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Review

Fruit Juices as Alternative to Dairy Products for Probiotics' Intake

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Abstract: In the context of functional foods and beverages, probiotic fruit juices offer a unique opportunity to combine probiotics' health benefits with fruit juices' nutritious properties. Since most probiotic food products are dairy products, fruit juices could be a viable alternative to dairy foods. Moreover, the variety of tastes with which these juices can be produced can expand the market for these products to consumers of all ages. Important challenges for the probiotic juice manufacturing industry are stability and sensory properties, which can be altered by probiotics or their metabolites. Any functional food must be healthy but still acceptable to consumers' tastes. To evaluate the safety of these products, and improve the efficacy of probiotics in fruit juices, innovative technologies have been proposed, such as microencapsulation and the contemporary use of antioxidants.

Keywords: human health; functional foods; probiotic food products; microorganisms

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1. Introduction

In the last two decades, the prevalence of people who developed chronic diseases has drastically increased [1], especially in high-income countries [2], and similar trends are emerging in low- and middle-income countries [3]. The role of diet in promoting health and preventing chronic diseases has been proven by scientific evidence consolidated over the years. Numerous studies demonstrate how specific foods, nutrients and natural bioactive compounds can significantly influence the physiological processes underlying the pathogenesis of diseases [4]. Advances in nutritional science and food technology have highlighted the effects of functional foods, nutraceuticals, dietary components and supplements for chronic diseases. Functional foods, appreciated by large segments of the population, demonstrate great health and commercial interest; they lead to a significant improvement in the state of health and well-being or a reduction in the risk of disease. Currently, consumers are showing a growing awareness of health and interest in the benefits of food, incentivizing producers to promote functional foods. The concept of functional foods originated in Japan in the 1980s. Generally, functional foods are foods, whether natural or processed, that, when included regularly in the diet and consumed at appropriate dosages, can offer health benefits beyond those associated with basic nutrition alone. It is important to emphasize that to support such claims, it is essential to conduct rigorous, randomized, double-blind, placebo-controlled clinical trials to demonstrate actual functionality. Functional foods, in addition to their basic nutritional value, offer specific health benefits by the presence of bioactive components, such as antioxidants, fiber, phytonutrients, with scientifically proven positive effects. In this way, they can support the maintenance of health and reduce the risk of developing diseases such as dyslipidemia, cancer, type 2 diabetes, stroke and cardiovascular disease. Functional foods must remain foods and must demonstrate their effects in the quantities normally expected to be consumed in the diet. In fact, in order to consider food as functional, its efficacy must be proven by clinical intervention studies

that meet the regulatory requirements of the relevant country. For example, in Brazil, it is the National Health Surveillance Agency (ANVISA) that regulates its approval, in the European Union, the European Food Safety Authority (EFSA), and in the United States, the Food and Drug Administration (FDA) [5]. Several strategies can be used for their production. The development of functional foods plays a crucial role for food companies. This process involves creating, optimizing and testing various formulations and implementing specific processing techniques to be applied to products before they are distributed on the market. Generally, functional foods can be created in two ways: by using technologies that generate or improve the bioavailability of functional ingredients during production, or by adding ingredients that enhance the efficacy of those already present [6]. In this context, probiotics, live microorganisms that are added to foods to improve their nutritional aspect, are widely used. The production of new functional foods containing probiotics is rapidly growing due to their role in the gastrointestinal system [7]. Probiotics have been defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [8]. A microorganism is considered a probiotic if it meets certain criteria, such as resisting acidity and bile salts in the human body. It must also be well absorbed in the intestine. Also, it must demonstrate a clear link to health indicators in clinical studies [9]. For this reason, the viability of the product is considered an important requirement for obtaining health effects. Food products on the market already contain probiotics, such as fermented dairy products [10]. The suggested minimum levels of viable cells in dairy products to achieve benefits are 10^5 CFU/g, 10^6 CFU/g and 10^7 CFU/g [11]. The fermentation process plays a crucial role in the survival of probiotic bacteria, protecting them from adverse intestinal conditions such as acid pH and bile salts. In general, fermented dairy products have higher concentrations of lactic acid bacteria; in some cases, up to 10^9 cells per milliliter or gram [12]. However, the increasing demand for non-dairy products by consumers, either due to widespread problems of allergies and intolerances [13], or due to choices to adhere to specific dietary regimes, such as veganism [14], has led food research to find new matrices as delivery systems for probiotics [15].

The goal over the years has been to satisfy all the needs of a wide range of consumers. Hence, this has led to the idea of developing probiotic fruit juices, owing to the multiple benefits they bring to the great variety of fruits present in nature since they are widely consumed by the entire population. Today, the goal is to create probiotic fruit juices appreciated by consumers, with added nutritional value, without influencing the sensory properties. The sensorial properties of these juices and the viability of the bacterial strains currently represent the main challenges in the production of these innovative functional foods [16].

2. Probiotics

Probiotics are live microorganisms that, if added in the right quantities (greater than 10^6–10^7 CFU/mL or g of product), bring various benefits to the body [8]. Probiotics are identified by the specific strain, which includes genus, species, possible subspecies and a unique alphanumeric code. The main genera used in probiotic products are *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Streptococcus*, *Enterococcus*, *Escherichia* and *Bacillus* [17]. Probiotics act mainly in the gastrointestinal tract. Their beneficial effects on human health result from different mechanisms, which may be generic, species-specific or strain-specific. Generic mechanisms include inhibition of the proliferation of pathogenic microorganisms, regulation of intestinal transit, restoration of altered microbiota and production of bioactive substances such as short-chain fatty acids, which also contribute to pH reduction in the colon. Species-specific mechanisms include vitamin synthesis, strengthening of the intestinal barrier, management of bile salts and neutralization of toxins. Less common strain-related mechanisms include cytokine production, immunomodulation and influence on the endocrine and nervous systems. Owing to this wide range of mechanisms of action, probiotics can play a crucial role in supporting health and preventing numerous diseases. However, as not all bacterial strains have the same effects on the human body, the amount

needed varies depending on the strain and the expected beneficial effect [18]. To date, research is focusing on identifying new probiotic species [19]. The probiotic products on the market nowadays are mainly foods, supplements and pharmaceuticals. In recent years, the study of the effect of probiotics on foods has intensified. Sometimes, probiotics associated with food matrices have the potential to resist conditions of the gastrointestinal tract and are therefore more effective than those of other products [20].

2.1. Role of Probiotics for Human Health

Probiotics have the potential to play multiple roles in human health, improving microbiota homeostasis and maintaining human gut health (Figure 1). Several studies have shown that this function of probiotics can be exploited to prevent or treat several diseases associated with the human intestinal microbiome due to their close association with intestinal microbiota dysbiosis. Probiotics are also useful in the treatment of different types of diarrhea caused by the use of antibiotics or inflammatory bowel disease (IBD), and even in the prevention of colon cancer [21,22]. At the level of the gastrointestinal tract, they also play a role in metabolism, improving the digestibility of lactose [23], reducing cholesterol [24], and reducing urinary excretion of oxalate, which is important in cases of nephrolithiasis [25]. Probiotics have been reported to reduce risk factors for infection, for example, in cases of *Helicobacter pylori*, urinary tract and respiratory tract infections [26,27]. In the study of Sundararaman et al., probiotics have been highlighted as a potential adjunctive therapy in patients with COVID-19 due to their proven immune system regulatory function [28]. For the same reason, probiotics have found application in reducing the symptoms of allergic diseases such as eczema, atopic dermatitis, allergic rhinitis and asthma [29–31]. Consequently, research is investigating if probiotics can be used for autoimmune diseases [32]. Finally, probiotics have also proven useful for the prevention of osteoporosis and oral health, such as the treatment of dental caries [33,34]. Research today is also focusing on other potential health benefits of probiotics, including those on brain function, such as reducing perceptions of stress and anxiety and treating symptoms of depression and autism [35,36]. However, all the health benefits potentially attributable to probiotics are strain-specific; so, even strains of the same species will not be effective in all areas.

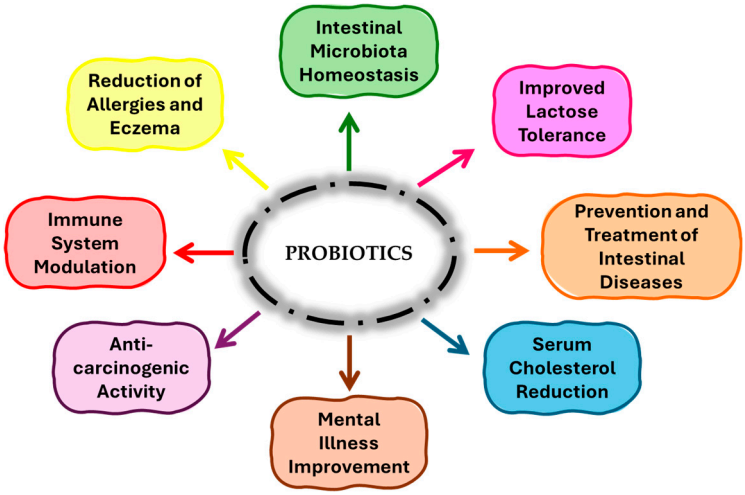

Figure 1. Main functions of probiotics in human health.

2.2. Safety Claims of Probiotics

In recent years, some studies have identified various risks in the improper use of certain probiotic strains [37], but other clinical research has confirmed the safety of probiotics due to the proven absence of toxicity in different populations [38]. Probiotics, according to the definition, are non-pathogenic and should not cause or aggravate disease in humans, regardless of the source of intake [8]. However, the efficacy of probiotics varies between

individuals; so, it is important to consider not only the probiotic strain and dosage but also the consumer's medical history, nutritional status, presence of specific diseases or concomitant use of drugs [37]. In fact, it has been recognized that the gut microbiota has a significant impact on the bioavailability of drugs and their effects [39]. Consequently, taking probiotics could interfere with the absorption of drugs and their effect, which is especially dangerous for drugs that have a narrow therapeutic index [40]. Although probiotics are generally considered safe and beneficial for healthy individuals, they may pose a risk for certain categories of individuals, such as hospitalized or immunocompromised patients [41]. The efficacy and benefits of probiotics depend on several factors and there is no one-size-fits-all approach to clinical evaluation and intake recommendations. Despite the scientific evidence in favor of probiotics, consumers constitute a heterogeneous group; so, a generalized assessment of their safety cannot be made. Cases of serious or fatal infections have been reported in premature infants administered probiotics, prompting the US Food and Drug Administration (FDA) to warn healthcare professionals of this potential danger [42]. Possible adverse effects associated with the use of probiotics include infections, the production of harmful compounds by probiotic microorganisms, and the transfer of antibiotic resistance genes to other bacteria in the digestive tract [43]. In addition, some probiotic products have been found to contain microorganisms that are not declared on the label, with potential health risks related to contamination [44]. Therefore, an individual clinical assessment before using probiotics is recommended, as they are generally considered safe for a healthy population but may pose a potential threat to at-risk populations [37].

3. Probiotic Food Products

Functional foods, enriched by the presence of "biologically active ingredients" such as probiotics, have been gaining popularity in recent years, motivated by the potential human health benefits associated with probiotics. Probiotic food products represent approximately 60–70% of the total functional food market [45]. The most common probiotic bacteria used by the food industry are *Lactobacillus* spp. and *Bifidobacterium* spp. because they are accepted as "Generally Recognized As Safe" (GRAS) by several regulatory agencies [46]. Several probiotic properties have also been identified for certain yeasts, such as *Saccharomyces cerevisiae* and *S. Boulardii* [47]. Furthermore, a variety of food matrices can be used as probiotic delivery systems [15].

Most commercially available probiotic foods are dairy products [48]. The main probiotic strains contained in these are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Several studies in the literature demonstrate the protective effect of dairy products at the digestive level on probiotics, in particular, on milk proteins, sources of bioactive peptide precursors, also owing to their pH, buffering capacity and fat content [48,49]. Milk fats and proteins provide a physical shield for probiotics, protecting them from digestive enzymes and improving their resistance during intestinal transit [50]. Milk proteins are occasionally used as a delivery system for probiotic encapsulation techniques [51]. In the context of dairy products, cheese is considered an excellent carrier for probiotics due to its higher pH and high fat content, which protect probiotic cells during transit through the gastrointestinal tract [50]. The pH is one of the main factors influencing the viability of probiotics. Fermented milk products, particularly yoghurt products, are the most popular probiotic functional products. Fermented food products are created through the metabolic activity of various live microbial cultures, and many of these products are rich in potentially beneficial microorganisms These products contain nutrients that support the growth and viability of probiotics by providing sugars, amino acids and peptides as energy sources [52]. These nutrients help keep probiotics active during storage and gastrointestinal transit. In fermented products such as yoghurt and kefir, probiotics coexist with starter lactic bacteria that produce organic acids and antimicrobial substances. These compounds inhibit pathogenic and competitor bacteria, providing a suitable environment for probiotics. In general, the probiotics in these products can survive for the entire shelf life of the product [53]. However, they do not always withstand the acidic conditions of the stomach and can be degraded

by enzymes and bile salts in the small intestine, which prevents them from reaching the distal intestine.

3.1. Non-Dairy Probiotics Food Products

Due to consumer demands, food industries have focused not only on the production of dairy probiotic foods, but also on different functional foods with different food matrices, but with health benefits, such as non-dairy probiotic foods [54,55]. These needs may derive from personal choices, health reasons (cardiovascular disease and obesity), or lactose intolerance [13,14]. *Lactobacillus, Streptococcus, Propionibacterium, Enterococcus, Pediococcus* and *Saccharomyces* can be used as sources of probiotic microorganisms for non-dairy probiotic foods [56]. Therefore, over the years, various food matrices have been used as probiotic carriers [57]. Examples of commercially available non-dairy probiotic foods are kimchi (traditional Korean cabbage dish), kombucha (fermented tea), sauerkraut, miso (fermented soybean paste), pickles and unfiltered apple cider vinegar [58,59]. These contain live ferments but do not always have probiotic strains with proven benefits. Other non-fermented products, such as juices, smoothies, cereals, nutritional bars and baby formulas, may contain added microorganisms. To be considered probiotic, these foods must contain an adequate amount of live microorganisms at the time of consumption, capable of surviving passage through the digestive system and providing health benefits from specific probiotic strains and species [60]. Cereals and their components can be used effectively to supplement probiotics in the human diet. β-glucan, a fiber present in large quantities in barley and oats, promotes the growth of probiotic microbes such as *Bifidobacteria* in the gastrointestinal tract [61]. Chocolate can be an effective vehicle for probiotic bacteria, since the lipids in cocoa butter protect these bacteria during digestion in the upper gastrointestinal tract and storage of the product [62]. This decision also stems from the food industry's desire to improve the nutritional value of non-dairy foods, especially cereals and fruit and vegetable foods [63]. Studies on beet products enriched with *L. plantarum* revealed 8–9 logs of probiotic cells per 100 g, maintaining over 7 log CFU/g viability after 21 days at 4 °C. These results indicate that beetroot is a suitable food matrix for the survival of probiotics [64]. Meat can be used as a carrier for probiotic bacteria. It was demonstrated that the addition of probiotics in meat products could prevent and inhibit their spoilage and the growth of pathogens [65]. In a recent review by Karbowiak et al. [66], it was shown that the incorporation of probiotic cultures and their bioactive metabolites in meat foods is a promising strategy for inhibiting their deterioration. Abdallah et al. reported that inoculation of *Lactobacillus rhamnosus* and *Bifidobacterium bifidum* strains into meat can extend its shelf life during refrigerated storage [67].

3.2. Fruit-Based Drinks with Probiotics

Among various food products that can be functionalized, fruit juices deserve more attention. Fruit juices are already considered functional foods since they contain several bioactive compounds that are good for health, such as antioxidants, fiber and vitamins [68]. They are widely consumed by people of all ages because they can be produced with different fruits and flavors capable of satisfying the tastes of the world's population. Furthermore, they do not contain allergens or cholesterol, unlike dairy products. In recent years, an increasing number of consumers are opting for non-dairy food alternatives for a variety of reasons, such as food intolerances, dietary choices and personal preferences. Fruit juices can therefore be a viable alternative for supplementing probiotic cultures for these consumers. One of the main reasons is lactose intolerance or milk protein allergies, which limit the dietary options of those concerned [13]. In addition, many people today adopt vegan or vegetarian diets for ethical, environmental or health reasons [14]. These regimes exclude animal products, including dairy products. Probiotic juices fit well with these food preferences, providing the benefits of probiotics without clashing with the fundamentals of these diets. Furthermore, those who prioritize a healthy lifestyle and aim to reduce their intake of saturated fats and cholesterol would find probiotic juices a light and healthy

option. In addition, probiotic juices also contain fruit-specific nutrients that contribute to a balanced diet and general well-being. Continued innovation in the industry promises to make these products more effective and accessible, meeting the growing demand for healthy and inclusive dietary alternatives.

Several studies in the literature demonstrate how the bioactive substances present in fruit juices can protect probiotics and increase their vitality, and therefore bring benefits to human health [69]. It has been shown, how probiotics can utilize the sugars in fruit for their metabolism and maintain their viability [70]. In the work of Kardooni et al., it is demonstrated how vitamin C in orange juice can help to increase the viability of *L. acidophilus* cultures [71]. The ability of the probiotics to adhere to the fibers in the fruit is another way of protecting them from damage caused by the surrounding environment [72]. Moreover, the transit of juices in the gastrointestinal tract is relatively fast, which reduces the exposure time of probiotics to hostile conditions in the gastrointestinal tract, such as stomach acidity [73].

4. Probiotic Fruit Juice Production

The production steps of probiotic juices are very similar to those of traditional juices, with minimal differences in the timing of the process (Figure 2). However, the preparation of juice functionalized with probiotics requires a heat treatment in order to inactivate the microbiota and enzymes of the fruits used. Since probiotics are sensitive to heat, they are added to juices after heat treatment [74]. Heat treatments are classified into three types according to temperature: pasteurization (<100 °C), canning (ca. 100 °C) and sterilization (>100 °C). Pasteurization is the most common method for producing juices and beverages [75]. Probiotic fruit juice has the potential to provide real health benefits only if the number of viable and active cells is at least 10^6–10^7 CFU/mL of product [8]. Probiotic cells can be added into a food product either as fresh, dried or frozen-concentrated cultures [76]. The production of food probiotics involves several crucial steps to ensure the quality and efficacy of the final products [77]. Initially, a specific bacterial strain is selected based on its probiotic properties and its ability to survive in the gastric environment and colonize the gut. Subsequently, the selected strains are cultivated under controlled conditions, e.g., in fermenters, using suitable nutrient substrates such as milk, whey or glucose solutions. During the fermentation phase, the probiotic bacteria metabolize the nutrients in the substrate, producing beneficial compounds such as organic acids and bioactive metabolites. Once the fermentation phase is complete, the probiotics undergo concentration and purification processes to obtain an optimal concentration of live cells [78]. These processes may include centrifugation, filtration and freeze-drying, which allow for a stable and easily storable form of probiotics. Finally, concentrated probiotics are incorporated into food products such as yoghurt, fermented beverages, capsules or tablets, ensuring effective delivery into the gastrointestinal tract and maximizing gut health benefits. The production of food probiotics is then subject to strict food safety regulations and standards to ensure the quality, safety and efficacy of products for human consumption.

The effectiveness of probiotics depends on their stability in foods and their ability to remain in high concentrations in the product during production, storage and after ingestion. To keep this value stable, it is necessary to evaluate all those factors that could compromise the vitality of the bacterial cells during these phases, such as the strain of the microorganism, the composition of the fruit chosen for the juice, the pH and the concentration of oxygen [79]. In addition, juices usually contain antimicrobial compounds, dyes and artificial flavors, which contribute to the loss of viability of probiotic cultures. Therefore, the production of probiotic juice could be difficult for industries because there is a high risk of placing an ineffective product on the market. It is therefore necessary to consider the various production and storage phases. It is also important that probiotic cells remain alive even after ingestion and adhere to the mucosal surface to perform their function [80]. Temperature and storage time are two important factors that can influence bacterial viability. Probiotic juices should be stored at a temperature between 5 and 10 °C [81]. In the study

conducted by Nematollahi et al., the number of viable cells of *L. rhamnosus* was higher during cold storage at 4 °C for 4 weeks in grape probiotic drink [82]. Similar results were shown in the study by Pimentel et al., in which cells of *L. paracasei* were incorporated into apple juice [83]. Furthermore, to avoid the entry of oxygen, the packaging material must be carefully selected. Oxygen produces metabolites that subject cells to oxidative stress and death [84]. A recent study reported that high-barrier plastic packaging materials are a better option for increasing the shelf life of probiotic food products [85].

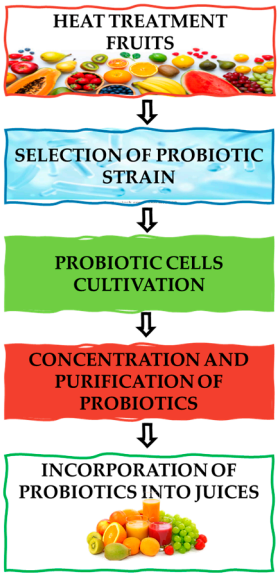

Figure 2. Main production steps of probiotic fruit juices.

4.1. Market of Probiotic Fruit Juice

The probiotic juice market has expanded rapidly in recent years in response to the growing demand for functional and beneficial alternatives for gut health. Numerous food companies in different parts of the world have introduced probiotic-enriched juices in the global market, offering a wide range of options for consumers, although it is not yet developed at the European level [74]. A prominent product is the "GoodBelly" brand, which offers a line of probiotic fruit juices in different flavors, such as mango, orange and berries. GoodBelly brand probiotic formulations are produced by NextFoods, a company based in the United States. GoodBelly is a well-known brand for probiotic drinks and supplements containing Lactobacillus plantarum 299v (LP299V), a probiotic strain that is particularly effective for gut health. The range offers probiotic drinks, including concentrated juices and shots, which can be found in many grocery stores and supermarkets. These products are formulated with clinically proven probiotic strains aimed at improving digestive health and strengthening the immune system. Another example is the "Kombucha Town" brand, known for producing kombucha, a fermented drink rich in probiotics and benefits for gut health. These probiotic formulations are also produced in the United States, more precisely in Bellingham, Washington. In addition to its traditional line of kombucha, the company has launched a series of probiotic fruit juices, combining the benefits of fermentation with the fresh, natural taste of fruit juices. KeVita brand probiotic formulations are manufactured in the United States, primarily in facilities located in California. KeVita is a brand known for its fermented probiotic beverages, such as kombucha and coconut water and vinegar probiotics. The company was founded in 2009 and acquired by PepsiCo in 2016, which expanded its distribution and manufacturing capabilities while maintaining its focus on natural ingredients and traditional fermentation processes. Another American brand is "KeVita", whose probiotic formulations are primarily produced in facilities located in California. KeVita is a brand known for its fermented probiotic beverages, such as kombucha and probiotic coconut water and vinegar. The company was founded in 2009

and acquired by PepsiCo in 2016, which expanded its distribution and manufacturing capabilities while maintaining its focus on natural ingredients and traditional fermentation processes. The "KeVita" brand offers a wide range of probiotic juices in different flavors, such as lemon, ginger, pomegranate, and apple. The widespread presence of KeVita products in supermarkets and health food stores has helped to increase the accessibility of probiotic juices, especially in the United States. In Europe, the "Biotiful Dairy" brand is the best-selling brand in the UK, while in Sweden "ProViva" is popular [86]. This shows that the global probiotic juice market is experiencing dynamic growth, with a wide range of brands and products and an increasingly wide geographical distribution.

The development of functional foods with probiotic activity is a key research area for the future food market. Statistical forecasts have shown that the global probiotic food market value has increased from USD 3.3 billion to USD 7 billion from 2015 to 2025, and is expected to reach over USD 85 billion by 2027 [87,88]. In the European context, the probiotic fruit juice market is part of the broader functional beverage industry. The European probiotic market is estimated at USD 13.78 billion in 2024, and it is expected to reach USD 16.36 billion by 2029, with a compound annual growth rate of 3.49% between 2024 and 2029, with a strong dominance of major players such as Yakult, Danone, PepsiCo, Morinaga, and Nestlé. Dairy dominates probiotic foods, such as fermented milk drinks, probiotic yoghurts, and probiotic yoghurt drinks, but new launches by food and beverage players are fueling the market growth [89]. Min et al. estimated in 2020 that their sale would represent about $5.5 billion [90,91]. The food and beverage segment held the highest revenue share of 73.18% in 2021 and it is expected to register a growth of over 8.0% during the forecast period. Consumers prefer the intake of probiotic ingredients in various types of food and beverages as taste is a primary concern for them. Rising disposable income coupled with growing awareness of healthy lifestyles is likely to promote the adoption of probiotic ingredients in various food and beverage products [92].

4.2. Fruit Selection for Probiotic Fruit Juice

The great variety of fruit is one of the aspects that make a juice very suitable for consumers of all ages since it can have different flavors. However, to design a fruit juice that contains probiotics, the type of fruit must also be chosen correctly, since this can vary all the parameters involved in the survival of probiotics, such as pH. In particular, the reduction in pH during the storage of probiotic juice is fundamental for maintaining the quality of probiotic juices [16]. Furthermore, fruits may naturally contain antimicrobial components that impair the viability of microbial cells [93]. Fruit juices generally have a pH range of 2.5 to 3.7. It is therefore essential to select microbial strains capable of maintaining their vitality during the juice storage period in order to guarantee effective benefits for the consumer. These low pH values also increase the concentration of the non-dissociated form of organic acids, which may contribute to a synergistic action between the acidic conditions and the intrinsic antimicrobial effect of the acids, resulting in reduced survival of probiotic cultures in the environment [94].

Therefore, not all types of fruit are suitable for the production of functional probiotic drinks. Orange juice has shown the most promising results as a medium for the addition of probiotics because it already contains vitamin C, which contributes to oxygen reduction, increasing the survival of probiotic cultures [71]. For the same reason, pineapple juice also showed positive results on the growth and viability of probiotic LAB (Lactic Acid Bacteria) [95]. Apple juice proved to be an excellent substrate for the production of functional probiotic beverages [96]. Furthermore, the fibers contained in these fruits can act as a substrate for the probiotics to protect them from the harsh conditions of the gastrointestinal tract after ingestion of the juices [97]. Pomegranate juice did not show excellent results due to its acidity, with different effects based on the probiotic strains examined [98]. Cornelian cherry juice was very harmful to probiotic strains; in fact, even the most resistant strains were completely reduced after about 7 days of storage in the refrigerator [82]. Cranberry juice also reduced probiotic viability due to its low pH value and high concentration of

benzoic acid, usually used for preserving perishable foods [99]. Tropical mango proved to be a good matrix for *L. rhamnosus* and *L. plantarum*. In the research of Furtado et al., the pH changed positively over time, increasing the resistance of microorganisms to the in vitro test of the gastrointestinal tract [100]. In the study conducted by Monteiro et al., the combination of probiotic properties with bioactive compounds in passion fruit pulp improved the overall quality of the juice [101]. The chemical composition of the juice, then, is very important to ensure the viability of the bacterial strains and, consequently, the real beneficial effect of these in the human body. The reported studies show, therefore, that orange, pineapple and mango should be the best substrates to produce probiotic fruit juices. These fruits have a slightly acidic natural pH (between 3.0 and 4.5), which creates an optimal environment for the growth and stability of probiotic bacteria. This acidity balance promotes the viability of probiotics and inhibits the growth of pathogenic bacteria while maintaining good palatability of the juice. In addition, they are rich in bioactive compounds that support the growth and efficacy of probiotics.

4.3. Strains for Probiotic Fruit Juices

To guarantee the effectiveness and safety of these products, the choice of the strain used in fruit juices is important because this influences the stability and chemical–sensory characteristics of the product. Probiotic strains must present several characteristics, including resistance to the acidic pH of the juice, the ability to survive during product storage and tolerance to antimicrobial agents naturally present in the juice. There are differences between the strains in terms of acid and oxygen tolerance.

In general, Lactobacillus strains have proven to be much more resistant than Bifidobacterium ones [102]. The Lactobacillus strains that can tolerate more acidic environments and can therefore be included in a probiotic juice are *L. plantarum*, *L. acidophilus*, *L. casei* and *L. rhamnosus* [103]. *L. acidophilus* is widely studied for its beneficial effects on digestive and immune health. Its ability to survive in acidic environments, such as those typically found in fruit juices, makes it an ideal candidate for the enrichment of such products [104]. *L. plantarum* is known for its resistance to storage conditions and its ability to produce beneficial metabolic compounds [103]. *L. paracasei* has been identified as an extremely versatile probiotic strain due to its ability to remain viable in the low pH juice matrix at commercially acceptable levels. These robust strains remained viable in orange juice at levels above 10^7 CFU mL^{-1} and in pineapple juice at levels above 10^6 CFU mL^{-1} for 12 weeks [103]. In their study, de Oliveira et al. showed that Pediococcus acidilactici not only has excellent probiotic capacity, but also that it may be ideal to add to probiotic orange juice for its strength [105]. *L.rhamnosus* cells remained viable in pineapple and jussara juice throughout the product's shelf-life, with counts exceeding 9 log CFU per 100 mL portion. Furthermore, in vitro and in vivo tests conducted on this juice showed that at least 5.6 log CFU/mL of LGG reached the simulated large intestine [106]. In addition, the possibility of including certain yeasts with probiotic activity, such as *Saccharomyces boulardii*, in fruit juices is also being evaluated, but there are still not enough results [107].

4.4. Sensorial Properties of Probiotic Fruit Juices

The sensorial qualities of probiotic fruit juices represent a challenge for industrial production. In most studies conducted on probiotic fruit juices, sensory qualities have always been a limitation for the production of these products [16,108]. Probiotics can have a significant influence on the sensory characteristics of fruit juices. This is attributable to the metabolism of these, which can generate components that can negatively impact the aroma and taste of products [109]. Some probiotics can generate organic acids, such as lactic acid, which can change the taste profile of the juice, giving it a more acidic or slightly spicy flavor [110]. Consequently, probiotic-enriched juice may be less palatable than their conventional equivalents due to a distinctive sensory profile. Some probiotics may also produce enzymes that affect the texture of the product, leading to variations in the consistency of the juice [111]. Other probiotic strains can produce pigments that

influence the color of the juice, while fermentation can lead to changes in the concentration of compounds responsible for the natural color of the fruit [112]. These changes can be perceived positively or negatively by consumers and may influence their perception of the product. Furthermore, regarding the sensory properties of final products, changes affecting taste, odor and texture have been observed, as alterations in the profile of compounds, such as volatiles and polyphenols, occur as a result of probiotic-culture-induced reactions [113]. Although probiotic fruit juices may represent a new functional beverage with higher nutritional value, it is important to maintain a balance between the beneficial effect of probiotics and the sensory acceptability of the final product. It is important to maintain the acceptability of products with probiotics. Indeed, consumers avoid functional beverages if the added ingredients compromise their quality, even with health benefits. This is one of the aspects still to be improved. However, a combined choice of the type of fruit with the correct strain of probiotics could solve this problem. Promising results have been obtained in some studies. For example, in the research of Furtado et al., all mango juices enriched with *L. plantarum* and *L. rhamnosus* showed good acceptability values on sensory analysis, indicating that the addition of the probiotics had no effect on the sensory quality of the product [100]. Pineapple and orange juices have been a great base for adding different strains of probiotics, as these have demonstrated positive results in sensory evaluation [106,114].

5. Strategies to Improve the Viability of Probiotics in Juices

Since the survival of probiotics in fruit juices, and thus their effect, can be compromised by several factors, over the years, various strategies have been developed to try to improve the survival of bacterial strains, and these are shown in Figure 3. Furthermore, compared to dairy products, fruit juices do not contain compounds essential for the viability of probiotic cultures, such as amino acids and small peptides. The selection of the right strain of probiotics plays a crucial role in their survival in fruit juices. It is important to choose resistant strains that possess a high level of resistance depending on the type of fruit juice to be produced [103].

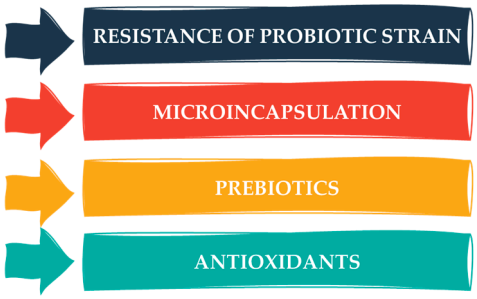

Figure 3. Strategies generally used for improving probiotics' viability.

Microencapsulation of probiotics has proven to be an effective strategy to protect them from the external environment during storage in juices, but also from the hard conditions of the gastrointestinal tract [115]. This technique involves encapsulating probiotics in biocompatible materials that provide a protective barrier against factors harmful to them. Microencapsulation effectively protects probiotics during juice storage, allowing them to maintain high viability and metabolic activity [116]. Microencapsulation could provide a more favorable anaerobic environment for sensitive probiotic bacteria as well as a physical barrier from the harsh acidic conditions of fruit juice. In addition, microencapsulated capsules may improve the delivery of probiotics into the gut, protect them from the acidic stress of the stomach and promote their survival in the gastrointestinal tract. Several studies have shown that microencapsulation significantly improved the survival of probiotics in fruit juices. In the study by da Silva et al., it was shown that the complex coacervation technique combined with transglutaminase cross-linking increased the viability of *L. acidophilus* in apple juice [117].

Another possibility to improve the viability of probiotics in juices involves the addition of prebiotics [118]. Many fruits already naturally contain prebiotics. Prebiotics are non-digestible compounds that promote the growth and activity of probiotics in the gut [119]. Adding prebiotics to probiotic juices can promote the survival of probiotics through their selective fermentation by gut bacteria, which produce metabolites that improve the gut environment [120]. In addition, prebiotics can act synergistically with probiotics to improve stability and functionality in juices, creating a favorable environment for the growth and colonization of probiotics in the gastrointestinal tract. Examples of commonly used prebiotics include fructo-oligosaccharides (FOS), inulin, oligofructose and galactose-oligosaccharides (GOS). The addition of prebiotics to probiotic-enriched juices can enhance the health benefits associated with consuming probiotic-enriched juices [70]. Several studies have shown that the use of prebiotics had a positive influence on microbiotic viability and did not affect the organoleptic characteristics of the final products, which is extremely important in the case of these products, which often suffer a change in their sensory properties [121].

The addition of protective agents such as antioxidants can preserve the viability of probiotics in fruit juices [88]. These agents can reduce oxidative stress and inhibit the growth of undesirable microorganisms, thus favoring the survival of probiotics. The protective effect of antioxidants on probiotics in fruit juices can be attributed to their ability to neutralize free radicals that can damage the cell membranes of probiotics and compromise their structural integrity [122]. In addition, antioxidants can interact with heavy metals and other oxidants in fruit juices, thus reducing the potential damage to probiotics [123]. The use of antioxidants in probiotic juices can therefore help maintain high concentrations of probiotics during storage and ensure the delivery of effective amounts of probiotics to the host gut, where they can exert their beneficial health effects.

In conclusion, the survival of probiotics in fruit juices can be improved through the adoption of several strategies. All these techniques can protect probiotics from the external environment, reduce oxidative stress and promote their growth and activity in the host gut, thus helping to maximize the health benefits associated with probiotic juice consumption.

6. Future Perspectives

Over the past few years, there has been growing interest in the development and innovation of probiotic fruit juices, with an emphasis on improving the survival and functionality of probiotics and expanding the range of probiotic strains and fruits used. The adoption of new technologies and methodologies represents an important future perspective for probiotic fruit juices. Technologies such as microencapsulation, the use of mixed cultures, controlled fermentation and the use of specific delivery systems can help improve the stability and functionality of probiotics in fruit juices [16,74]. Recent studies have shown that the exploitation of advanced technologies can significantly increase the survival of probiotics during juice production and storage. A promising direction for the future development of probiotic fruit juices concerns the exploration of the potential new probiotic strains [124].

This investigation requires an accurate evaluation of the resistance of these strains to the chemical characteristics of fruit juices, such as acidic pH, water activity, the presence of additives such as salts and sugars and oxygen concentration, to ensure their survival and functionality during storage and consumption of probiotic fruit juices. If strains are found to be less resistant, it may be necessary to use genetic engineering techniques to optimize their properties [125]. This could involve the targeted inclusion or deletion of specific genes to increase the probiotics' ability to survive and colonize in the gastrointestinal tract. However, the efficacy and safety of engineered strains must be carefully evaluated before their practical implementation in food products. In summary, the exploration of new probiotic strains and the application of genetic engineering techniques represent crucial prospects for improving the stability and efficacy of probiotic juices. Another future perspective for probiotic fruit juices is the customization of products according to consumer needs and preferences. This could include the selection of specific probiotic strains for therapeutic

purposes, or the formulation of probiotic juices enriched with additional functional ingredients, such as prebiotics, antioxidants or vitamins [109]. The customized approach could enable consumers to obtain maximum health benefits according to their individual needs. The future development of probiotic fruit juices should require a multidisciplinary approach involving experts from microbiology, food technology, nutrition, medicine and engineering [126]. Interdisciplinary collaborations could, in fact, facilitate the development of new production strategies, the evaluation of health effects and the understanding of the mechanisms of action of probiotic fruit juices. Finally, an important goal for industries producing these products is the exploration of new markets and consumer segments [127]. Increasing awareness of the health benefits of probiotics and growing interest in functional nutrition are stimulating demand for innovative products such as probiotic fruit juices. This offers opportunities for the expansion of probiotic juices into new sectors, such as the catering, fitness industry and wellness market. In addition, the adaptation of probiotic juices to the cultural and dietary preferences of different global markets can facilitate penetration and commercial success in new regions. The expectation is that probiotic foods will become popular in many people's diets soon. In fact, these foods have better nutritional characteristics than the original product, which will make them a more attractive choice for consumers.

The additional costs that might occur from adopted strategies to improve the viability of probiotics must be estimated so they can be minimized. In this way, the consumer may be more inclined to buy a healthier innovative product. Cost reductions may result from the choice of simpler production technologies and less waste of probiotic cells. For instance, microencapsulation of probiotics by the spray-drying method could be considered an efficient technique in the food industry [128]. This technique, in fact, appears to be a viable alternative for better utilization of probiotics in fruit juices, leading to less waste associated with the possibility of industrial applications due to a continuous and cost-effective process [116].

7. Conclusions

Through the evaluation of recent studies and trends, it emerged that probiotic fruit juices represent a promising category of functional foods, combining the health benefits of probiotics with the nutritional and antioxidant properties of fruit juices. Studies have shown that probiotic fruit juices can improve intestinal health, boost the immune system and promote general well-being. However, some challenges were also identified, such as the stability and survival of probiotics during juice production, storage and consumption. To address these challenges, several strategies have been proposed, including the use of robust probiotic strains, microencapsulation, the addition of prebiotics and antioxidants, and the adoption of innovative technologies. These strategies can help improve the survival and efficacy of probiotics in juices, allowing them to fully exert their health benefits. However, it is important to emphasize that further research is needed to evaluate the efficacy and safety of probiotic fruit juices and to explore new opportunities for development and innovation. Probiotic fruit juices can represent a valid alternative for all those consumers who want healthier food without consuming dairy products. With further research and development, their beneficial potential can be fully exploited, and consumers can be provided with safe, effective and high-quality food products.

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