



Trends and challenges for the application of probiotic lactic acid bacteria in functional foods

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ABSTRACT: It is increasingly challenging for the food industries to develop products which meet the consumers' demands. They seek foods that are innovative and present health benefits. In this review, the main objectives are to show the tendencies and innovations in the dairy food market and to indicate the challenges to apply probiotic bacteria to non-dairy products. Moreover, the safety of probiotic lactic acid bacteria (LAB) to be applied to food products and the beneficial effect of probiotic bacteria on the intestinal microbiota and overall human health were also discussed. We considered that the development of probiotic fermented products added with fruits and fruit by-products, cereals or other vegetables aligns with the market tendencies and the consumers' demands.

Key words: health, innovation, nutrition, fermentation, vegetables.

Tendências e desafios para aplicação de bactérias lácticas probióticas em alimentos funcionais

RESUMO: Para as indústrias alimentícias é cada vez mais desafiador desenvolver de produtos que atendam às demandas dos consumidores. Eles buscam alimentos que sejam ao mesmo tempo produtos inovadores e apresentem efeitos benéficos à saúde. Neste trabalho, os objetivos principais são mostrar as tendências e inovações no mercado de lácteos e apontar os desafios para aplicação de bactérias probióticas em produtos não lácteos. Adicionalmente, a segurança das bactérias ácido-lácticas (BAL) probióticas para uso em alimentos, e os efeitos dos probióticos na microbiota intestinal e na saúde humana também foram discutidos. Consideramos que o desenvolvimento de produtos fermentados probióticos com adição de frutas e subprodutos, cereais ou outros vegetais atende as tendências do mercado e a demanda dos consumidores.

Palavras-chave: saúde, inovação, nutrição, fermentação, vegetais.

INTRODUCTION

Milk plays a remarkable role in Brazilian market both due to its production and to the diversity of dairy products. Fermented dairy products stand out, since they are consumed regularly, present high nutritional value and many health benefits, in addition to being recognized as healthy food [by the consumers]. However, there is a growing the interest in probiotic and fermented non-dairy products, including functional juices, teas and plant-based beverages (VALERO-CASES et al., 2020; KAUR et al., 2022).

In general, to produce fermented products, including dairy, meat and vegetables, lactic acid bacteria (LAB) are used. LAB ferment the food matrix and can improve the sensory properties, the nutritional value, and play an important role in food safety. Moreover, some of them can beneficially modulate the host' metabolism; those are known as probiotics (PENNA et al., 2015; BORGES et al.,

2019). Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host (HILL et al., 2014). However, the Brazilian Agency of Sanitary Surveillance (ANVISA), the European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA) do not attribute the ability to prevent or treat diseases to the administration of probiotics (GIBSON et al., 2017; BRASIL, 2018; PLAZA-DIAZ et al., 2019).

Strains of *Lactobacillus*, *Enterococcus*, *Bifidobacterium* (*B.*) and *Saccharomyces boulardii* and *Escherichia* (*E.*) *coli* have a long history of safety and are the most widely used probiotic bacteria (PLAZA-DIAS et al., 2019). Recently, the *Lactobacillaceae* and *Leuconostocaceae* families were reclassified, and the *Lactobacillus* (*Lb.*) genus was divided into 25 genera; *Lacticaseibacillus* (*Lc.*), *Lactiplantibacillus* (*Lp.*), *Levilactobacillus*, *Ligilactobacillus* and *Limosilactobacillus* are examples of the new genera

with probiotic strains (ZHENG et al., 2020). Probiotic bacteria can (i) modulate the microbiota; (ii) promote the strengthening of the intestinal barrier; (iii) reduce pathogen competition; (iv) relieve constipation; (v) improve the symptoms of colitis and necrotizing enterocolitis; (vi) contribute to reduce overweight, obesity and blood glucose; (vii) modulate the immune system; (viii) decrease atopic and food allergies; (ix) regulate the central nervous system; (x) alleviate some of the symptoms of autism, depression and anxiety, among others. It is important to emphasize that the functionality of probiotics in health is specific to the strain used; therefore, it cannot be generalized for all the species of the genus or to the genus. Moreover, these therapeutic properties are effective only if the probiotic products are consumed regularly (LEO et al., 2019; XAVIER-SANTOS et al., 2020).

To have positive physiological effects, probiotic strains need to be viable at a minimum concentration ($> 6 \log$ CFU/mL) in the final product throughout its shelf life (COSTA et al., 2019; PIMENTEL et al., 2019; STAVROPOULOU et al., 2020). Although, many commercial products present probiotic strain quantities ranging from 10^9 to 10^{10} CFU/dose, there are products which demonstrate beneficial effects in lower levels, whereas other products require large amounts; therefore, it is not possible to establish a general dose for products containing probiotic strains (LEO et al., 2019; XAVIER-SANTOS et al., 2020).

New probiotic strains have been studied, as well as the issues regarding their efficacy, safety and technological aspects in large-scale food processing. Most intestinal bacteria like *Roseburia* spp., *Akkermansia* spp., *Propionibacterium* spp. and *Faecalibacterium* spp. have shown promise as next generation probiotics (NGP); however, they are nutritionally demanding and very sensitive to oxygen (SAARELA, 2019, SANDERS et al., 2019; DO CARMO et al., 2020).

Besides probiotics, prebiotics, postbiotics and paraprobiotics also act by modulating the host metabolism and have direct effects on the immune response. The prebiotics are defined as substrates used selectively by host microorganisms, conferring a health benefit (GIBSON et al., 2017). The definitions of postbiotics and paraprobiotics continue to be a topic of debate within the scientific community. The International Scientific Association of Probiotics and Prebiotics (ISAPP) has proposed a standardized terminology, suggesting the use of the term “postbiotics” to encompass both inactivated microorganisms and their components that provide

health benefits to the host (SALMINEN et al. 2021). However, there are scientists who argue in favor of maintaining a distinction between these two terms. In this view, postbiotics are considered to be well-defined microbial substances with beneficial health effects. In contrast, paraprobiotics encompass inactivated and/or dead microorganisms for which the exact molecular factors responsible for conferring health benefits are unknown (AGUILAR-TOALÁ et al. 2021).

Improving human health through the modulation of microbial interactions during all stages of life is increasingly important to consumers, food manufacturers, healthcare professionals and regulators. In this scenario, the objectives of this review are to show the market tendencies for dairy products and the challenges to apply probiotic bacteria to non-dairy products. To provide a more comprehensive context for this review, we also presented the characteristics of probiotic LAB and their health benefits.

Lactic acid bacteria

LAB are facultative anaerobic or microaerophilic, fastidious, and acid tolerant microorganisms, which produce lactic acid as the main product of carbohydrates fermentation. Most LAB belong to the *Lactobacillus* and to the new genera, *Lactococcus*, *Leuconostoc*, *Streptococcus*, *Enterococcus* and *Pediococcus*. They are naturally present in nutritionally rich environment, as vegetables, dairy products, meat products and silages; they are included in the autochthone's microbiota of the oral cavity, vagina, and gastrointestinal tract of humans and animals (POON et al., 2020; ZHENG et al., 2020).

In addition to the organic acids, LAB can also produce many types of compounds, like enzymes, exopolysaccharides, aromatic compounds (diacetyl and acetoin), bioactive and antimicrobial compounds and vitamins (MELINI et al., 2019; ALAMERI et al., 2022). The production of B-group vitamins (thiamine, folic acid and riboflavine) and vitamin K by LAB improve the nutritional profile of fermented products, which can be used to help the reduction of the incidence of vitamin deficiencies in different population groups. Additionally, the use of vitamin-producing LAB may represent a more natural and consumer-friendly alternative to fortification using chemically synthesized vitamins (ALBUQUERQUE et al., 2020). LAB also produce antimicrobial substances, such as fatty acids, hydrogen peroxide, carbon dioxide, ethanol, acetaldehyde, and bacteriocins, which are responsible for bactericide or bacteriostatic effects against pathogenic and spoilage

microorganisms in food (REIS et al., 2012; LA FATA et al., 2018). So, these LAB should be more explored by food industries to develop innovative food.

Probiotic LAB are applied into a wide variety of food matrices, including dairy and meat products, beverages, cereals, vegetables, fruit and bread products (PIMENTEL et al., 2021, RASIKA et al., 2021); however, most of the probiotic products on the market are milk-based. Probiotic LAB are also present in nutritional supplements, as capsules, pouches or tablets (COSTA et al., 2019).

In order to promote the beneficial effect on the host, the maintenance of the activity, viability, and growth efficacy of the probiotic under technological treatment, storage and resistance to the passage through gastrointestinal tract (GIT) should be demonstrated (PLAZA-DIAZ et al., 2019). Additionally, the growth and viability of probiotic bacteria in the food matrix depend on the species and strains of the bacteria used, pH, presence of hydrogen peroxide and dissolved oxygen, concentration of metabolites, such as lactic and acetic acid, average buffering capacity, storage temperature, nature of the ingredients added, and the presence of competing and inhibiting microorganisms (MEYBODI et al., 2020; RAMOS et al., 2020). Furthermore, probiotic strains must also attend many technological criteria, such as to be easily cultivated in large scale, present genetic stability and be able to maintain its viability in food products and supplements (PENNA et al., 2015). During fermentation, the growth of probiotic bacteria in milk is slow and their surviving rate during storage is low. So, the addition of prebiotic substances, the suitable selection of starter cultures and the inoculum level to improve its growth need to be evaluated (MEYBODI et al., 2020).

Safety of probiotic LAB for food use

Although LAB are generally considered safe and offer health benefits to the host, concerns about their safety properties have been increasing, since some strains from different genera can present virulence factors, resistance to antibiotics or were associated to the risk of development of diseases (CASAROTTI et al., 2017; XAVIER-SANTOS et al., 2020). Thus, to guarantee the safety of a strain used in food, it is mandatory to check some characteristics to demonstrate that the strain is safe for human consumption and considered GRAS (*Generally Recognized As Safe*), such as: (i) unequivocal taxonomic classification in species level, strain and origin; (ii) complete characterization by total genome sequencing analysis in order to detect

possible genes related to virulence, resistance to antibiotics and potential for horizontal transference, or other potential adverse metabolic effects, and the production of biogenic amines; (iii) initial indication of presumed safety in relation to known probiotic strains; (iv) absence of harmful enzymes activity; (v) absence of history of pathogenicity, infectivity and toxicity; (vi) studying potential invasive adherence and strain pharmacokinetics; and (vii) interaction between strain from intestinal microbiota and the host (BRAZIL, 2018; LEO et al., 2019; SAARELA, 2019). Checking all these factors must guarantee that the probiotic use is safe in the dose recommended, the period of supplementation, the duration of effects and their potential for contraindications (XAVIER-SANTOS et al., 2020).

In many studies available in the literature, the safety of probiotic strains isolated from food matrices was evaluated for future application to food production, such as *Enterococcus* sp. and *Lactobacillus* sp. isolated from water-buffalo mozzarella cheese (NASCIMENTO et al., 2019) and from cheese whey (LOZANO et al., 2022), *Lactiplantibacillus (Lp.) plantarum* isolated from Bulgarian Shpek salami (TODOROV et al., 2017), *Lactobacillus* sp. and *Saccharomyces* sp. isolated from Pupunha fruit (SILVA et al., 2021), *Enterococcus faecium* isolated from human milk (KHALKHALI & MOJGANI, 2018) and *Lactobacillus* strains isolated from fermented vegetables (CHEN et al., 2021).

Beneficial effects of probiotic bacteria

The microbial colonization of the gut in infants occurs during and after birth; it includes vertical (maternal factors and birth mode) and horizontal transfers (from the immediate environment, such as breast or formula feeding, diet, use of antibiotics, hormonal and metabolic statuses, genotype, and immune system) (JIAN et al., 2021). This intestinal microbiota plays a pivotal role in several metabolic pathways in the host. Alterations in intestinal microbiota composition and function are widely associated with the development of metabolic diseases (REGNIER et al., 2021). Chronic metabolic diseases include a series of clinical metabolic syndromes, such as obesity, diabetes, hypertension, dyslipidemia, non-alcoholic fatty liver disease, kidney stone disease, and cardiovascular diseases (TIAN et al., 2023; KNEZ et al., 2023). These conditions primarily arise from a lack of physical activity and an unhealthy diet characterized by excessive consumption of saturated fats and refined sugars (KNEZ et al., 2023).

Recent studies showed that some probiotic strains have been applied to reduce or prevent these metabolic diseases. As an example, *Lp. plantarum* has exhibited the capacity to alleviate the symptoms of chronic metabolic diseases by effectively controlling inflammatory and oxidative stress levels. This is achieved by modulating the production of cytokines and short-chain fatty acids (SCFAs), regulating the activity of antioxidant enzymes, and maintaining a healthy balance in the microbial communities of the intestines (TIAN et al., 2023). KNEZ et al. (2023) demonstrated that fermented vegetables, such as pickled vegetables and legumes, may influence metabolic diseases by triggering epigenetic changes that lead to the inhibition of lipogenesis and decreased appetite. Additionally, fermented vegetables presented higher bioavailable antioxidant contents and the microorganisms used for the fermentation processes produced enzymes, which reduced the antinutrient compounds.

When the gut microbiota is stable or balanced (eubiosis), it avoids that potentially pathogenic microorganisms cause harmful effects. In contrast, a disruption or imbalance of the homeostatic state of the gut microbiota (dysbiosis) is characterized by quantitative and qualitative changes in the microbiota composition. These changes can result in different interactions in the host microbiota and can contribute to the development of non-communicable diseases or other diseases, often with inflammation (PLAZA-DIAZ et al., 2019). Although, no definition for a healthy gut microbiome was proposed (CANI et al., 2021), gut dysbiosis triggers a chronic low-grade pro-inflammatory status in the host by increasing the permeability of the gut barrier (“leaky gut”) and by facilitating the endotoxemia, which is characterized by translocation of bacterial antigens into the bloodstream (SAFADI et al., 2022). However, gut dysbiosis cannot be used as a unique marker of disease or as a unique risk factor. Dysbiosis is influenced by endogenous and exogenous factors, which include diet, lifestyle (physical activity, alcohol consumption, medication, stress, smoking) age, genetics, hygiene, environmental factors, human pathologies (immunological or metabolic and neoplastic disorders, neurodegenerative and neuropsychiatric conditions), among others (CANI et al., 2021).

The probiotic effects were supposed to be mediated through a direct interaction with commensal microbiota, favoring eubiosis. However, nowadays, the probiotic effects are not considered to be only microbiota-mediated, and, indeed, other types of mechanism are known (SANDERS et al.,

2019). The main beneficial effects provided by the consumption of probiotic fermented products or probiotic supplements are summarized in table 1, although not all mechanisms of action of probiotics on the microbiota have been confirmed in humans. The mechanisms of action of probiotics involve (i) colonization and normalization of perturbed intestinal microbial communities in both children and adults; (ii) competitive exclusion of pathogens and bacteriocin production; (iii) modulation of enzymatic activities related to metabolization of a number of carcinogens and other toxic substances; and (iv) production of volatile fatty acids, namely, SCFAs and branched chain fatty acids (BCFAs) that maintain energy homeostasis and regulate the functionality of peripheral tissues. Probiotics increase intestinal cell adhesion and mucin production, modulate the activity of gut-associated lymphoid tissue and the immune system (PLAZA-DIAZ et al., 2019; RANADHEERA et al., 2019).

Probiotic bacteria also produce neurochemicals with the potential to act on the brain via the vagus nerve. Gut-brain signaling influences metabolic, immunological, hormonal and neural mechanisms and potentially affects mood, pain and cognition. Evidence suggests that probiotics may positively affect responses to stress and anxiety among human subjects in experimental settings. However, more research is required to confirm these preliminary findings (CHEN et al., 2022).

Data available in the literature have shown that eating habits are positively correlated with the human intestinal microbiota, since dietary changes can alter its diversity and composition. Thus, beneficial modulation of the human intestinal microbiota by food can occur through the ingestion of probiotics, prebiotics and synbiotics. The amount and frequency of consumption of probiotics necessary to ensure the functional benefits attributed to them is still an issue under discussion (ZMORA et al., 2019). Although stable colonization is not necessarily required for probiotics to exert benefits on host health, a substantial population must be present, at least transiently, for them to have a metabolic impact on the host and the resident gut microbiota (OJIMA et al., 2022).

The effect of a single strain or multiple strains of probiotics on human health has been the topic of some studies. As an example, the effects of single strains and mixtures of probiotic bacteria on the immune profile in liver, spleen, and peripheral blood of C57BL/6 mice were studied by FONG et al. (2022). Healthy mice received orally the probiotics *Lc. rhamnosus* strains GG (LGG) and

Table 1 - Main health benefits provided by the consumption of probiotic product.

Beneficial effects	Description	Reference
Interaction with gut microbiota	Enhancing the production of antimicrobial compounds, promoting substrates transformation and cross-feeding, as well as supporting microbiota stability. Increasing the colonization resistance by the competition for nutrients and location. Stimulating the production of organic acids, as lactate, propionate and acetate, as well as butyrate through cross-feeding, and decreasing the intestinal pH, which inhibits the growth of pathogens.	(SANDERS et al., 2019)
Immunomodulation	Improving the gut microbial population, increasing mucus secretion, and preventing the destruction of tight junction proteins by decreasing the number of lipopolysaccharides. Decreasing gut dysbiosis and intestinal leakage. Improving the differentiation of T-cells against Th2 and development of Th2 cytokines IL-10.	(CRISTOFORI et al., 2021)
Physiological	Releasing small molecules with systemic effects, such as neurochemicals (cortisol, serotonin and gamma-aminobutyric acid - GABA), tryptophan and histamine derivatives and conjugated linoleic acid, which are related to brain function. Increasing nutrient absorption and mucus secretion, improving intestinal barrier function, and reducing blood glucose and cholesterol. Increasing the production of β -galactosidase and bile salt hydrolase enzymes, which improve lactose digestion and blood lipid profiles in humans.	(SANDERS et al., 2019)
	Increasing the production of SCFAs by intestinal microbiota, and reduction of the obesity by stimulation of the intestinal release of the satiety-inducing hormones peptide YY and glucagon-like peptide-1 (GLP-1) by decreasing systemic inflammation or by signaling to the brain.	(SILVA et al., 2020)
	Decreasing the toxic effect of heavy metals and xenobiotics mainly by intestinal sequestration and intestinal barrier protection and by alleviating oxidative stress.	(FENG et al., 2020)
	Reducing gut inflammation and preventing allergies, such as atopic dermatitis (eczema) and allergic rhinitis in infants.	(OJIMA et al., 2022)
Others	Psychobiotics can produce or stimulate the production of neurotransmitters and enteroendocrine hormones which can be useful for therapeutic neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, autism spectrum disorder, stress, depression and anxiety, among others.	(SHARMA et al. 2021)

LC705, *B. breve* 99 (Bb99), *Propionibacterium freudenreichii* subsp. *shermanii* JS (PJS) and *E. coli* Nissle 1917 (EcN), and two of their mixtures. The probiotic mixtures had different immunological effects than the single strains, confirming that the immunomodulatory potential of probiotics is strain- and organ/tissue-specific, and the effects of probiotic mixtures cannot be predicted based on their single constituents.

Different probiotic strains (*Lb. acidophilus* La-3 and La-5; *B. animalis* subsp. *lactis* BB-12 or *Lc. casei*-01) used to produce probiotic fermented whey-milk beverages were evaluated on the physicochemical characteristics, biological activity, and bioactive peptides. *Lc. casei*-01 exhibited increased metabolic activity and demonstrated the presence of anti-hypertensive peptides. Conversely, *Lb. acidophilus* La-5 and *B. animalis* subsp. *lactis* BB-12 displayed higher α -glucosidase inhibition, leading to improvements in the high saturated hypercholesterolemic index. Moreover, these strains produced peptides with ACE-inhibitory, antimicrobial, immunomodulatory, and antioxidant activities (ROSA et al., 2023).

The administration matrix also influences the beneficial effects on human health. The probiotic properties of *B. animalis* subsp. *lactis* (BB-12) and *Lb. acidophilus* (LA-5) in different administration forms (capsules and yogurt) were evaluated by PÁPAI et al. (2021). The probiotic bacteria survival rates to gastrointestinal conditions were slightly higher and the anti-inflammatory effects (reduction of colitis and secretion of pro-inflammatory cytokine IL-8) were limited when mice were treated with the probiotic strain on a yogurt matrix.

In recent years there has been a significant increase in studies assessing the interaction between foods (including probiotics and prebiotics), microorganisms and their metabolites, and the use of probiotics in medicine, aiming to reduce dysbiosis and symptoms of various diseases. Treatments with antibiotics, inadequate diet, stress, and malfunction of the lymphoid tissue system lead to dysbiosis and increased intestinal permeability, and infectious and immune-mediated diseases (allergies and autoimmune disorders) can occur. In contrast, the administration of probiotics improves the immune system in several ways: it stimulates the production of IgM natural

antibodies and IgG levels, increases local IgA antibodies and increases the phagocytosis capacity that modulates cytokine presence. Probiotics have a beneficial impact on the immune system, stimulate non-specific immune response, improve various diseases and alleviate allergies (WANG et al., 2019; STAVROPOULOU et al., 2020). In fact, patients with diabetes underwent treatment with synbiotics containing the probiotics (*Lactobacillus* sp., *Bifidobacterium* sp. and *Streptococcus thermophilus*), prebiotics (FOS), group B vitamins, lactose, maltodextrin and magnesium. The consumption of synbiotics reduced the level of fasting glucose, glycated hemoglobin, and the lipid profile remained constant (EBRAHIMI et al., 2017).

Trends in the dairy market

The current trend for the food market considers new foods and drinks that fall into 6 categories: nutritional density and convenience; digestibility and well-being; functionality and prevention; control and adequacy; exclusivity and sensoriality; sustainability and naturalness (FOOD INGREDIENTS FIRST, 2019a). The dairy sector aligns with this trend and has developed several products to meet consumer expectations (REGO et al., 2017).

Considering the *Nutritional density* category, milk is food of excellent nutritional value, considered essential to humans (RODRIGUES et al., 2019). In this regard, many dairy companies have invested in the development of products that meet the categories *Digestibility and well-being, functionality, and prevention*, mainly with the launch of lactose-free products, with sugar and/or fat reduction, with high protein content and/or mineral addition. Some foods, in addition to nutrition, may have a beneficial effect on health and are known as functional food (GRANATO et al., 2020). Probiotic and prebiotic ingredients are usually used to produce functional food (XAVIER-SANTOS et al., 2020). The appropriate dose of prebiotic must be sufficient to generate the prebiotic effect, but without inducing undesirable or adverse effects, as excessive gas formation or non-selective use (GIBSON et al., 2017). They are now recognized for their system-wide metabolic and physiological effects. Prebiotics are applied to induce modification of the gut microbiota to improve host health because they are affordable, effective, safe, and accessible (SANDERS et al., 2019).

Various prebiotic substrates have been studied; oligosaccharides, such as fructooligosaccharides (FOS) and galactooligosaccharides (GOS), have been reported to stimulate the growth of *Bifidobacterium* sp.

and *Lactobacillus* sp. Other potentially prebiotic ingredients include lactulose, resistant dextrin, polysaccharides, as polydextrose, arabinoxylans, xylooligosaccharides, and resistant starches, as well as some polyols, as lactitol and isomalt. Prebiotics such as human milk oligosaccharides (HMOs) can confer a fitness advantage to probiotic strains and facilitate colonization in the human gut, but only if the genes to utilize them are also present in the bacterial genomes (OJIMA et al., 2022). Polyphenols are prebiotics that favor the development of beneficial microorganisms in the microbiota and contribute to potential health effects for individuals (XAVIER-SANTOS et al., 2020). Symbiotic products are obtained by the combination of probiotic microorganisms and prebiotic ingredients (COSTA et al., 2019; XAVIER-SANTOS et al., 2020, CHEN et al., 2022).

For the development of these functional foods, milk and dairy products are among the main matrices used, and among them, fermented dairy products are singled out as the most important type. Another trend in developing functional dairy products is to add vegetables (carrots, spinach, beets, ginger, etc.), fruit or fruit pulp, cereals, nuts and leafy greens, or their by-products (peel, stalk and seeds). These combinations of dairy products with vegetables provide the creation of new experiences and sensations for consumers, in addition to increasing the nutritional value (ZAHID et al., 2021). Fruits, vegetables, legumes, and cereals have a high content of nutrients, such as antioxidants, fiber, minerals, vitamins, carotenoids, which can make them ideal substrates for the growth of probiotic bacteria. Their by-products can be used to develop new products, as a source of dietary fibers, bioactive compounds or prebiotics, which can improve sensory properties, such as flavor, aroma, texture and color, diversifying the products available on the market (CASAROTTI et al., 2018; RASIKA et al., 2021). Some studies indicate beneficial effects of the incorporation of vegetables or their by-products in fermented milks. Skimmed probiotic yoghurts added with buriti showed a better profile of fatty acids, higher content of total phenolic compounds, β -carotene, lycopene and antioxidant activity (BORGONOVI et al., 2021). In a study using the Human Microbial Ecosystem Simulator (SHIME®), probiotic fermented milk was able to modulate the human microbiota (CASAROTTI et al., 2020; BORGONOVI et al., 2022).

Probiotic fermented milks containing fruits and vegetables meet the *Exclusivity and sensoriality* category. The same can be affirmed for fresh, premium and gourmet dairy products, and products made with

milk from other species, such as sheep, goat, buffalo and camel milk (REGO et al., 2017; AYYASH et al., 2021; KOWALCZYK et al., 2021). Furthermore, original and innovative products serve to reduce daily stress and provide moments of relaxation and pleasure and meet consumers' desire to try products that provide incomparable sensory experiences. This trend favors the launch of products with different flavors, such as probiotic sheep milk ice cream with apple fiber (KOWALCZYK et al., 2021).

For the development of fermented products with exotic or innovative ingredients, such as some typical fruits from the Brazilian Amazon, Cerrado and Caatinga, or unconventional food plants, the concern is to ensure that the added products do not affect the survival and/or activity of the probiotics (MONTEMURRO et al., 2021). In the Brazilian market we already have several examples of dairy products that fall into this category. In addition to products available on the international market, such as yogurt with honey, almonds, sesame and orange blossom, and Greek yogurt, natural and beetroot flavored, or added with cumin seeds and dill, we also have fermented milks and yogurts with apricot, blueberry, and ginger flavor, and blackberry flavored sheep's milk yogurt (REGO et al., 2017; FOOD INGREDIENTS FIRST, 2019a). In Europe, products formulated with a mix of vegetables are available: (i) strawberry, blueberry, blackberry, elderberry and chai spices, (ii) mango, orange, peach, tangerine, carrot and turmeric, and (iii) apple, kiwi, pear, cucumber, spinach, spirulina and mint (SATIN, 2019).

The addition of fruit pulp to fermented milk can have a positive or negative effect on bacterial growth and this depends mainly on the strain, the type of fruit and the concentration of bioactive compounds, especially polyphenols, found in the fruit (RAMOS et al., 2020). BORGONONI et al. (2021) compared the effect of passion fruit and buriti pulps on probiotic and starter LAB growth and on fermented and acidified milk. *Lactocaseibacillus (Lc.) casei* SJRP38 showed the best results in the presence of passion fruit and the lowest reduction in the presence of buriti. The addition of fruit pulps into fermented and acidified milk increased the total phenolic compounds. Fermented milk with buriti pulp stood out for its high amount of total phenolic compounds, which indicates its high potential to be used as a functional food.

In the *Control and adequacy* category are included the products with reduced fat, sugar or salt contents, aiming to meet the need for products to favor the reduction of blood pressure (hypotensive), reduction

in the incidence of cardiovascular diseases and related diseases, products to reduce weight or cholesterol, or even lactose-free products due to intolerance, prejudice or personal choice. Several products have been studied in this category, such as probiotic fermented blueberry juice with antidiabetic potential and probiotic yogurt for patients with lactose intolerance (MASOUMI et al., 2021; ZHONG et al., 2021).

In the category *Sustainability and naturalness*, consumers look for natural products, with fewer industrialized ingredients, also called "clean label" products, which strengthens the positioning of dairy products in the food market (REGO et al., 2017). These *clean label* products must contain simple and known ingredients, made only with natural ingredients, which do not look like or feel like chemical products and that have been subjected to minimal processing and with a pure, healthy nutrient profile (REGO et al., 2017; FOOD INGREDIENTS FIRST, 2019b). The natural food preservation method is considered favorable, since it does not negatively affect consumers' health and they may have a smaller impact on food nutritional and sensory properties. Studies have confirmed that an excessive consumption of synthetic food additives, like artificial sweeteners, preservatives like sulfites, nitrates, sorbates, or the use of antibiotics in animal feed for meat production, can cause gastrointestinal, respiratory (asthma), dermatological (allergy) and neurological diseases and cancer (SUEZ et al., 2014; SINGH et al., 2021, ELSHREIF et al., 2023). Additionally, the use of bacteriocinogenic LAB as natural antimicrobials in food processing have been encouraged by researchers and explored by food processors to protect the safety and quality of food (TAYLOR, 2018). For instance, BORGES et al. (2019) applied a promising biopreservative function of *Leuconostoc mesenteroides* subsp. *mesenteroides* SJRP55 in fermented cream, due to its antibacterial activity against *Listeria (L.)* spp. This strain caused a reduction on the *L. monocytogenes* population and had distinguished effect on the fatty acid profile, causing an increased conjugated linoleic acid content and decreased α -linolenic and oleic acids contents.

Challenges for applying probiotic bacteria in non-dairy products

Currently, there is an increasing demand for plant-based and non-dairy probiotic products due to various reasons, such as vegetarianism, allergies to dairy products, lactose intolerance, search for new flavors, health benefits and environmental impacts of dairy production (RASIKA et al., 2021). Non-

dairy sources, such as fruits, vegetables, cereals and legumes, contain a high level of beneficial substances to human nutrition, that include antioxidants, vitamins, minerals and dietary fiber (PANGHAL et al., 2018; RASIKA et al., 2021). However, the application of probiotic cultures to plant-based products represents a challenge for the development of new products, since these matrices may contain phenolic compounds with antimicrobial activity or excessive acidity which may reduce the viability of probiotics (CASAROTTI et al., 2018; BORGONOVİ et al., 2021).

The manufacture of plant-based fermented products often has problems associated with appearance and texture, such as phase separation. The acidification of plant-based products promotes protein destabilization which results in the separation of whey (GRASSO et al., 2020). In addition, working with plant-based raw materials increases the concern about chemical residues, such as allergens, pesticides and mycotoxins that can be present in the plant (HASAN et al., 2022).

Some studies available in the literature evaluated the influence of fruit matrices on LAB viability. As examples, MALDONADO et al. (2017) studied the potential of tropical fruit (carambola, guava, mango and pitaya) for the development of fermented lactic beverages added or not of whey. The LAB tested had better growth (log 7-8 CFU/mL) and the highest sensory acceptance rates (70% for taste, 75% for flavor and 90% for color) utilizing mango or guava.

SEPTEMBRE-MALATERRE et al. (2018) reviewed fruit and vegetable phytochemicals, as bioactive non-nutrient compounds, their production through the fermentation process and their potential interest for human health. The fermentation process changes the profile and types of bioactive compounds, improving food components bioaccessibility and bioavailability, which results in modifications of health-related properties. However, these properties are highly dependent on the vegetable material. Another study evaluated the administration of food supplements (wheat flour; taro/wheat flour; taro/wheat flour and probiotic *Lp. plantarum* IS-10506 or taro/wheat flour and beet juice) on the gut of prediabetic individuals for two weeks. This resulted in total weight loss ranging from 0.5 to 11 kg. Additionally, the abundance of genus *Butyrivibrio* in the gut microbiota was significantly different between the conditions with and without taro flour (SURONO et al., 2022).

More recently, food-grade encapsulation systems have been used as a trend to increase the

resistance of probiotic bacteria to harsh environmental and food processing conditions, like heat, pH, oxygen, enzymes, water activity, bile salts, and others (GUO et al., 2022). Several studies have shown that encapsulation is the best way to maintain the viability of these bacteria, since it guarantees their stability without altering the properties of the native strain, protects probiotics from adverse stressful conditions, regulates their release in the gastrointestinal tract and improves the fermentation process (YAO et al., 2023; ZHU et al., 2023).

The industrial encapsulation methods for probiotics include spray drying, lyophilization, emulsion, lipid-based delivery system, coacervation, extrusion, emulsion, reflectance window drying, electrospinning and electrospraying (YOHA et al., 2022). Animal and plant proteins (milk or whey protein, gelatin, soy protein, pea protein, gluten), plant polysaccharides (starch, maltodextrin, pectin), microbial and animal polysaccharides (chitin, chitosan, xanthan, dextran), plant extrudates (arabic and acacia gums), marine extracts (agar-agar, alginate, carrageenan) are most used wall materials for probiotics' encapsulation (RAJAM & SUBRAMANIAN, 2022). As an example, YAO et al. (2023) incorporated peach gum polysaccharide into soy protein-based microparticles to encapsulate probiotics, and this combination maintained the survival of *Lp. plantarum* 550 during digestion and gastric storage. A combination of alginate, xanthan gum and β -cyclodextrin was used for microencapsulation of *B. animalis* subsp. *lactis* BB-12. Microencapsulated probiotic was highly resistant to simulated gastrointestinal conditions, and the viability was above 6 log CFU/g at $-18\text{ }^{\circ}\text{C}$ for 4 weeks (HAIN et al., 2022).

Extensive studies about enzyme activities present in plant-based matrices and interactions between ingested food, adequate starter cultures for fermentation processes, production of metabolites and their health benefits, including modulation of intestinal microbiota and related benefits are still necessary.

FINAL CONSIDERATIONS

The dairy market trend points to the development of healthy products, which can contribute to the health and well-being of the consumer. That market has great potential to be explored, including the development of innovative products, with probiotic strains, differentiated flavors and longer shelf life. A concern for the application of probiotic LAB in fermented products is to ensure

that the strains are safe for use in food and that they are viable in sufficient quantity to provide beneficial health effects. Recent studies indicate that the addition of plant ingredients in dairy products increases the nutritional value and survival of probiotic microorganisms, as well as providing health benefits. Thus, dairy companies can take advantage of this trend to develop probiotic dairy products containing typical Brazilian fruits, often considered exotic fruits, and launch products with typical characteristics, valuing exclusivity, sensoriality and naturalness. Moreover, non-dairy probiotic products are a promising field of research that has the potential to provide nutritious alternatives to dairy-based products for those who are lactose intolerant or prefer plant-based options.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

ASS prepared first draft of the manuscript. SNC and ALBP supervised and coordinated the writing and revised the whole manuscript. All authors critically revised and approved of the final version of the manuscript.

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