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Review

Probiotic Functional Yogurt: Challenges and Opportunities

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Abstract: This article aims to explore the challenges and opportunities inherent in producing probiotic functional yogurt through information on the benefits of consuming this food, emphasizing its nutritional characteristics and importance for health. It explains the advantages of regular consumption, including improved digestion, gastrointestinal health, immunity, and increased absorption of minerals. The discussion focuses on the perspectives within the production of functional probiotic yogurt, with emphasis on microbial dynamics, inhibition of undesirable microorganisms, and strategies to improve the quality of this product. It highlights the challenges in maintaining the viability of probiotics during production and storage, addressing factors such as pH, exposure to oxygen, temperature regulation, and interactions between strains and ingredients. Additionally, it highlights the integration of natural compounds in probiotic functional yogurt and their sensory impacts associated with health benefits. The article is a comprehensive overview that emphasizes the critical factors that influence the fermentation process of functional probiotic yogurt and the fundamental role of probiotic viability for consumer health.

Keywords: fermented milk; *Lactobacillus*; *Bifidobacterium*; stability; natural compounds

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1. Introduction

The consumption of fermented milk is related to the image of healthy and nutritious foods, as several biochemical changes occur in the raw material during fermentation, which provides beneficial effects for its consumers [1].

Yogurt is a fermented milk that is characterized as a source of mineral salts, including potassium, zinc, phosphorus, and calcium, the latter of which is essential for the formation of bones and teeth, benefiting the growth of children and reducing the risk of osteoporosis in adults [2,3]. However, other benefits are associated with the regular consumption of yogurt, such as better digestibility of proteins and sugars compared to milk; stimulation of peristaltic movements as a result of the presence of lactic acid, facilitating digestion; combating oral problems; colonization of the gastrointestinal tract by beneficial microorganisms; development and maintenance of the support system; combating of inflammation and stimulation of the immune system; stimulation of the production of hormones and enzymes; ease of absorption of mineral salts; etc. [4].

This food is produced through the fermentation of milk, where the bacteria used in its elaboration are known as starter cultures [5]. During the fermentation process, bacteria produce lactic acid, which acts on the milk protein, giving the product its texture and specific characteristics [6].

Yogurt is understood as the product resulting from the fermentation of pasteurized or sterilized milk, with fermentation carried out using protosymbiotic cultures of *Streptococcus salivarius* subsp. *Thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* [7]. Moreover, yogurt is considered a suitable vehicle for administering probiotic bacteria to consumers, typically incorporating starter bacteria along with probiotic cultures [8,9].

In the case of yogurt, starter culture bacteria do not survive gastrointestinal conditions or lack the ability to colonize the human intestine, making it unlikely that they provide therapeutic benefits [10]. For this reason, probiotic microorganisms are added along with yogurt starter cultures to achieve functional benefits for humans, as they are living microorganisms capable of passing through the gastrointestinal tract and benefiting consumer health [11].

Several species of the *Bifidobacterium* spp. and *Lactobacillus* spp. genera have been receiving attention as probiotics. Furthermore, in recent years, new microorganisms have been identified as probiotics, such as those belonging to the *Bacillus* genus (*Bacillus subtilis* [12] and *Bacillus coagulans* [13]) and *Saccharomyces* (*Saccharomyces boulardii* [14,15]). Among the benefits associated with these microorganisms are their contributions to a quicker recolonization of the intestinal microbiota after antibiotic administration, treatment and prevention of diarrhea, relief of constipation, possible treatment of inflammatory bowel disease, reduction in lactose intolerance in some individuals, reduction in serum cholesterol levels, increased resistance to microbial infections, impact on immune function, and a potential role in cancer prevention [16,17].

In contemporary times, various food components have been added to foods to promote health [18], as is the case for functional probiotic yogurt. A food that, in addition to its basic nutritional composition, has a beneficial effect on one or more physiological functions, such as improving health or reducing the risk of developing diseases, can be considered functional [19]. Therefore, functional foods possess nutrient-rich ingredients but can also be enriched with vitamins, minerals, probiotics, prebiotics, and fibers [20].

In this context, this research aims to explore the challenges and opportunities inherent in producing functional probiotic yogurt through an existing literature review. We seek to understand the microbial dynamics in the presence of probiotic strains and evaluate alternatives to inhibit the growth of unwanted microorganisms. The investigation aims to contribute to understanding the fermentation process of functional probiotic yogurt and identifying innovative strategies to enhance the quality of these products.

Therefore, this study provides an overview of the advances and challenges in the context of functional probiotic yogurt, highlighting its significant contribution to human health. The analysis addresses the interaction between microbial dynamics and the production of this functional food, emphasizing the need for innovative strategies to maintain the viability of probiotic microorganisms. The article underscores the importance of selecting and caring for probiotic strains, controlling fermentation processes, and incorporating natural compounds, demonstrating the influence of each factor on the final product's quality and the associated health benefits.

2. Yogurts and Functional Foods: A Contemporary Perspective

Functional foods can be defined as those in which concentrations of one or more ingredients are modified to enhance their contributions to a healthy diet [6]. They can be natural or processed foods but must contain biologically active compounds that confer proven health benefits [21] when consumed in adequate amounts, including probiotic foods.

Initially, a probiotic was defined as a live microbial food supplement capable of positively affecting the host by improving intestinal microbial balance [22]. Subsequently, this definition was modified by Havenaar and Huis in't Veld (1992), attributing this term to a "single or mixed culture of live microorganisms that promotes improvement in the properties of the native microflora of humans and other animals" [6]. However, a new reformulation of this definition occurred, and probiotics came to be defined as "live microorganisms that, when administered in adequate amounts and consumed regularly, confer a health benefit to the host" [23].

Since then, there has been a growing interest in the food industry to develop and market innovative dairy products containing probiotic microorganisms with potential for human health [24]. Probiotic products are divided into two categories: one comprises food supplements and fermented milk that use probiotic bacteria in their formulations, and the other includes dietary supplements, where probiotics are commercialized in the form of capsules, tablets, and sachets containing lyophilized bacterial cultures [6].

Among the most common forms of probiotic use are dairy products, such as fermented milk and cheeses, as these products promote the growth of these microorganisms during the fermentation phase, besides providing an excellent nutrient density [5,25]. Although each microorganism strain is unique, some aspects must be considered when selecting a probiotic: the technological properties of the probiotic microorganism and the food matrices and production processes, as both can affect the strain's viability in the final product and in the intestine [21].

In this regard, it is important to emphasize that the survival of probiotic microorganisms in the product throughout its shelf life is considered a requirement because it is how the beneficial effects of the strains are verified [4]. The authors of [26] highlighted the importance of maintaining the viability of probiotic strains and the desirable characteristics of fermented foods during processing and storage to ensure the safety and beneficial effects of these products when consumed.

Therefore, it can be said that the preparation of probiotic foods needs to be defined by the presence of viable microorganisms in sufficient numbers to alter the host's intestinal microbiota [6], meaning that, to provide health benefits, probiotic microorganisms must remain viable in the food at an effective dose throughout the manufacturing and shelf life of a product, and the recommended minimum daily intake of probiotics is 6–9 Log CFU/g or mL, or at least 8 Log CFU per serving [23,26,27].

However, the quantity of probiotic strains in foods depends on factors such as ingredients used in manufacturing, starter cultures used in conjunction with probiotic cultures, processing conditions, temperatures used during production, aeration conditions, packaging conditions, storage, and product transportation [4].

Moreover, [28] emphasizes that in a probiotic functional food, the microorganisms used should present the following properties: known source or origin, resistance to pH and bile salts, adhesion and colonization capacity, competitive exclusion of pathogens, immune regulation, safety, stability to food processing conditions, good sensory evaluation, and consumer acceptance and efficacy in human clinical trials.

Similarly, the authors [4,29,30] highlight that when adding probiotic strains to fermented foods, it is important to consider the physiological state of the added microorganisms (logarithmic or stationary growth phase), the microorganism concentration at the time of consumption, the physical storage conditions of the product, the chemical composition of the product where probiotic strains are added, and the possibility of interaction between probiotic strains and starter cultures.

Another aspect to be considered refers to the ingredients used in the formulation of the food matrix, as they can play a protective, neutral, or detrimental role in the stability of probiotic strains [31].

The compatibility of probiotic microorganisms with the different ingredients used has a significant role in their survival [32]. Thus, substances such as glucose, vitamins, minerals, casein, whey protein hydrolysates, yeast extract, and antioxidants can be used in dairy products to increase the multiplication rate of probiotic species such as lactobacilli and bifidobacteria [4,32]. Other compounds of interest for increasing the viability of probiotics in dairy products are prebiotics, which can be defined as compounds that are selectively used by beneficial microorganisms, bringing health benefits [33]. The simultaneous use of probiotics and prebiotics is called synbiotic [34].

Given this context, research on fermented dairy products containing probiotic cultures has been encouraged as it represents an option for the industrial production of functional foods with these ingredients. It also represents a lucrative and expanding market niche and stands as a promising alternative in positively influencing the composition and/or metabolic activity of the human intestinal microbiota.

Probiotic Microorganisms

Probiotic microorganisms consist mainly of strains from the *Lactobacillus* and *Bifidobacterium* genera, but species of *Bacillus*, *Pediococcus* and some yeasts have also been identified for their positive aspects related to health, as they play a significant role in protecting the body against harmful agents and strengthening the immune system [35].

In the fermented food and dairy industry, lactic acid bacteria (LAB), classified as Gram-positive, non-spore-forming, and catalase-negative, have been used due to their fermentative capacity and probiotic potential. For instance, species of the *Lactobacillus* genus are crucial for humans [36].

Lactobacillus species produce lactic acid through the fermentation of carbohydrates and colonize the digestive system and female genital mucosa in humans. The main microorganisms belonging to this group include *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus antri*, *Lactobacillus gastricus*, *Lactobacillus kalixensis*, *Lactobacillus reuteri*, *Lactobacillus ultunensis*, *Lactobacillus gasseri*, *Lactobacillus jensenii*, *Lactobacillus vaginalis*, *Lactobacillus iners*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, and *Lactobacillus rhamnosus* [37,38].

When administered in adequate amounts in the diet, *Lactobacillus* microorganisms provide benefits to the host, such as producing short-chain fatty acids that reduce luminal pH, stimulating the growth of beneficial intestinal bacteria, reducing pathogenic bacteria like *Clostridium difficile*, which is associated with diarrhea, colon cancer, atopic dermatitis, inflammatory bowel diseases, type II diabetes, and nervous system disorders, among other effects [23,39]. Authors such as [28] have highlighted that strains of *Lactobacillus acidophilus* can metabolize cholesterol, which is believed to reduce serum cholesterol levels in humans. Similarly, [40] emphasizes the role of these microorganisms in patients with chronic diseases such as obesity, diabetes, and hypertension.

Used in human diets for a long time, some bacteria in this genus have high resistance to the destructive effects of the digestive system, remaining viable in the intestine and promoting benefits through barrier protection, absorption of toxic substances, immune system stimulation, competition for nutrients, and production of antimicrobial compounds. This reduces the chances of infections, making therapeutic use favorable for correcting the host's microbiota balance [41].

However, there is a growing use of *Bifidobacterium animalis* subsp. *lactis* in conjunction with other bacteria such as *Lactobacillus acidophilus*. *Bifidobacterium* genus microorganisms are Gram-positive rods with varied shapes and sizes, found individually or forming chains, considered lactic acid bacteria and heterofermentative, non-motile, non-spore-forming, and anaerobic, although some can tolerate O_2 [28,42].

Among the most known species are *Bifidobacterium bifidum*, *Bifidobacterium animalis*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium angulatum*, *Bifidobacterium catenulatum*, *Bifidobacterium dentium*, and *Bifidobacterium adolescentis* [28,43], with most species inhabiting the mammalian intestinal tract [42]. The species *Bifidobacterium adolescentis* and *Bifidobacterium longum* are found in adult humans [43].

Bifidobacteria can be associated with immune modulation, improvements in intestinal function, prevention or relief of infectious diarrhea, contribution to the nutrition and metabolism process, and antagonism against pathogens through the secretion of antibacterial factors [44].

Some genera of *Bacillus* (*B. cereus*, *B. clausii*, *B. coagulans*, *B. licheniformis*, *B. polyfermenticus*, *B. pumilus*, and *B. subtilis*), both in vegetative and sporulated forms, have been highlighted due to their probiotic properties, as they present good technological characteristics (resistance to heat, acidity, and humidity), viability during passage through the digestive tract and antimicrobial, anticancer, antioxidant, anti-diabetic, and vitamin production properties. However, the possibility of producing toxic compounds (toxins-emetic or enterotoxin; bipartite exotoxins and biogenic amines) and resistance to antibiotics, as well as the limited study of their use in humans, means that these microorganisms require further studies to ensure their safe use [45,46].

Among yeasts, *Saccharomyces cerevisiae* subsp. *boulardii* stands out due to its impact on bowel micro-flora and on enteric pathogens: it is a unique probiotic known to survive gastric acidity, it is not adversely affected or inhibited by antibiotics, and it does not alter or adversely affect the normal microbiota in the bowel [47].

In this sense, the advantages [47–56] and disadvantages [57–59] of using probiotic microorganisms in functional yogurts stand out, as shown in Figure 1.

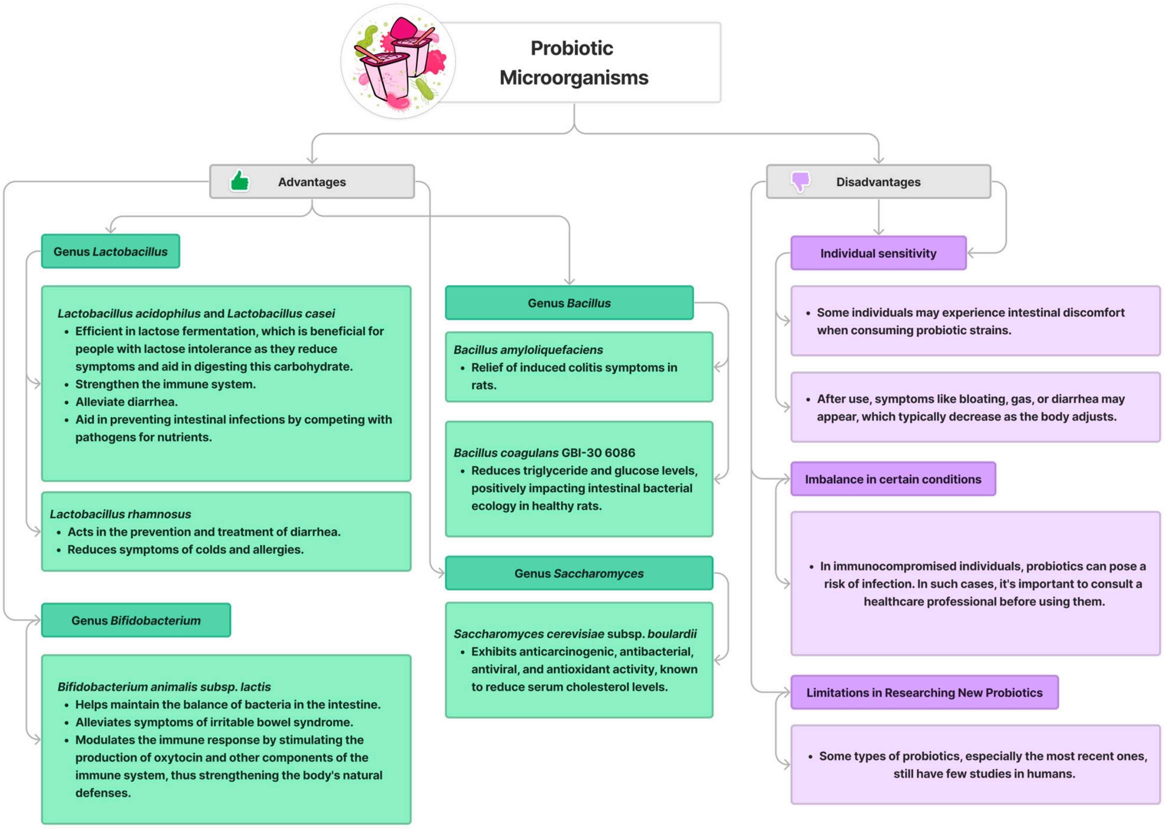

Figure 1. Advantages and disadvantages of probiotic microorganisms.

In summary, it can be inferred that probiotic microorganisms are a promising tool in health promotion; however, their use requires care and more significant investigation regarding their complexity, especially when considering individual factors.

Furthermore, although some characteristics of probiotics are known, there are gaps regarding their use: long-term effects of regular consumption of probiotics and their microbial diversity, especially concerning the combined use of strains; understanding and establishing the ideal dosage and survival capacity of these microorganisms during the manufacturing and storage processes; identification of the risk of developing antibiotic resistance mechanisms by probiotic strains; impact on individuals with autoimmune diseases, allergies, and neurological disorders; innovations for some probiotic species; studies with humans in probiotic dairy product formulations, aiming to maintain the stability of the strains and meet dietary restrictions.

These considerations highlight the importance of continued and more comprehensive research into probiotics, aiming to assess the risks of their use to develop mitigation strategies, improve product development, and explore the unknown potential of these microorganisms.

3. Probiotic Functional Yogurts Enriched with Natural Compounds

In recent years, the intersection of probiotics and functional foods has been growing in the dairy industry, with probiotic functional yogurts representing a significant advancement in this direction. Moreover, it is essential to highlight the incorporation of natural compounds in this food, which not only enhances sensory attributes but also aids in promoting health benefits for consumers.

Functional yogurts enriched with probiotics are a promising category in pursuing healthier dietary choices. However, beyond their benefits, the association with natural additives has become a subject of investigation.

Recent studies [60–63] have emphasized the significance of these additives, distinguishing them from synthetic chemical additives due to their natural sources and food safety.

In this sense, incorporating coffee extracts into functional yogurt deserves attention. Coffee, a globally appreciated beverage with documented health benefits [64], not only presents a distinctive flavor but also possesses antioxidant, anticarcinogenic, and anti-inflammatory properties [65]. Its composition includes caffeine, chlorogenic acids, and melanoidins [66], giving the yogurt matrix a unique flavor profile.

To extend shelf life and protect against undesirable microorganisms, researchers [67,68] advocate using natural preservatives. Adding plant-based antioxidants, such as those from coffee extract, proves effective in controlling free radicals and preserving dairy products. This dual role—flavor enhancement and food preservation—positions them as essential ingredients in functional yogurt formulations.

Researchers [69] studied the antimicrobial potential of arabica coffee (*Coffea arabica*) and robusta coffee (*Coffea canephora*) extracts on the growth of pathogenic microorganisms and lactic acid bacteria. They observed that the extracts did not affect the microbial growth of *Lactobacillus plantarum* and *Lactobacillus rhamnosus* but inhibited the biological activity of the tested pathogenic bacteria (*Salmonella enterica* serovar Typhimurium, *Escherichia coli*, and *Staphylococcus aureus*). They concluded that using coffee extracts could serve as a preservative in functional foods, enabling the growth of probiotics while inhibiting pathogenic microorganisms.

The effect of coffee extract on unwanted microbial growth is technologically intriguing as it can prolong the shelf life of foods complementarily to heat treatment. This not only ensures product safety but also paves the way to explore factors related to inhibiting deteriorating and pathogenic microorganisms in food.

However, other natural compounds are also making their mark in the formulation of probiotic yogurts. The *Spirulina* spp., known for its nutritional properties, joins this repertoire.

Spirulina platensis contains a protein content of 55–70%, in addition to vitamins (B1, B2, B12, E, and provitamin A), minerals (Fe, Mg, Ca, P, Cr, Cu, Na, Zn), pigments (phycocyanin, chlorophylls, and carotenoids), and essential fatty acids (γ-linolenic acid) [70,71]. It is recognized as safe (Generally Recognized as Safe-GRAS) for human consumption and has been added to food products [72,73]. Among *Spirulina's* bioactive properties is its antioxidant capacity attributed to compounds like α-tocopherol and phycocyanin [74]. Furthermore, evidence suggests that β-tocopherols and δ-tocopherols are associated with anti-inflammatory effects [75].

A study [76] emphasized *Spirulina's* antibacterial properties and its potential as a multifunctional bioactive agent. Authors [77] have also reported that *Spirulina* spp. promotes the growth of probiotic bacteria by providing essential compounds such as adenine, hypoxanthine, and free amino acids to these microorganisms.

However, the combinations of probiotics with compounds like cocoa polyphenols, rich in bioactives such as flavonoids, polyphenols (epicatechin, catechins, and theobromine), and alkaloids, also stand out [78–80]. The research by [81] highlighted the advantageous properties of cocoa polyphenols, including their antioxidant, anti-inflammatory, and cardiovascular protective effects. The conclusions of [80] contribute information on the neurological benefits of cocoa compounds, suggesting that these compounds could enhance brain function by improving blood flow to the brain, providing protection against neurodegenerative diseases. This highlights cocoa's potential not only for bodily health but also for cognitive well-being.

The interaction between cocoa polyphenols and the intestinal microbiota is explained by [82]. These authors highlight that cocoa polyphenols can modulate the intestinal environment, promoting the proliferation of beneficial bacteria while inhibiting the growth of harmful strains. Research [80] suggests that incorporating cocoa extract into probiotic yogurt formulations may contribute to improving intestinal health and reducing inflammation. Furthermore, [83] states that cocoa compounds play a protective role, safeguarding the integrity of probiotics in yogurt, protecting them from degradation, and emphasizing improvements in both sensory and health-related aspects of consuming probiotic yogurts infused with cocoa.

In this context, extracts of turmeric (*Curcuma longa*) and saffron (*Crocus sativus*) also warrant attention, not only contributing to flavor diversity but also conferring beneficial health properties.

Turmeric is recognized for its curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin), a group of bioactive compounds [84,85] that possess antioxidant capacity, protecting cells from the harmful effects of free radicals [86]. The integration of turmeric extract into yogurt, as demonstrated in the study by the latter author, enhanced the yogurt's antioxidant capacity, anti-inflammatory activity, and antimicrobial properties.

Regarding saffron, the carotenoids (crocin), aldehydes (safranal), and glycosides (picrocrocin) stand out as bioactive compounds [87–91]. Its anticancer properties were corroborated by [92], who reported that this extract contributes to yogurt stability. The same was observed by [93], affirming that saffron extract contributed to extending the yogurt's shelf life. The antimicrobial efficacy of saffron is further emphasized by [92,94] who identified an increase in the viability of probiotic bacteria in yogurt.

Considering the mentioned natural additives, the advantages and disadvantages of the use of coffee [64–66,68,95,96], cocoa polyphenols [79–82,97,98], turmeric [84–86,99], saffron [87–91,100,101], and *Spirulina* spp. [70,71,74,75,102–105] in foods are established, which can be visualized in Figure 2.

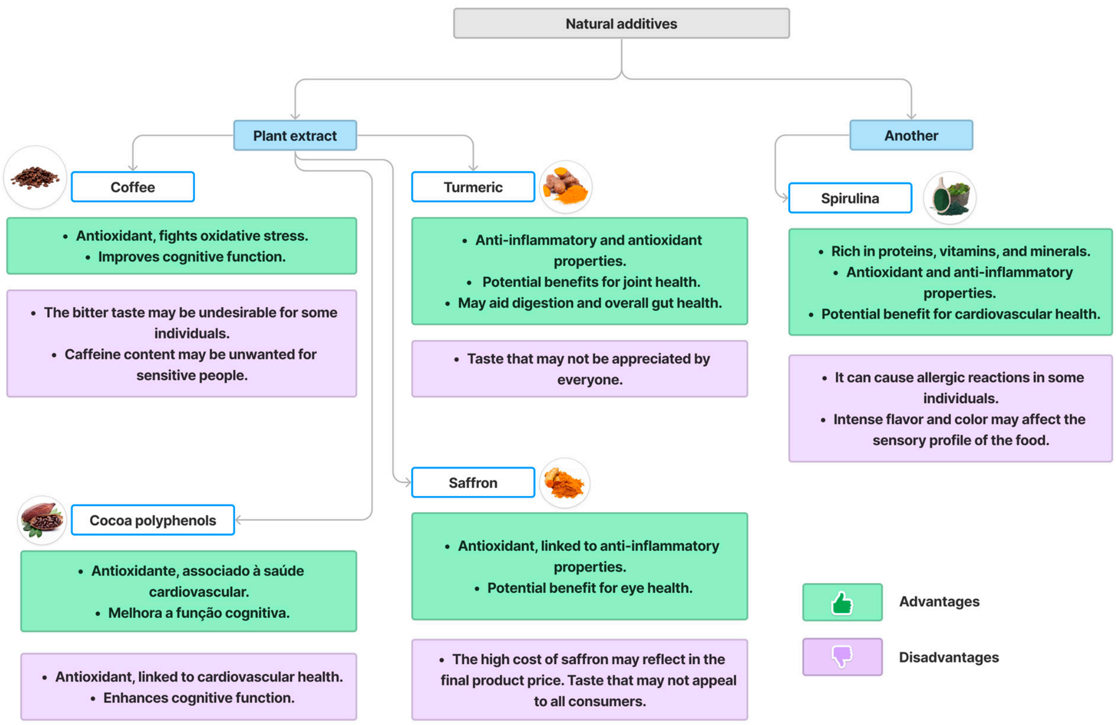

Figure 2. Advantages and disadvantages of natural additives in food.

This analysis indicates that natural additives can offer benefits. Still, manufacturers must evaluate their negative sides before incorporating them into food products because each extract offers advantages; it is crucial to consider consumer preferences and their sensitivity to flavor.

However, natural additives can serve multiple purposes, and their inclusion in foods can improve both organoleptic properties [106–112] and biological activity [113–119]. In this sense, the synergy of these factors in integrating plant extracts in probiotic yogurts stands out, as seen in Figure 3.

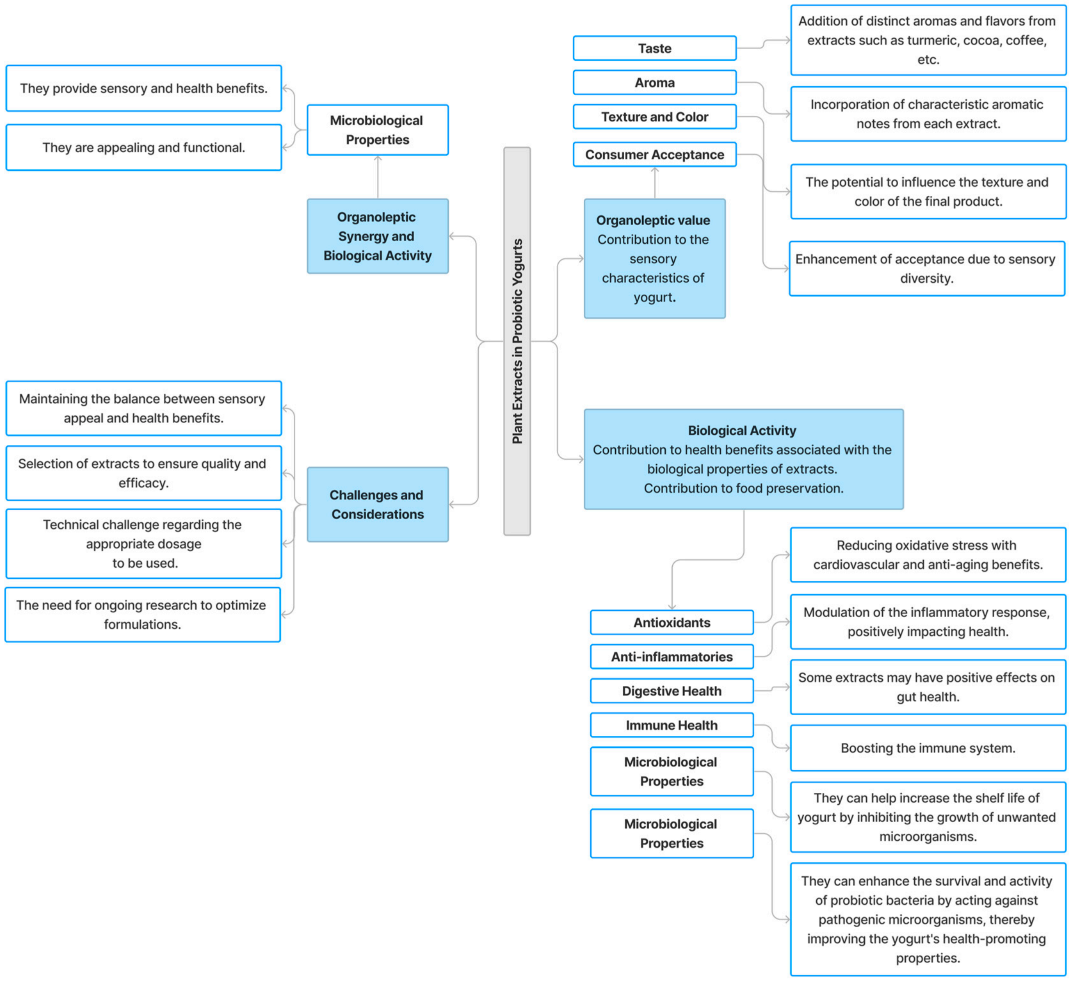

Figure 3. Integration of plant extracts into probiotic yogurts: organoleptic value, biological activity, and challenges.

Given the information presented, it can be inferred that the balance between organoleptic value and biological activity helps in the development of a product that is sensorially attractive to consumers and potentially beneficial to health; at the same time, the challenges inherent to this combination in functional probiotic yogurt are considered. However, the synergy between probiotics and natural compounds in functional yogurts needs further exploration. In this sense, [63] emphasizes the need for continuous innovation to improve methods of obtaining natural additives. This, in turn, can contribute to the functionality of probiotic yogurts and new dairy formulations.

Other studies related to using natural compounds in probiotic yogurt are in Table S1. Studies have indicated various benefits when enriching this food, including aspects related to health and consumer acceptance. In summary, it can be said that using natural additives in probiotic yogurts results in healthier and more attractive products.

Thus, after analyzing the research carried out concerning natural additives in functional probiotic yogurts, we highlight areas of knowledge to be deepened: specific interactions between these compounds and probiotic strains, stability and survival of strains over time, direct impact on human health, determination of ideal doses of the compounds, and exploration of less studied extracts. An improved understanding of these aspects will contribute to optimizing food production.

4. Aspects Related to the Fermentation of Probiotic Functional Yogurt

The production of yogurt begins with heating the milk (to around 85 to 95 °C), which in addition to destroying pathogenic microorganisms and reducing contaminating microorganisms, denatures the serum proteins so that they associate with the casein micelles, collaborating to improve technological and sensorial properties. Pasteurization and UHT processes are the most used in the dairy industry; however, studies have suggested the application of emerging technologies, such as ohmic heating (OH), moderate electric fields (MEF), pulsed electric fields (PEF), ultrasound (US), and high hydrostatic pressures (HHP). Most of these processes are non-thermal and could in addition, reduce fermentation time, guarantee or increase the viability of the probiotic, and improve taste and nutritional-quality properties, in addition to increasing shelf life [120]. Subsequently, the milk is cooled (to around 42 to 45 °C) so that starter and probiotic cultures can be added. This temperature must be maintained until a pH of 4.6 is reached, which may vary between four to seven hours of fermentation [4,6].

Yogurt fermented with probiotics presents unique challenges in ensuring the viability of beneficial bacteria during its production, storage, and consumption. To develop a successful product, critical steps must be carefully managed to maintain the viability and effectiveness of probiotics.

Among the factors that significantly impact the viability of probiotic bacteria in fermented dairy products like yogurt are pH and titratable acidity, exposure to oxygen, temperature control, variability of probiotic strains, and yogurt starter ingredients and cultures [32].

The pH and titratable acidity influence the growth and metabolic characteristics of probiotic bacteria, affecting their performance in yogurt [32]. Lactobacilli thrive in low-pH environments, while bifidobacteria require a higher pH for optimal growth [32].

Oxygen levels during fermentation and storage impact the survival of probiotics, as lactobacilli are microaerophilic while bifidobacteria are sensitive to oxygen [121]. Oxygen barriers have been employed in packaging to maintain probiotic microorganism levels [122].

The temperature during fermentation and storage plays a crucial role in maintaining probiotics, both in terms of optimal growth conditions and in reducing the counts of these microorganisms [123,124].

The variety of probiotic strains must be considered as each exhibits variations in their resistance characteristics to adverse conditions in yogurt, impacting the survival rates of these microorganisms differently [125]. Probiotic products may contain one or several probiotic bacterial strains, mainly *Lactobacillus* and *Bifidobacterium* [126]. Probiotic strains should undergo safety assessments, including genomic analysis, to identify antibiotic resistance genes and enzymatic activities [127].

Therefore, all ingredients used in yogurt formulation, including the fat content, can influence the growth of probiotics [122]. Moreover, interactions between starter and probiotic cultures during fermentation can affect the survival rates of each group of microorganisms [128].

Hence, it can be said that the production process of probiotic functional yogurt presents unique challenges and demands careful attention to a series of factors, aiming to optimize the viability of probiotic microorganisms during production, storage, and consumption. Therefore, to achieve a quality product, the critical steps mentioned must be managed to preserve the effectiveness of probiotics, which should be present in high counts in the final product.

4.1. Technical Challenges Associated with Probiotic Functional Yogurt Fermentation

The fermentation of yogurt with probiotics poses challenges in ensuring the viability of beneficial bacteria throughout the process from fermentation to commercialization.

Fermented milk stands as one of the primary carriers of probiotics in the dairy industry [129]. The combination of probiotics with traditional fermentation cultures has been explored, aiming for improvements in the sensory and physiological characteristics of food products [130]. Studies conducted by [131] showcased the successful use of probiotic strains in milk fermentation, resulting in foods with health benefits. However, the selection of starter cultures, temperature, and fermentation duration are important aspects defining the flavor and texture of yogurt [132].

Thus, probiotic yogurt fermentation faces technical challenges, from the careful selection of strains to the maintenance of stability during production and probiotic counts in the final product. To overcome these challenges, researchers and manufacturers have been exploring innovative methods, such as encapsulation techniques, that aim to protect probiotics during all stages of the production chain [133–135].

In this context, optimizing growth conditions is essential due to the possibilities of non-synergistic interactions between starter cultures and probiotic strains [136,137]. The appropriate choice of probiotic cultures allows for the production of fermented foods with desired sensory attributes of texture, aroma, and taste [138] and should consider safety criteria as well as technological and functional properties [139].

Maintaining probiotics in acidic environments also poses a challenge, but studies have shown promising results in the viability [140]. Furthermore, probiotic supplementation in yogurt can influence texture, aroma, and consumer acceptance, requiring strategies to maintain the desired consistency and flavor [140].

The production of probiotic yogurt is a promising area involving the integration of defined probiotic strains that must be kept viable in the final product and fermentation techniques that optimize sensory and nutritional attributes. However, technical challenges such as probiotic survival and preserving desired yogurt characteristics remain a focus in the dairy industry. With technological and research advances, more efficient production methods and improved probiotic strains can be expected, which can increase the health benefits and sensory characteristics of this food.

4.2. Challenges in Maintaining Viability and Microbiological Quality of Yogurt

Among the challenges in maintaining the viability and microbiological quality of yogurt are deteriorating and pathogenic microorganisms as well as sources of contamination [141–144], as can be seen in Figure 4.

Determining undesirable microorganisms in food is important to ensure the consumption of quality products and is established through legal microbial count limits in each country.

Firstly, it is important to differentiate between deteriorating microorganisms and pathogens since fermented products can become contaminated by them [145].

Most of the time, deteriorating microbial agents are part of the food's natural microbiota and are, therefore, mostly harmless to human health, but they promote sensory alterations in color, taste, odor, and/or texture in the food [4].

Microbial activity causing deterioration involves numerous mechanisms and directly impacts consumer product acceptance, either due to the visible growth of microorganism colonies, texture changes resulting from the degradation of proteins, carbohydrates, and lipids, or the perception of unpleasant odors and flavors [146]. It also leads to economic losses, damage to reputation, and legal penalties in the food industry [145].

Pathogenic microorganisms result from food contamination, mainly due to inadequate hygiene conditions during the production process [4]. This jeopardizes food safety as it represents a health risk for consumers since their ingestion can lead to foodborne illnesses [145,146].

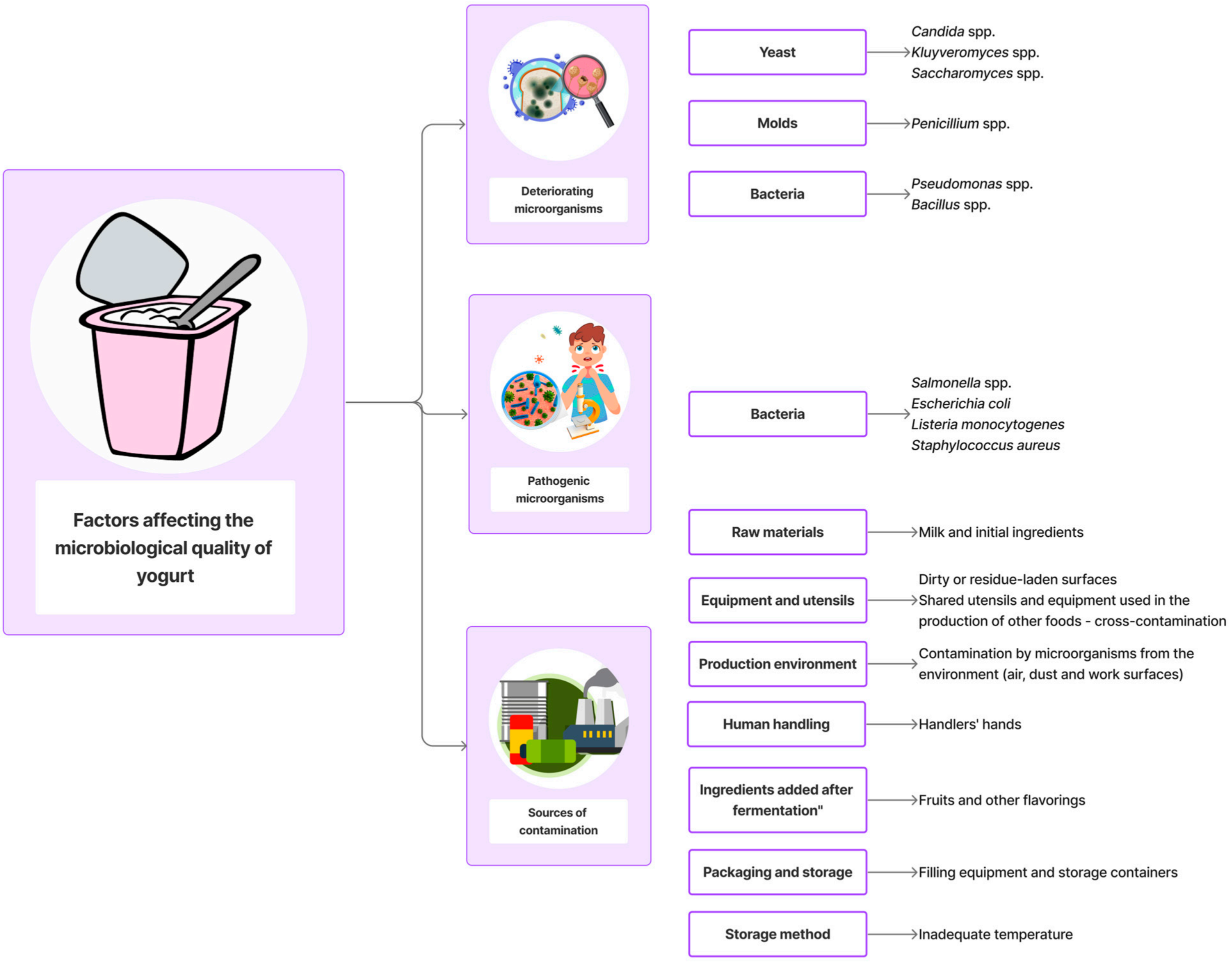

Figure 4. Factors influencing the microbiological quality of yogurt.

Sources of contamination can affect the microbiological quality of yogurt by introducing unwanted microorganisms during the production process, which can cause changes in the organoleptic characteristics of yogurt or even pose a threat to consumer health.

In this context, the main sources of yogurt contamination (Figure 4) include raw materials, equipment and utensils, the processing environment, handling, ingredients, packaging, and storage [143], as well as the method of conservation [144]. Given this context, it can be said that maintaining yogurt quality involves specific challenges that require constant attention: temperature control, contamination risks, storage conditions, preservation of viability, quality of ingredients, supply chain challenges, and awareness and consumer handling.

It is important to emphasize that microbial cultures require specific conditions in all processing phases (production, storage, and transport) of yogurt, and temperature fluctuations can compromise their viability, which impacts the final quality of this food. Contamination risks are also ongoing concerns, as unwanted bacteria can affect the flavor, texture, and safety of yogurt, making it essential to establish strict hygiene protocols during production. Storage conditions, including post-production temperature, are essential to prevent starter and probiotic culture degradation. Preserving bacterial viability during the shelf life of the food is another challenging aspect, as it is influenced by factors such as acidity, additives, and storage conditions.

Last but not the least of the challenges are the quality of ingredients, especially milk, which can impact crop growth and overall product quality; challenges in the supply chain, during transport and distribution, which make it difficult to maintain ideal microbiological conditions; and awareness and proper handling by consumers, which can harm the viability of microbial cultures in cases of inadequate storage. Therefore, maintaining yogurt's viability and microbiological quality involves challenges in controlling and preserving beneficial microbial cultures while mitigating contamination risks.

Given that yogurt serves as an appropriate substrate for the action of deteriorating and/or pathogenic microorganisms [1], the presence of unwanted microorganisms in these foods remains a problem. To reduce these risks, yogurt manufacturers implement strict hygiene practices, microbiological monitoring, quality control, and adequate employee training. Furthermore, regular testing during the production process helps identify and correct problems affecting this food's quality. However, consumers also play an important role by following storage guidelines and consuming products before expiration.

Suggestions to overcome these challenges include continuous investment in research and development, exploration of production techniques, packaging innovations, storage method improvement, and strict quality control measures. Furthermore, using advanced technology to monitor and maintain ideal conditions throughout production and distribution is an effective strategy to mitigate such obstacles. The establishment and adherence to strict quality assurance protocols, following regulatory standards required by each country, also play a decisive role both from the point of view of microbiological quality and the food safety of products such as yogurt.

5. Conclusions

Integrating probiotics into functional foods such as yogurt represents a promising technological alternative. However, ensuring the quality of these products requires the consideration of various critical factors, such as the viability of probiotic microorganisms throughout the food's shelf life, emphasizing the importance of rigorous hygiene measures in the manufacturing processes, storage conditions, and food matrix formulation.

Research highlights that probiotic strains must meet minimum recommended levels to provide the expected health benefits to consumers. However, this condition is affected by several factors, such as the physiological state of adverse microorganisms, food matrix composition, and interactions between probiotic strains and starter cultures.

Moreover, the incorporation of natural compounds such as coffee extract, *Spirulina* spp., cocoa polyphenols, turmeric, and saffron into yogurt formulations has not only improved sensory attributes but also imparted beneficial characteristics to human health. These natural additives possess bioactive properties, including antimicrobial, antioxidant, anti-inflammatory, and even neuroprotective attributes.

Nevertheless, challenges in processing functional probiotic yogurt persist, requiring improvements from strain selection to maintaining stability during production, as probiotics are susceptible to pH variations, exposure to oxygen, temperature fluctuations, and interactions within the yogurt matrix.

Microbiological quality and safety in yogurt are also critical, considering the presence of deteriorating and pathogenic microorganisms, as well as identifying sources of contamination. Strategies to inhibit unwanted microbiota while preserving probiotics are being explored, highlighting the use of natural antimicrobial substances derived from vegetables.

Therefore, the urgency for continuous research to overcome technical challenges, such as probiotic stability and predictions, along with effective microbiological management in production environments, is confirmed. Finally, this study underscores the need for future investigations focused on innovations that drive the development of functional probiotic yogurts, enhancing both their effectiveness and market accessibility with a positive impact on consumer health.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation10010006/s1, Table S1: Effect of different plant extracts on probiotic yogurt [106–119,147–151].

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