

# Fruit-Based Non-Dairy Beverage: A New Approach for Probiotics

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## Abstract

The growing interest of consumers in using foods that improve health has motivated researchers and the food industry to develop new functional products such as foods with probiotics. Probiotic cultures, for example, from lactic acid bacteria and bifidobacteria have been highlighted for their ability to promote balance in the intestinal microbiota as well as other benefits such as anticarcinogenic and antimutagenic effects, reduced plasma cholesterol levels, decreased symptoms of lactose intolerance, and stimulation of the immune response. Traditionally, probiotics are incorporated into dairy products. However, because of the growing number of individuals affected by lactose intolerance and/or vegans, other food matrices have been studied as potential carriers for these microorganisms. Considering all the facts mentioned above, cereals, legumes, fruits, and vegetables could be potential substrates, where probiotic bacteria can be used for the development of non-dairy beverages. This review aimed to highlight the research carried out on 1) probiotic micro-organisms, including the more recent reclassification according to their phylogenetic position, 2) probiotic beverages from non-dairy sources which emerged as an alternative for lactose-intolerant consumers and, 3) the aspects of improving the gut microbiota.

## Keywords

Functional Foods, Fruit Juice, *Lactobacillus*, Non-Dairy Beverages, Gut Microbiota

## 1. Introduction

The term “functional food” seems generic, but the history of the term can be

highlighted in the late 1960s, for example, with researches that showed that polyunsaturated fatty acids could control the level of cholesterol in the blood [1].

Initially, the concept of functional foods was to seek food with the ability to treat diseases [2]. The concept of functional food emerged in Japan in 1984, with the disclosure of information from the beneficial effects of foods enriched with special components, such as probiotics by Japanese scientists [3]. From 1984 until now, functional food has changed its meaning due to different cultural origins [2]. In 1991, the Japanese Ministry of Health, Welfare, and Labor established “Food for Specified Health Uses” (FOSHU) as a regulatory system for functional foods. FOSHU was the result of a program financed by the Japanese authorities which aim at the reduction of the financial resources spent on public health, containing the progress of chronic diseases. After the introduction of the FOSHU regulation, the number of functional food products increased, especially between 1997 and 2007 [4].

In 2014, at the 17th International Conference on Functional Foods in Health and Diseases, functional foods were granted a new definition as natural or processed foods, containing known or unknown bioactive components in the non-toxic efficacy of performing clinically proven or documented health benefits [3].

In 2015 a new regulatory system for functional foods was established based on the system of the Food Supplement Health and Education Act, already established in the USA. With the introduction of this system, many new functional foods were developed due to the more flexible health claims when compared to FOSHU. This fact provided a growing increase in functional foods, which in 2018 reached an appreciation of around 1.8 billion dollars and this market is still undergoing an exponential and impactful expansion [4].

The European Commission has adopted a definition stating that “a food can be considered “functional” if it is satisfactorily determined to benefit one or more target functions in the body, in addition to having adequate nutritional effects to improve health, health, and well-being and/or reduced risk of disease” [5].

In Brazil, the current legislation on functional foods, approved by the National Health Surveillance Agency (ANVISA) in 1999, does not define the term “functional foods”, but rather a functional property claim that is “related to the metabolic or physiological role that the nutrient or non-nutrient has in the growth, development, maintenance and other normal functions of the human organism”. As long as ANVISA’s General Management of Food and its safety of use evaluate it and its efficacy is proven, the food that bears the claim may be made available on the consumer market [6]. Brazilian legislation prevents the attribution of medicinal and therapeutic effects to foods; therefore, claims cannot be associated with prevention, treatment, or cure of diseases. Specific claims are desirable as they communicate the claimed benefit more clearly to the consumer. This type of allegation should also not be too general, at the risk of not being able to obtain evidence capable of proving the effect and properly communicat-

ing about the claimed benefit [6].

The fastest-growing sector of functional food worldwide contains probiotics. Probiotic foods consist in represent 60% to 70% of the functional food market [7]. In 2015, it was predicted that the food market containing probiotics would increase from \$35 billion up to \$48 billion in 2020 [8].

Currently, the demand for functional foods containing bacteria with probiotic properties is growing rapidly due to increased public awareness of the benefits of probiotics for health, maintaining the balance of the intestinal microbiota, and improving mucosal defenses against pathogens [9].

This study aimed to summarize the current state of non-dairy beverages with the addition of probiotics, as well as to demonstrate the potential of the application of probiotics in juices from fruits.

## 2. Probiotics

The intestinal microbiota is a complex ecosystem, which is composed of microorganisms associated with various nutritional, metabolic, endocrine processes, immunological and psychological mechanisms. This complex of microorganisms maintains and regulates some endogenous functions such as nutrient metabolism, immunomodulation, synthesis of bioactive compounds and vitamins, and the fermentation of non-digestible carbohydrates, in short, they serve as a more efficient intestinal selective barrier. Thus, due to the importance of the intestinal microbiome in maintaining health, the search for new probiotics products with single, multi-strain or multi-species strains, associated or not with prebiotics, are sure bets on the market [10].

Romans and Greeks in the past used fermented dairy foods to ensure and maintain health [11]. The definition of probiotics (Greek; Pro: promotion, biotic: life) as living microorganisms, which when administered in adequate quantities, can offer a benefit to the health of the host, was established by the United Nations Food and Agriculture Organization (FAO) and by World Health Organization (WHO) in 2001 [12].

In Brazil, the National Health Surveillance Agency (ANVISA) defines probiotics as live microorganisms which are capable of improving the intestinal microbial balance, producing beneficial effects on the health of the individual when administered in adequate doses [13] [14].

Probiotics are defined by The World Gastroenterology Organization as live microorganisms that, when administered in quantity, confer health benefits on the host. The species of *Lactobacillus* and *Bifidobacterium* are the most used as probiotics, but the yeast *Saccharomyces boulardii* and some species of *E. coli* and *Bacillus* are also used. The new agents also include *Clostridium butyricum*, recently approved as a novel food in the European Union. Government regulations differ between countries, however, the status of probiotics as a component in foods is not currently established on an international basis. For most countries, probiotics come in dietary and dietary supplements because most come in the form of foods [15].

Probiotics also called living biotherapeutic products (LBP), are products that contain living organisms, such as bacteria, found naturally in humans. Government regulation of probiotics in the United States is complex. Depending on the intended use of a probiotic product, the Food and Drug Administration (FDA) may regulate it as a dietary supplement, food ingredient, or drug [16].

The mechanisms of action of probiotics are not always well understood, which are one of the problems considered by the European Food Safety Authority (EFSA), which in 2014 rejected the health claims of marketed probiotics due to lack of sufficient evidence [17].

Historically, the concept of probiotics was developed around 1908 by the Nobel Prize winner Elie Metchnikoff, who discovered that the consumption of live bacteria (*Lactobacillus bulgaricus*) in yogurt or fermented milk could improve some biological characteristics of the gastrointestinal tract [7] [12]. In his study, Dr. Metchnikoff concluded that a bacterium helps control the effects caused by enteric pathogens and toxemia, which play an important role in aging and mortality. This research resulted in an increase in the production and consumption of yogurt all over the world [11].

In 1965, Lilly and Stillwell used the term probiotic for the first time. Over the next decade, the term was used by Fujii and Cook in 1973 and denoted chemicals in mice that protected against *Staphylococcus aureus* infection. In 1974, the term was used by Parker in a broader sense to refer to interactions of microorganisms with the animal or human host, that is, “organisms and substances which contribute to balance microbial proliferation”. Finally, in 2013, the consultation of experts from international scientists at the meeting of the International Scientific Association of Probiotics and Prebiotics provided minor grammatical corrections and reformulated the previous definition as “living microorganisms that, when administered in adequate quantities, confer a benefit to the health of the host”, which is now widely accepted and used. Several studies on probiotics have been published since this discovery [11].

The daily recommendation for ready-to-eat probiotics must contain at least a minimum amount of viable cells in the range of  $10^8$  to  $10^9$  colony forming units (CFU). Smaller values are acceptable, as long as its effectiveness is proven [18]. The viability of probiotics should be ensured during processing and storage, aiming to keep their counts at high levels ( $10^6$  -  $10^7$  CFU/mL or g of food) until consumption [19].

The World Gastroenterology Organization considers that a required dose of probiotics varies greatly depending on the strain and the product. Although many products provide between 1 - 10 billion CFU/dose, some products are effective at lower levels, while others are at higher amounts. It is not possible to establish a general dose for probiotics; since the dosage has to be based on human studies that show a health benefit [15].

In Brazil, the ANVISA legislation on foods with alleged functional and/or health properties, new foods/ingredients, bioactive substances, and probiotics has such probiotic microorganisms approved for use in food: *Lactobacillus acido-*

*philus*, *Lacticaseibacillus casei shirota*, *Lacticaseibacillus casei* variety *rhamnosus*; *Lacticaseibacillus casei* variety *defensis*; *Lacticaseibacillus paracasei*; *Lactococcus lactis*; *Bifidobacterium bifidum*; *Bifidobacterium animalis* (including the subspecies *B. lactis*); *Bifidobacterium longum*; *Enterococcus faecium* [20].

All probiotic species are considered safe for the general population by the European Food Safety Authority (EFSA). The U.S. Food and Drug Administration (FDA) classifies probiotics individually but also classifies them as safe for food use [10]. For a single strain to reach probiotic status, it is necessary to assess its resistance to the digestion process and its ability to promote health benefits [10].

Probiotics have been used in food with the main objective of strengthening the natural intestinal microbiota. Its effectiveness in improving health status depends mainly on its ability to provide viable functional bacteria, overcoming the harsh effects of the intestinal tract [6]. Raising their health benefits, probiotic bacteria with activity have been increasingly added to a range of products, including yogurts, cheeses, ice cream, powdered milk, and frozen desserts [6]. Viable microorganisms such as lactobacilli and bifidobacteria that benefit the host by improving intestinal bacterial balance are the most used by the industry, as they have several typical characteristics such as metabolic stability, adherence to intestinal cell walls, without promoting antibiotic resistance and not pathogenic, safe for the consumption and effective. Besides that, these bacteria must be active in the product, survive throughout the upper digestive tract, resist gastric juice, oxygen, and enzymes, and can co-aggregate as a part of the natural intestinal microbiota and have beneficial effects after adhering to the host's intestine [21] [22].

The world market for probiotics including their use as an ingredient, supplements, and their incorporation in food products is increasingly expanding [11] and according to Transparency Market Research was estimated to reach U\$12,753 million by 2026.

Preclinical and clinical studies on the benefits of probiotics to gastrointestinal health has shown effects on 1) prevention of acute diarrhea associated or not with antibiotics, 2) symptomatic relief in irritable bowel syndrome, 3) treatment of hepatic encephalopathy, and 4) prevention of necrotizing enterocolitis in premature babies [23]. Probiotics act as antagonists to pathogens such as *Enterococcus faecalis*, *Salmonella enterica* subsp. enteric serotype Enteritidis, *Listeria monocytogenes*, *E. coli* and *Staphylococcus aureus* by immunological, hormonal and neuronal manipulation [24]. Specific probiotics also have gained a place in the treatment of ulcerative colitis and are useful to combat overweight, obesity and are recommended as options in the main clinical guidelines [25].

To avoid miscommunication about all living species, biological knowledge on taxonomy and nomenclature should be considered when choosing probiotics. [26]. In the strictest sense, taxonomy deals with the theory and classification practice, including principles, rules, and methods. When new species or higher taxa are discovered or in case of revised or reclassified taxon, taxonomists publish a formal description of each change to establish names and circumscribe the

corresponding taxonomic concepts or to amend existing ones to reflect their discoveries [27].

When DNA-DNA hybridization method, used since 1960 for bacterial taxonomy, suffers from reproducibility problems and cannot provide an accurate measurement of the actual sequence identity between genomes [28], new strategies need to be found. For example the genus *Lactobacillus* was proposed by Beijerinck in 1901 and includes Gram-positive microorganisms, fermentative, optionally anaerobic, and non-spore-forming, which can also be mobile, catalase-negative when cultivated without heme on the medium, usually oxygen tolerant, aciduric or acidophilic, mandatorily saccharolytic with at least 50% of the final carbohydrate product being lactate and other fermentation products consisting of acetate, ethanol, CO<sub>2</sub> and succinate. Several types of fermentation can be recognized, such as metabolisms that are mandatory homofermentative, optionally heterofermentative, and mandatorily heterofermentative, based on the types of fermented sugars (hexoses and pentoses) and fermentation products [29]. The genus is classified in the phylum Firmicutes, class Bacilli, order Lactobacilales, family Lactobacillaceae, which contains the genera *Lactobacillus*, *Paralactobacillus*, and *Pediococcus* [30]. The Lactobacillaceae family contains the genera *Lactobacillus* and *Pediococcus*, which are phylogenetically mixed. The ancient genus *Paralactobacillus* was recently included in the genus *Lactobacillus*, although this inclusion is questionable. More than 150 species are recognized in the genus *Lactobacillus* and are heterogeneous in several properties [29].

In the past two decades, sequencing of entire bacterial genomes has become widely available and the mean values of the nucleotide identity (INA) of the genes shared between different bacterial genomes has been introduced as the “gold standard” for the design of new bacterial species [30]. Due to the relatively small size of the bacterial genome and the greater availability of high-throughput sequencing DNA technology, phenotypic testing has now been replaced by genome sequencing as the main source of taxonomic information [26].

Since 1983, the similarity between the 16S rRNA genes has been used in bacterial taxonomy to provide phylogenetic schemes as a backbone for classification and nomenclature. Limitations to the 16S rRNA gene approach; for example, many recently divergent species that have undergone intense evolutionary pressures may have highly similar 16S rRNA gene sequences that can, however, ignore a wide phylogenetic gap between such taxa [28]. Based on parameters as the Average Aminoacid Identity (AAI) and the Percentage of Conserved Proteins (PCPO), this genus has a wide range that far exceeds the normal spread of a genus [26]. From the available genome sequences, 16 groups were discriminated within the *Lactobacillus* genus. Fourteen (14) stable phylogenetic groups were described within the genus, based only on 16S rRNA sequence similarity. According to the taxonomic subcommittee on *Bifidobacterium*, *Lactobacillus* and related organisms this taxonomic aspect was discussed and they decided for a formal division of the genus, creating a working group to collect all available genotypic and phenotypic information that would allow defining a new, reliable and

stable structure for the genus *Lactobacillus* [26].

In the First International Taxonomy Congress, held in 1930 in Paris, France, the first “Bacterial Code” was developed. This code updates qualitatively the bacterial nomenclature, reducing the duplicate names and including a better description of the different species and corresponding Type strains [26]. The Prokaryotes International Nomenclature Code defines rules for the nomination of bacterial taxa based on their taxonomic classification. Although it covers many exceptions and particular cases, its principles are relatively simple; but, as the Code stands, it is not easy to find reasons for name changes of the genus *Lactobacillus* [26]. In the *Lactobacillus* genus the species were included based on several common phenotypic characteristics. For a reclassification of the genus, the demonstrated genetic distinctions within the genus should be supported by a discriminative set of parameters which, in addition to genome sequence, will allow a reliable and consistent description of a new genus. So, it is necessary to collect available information on all known *Lactobacillus* species, exploring different bioinformatic tools, and eventually arrive to a possible reclassification for the genus [26].

By March 2020, 261 species of *Lactobacillus* had already been described. The genus *Lactobacillus* is very heterogeneous and in the last decades, more than 250 species have been attributed to this genus [30].

The previous taxonomy of lactobacilli was based on phenotypic factors and characteristics such as the ideal temperature of growth, use of sugar, and range of metabolites produced [30]. Later in the 20th century, genotypic and chemotaxonomic criteria (e.g., chemical structure of peptidoglycans) were used to design new bacterial species [30].

A new perspective for taxonomy, the Total Nucleotide Identity (TNI) was found between the expected values between order and family and the INA values between Order and Class for the 237 species of *Lactobacillus* (208, excluding synonyms and subspecies). In the same study, Pot *et al.* [26] showed that representatives of the *Pediococcus* genus, and members of Leuconostocaceae family are mixed with species within the *Lactobacillus* genus. But the genus continues to grow, and new isolates are continuously being added, which the latter authors find scientifically unacceptable. rRNA gene sequence analysis introduced a tool for a more exhaustive and robust taxonomy for the genus with the introduction of 16S, but ended by revealing fewer correlations between traditional classification based on phenotype and the new phylogenetic one. The continuous description of new species of *Lactobacillus*, led to the recognition of an increasing number of variable phylogenetic subgroups [26].

Zheng *et al.* [30] states that it is recognized according to the level of genetic diversity found for the *Lactobacillus* genus exceeds what is commonly found for other bacterial genera and even for bacterial families. The availability of complete genomes of *Lactobacillus* strains, representing the main families of the Lactobacillales order, has allowed a more definitive analysis of their evolutionary relationships [29].

Recommendation 30b of the Bacteriological Code of Nomenclature (1990 Review), as modified at the 1999 meeting of the International Committee for Systematic Bacteriology (ICSB) and its Judicial Commission, calls for the definition of minimum standards to describe new bacterial taxa [29].

With the description of the new genus, the researchers suggested keeping the initial “L” for the new genus names to minimize confusion. This way, considered commercially important species such as *Lacticaseibacillus casei*, *Lactiplantibacillus plantarum*, and *Limosilactobacillus reuteri*, which will no longer be lactobacilli and will be included in a new genus, will be abbreviated as *L. casei*, *L. plantarum*, and *L. reuteri*. Other commercially important species of *Lactobacillus*, including species and strains with fermentation capacity and others with proven probiotic activity, will be mainly found in the newly defined genera. In this way, the number of species of the genus *Bifidobacterium*, *Lactobacillus*, and related genera has increased considerably over the past 10 - 15 years [29].

Thus, some guidelines have been defined to recommend the labeling of probiotics using a current nomenclature for genera and species, although there is a greater concern from a practical and financial standpoint, there is a clear demand for stability in the new classification system [26].

## 2.1. Probiotic Products Availability on the Market

After *in vitro* and pre-clinical research, or after large-scale clinical trials, a substantial number of microbial species have been revealed to exhibit potential probiotic properties, however, only the most documented and robust strains can reach the market [31]. Most of the current probiotics are lactic acid bacteria (LAB), which belong to the genus *Lactobacillus* and *Bifidobacterium*, with a smaller number of *leuconostocs*, *pediococci*, *lactococci*, *enterococci*, and *streptococci*. A variety of species of LAB are listed as generally recognized as safe (GRAS) and comprise the probiotic species that are the most used in supplements or food matrices. The most commonly used probiotics are species of *Bifidobacterium* (*B. animalis*, *B. bifidum*, *B. breve*, *B. infantil*, *B. longum*, *B. lactis*), and species of *Lactobacillus* (*L. acidophilus*), *Lacticaseibacillus* (*L. casei*, *L. rhamnosus*), *Lactiplantibacillus* (*L. plantarum*), *Ligilactobacillus* (*L. salivarius*) and *Limosilactobacillus* (*L. fermentum*, *L. reuteri*). Other species as *Streptococcus* and *Bacillus* or the yeast *Saccharomyces cerevisiae* are also used as probiotics, and incorporated in non-dairy foods. There has recently been a reclassification of *Lacticaseibacillus*, *Lactiplantibacillus*, *Ligilactobacillus*, and the genus *Limosilactobacillus*, which previously comprised the genus *Lactobacillus*. Although the use of some *Bacillus* or *Clostridium spp.* may seem to be controversial from a security perspective, the technological advantage for using spores compared to the most vulnerable plant cells explains the increased interest in research and commercial development for these species [31] [32].

A beneficial effect on the host is obtained if a minimal amount of viable probiotic cells reaches the intestine. The suggested minimum amount in food at the time of consumption is around  $10^8$  viable cells per mL or g of food, so that the

observed survival during exposure in the gastrointestinal tract (GIT) cannot compromise the functionality of probiotics. Despite the differences between the daily amounts recommended by American or European agencies for making health claims, it was proposed that the daily intake should be from 6 to 9 log CFU probiotic g<sup>-1</sup> or mL<sup>-1</sup> for its effectiveness. However, to claim specific health effects, the required dose may be lower and it is specific to each strain [32].

Dairy industries and LAB preparation companies in Europe, Japan, and the United States have developed their own internationally renowned strain brands, as well as product brands. And, to assess the probiotic functions, these strains have been submitted to many clinical trials. *Bifidobacterium lactis* BB-12 strain, developed by Chr. Hansen (Denmark), is reported as the most studied Bifidobacterium strain in the world. Over the past 80 years, the strain *Lactocaseibacillus casei* Shirota from Yakult Company has undergone a large number of scientific studies and clinical trials. In late May 2015, its survival, efficacy, and safety in the gut were scientifically verified in China, Japan, Thailand, UK, and elsewhere [33].

Large differences are observed between strains from the same species, as they may have different phenotypes and properties that can lead to different clinical effects [31].

Many studies have found out that the addition of probiotic cultures in foods has not influenced the sensory acceptance of the products [32].

## 2.2. Dysbiosis

Dysbiosis, an imbalance in the intestinal microbiota composition after the use of antibiotics, has largely boosted the therapeutic application of probiotics [24]. A consideration of the intestinal microbiota is necessary to understand its relevance to human health and the concept of probiotic food. Each individual has in its gastrointestinal tract a unique signature with more than 1000 microbial species. The bacterial cells comprehend half the wet weight of the colon material and its number reaches 10 times over the number of cells in the tissue that forms the human body.

Normally, the stomach contains 10<sup>3</sup> different bacterial species, while the total microbial population of colon bacteria is around 10<sup>11</sup> and 10<sup>12</sup> CFU/g. The bacterial colonization of the intestine begins at birth when newborns are exposed for the first time to a non-sterile environment. Thereafter, it evolves and transforms throughout life, depending on a complex and dynamic interaction between the host diet, genome, and lifestyle, as well as the use of antibiotics. The composition of the intestinal microbiota is generally considered to be essentially stable throughout adulthood [25].

The vast majority of clinical trials of probiotics reported in the literature have not given rise to major concerns. However, some examples of serious adverse effects of probiotics have been documented regardless of formulation, dosage, and daily consumption [12].

*Lactobacillus* and *Bifidobacterium* are common genera of the endogenous mam-

malian gastrointestinal tract. They induce host immunomodulation and reduce symptoms of a wide range of gastrointestinal disorders, and therefore have been widely used as probiotics. The cell membrane integrity, the intracellular pH, and the functional enzymes of probiotic cells can be constantly attacked by stress factors such as bile acids, digestive enzymes and gastric acidity during their passage through the gastrointestinal tract. As a consequence, probiotic cells can be viable (active and cultivable), inactive (inactive, but cultivable), active (but not cultivable), or dead (inactive and non-cultivable). Cells that experience a high level of stress may still have some metabolic activity, but they may no longer be cultivable [10].

Even inactive probiotic cultures or their fragments are also being investigated by researchers and have been shown to have effects such as an improvement in adverse behaviors (mental health) related to sleep disorders, stress, regulation of intestinal function, and positive effects on the administration of immunity and allergy. These non-viable cells also present advantages for the food industry, making products safer and more stable. These types of applications deserve special attention for patients in critical states for whom the risks of consuming active cultures are quite high [10].

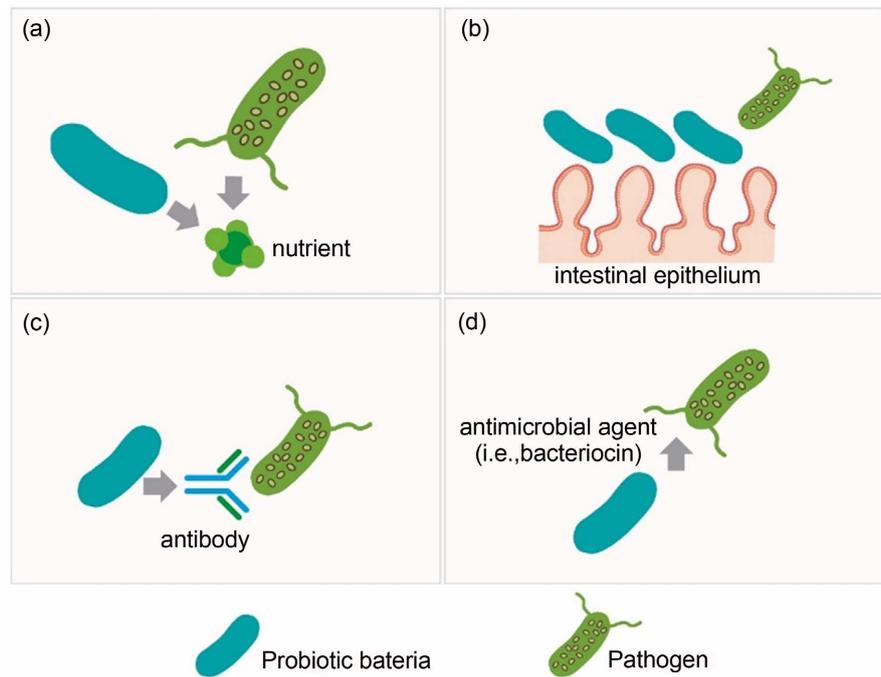
### 2.3. Mechanism of Action and Probiotic Activity

Probiotics create a favorable environment for the intestine, through their mechanisms of action, which will depend on a variety of factors, such as the type of strain used, the host, and the food. The probiotics can improve the epithelial barrier promoting mucus secretion. Furthermore, some probiotic strains release active peptides, also known as bacteriocins, against bacteria, fungi, and viruses, which may stabilize the intestinal barrier. Other antimicrobial substances are also produced by probiotics, such as lactic and acetic acid, that present an inhibitory effect on Gram-negative bacteria [6].

In this way, the probiotics act in several ways, interfering with the adhesion of pathogens to the intestinal mucosa [6]. As there are different strains and product formulations, there is not a single answer. An intriguing aspect of probiotic strains is the ability of some to confer distant effects at the site of administration. This can occur through the transfer of organisms, for example, from the intestine to the mammary glands of breastfeeding women [34].

The mechanisms by which probiotics exert their effects, in general, are largely unknown, but they may involve changing the intestinal pH, antagonizing pathogens through the production of antimicrobial compounds, competition for binding sites and pathogen receptors, as well as nutrients and factors of available growth, stimulation of immunomodulatory cells and lactase production [23]. As described in **Figure 1**, there may be four different mechanisms in which probiotics can defend the body against pathogens.

Bacteria with probiotic properties are now widely available in the form of foods, such as dairy products and juices, as well as capsules, drops, and powders [12] [25].



**Figure 1.** Probiotics functions against the pathogen in the intestine by: (a) competing against pathogens for the same essential nutrients, thus making it less available for the pathogen to use, competition for nutrients; (b) attachment to the adhesion sites and, therefore, preventing attachment of the pathogen by reducing the surface area available for colonization of the pathogen, Blocking of adhesion sites; (c) sends a signal to the immune cells that result in the secretion of cytokines, which target the pathogen for destruction, Immune stimulation; (d) attacking pathogenic organisms by releasing antimicrobial agents, such as bacteriocins, that kill them directly (adapted from Fazilah *et al.*, 2018), direct antagonism.

Traditionally, a wide range of fermented foods such as yogurt, kefir, kimchi, sauer-kraut, tempeh, miso, and kombucha are part of the regular diet in different cultures and ethnicities, ranging from eastern to western, serving as a conventional source of probiotic strains [24].

Studies have shown that the specific food matrix can affect probiotics, by allowing their multiplication and protecting the product's shelf life, as well as providing protection in the passage through the gastrointestinal tract. Foods with high pH or high buffer capacity can reduce stomach acidity in humans, promoting the survival of probiotic micro-organisms. In addition, liquid foods are digested more quickly than solid foods, consequently reducing the exposure of probiotics to agents of stress, such as stomach acid [35]. Probiotics demonstrate the beneficial effects as shown in **Table 1**.

Dairy beverages have been the most popular source of probiotics. However, due to the market demand for plant-based beverages (e.g., vegetarianism and veganism) and to consumer awareness of adverse reactions to dairy products (intolerance and malabsorption) functional non-dairy beverages became an alternative carrier for probiotics [43]. It is well known the association for the intake of dairy products with lactose and milk protein intolerance. Besides that,

**Table 1.** Probiotic functions.

Strain	Action	Reference
<i>Lactobacillus</i>	Significant decrease in gastrointestinal symptoms, including nausea, postprandial fullness, and gastrointestinal pain	[36]
<i>Lactobacillus</i>	Decreased inflammation related to <i>H. Pylori</i>	[36]
<i>Lactobacillus</i>	Relief of symptoms of lactose intolerance, cholesterol reduction	[37]
<i>Sacharomyces boulardii</i>	Anti-inflammatory activity	[38]
<i>Saccharomyces boulardii</i>	Recommended for the treatment of acute gastrointestinal diseases, such as rotaviral and bacterial diarrhea and chronic conditions, such as inflammatory bowel disease	[39]
<i>Lactobacillus acidophilus</i>	Changes in the profile of inflammatory cytokine production and regulation of proinflammatory pathways, inhibition of the adhesion of pathogenic bacteria, modification of the microbiota by acidification of the colon due to fermenting agents, improvement in the function of the epithelial barrier, and protection against physiological stress	[40]
<i>Bifidobacterium longum</i>	Associated with carbohydrate metabolism	[41]
<i>Bifidobacterium longum</i>	Protection against autoimmune diseases such as inflammatory bowel disease, metabolic syndromes, irritable bowel syndrome colitis and brain disorders	[41]
<i>Lactocaseibacillus rhamnosus</i>	Beneficial for vaginal and postpartum female health	[42]
<i>Lactobacillus acidophilus</i>	Decrease in toxins produced by the kidneys	[43]

the higher saturated fat and cholesterol content in dairy based products compared to plant-based ones is also an inhibiting factor among some health-conscious consumers. The aforementioned concerns have opened a path for probiotic non-dairy products. The flavor and refreshing nature are the main advantages of non-dairy probiotics, as they include fermented products from cereals, soy, meat, fruits, and vegetables, enriched with various nutrients, vitamins, and antioxidants [44].

#### 2.4. Clinical Effect of the Consumption of Probiotics

The World Health Organization stated that up to 38 million people died from chronic diseases worldwide in 2012, of which more than 40% died of premature death, and this value was much higher than in 2000 (14.6 million). Inadequate

dietary fiber intake is another major cause of chronic diseases, which can lead to the loss of some intestinal microorganisms, which can later result in various chronic diseases [33]. The growing number of studies shows the association between intestinal microbiota and chronic diseases that helped to develop the hypothesis that modulation of the intestinal microbiota may be a factor that links the environment with the genetics and diseases of the host [45] [46].

The intestine is one of the organs most important organs in the human body. It contains more than 70% of the body's mucosal immunity. In addition, the intestine is closely related to various parts of the body through complex immune mechanisms. Therefore, considerable attention must be paid to intestinal health to achieve a healthy lifestyle [33].

More than 300 scientific publications describe the benefits of using probiotics, of which more than 130 are related to clinical studies in humans [33]. One study demonstrated that tomato and bean juices fermented with *Lactiplantibacillus plantarum* LP DSM20205 (formerly known as *Lactobacillus plantarum* LP DSM20205) could have an important effect on the integrity and adherence of the barrier, being the most pronounced effect for fermented tomato juice. Probiotic cultures isolated from plant products could also have *in vitro* effects. For instance, Lactic acid bacteria (LAB) isolated from fermented cocoa juice and their metabolites have demonstrated antagonistic activity against *Helicobacter pylori*, which is associated with gastric ulcers. Also, *Pediococcus pentosaceus* SC28 and *Levilactobacillus brevis* KU15151 (formerly known as *Lactobacillus brevis* KU15151) from traditional Korean food (jeotgal octopus and kimchi radish) showed adherence rates of 4.45% and 6.30%, respectively, to HT-29 cells, which is a human colon adenocarcinoma cell [32].

Vegetable products with the addition of vegan probiotics could have hypocholesterolemia *in vitro* and anticarcinogenic effects. The addition of *Lactiplantibacillus plantarum*-1 (formerly known as *Lactobacillus plantarum*-1) and *Lactocaseibacillus rhamnosus* GG (formerly known as *Lactobacillus rhamnosus* GG) on the blueberry bagasse presented potential benefits on the cholesterol reduction this fact is to the hydrophobic bonding, increasing the excretion of cholesterol [32] [47].

Immunomodulatory and control of diabetes properties have also been associated with plant products added with probiotics. Lychee juice fermented with *Lactocaseibacillus casei* FL (formerly *Lactobacillus casei* (FL) was used to investigate the effects on immunity and intestinal microbiota in mice. Also, consumption of soy milk containing *Lactiplantibacillus plantarum* A7 (formerly *Lactobacillus plantarum* A7) resulted in antioxidant properties and decreased the risk of incompatible base pairs in DNA among patients with type II diabetes [32].

### 3. Non-Dairy Products with the Addition of Probiotics

Probiotic cultures are usually added to dairy products; besides that, consumers

are used to the presence of microorganisms in this type of product [15]. However, lactose intolerance, veganism, high cholesterol content, and allergy to milk proteins are limiting factors in the growth of dairy products with probiotics. In total, 75% of the world's population suffers from lactose intolerance [48]. According to previous studies, higher milk fat content has shown inhibitory effects for probiotic cultures, particularly *B. bifidum* in yogurt [49].

The use of probiotics in non-dairy products has increased, probably, due to the growing number of adherents to veganism, thus opening room for demand for products free of ingredients from animals [50]. Therefore, other food matrices are being evaluated as carriers of bio-culture, aiming to provide other options to the market, especially to consumers who do not appreciate or cannot consume milk products, including people who are lactose intolerant, allergic to proteins milk or strict vegetarian (vegan) [13].

Probiotic beverages can be made from various raw materials, such as vegetables, corn, legumes, and fruits [51] [52].

Juices (from fruits) can represent an alternative means for adding probiotic cultures because they are considered healthy products and are regularly consumed. In addition, fruit juices are rich in sugars, minerals, and vitamins, which are used as a substrate by probiotics and in combination with a rapid passage through acidic stomach conditions result in the high viability of probiotic cells [13] [37] [52].

Unlike dairy products, fruits and vegetables do not have allergens, lactose, and cholesterol, which adversely affect certain population groups [37]. They are healthy, refreshing, have a good taste, and may be suitable for probiotics. Because they are considered perishable products, fruits require immediate processing to reduce post-harvest losses and the development of probiotic products can be an approach to increase the product's availability and market value. Fruit-based probiotic products are made from pineapple, blackberry, apple, strawberry, lemon, mango, grape, cashew, oranges, carrot, beet, etc., as shown in **Table 2** [48].

A variety of types of probiotic fruits and vegetables have been developed and marketed including fruit and vegetable juices, dried fruits, fermented vegetables, and desserts for vegetarians. However, studies that show the feasibility of incorporating probiotic bacteria in fruits and vegetables, and found that their feasibility and stability in these foods are highly dependent on several factors [37]. Fruits, such as apples, guava, bananas, and melons, are potential carriers of probiotic bacteria and strong adhesion of these bacteria to fruit tissue [64].

Considering that fruit and vegetable beverages are an excellent source of vitamins, antioxidants, minerals, and bioactive compounds and represent a good alternative to dairy matrices and a good choice. Different fruits and vegetable juices in the fermentation process can increase the nutritional and functional properties, with beneficial effects on health, in addition to increasing the shelf life of beverages [19].

**Table 2.** Probiotic fruit drinks.

Strain	Fruit	Shelf life	Reference
<i>Saccharomyces cerevisiae</i>	Cherry	21 days	[43]
<i>Bifidobacterium animalis subsp. lactis</i> and <i>Lactobacillus acidophilus</i>	The mix of banana, strawberry, and juçara beverage	90 days	[52]
<i>Lactiplantibacillus plantarum</i>	Apple	3 days	[53]
<i>Lactiplantibacillus plantarum</i> , <i>Bifidobacterium breve</i> and <i>Streptococcus thermophilus</i>	The mix of orange, carrot, apple	21 days	[54]
<i>Lactiplantibacillus plantarum</i> , <i>Lactobacillus delbrueckii</i>	Cabbage juice	-	[55]
<i>Lactobacillus</i> spp., <i>Leuconostoc mesenteroides</i> , <i>Bifidobacterium longum</i>	Carrot and orange juice	-	[56]
<i>Lactiplantibacillus plantarum</i> , <i>Lacticaseibacillus casei</i> , <i>Lacticaseibacillus paracasei</i> , <i>Lacticaseibacillus rhamnosus</i>	Cherry	12 days	[57]
<i>Lactiplantibacillus plantarum</i>	Cornelian cherry	28 days	[58]
<i>Lacticaseibacillus casei</i>	Pineapple	42 days	[59]
<i>Lacticaseibacillus casei</i>	Apple	42 days	[60]
<i>Lacticaseibacillus casei</i>	Lychee	28 days	[30]
<i>Lactiplantibacillus plantarum</i> e <i>Lactobacillus delbrueckii</i>	Pomegranate	28 days	[61]
<i>Lactiplantibacillus plantarum</i> e <i>Lactobacillus acidophilus</i>	Orange	3 days	[62]
<i>Lactobacillus</i> e <i>Bifidobacterium</i>	Orange, pineapple, and cranberry	84 days	[63]

Researches with non-dairy symbiotic beverages fermented beverages, including different types or mixed vegetables or fruits, with different concentrations of inulin, pomegranate juices, and cherry beverages using wheat bran, apple juice with oligofructose, orange juice with oligofructose, orange juice and hibiscus tea mixed with oligofructose and berry (strawberry, blackberry, and papaya) supplemented with three separate prebiotics: FOS, inulin, and galactooligosaccharides has been a constant search [13] [19]. In some cases, LABs can bio-transform polyphenols into phenolic compounds with better bioavailability and bioactivity during the fermentation time [65].

However, adding probiotics to fruit juices is more complex when compared to adding to the dairy matrix. The main challenges faced this, are due to some intrinsic properties of these products, such as low pH and high concentration of organic acids, associated with other important factors like storage time and con-

ditions for maintaining the viability of probiotics. In addition, fruit juices are considered highly perishable products and contain a large amount of water, which leads to a higher cost of transportation and production [53]. The disadvantage of non-dairy beverages is some unpleasant flavors caused by probiotics are almost perceived by the consumer [49].

Patents involve an admirable transfer of knowledge, both in terms of dispersing information about the deposits, and through the diversified use of scientific and technological knowledge necessary to produce the patented technology [66]. Patent-based statistics assume the innovative performance of a country, company, or institution, as well as other aspects that involve the innovation process. There was an effective start of patent filings from the year 2000, this fact may be related to the growth due to the increasing demand for functional foods since they are important for nutrition that helps in improving health and quality of life. Dairy beverages grew by 2.5% between 2008 and 2011 [66].

Currently in Brazil, the panorama regarding the protection of new products characterized as probiotics, focusing on non-dairy food matrices indicates that the products already patented on this topic fall on the production of non-alcoholic beverages fermented with probiotics, mainly of the genus *Lactobacillus* and/or *Bifidobacterium*, with fruits as a food matrix, and a patent on a smoothie, using the same genera of microorganisms and food matrix as the others [67]. It is necessary to encourage mainly in Brazil, the development of technological innovations aimed at patenting of the methods, the preparation of probiotic products, given that these are influencing the quality of life and preventing diseases that affect the world population [68].

Spray drying is the most used technique in the production of juice powders. In addition, it is widely used for microencapsulation of bioactive components, including probiotics, providing protection against adverse environmental conditions and improving processing and stability during storage [52]. In addition to being a quick-drying process, this technique has other advantages, such as relatively low cost, simplicity of use, and continuous operation capability. Although in some cases high temperatures used on processing and the low moisture content can lead to the decreased survival of the probiotic cells, to overcome these limitations polysaccharides especially those with prebiotic properties, such as inulin and oligofructose, can be used for the microencapsulation of probiotics increasing the viability of probiotic cultures [52] [66].

During the processing and storage of the products, oligofructose is the available substrate for the metabolism of these microorganisms and, thus, could increase the stability of probiotics in fruit juices during storage. In addition, oligofructose can be used as a sugar substitute, as it has a sweet taste similar to sucrose [13].

### 3.1. The Market of Vegetable Beverages with Probiotics

Combining probiotics with fruits and vegetables can be interesting as it provides the probiotics and dietary fiber the body needs, indicating an important direc-

tion of development for the probiotic industry in the future [48]. The combination of “probiotics + fruits and vegetables” takes many forms; of these, the direct addition of probiotics to existing traditional fruits and vegetables is the simplest way and the best approach is to ferment raw fruits and vegetables using probiotics strains [33].

The probiotic industry is expanding rapidly and new probiotic products are constantly been developed. The global probiotics market is estimated to be worth \$15 billion a year and is growing at an estimated 7% annual rate [45]. This increase has led to a large number of new products including probiotics on supermarket shelves as well as in drugstores [31].

In recent years, there was an increase in vegetarianism and veganism and with that, the consumer demand for products with high nutritional value has increased too. People are increasingly avoiding products derived from animals (*i.e.*, vegetarians and/or vegans) and this has become a growing trend in modern lifestyles. In addition to that, many consumers started to demand plant-based milk alternatives for sustainability, health, dietary, and lifestyle issues or broader social or political reasons, resulting in an abundance of fruits, seeds, nut products or beans. Also, the global market for alternatives to non-dairy beverages has become a multi-billion-dollar business and will account for approximately U\$26 billion in 2023 [32] [34].

In this scenario, the development of new products that are nutritionally balanced and/or add value stands out for their practical use as probiotics should be emphasized because of its proven effectiveness and wholesomeness, and the adaptability of probiotic cultures in different food matrices. Teas, fruit juices, or fermented drinks are matrices composed of bioactive compounds, such as vitamins, minerals, and polyphenols, representing interesting matrices for the addition of probiotics. However, there is always a need to assess the survival of the probiotic culture and its impact on the quality characteristics of the product [32].

It is necessary to exhaustively select excellent strains for the fermentation of different fruits and vegetables, as LAB derived from plants such as *L. plantarum* and *L. acidophilus* have gradually come to be used for the fermentation of fruit and vegetable juices. There is also a lack of high-density cultivation technology for fruit and vegetable fermentation varieties suitable for industrial production that needs to be incremented [34]. The industry still faces several important scientific and technological issues. More strains with excellent fermentation performance are needed to develop and the effects of prebiotics, probiotics, and fermented fruit and vegetables on human health, in addition to their mechanisms of action, should be better understood. Research and industries in the field of fruits and vegetables fermented with probiotics will have greater development opportunities if these problems can be effectively addressed [34].

### 3.2. Study for the Viability of Probiotics

In the development of functional foods with probiotic microorganisms, the for-

mulation, processing, and storage should favor the survival of the microorganisms. Both technologies and the food matrix must aim to protect the microorganism's cells against external stress factors. In addition, once the food is consumed, the effect of digestion through the gastrointestinal system must be taken into account [69]. Foods that contain probiotic microorganisms with beneficial properties represent the largest segment of functional food on the market. For benefits to be obtained, foods containing probiotics must be consumed regularly and the food matrix must contain a minimum amount of viable probiotic microorganisms. The use of fruits and vegetables as vehicles for probiotic microorganisms represents a challenge. However, several factors can influence the viability of the probiotic microorganism. These factors can be inherent to the food matrix, such as fat and protein content, sugar composition, pH, and presence of antimicrobial substances, in addition to those linked to the process (oxygen level, presence of preservatives, storage time, and temperature) [70]. In this regard, probiotic microorganisms must survive not only the shelf life of the food product but also the passage through the gastrointestinal tract (GIT) [35]. The low pH of the stomach combined with the presence of bile in the intestine can affect survival, which could directly influence the proliferation and colonization of probiotics in the intestinal tract [71].

Although the *in vitro* test has limitations to assess the viability of a probiotic strain in humans, it is very useful for selecting the strains that, when introduced into a food matrix, behave more satisfactorily [35] [71]. **Table 3** shows some studies focused on the gastrointestinal viability of some strains with probiotic activity.

The main concern of the industry is ensuring the viability of probiotics is essential, due to the adverse conditions of food matrices, which can affect the viability and gastrointestinal resistance of these microorganisms. Therefore, the results found in several studies strengthen the processing and marketing of these products, ensuring the transmission of probiotics to consumers [71].

**Table 3.** Gastrointestinal viability tests of probiotic strains.

<i>Strain</i>	<i>Food Matrix</i>	<i>Study</i>	<i>Reference</i>
<i>Bifidobacterium animalis</i>	Juçara juice	<i>In vitro</i>	[53]
<i>Ligilactobacillus salivarius</i>	Apple juice	<i>In vitro</i>	[70]
<i>Lacticaseibacillus rhamnosus</i>	Pineapple juice with juçara	<i>In vitro</i>	[71]
<i>Lacticaseibacillus rhamnosus</i>	Guava juice	<i>In vitro</i>	[72]
<i>Lacticaseibacillus casei</i>	Mao luang Juice	<i>In vitro</i>	[73]
<i>Lactobacillus acidophilus</i>			
<i>Lacticaseibacillus rhamnosus</i>	Apple juice	<i>In vitro</i>	[74]
<i>Lactiplantibacillus plantarum</i>	Apple juice	<i>In vitro</i>	[75]

#### 4. Effects of the Food Matrix on Cell Viability

In dairy-based probiotic foods, the physical-chemical composition of the milk, which is rich in proteins and lipids (fats), acts as a protective matrix for probiotics and these factors help the survival of probiotics from adverse conditions of the stomach and small intestine. However, matrices of non-dairy foods are very different from those based on dairy products; they are more versatile and less understood [65].

The big challenge is the application of probiotic cultures in different beverages based on food matrices. Different probiotic species show different sensitivities concerning substrate acidity, dissolved oxygen, post-acidification in fermented beverages, metabolism products, temperatures, and conditions of the gastrointestinal tract [67].

To exercise their probiotic activity, the live microorganisms must be in an adequate quantity, resisting the adverse conditions of digestion and reaching the intestine in a sufficient dose to effectively develop and promote the benefits to the host. The viability and metabolic activity of the bacteria are important characteristics of the inclusion of probiotics in beverages. This occurs because the bacteria need to survive in the beverage during the expiration date and on the gastrointestinal digestion [67]. Therefore, the choice of the food matrix is an essential part to maintain the probiotic viability in the final product. Fruit juices and smoothies can be challenging matrices and their effects on probiotic viability are worth investigating since organic acids and phenolic compounds commonly present in fruits can exhibit microbiological properties. On the other hand, the content of the phenolic compounds can contribute to the survival of probiotic bacteria in food and even exert an effect similar to a prebiotic in the human intestine [53].

The formulation of beverages can favor their stability. Storage temperature is also a relevant factor in maintaining probiotic activity. There is a general recommendation that probiotic foods should preferably be stored between 4°C and 5°C [53].

Sugar supports the multiplication of probiotics, so, in theory, the use of sugar by the probiotic in juices will decrease the sugar content and increase the acidity of the juice [76]. In general, according to previous studies, the growth and viability of probiotic bacteria in fruit and vegetable beverages depends on the species and variety of bacteria used, the pH, and the concentration of lactic and acetic acid in the final production [77]. The applicability of probiotics in food products generally depends on factors such as water activity, processing, and storage temperature, expiration date, oxygen content, pH, mechanical stress, salt content, and content of other harmful or essential ingredients [78]. The non-dairy sources are fortified with acidulants that can increase the shelf life by creating an anaerobic environment that is ideal for probiotic cultures, which is achieved by eliminating the available oxygen. One more advantage is that these juices stay much less time in the stomach and, therefore, probiotic species spend much less

time in the acidic environment of the stomach [65].

Strategies can be used to improve the viability of probiotic microorganisms, such as proper selection of acid and bile resistant strains, use of oxygen-impermeable containers, two-stage fermentation, microencapsulation, and incorporation of micronutrients such as peptides and amino acids [37].

Technological advances have made it possible to alter some structural characteristics of the matrices of fruits and vegetables, modifying components of these foods in a controlled manner, which can make them ideal substrates for probiotic strains [78].

The survival of the bacteria within the host and the preservation of dedicated properties remains a problem, even with the use of encapsulation. The optimization of the process and product design, cell viability and probiotic functionality, and strict fermentation quality control (culture medium or food matrix, pH, temperature, carbon source composition, and fermentation time) and post-fermentation processing (spray drying, lyophilization, homogenization, mixing and high-pressure tablets, packaging, etc.). Sublethal stress during production can be useful to improve resistance to probiotics in foods and food additives can be avoided by encapsulation process [31].

## 5. Consumer Study and Potential Market

Sensory evaluation is a very important issue and has a direct association with product quality, processing characteristics, and consumer acceptability. Therefore, an appropriate selection of substrate composition and formations is necessary [77]. In addition to the above challenges, the sensory characteristics and general acceptance of non-dairy probiotic products also have some limitations. Thus, sensory evaluation of probiotic microorganisms in non-dairy products and consumer acceptance testing are of vital commercial importance [37].

It is important to consider the sensory acceptance by consumers during the development of non-dairy probiotic products, concerning appearance, aroma, texture, or flavor, to convey the direction for the production and ideal formulation of these products, always observing which are the expectations of consumers about these products. The attractive taste and the refreshing profile offered by fruit juices have stimulated a genuine interest in the industry for the development of fruit juices with the addition of probiotics [65]. Interactions between different probiotic strains and food substrates, where textures, flavors, aromas, and colors can be improved or aggravated by the production of different metabolic compounds, such as lactic acid and other metabolites during processing and storage, can influence the sensory properties of probiotic foods non-dairy. When preparing food with the addition of probiotics, the probiotic bacteria ferment the carbohydrates present in fruits, vegetables, cereals, and vegetables, releasing gases and alcohol. Some individuals report that the addition of probiotics to fruit juices can result in flavors described as “milky”, “medicinal”, “acidic”, “salty”, “bitter”, “astringent”, “artificial”, or “earthy”.

Some studies show that probiotics do not affect the general acceptance of fruit juices, this can happen depending on the type of fruit, the probiotic organism, the temperature at which they are stored, and the supplementation of prebiotics [37].

However, inadequate content of aromas (perfumery, dairy products) and flavors (sour, salty) have been reported when *Lactobacillus plantarum* was added to juices. A sensory impact study showed that consumers prefer the sensory characteristics of the conventional orange juice to their functional equivalent (juice containing probiotics), but if their information on health benefits is provided, preference increases over conventional orange juice [78].

The perception of unpleasant flavors in juices, resulting from the addition of probiotics that contribute to consumer dissatisfaction, can be overcome by adding 10% (v/v) of tropical fruit juices [65].

The value of the global market for probiotics is around \$15 billion per year and is increasing by 7%. Today, these probiotic products represent between 60% and 70% of the total functional food market, demonstrating their importance. The global probiotic food and beverage market was worth around 24.8 billion euros in 2011 and more than 31.1 billion euros in 2015 [37]. This value is expected to reach up to \$69.3 billion by 2023, which also represents the driving force behind the functional beverages market. The estimate in 2019 was that the market for supplements containing probiotics would increase from \$48 billion to \$62 billion in 2022.

Non-dairy food products have gained popularity in the past decade. It is expected that the food products business reach approximately \$26 billion over the next five years. However, the manufacture of fermented probiotic foods at the commercial level faces many challenges, including the selection and identification of economical and abundant substrates, reducing operating expenses, and improving probiotic viability [44]. The willingness of the consumers to buy products incorporated with probiotics explains why they use functional beverages to improve their health [43].

The first non-dairy probiotic was produced by a Swedish company called Skane Dairy in 1994, since then many non-dairy probiotic drinks are already on the market. The basis of this product was oat flour fermented by *Lactiplantibacillus plantarum*. A similar product Good Belly (another company), prepared from oats and *Lactiplantibacillus plantarum*, was the first non-dairy probiotic launched on the US market in 2006 [37].

## 6. Technological Challenges in the Food Industry with the Addition of Probiotics

The selection of the appropriate probiotic strains in an appropriate dose and food matrix is the first requirement for the development of a food product with the addition of probiotics. Fruits, vegetables, and cereals represent a good matrix of probiotic bacteria with good nutraceutical components. However, some limitations can prevent the production of non-dairy probiotics at an industrial level,

such as sensory characteristics, general acceptance, and, most importantly, the survival of probiotics over storage [79] [80]. To achieve these health benefits, the viability of probiotics through different conditions after consumption is crucial. The selected microorganisms must be able to tolerate bile and acid, colonization in the human intestine, good adhesion to people's epithelial cells; good growth characteristics, not being pathogenic and good impact on people's health [48].

The probiotic strains selected for use in the food industry must be stable during storage as chilled, frozen, or dried crops, and suitable for large-scale industrial production with the ability to survive and maintain their functionality [10].

The greatest difficulty in the production of dairy beverages with probiotic properties is the preservation of the product's physical stability. The optimization of the process of these beverages needs more care, including a selection of concentration and type of stabilizer and optimization of pretreatment conditions, such as homogenization regimes. Storage at room temperature, which is common in many types of non-dairy products, can create a major challenge for probiotic viability [37].

The proper scientific validation of claims for functional food products remains a critical issue for food science. Especially issues of safety, biocompatibility, and health claim of functional milk-based and/or non-dairy beverages are often recorded or evaluated with inadequate/insufficient methodologies. An interdisciplinary approach must be followed to create a solid scientific base for the functionality of the beverages in question [78].

New products need to take advantage of emerging technologies based on nanotechnology, high-pressure homogenization during processing, and methods that better preserve viability during storage, to increase the manufacture and consumption of products with the addition of probiotics [49].

Non-dairy foods must be developed with the addition of probiotics, allowing the consumption of these beneficial microorganisms by people who do not like dairy products or who are intolerant or allergic to milk components. There are two main challenges with a probiotic product: the maintenance of the physical-chemical and sensory characteristics equivalent to conventional products without probiotics and the maintenance of the viability of the microorganism during the lifespan of these products with the guarantee of the passage of the probiotic through the gastrointestinal tract, reaching in a sufficient quantity in the intestine to exert its probiotic activity [81]. Despite the challenges, the future of non-dairy products with probiotics is promising [37].

## 7. Conclusions

Since fruits and vegetables can be used as raw materials for a probiotic fermentation, this takes into account the biochemical and physicochemical composition of these raw materials. In addition to the content of health-promoting phytochemicals in fruits and vegetables, they also offer several advantages such as adding value to products and extending the shelf life of processed foods.

The development of new technologies that are more economically appropriate, and of matrices with technological potential for the industry is extremely important for the supply of non-dairy probiotic foods according to the demand they have. Although there is great potential for the use of fruit juices as probiotic products, there are few reports on their preparation and production and this needs to be intensified.

Innovative technologies for the preparation of probiotic food products to improve their nutritional value need to be an urgent priority area.

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## Conflicts of Interest

The authors declare no conflict of interest.

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