can achieve the desired taste, texture, and nutritional benefits in the final product [35]. The baking process transforms the dough into the final product with the desired sensory and nutritional characteristics. The baking conditions, including temperature and time, vary depending on the bread type and baking method. Two phases of baking are recognized: the first phase, involving rapid volume increase and biochemical changes, and the second phase, focusing on shape and texture stabilization, flavor development, and crust formation [36]. The survivability of probiotic microorganisms, crucial for their functionality, is influenced by various technological parameters during production, processing, and storage. The ability of probiotics to survive stressful conditions enhances their colonization potential in the gastrointestinal tract, ultimately promoting their health benefits [37]. The primary technological goal during production, which must be achieved

to obtain a probiotic, is to ensure its viability. Only in this form can it fulfill its purpose of being added to food, namely exerting a beneficial impact on the host's health [38].

Ensuring the viability of probiotics in the production process is crucial not only for the effectiveness of these products but also from the perspective of sustainable development. Optimal technological conditions that promote the survival of microorganisms help reduce losses and decrease raw material consumption, aligning with the principles of sustainability. This emphasis on efficiency and resource conservation underscores the importance of technological advancements in fostering a more sustainable approach to probiotic production, aligning with broader efforts to promote environmental responsibility and minimize waste.

5. Enhancing Probiotic Viability through Technological Processes

Figure 1 illustrates the factors influencing the viability of probiotics throughout the production process, storage, consumption, and until reaching the large intestine.

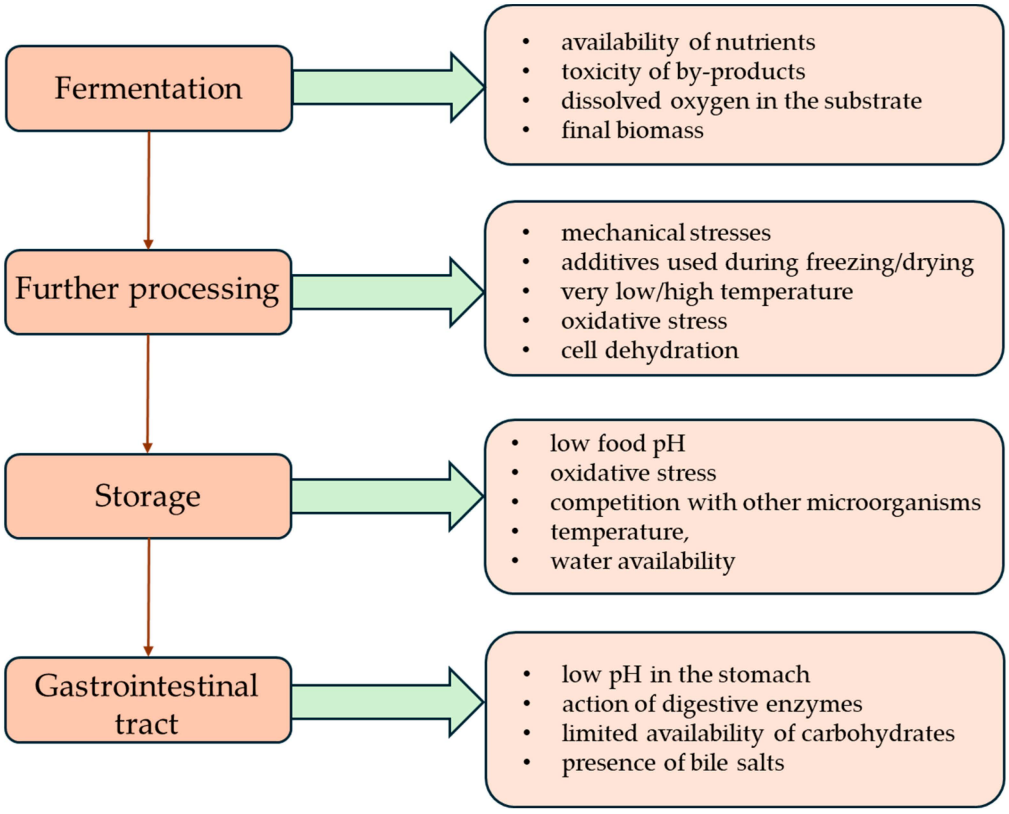

Figure 1. Factors affecting the viability of probiotics during production, storage, consumption, and until arrival in the large intestine [38,39].

To mitigate the loss of probiotic viability during production and storage, several methods can be employed. Carefully selected substrates containing easily assimilable energy sources, optimal pH for the specific strain, and the addition of antioxidants, growth factors (e.g., yeast extract, casein hydrolysates, acetates, vitamins), and biocompatible additives (including flavors, preservatives) can strengthen microbial cultivation.

To enhance the viability of probiotics, several methods are employed. These include the use of appropriately selected substrates to provide an optimal growth environment for probiotic cells. Exposing cells to sublethal stress prior to their application can increase their resistance to adverse conditions. Modification of the technological processes involved in probiotic production is another strategy, allowing for better survival rates during processing and storage. Finally, immobilizing probiotics on or within suitable carriers can offer protection and stability, further improving their viability and functionality [38].

5.1. Cell Exposure to Sublethal Stress

Enhancing viability can be achieved by prior exposure of cells to sublethal stress, i.e., unfavorable factors for growth and development that do not immediately lead to cell death. This procedure aims to adapt probiotics, improving their survival through subsequent production stages, storage, and passage through the gastrointestinal tract.

Examples of factors inducing sublethal stress include incubating cultures under nutrient-deficient conditions, exposing them to sublethal temperatures, subjecting them to the presence of bile salts, adjusting pH levels to sublethal ranges (either low or high), and introducing hydrogen peroxide. Additionally, methods such as immobilization on or within suitable carriers, pre-exposure of cells to sublethal stress, the selection of carefully chosen substrates, and the modification of technological processes can also induce sublethal stress to enhance probiotic viability and functionality [39].

Table 2 presents examples demonstrating enhanced survivability of probiotic strains following exposure to sublethal conditions. Studies also show increased survival rates of lyophilized microorganisms after mild thermal treatment [40] and improved survivability at high [41] and low temperatures [42] following exposure to sublethal shock induced by acidic environments.

Table 2. Examples of improved survivability after exposure to sublethal conditions [17,20,25].

Strain	Goal Achieved	Applied Exposure
Bifidobacterium longum	Increased survivability at 6 °C	Starvation conditions for 30 or 60 min
Lacticaseibacillus paracasei NFBC 338	Production of heat shock proteins. After reheating (60 °C, 30 min), the bacterial count decreased by 0.5 log CFUs/mL, whereas in the control sample, the decrease was 2 log CFUs/mL	Thermal stress at 52 °C for 15 min
Bifidobacterium breve 99	Improved strain tolerance to organic acids	Exposure to UV radiation and incubation in acidic environment
Lactobacillus delbrueckii subsp. *bulgaricus*	Increased cell viability up to 65 °C	Subjecting cells to initial heat treatment (50 °C) or hyperosmotic conditions

5.2. Production Process Modification

Adapting process conditions to microbial requirements is not as straightforward as it may seem. Several parameters contribute to the proper course of the process. Modifications to enhance probiotic viability and functionality may include lowering process temperatures, employing vacuum or packaging in a modified atmosphere (MAP) with nitrogen gas to maintain anaerobic conditions, and adjusting fermentation parameters. This adjustment involves selecting compatible starter cultures, enzymes, and an appropriate dose of microorganisms for inoculation. Combining certain modifications (e.g., lowering temperature and fermentation time) may result in an improper production process, leading to either the absence of the target product or very high production costs [43,44].

5.3. Encapsulation

Traditional probiotic preparations consist of dried microbial biomass. Drying, whether by freeze-drying or spray-drying methods, may reduce the durability of preparations when exposed to atmospheric conditions. The viability of probiotics is greatly influenced by three main factors: temperature, oxygen presence, and relative humidity [45,46]. Probiotics produced using conventional methods may undergo significant strain reduction during passage through the gastrointestinal tract. Therefore, exploring new probiotic production methods is crucial for obtaining a product with high bioavailability, translating into microbial effectiveness [47].

Probiotics can be introduced into food or pharmaceutical preparations along with a carrier. Depending on the location of microorganisms, immobilization (entrapment) on or within the carrier is distinguished. In the case of immobilization on the carrier, which can

be single-use straws or bottle caps, probiotics come into direct contact with food just before consumption [38,48].

The encapsulation of probiotics within a carrier is a promising technology for achieving bioavailable probiotic products, including non-fermented ones, which were previously unattainable due to unfavorable conditions during raw material processing in the technological process or insufficient strain stability [44,49]. Functional food containing probiotic microorganisms is already available on the market, including fruit juices and non-fermented milk, and ongoing research is being conducted to introduce a new assortment with probiotic cultures added, such as chocolates, sausages, grain products, dried products, and vegetables [44].

One of the latest methods to improve probiotic delivery is microencapsulation. Microencapsulation is a technology based on immobilization, during which material in liquid, solid, or gaseous form is coated with another substance with different physicochemical properties. The less durable material is enclosed in a more resistant capsule. Under certain conditions (e.g., temperature, pH, fermentation, etc.), the content is released from the shell. The concept of microencapsulation allows the functional core (in this case, the probiotic) to be separated and protected from the destructive action of the environment by a protective coating. Colonization of the intestinal walls by probiotics in microcapsules compared to those without a shell is more effective because the shell does not dissolve in the acidic stomach environment, allowing the probiotic strain to survive inside the capsule. Only in the alkaline pH of the intestines does the capsule dissolve. Therefore, it can be estimated that at least 90% of microencapsulated microorganisms can survive passage through the stomach and duodenum unchanged [49].

Probiotic microencapsulation is a promising technique applicable across various food matrices, including dairy products, beverages, and functional foods. In dairy products like yogurt and cheese, microencapsulation helps maintain probiotic viability, ensuring their efficacy until consumption [50]. Similarly, in beverages such as juices and smoothies, microencapsulation prolongs probiotic survival, preserving their functionality [51]. Microencapsulated probiotics can be seamlessly integrated into functional foods like cereal bars and snacks, providing convenient and palatable means to deliver probiotic benefits to consumers [51].

There are several microencapsulation methods, including the following:

- Extrusion—a mixture of the encapsulated substance (in this case, probiotic microorganisms) and encapsulating agent (hydrocolloid solution) is pumped under high pressure in droplet form into a hardening substance [52].
- Emulsification—a two-step method involving dispersion and hardening. A watery suspension of the encapsulated substance with the polymer is emulsified in a larger volume of oil (forming an oil-in-water emulsion). The dispersed solution undergoes solubilization to form small spheres in the oil phase. Hardening occurs through slow addition of $CaCl_2$ [53].
- Coacervation—polymer precipitation via phase separation. The method involves salting out the polymer using various mechanisms, e.g., temperature change or pH adjustment and adding salts with greater affinity for the aqueous phase. The process requires two oppositely charged colloids at a specific pH, causing phase separation and encapsulation of solid particles or liquid droplets. The next step is hardening [54].
- Lyophilization—dehydration process. This method involves freezing the encapsulated substance with the shell material, followed by removing the solvent under reduced pressure at a lowered temperature [54].
- Spray drying—the method involves dispersing the core in the shell material, similar to emulsification, and then spraying the mixture into a heated air stream. The hot air evaporates the solvent from the shell, forming microencapsulated products [54].

The vast majority of microcapsules produced using immobilization are made by microencapsulation in gel beads (ME). The technology is based on extrusion or emulsification techniques. Polymers such as alginate, gellan gum, xanthan gum, carrageenan, and bread-

fruit meal are used as gelling materials to form capsules. The capsule can then be coated with various layers to give it specific properties (e.g., increased thermostability) [55]. A more modern alternative method to ME is spray drying. The method allows for the formation of a capsule with simultaneous drying in one step. In the case of extrusion or encapsulation, drying of the capsules occurs only after their formation [56]. Drying the obtained microcapsules extends their storage time. However, elevated temperatures and rapid dehydration may contribute to greater losses of live cells.

Different encapsulation materials and methods affect how well probiotics survive and are released. Polysaccharides, proteins, and lipids are commonly used materials. Polysaccharides protect probiotics from moisture, while proteins offer strength. Lipids, like liposomes, improve stability and controlled release. Methods like spray drying and emulsion also impact probiotic survival and release. Each method has its strengths, cost, and effectiveness, influencing how probiotics are used in foods and supplements [57]. Stability testing and quality control are crucial for evaluating how well microencapsulated probiotic products perform. These assessments help determine if the formulations can maintain the probiotics' viability and functionality over time, especially under different storage conditions. Rigorous stability tests involve subjecting the formulations to controlled environments, monitoring factors like temperature, humidity, and oxygen levels, and periodically assessing probiotic survival. Quality controls include various analytical techniques to ensure consistency, safety, and effectiveness. These methods typically include microbial enumeration tests, particle size analysis, encapsulation efficiency determination, and microbiological safety evaluation. By following strict stability testing and quality control protocols, manufacturers can ensure the delivery of durable and reliable microencapsulated probiotic products to consumers [58].

6. Challenges and Strategies for Incorporating Probiotics into Bread Production

Factors adversely affecting the survival of probiotics during the production, storage, and consumption of bread encompass various challenges [56,57]. These include the harsh conditions encountered during the technological process, notably the mechanical processing during kneading, which can subject the probiotic cultures to shearing forces. Moreover, the baking process, characterized by extremely high temperatures and high humidity, poses a significant threat to the viability of probiotics. Subsequent storage of bread at room temperature further compounds this issue, as it exposes the probiotics to conditions that may not be conducive to their survival. Within the bread itself, a specific microenvironment exists, influenced by the presence of other microorganisms, such as yeast, which can potentially compete with or interfere with the viability of probiotics. Additionally, the variable and often unfavorable conditions within the gastrointestinal tract present another obstacle to the survival of probiotics, further complicating their journey from production to consumption.

Several attempts and studies have been made to produce bread containing probiotic cultures, as detailed in Table 3, which outlines the strain, final product mass, baking conditions, coating, initial and post-encapsulation population counts, microcapsule dimensions, population counts after baking and storage, reductions in log CFUs/g, and relevant references.

Various strategies have been explored to enhance the survival of probiotics in bread production [56,57,59–62]. These include the utilization of lactic acid bacterial spores, such as *Bacillus coagulans* and *Bacillus subtilis*, which offer resilience against adverse conditions. Additionally, applying edible coatings containing probiotics, like *Lactobacillus rhamnosus* GG, directly onto the surface of loaves has been investigated as a means to protect probiotics during baking and storage [61]. Encapsulation techniques have also been employed, such as coating *Lactobacillus acidophilus* with starch on partially baked bread surfaces [14], or using calcium alginate, high-amylose maize resistant starch, and alginate-chitosan coatings for *L. acidophilus* and *L. casei* [62]. Moreover, the use of successive layers of stearic acid, sodium alginate, and cellulose as capsules for *Bifidobacterium animalis* spp. lactis has

shown promise in maintaining probiotic viability [56]. Another approach involves directly adding free *Bifidobacterium lactis* Bb12 bacteria to the dough at the initial stage of bread production, aiming to ensure their survival throughout the baking process and subsequent consumption [57].

These attempts primarily focused on preparing probiotic cells with a protective layer to shield the core from environmental influences. Only Zhang et al., 2014 [57], have attempted to bake bread containing free cultures that participate throughout the production process. Despite these efforts, it has not yet been shown to be possible to introduce bread containing probiotic microorganisms to the market [56].

The imperative for sustainability underscores the drive towards probiotic bread production. Hence, ongoing endeavors are directed towards methodological advancements aimed at harmonizing ecological imperatives with nutritional advancements. Various methods for producing probiotic-enriched bread exist, ranging from using edible coatings [14,56,61,62] to incorporating free bacteria [57]. These methods differ in bacterial strains, coating compositions, encapsulation techniques, and the timing of probiotic addition during production.

Table 3. Attempts to obtain probiotic bread.

Strain	Final Product Mass [g]	Baking Conditions	Coating	Initial Population Count (CFUs/g)	Population Count after Encapsulation (CFUs/g)	Average Microcapsule Dimensions (μm)	Population Count after Baking (CFUs/g)	Reduction (log CFUs/g)	Population Count after Storage (CFUs/g)	Reduction (log CFUs/g)	References
Bifidobacterium animals spp. *lactis* NH019	700	40 min, 180 °C	Stearic Acid—Sodium Alginate—Cellulose Solutions	1.5×10^{11}	1.4×10^{9}	50–300	8.15×10^{7}	1.66	-	-	[56]
L. acidophilus LA-5	54	15 min, 180 °C	Alginate	10^{11}	10^{11}	216.6	1.19×10^{10}	0.66	1.13×10^{10}	0.68	[62]
L. acidophilus LA-5	54	15 min, 180 °C	Alginate + Chitosan	10^{11}	10^{11}	374.4	2.27×10^{10}	0.38	2.21×10^{10}	0.39	[62]
L. acidophilus LA-5	405	25 min, 180 °C	Alginate	10^{11}	10^{11}	216.6	6.48×10^{9}	1.80	5.67×10^{9}	1.85	[62]
L. acidophilus LA-5	405	25 min, 180 °C	Alginate + Chitosan	10^{11}	10^{11}	347.4	6.08×10^{10}	0.82	4.86×10^{10}	0.92	[62]
L. casei 431	54	15 min, 180 °C	Alginate	10^{11}	10^{11}	352.8	1.89×10^{10}	0.46	1.73×10^{10}	0.49	[62]
L. casei 431	54	15 min, 180 °C	Alginate + Chitosan	10^{11}	10^{11}	512.6	4×10^{10}	0.13	3.83×10^{10}	0.15	[62]
L. casei 431	405	25 min, 180 °C	Alginate	10^{11}	10^{11}	352.8	1.66×10^{10}	1.39	1.38×10^{10}	1.47	[62]
L. casei 431	405	25 min, 180 °C	Alginate + Chitosan	10^{11}	10^{11}	512.6	1.09×10^{11}	0.57	9.32×10^{10}	0.64	[62]
L. acidophilus	70	16 min, 180 °C	1 layer: 5% *w*/*v* starch containing 1% *w*/*v* microcapsules	4.83×10^{7}	4.83×10^{7}	77.67	2.40×10^{7}	0.20	1.70×10^{6}	1.45	[14]
L. acidophilus	70	16 min, 180 °C	2 layers: • 5% *w*/*v* starch containing 1% *w*/*v* microcapsules • 5% *w*/*v* starch	4.83×10^{7}	4.83×10^{7}	56.89	3.05×10^{7}	0.30	1.15×10^{6}	1.62	[14]
L. acidophilus	70	16 min, 180 °C	3 layers: • 5% *w*/*v* starch • 2% *w*/*v* microcapsules • 5% *w*/*v* starch	9.66×10^{7}	9.66×10^{7}	66.78	2.75×10^{7}	0.55	1.22×10^{6}	1.90	[14]

7. Conclusions

Despite the ubiquity and availability of bread, there is a growing need to enhance its nutritional value beyond mere sustenance, driving the quest for innovative solutions and technologies. Probiotic bread, containing live microbial cultures with documented health benefits, is one such product characterized in the study. However, free-form microorganisms are susceptible to harsh environmental conditions during production, storage, and exposure to the low pH of the stomach. Hence, ensuring their protection within bakery products is crucial. Encapsulation emerges as a promising method, demonstrating high efficacy in preserving probiotic viability in the final product and through the gastrointestinal tract, where conditions are unfavorable. Encapsulation involves coating microorganisms with a specialized layer, with multilayered alginate-chitosan coatings proving most effective.

Based on available research, the *L. casei* 431 strain enclosed in such coatings within small-sized products with shorter baking times exhibited minimal reduction. Incorporating microcapsules into the dough, as opposed to surface spraying post-baking, resulted in fewer bacterial losses during storage. Encouragingly, encapsulation enables the production of probiotic-enriched products. However, further studies are necessary to select strains least affected by technological processing, storage, and consumption and to assess sensory attributes to meet consumer preferences.

In conclusion, research on probiotic bread production is directed towards a sustainable approach that considers not only nutritional aspects but also ecological and social factors. Implementing new technologies, such as the encapsulation process, enables the production of more sustainable products that not only provide nutritional value but also protect probiotics from degradation in challenging environmental conditions. This approach aligns with the growing consumer demand for products that are not only healthy but also environmentally friendly and socially responsible.

Further research in the field of probiotic bread production should focus on exploring novel encapsulation techniques and combinations of encapsulation materials to enhance probiotic survival and efficacy. Additionally, interdisciplinary collaboration between food scientists, microbiologists, nutritionists, and consumer behavior experts is crucial for fostering innovation and addressing multifaceted challenges in developing probiotic-enriched bakery products, paving the way for future advancements in this area.

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