



Fruit based probiotic functional beverages: A review

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ABSTRACT

Probiotics are living bacteria and yeasts that nourish the human body, particularly the digestive system. Fruit-based beverages are an excellent vehicle for delivering probiotics to the body since they are frequently taken in large quantities and include a number of nutrients that promote the growth of these beneficial microbes. When adding probiotics to a fruit-based drink, the probiotics must survive and remain viable in the drink until ingested. This may be accomplished by choosing probiotic strains that are known to be substantial, as well as keeping the drink at the appropriate temperatures. It's also worth noting that not all probiotics have the same characteristics, and different strains of bacteria and yeast may provide varied health advantages. Fruit-based probiotic drinks, fermented vegetables like sauerkraut and kimchi, kombucha, water kefir, and non-dairy yogurt prepared from plant-based milk are all prominent non-dairy sources of probiotics. These commodities are high in beneficial bacteria and can be used in a balanced diet to help with digestion. Individuals who are lactose intolerant or follow a vegan diet prefer these non-dairy probiotic alternatives. The purpose of this review to comprehensively cover the mechanism of fruit-based probiotics and sum-up the recent advances fruit-based probiotic beverages and value-added products from these probiotics. It covers the notion of probiotics, fruits as prebiotics, the comparative fermentation mechanism of dairy and non-dairy probiotics, and the physiochemical and sensory alterations that occur following fermentation.

1. Introduction

In recent years, consumers have grown more conscious of the link between health and food. Customers are now seeking healthier goods with higher nutritional qualities and particular ingredients in order to prevent health issues, enhance their quality of life, and extend their lifespan. Culminating in the creation of “functional foods” and “nutra-ceuticals,” are foods that improve consumer health and lower their chance of developing chronic illnesses [1]. Due to growing public knowledge of the advantages of probiotics for health, preserving the balance of the intestinal microbiota, and enhancing mucosal defences against pathogens, the demand for functional foods containing micro-organisms with probiotic qualities is rising quickly. Probiotics are advantageous bacteria that have therapeutic benefits on host organisms that consume them. Probiotic products include live microorganisms at concentrations greater than 10^6 to 10^7 cfu/ml to have a positive impact on the human body, and also it must be able to tolerate the challenging

circumstances found in the human digestive system [2].

Among other health benefits, probiotics enhance immunity, reduce inflammation, relieve gastrointestinal pain, and prevent diarrhoea. It works through a variety of different mechanisms. They produce anti-microbial substances like organic acids or bacteriocins, control the immune response by secreting immune-globulin A (IgA) against potential pathogens, lower the risk of allergy development, enhance the function of the intestinal mucosal barrier, increase the stability or promote the recovery of the commensal microflora when it is disturbed, modulate the expression of host genes, and release functional proteins [3].

In the market, dairy products often fermented with lactic acid bacteria (LAB) are widely employed as probiotics, and the dairy business has been growing steadily. Probiotics have typically been eaten through dairy products that have undergone fermentation, such as yogurt, kumis, kefir, and sour milk. Lactase enzymes, which lactose intolerant person lack, are required to break down the lactose sugar component of milk. High milk consumption is anticipated to increase the necessary

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number of low-density lipoproteins (LDLP). LDLP is a kind of poor cholesterol found in blood and linked to an increased risk of heart disease, cardiovascular disease, and obesity [4]. These factors highlight the necessity for research into non-dairy probiotics. The movement of veganism gaining popularity is known to eliminate animal products. Veganism could be a possible reason for the expansion of fruit-based probiotics.

Fruit juices, which are generally consumed and are regarded as healthful goods, might serve as an alternate medium for incorporating probiotic bacteria. Fruit juices also include a lot of carbohydrates, minerals, and vitamins that probiotics use as a substrate. This together with the fact that probiotic cells pass through the stomach's acid quickly, results in a high viability for probiotic cells. Fruits are beneficial for health and energy supply taste, and they could be probiotic-friendly. They are regarded as perishable goods, and need to be processed right away to minimize post-harvest losses, and the creation of probiotic products may be a strategy to improve the market value and accessibility of fruits and their value-added products [5]. Production of probiotics is an example of value addition to fruits which can help to prevent post-harvest losses. Fruit drinks that have been prebiotically modified may sell for high price since they include healthy bacteria. As the cell culture breaks down fermentable carbohydrates to create by-products like lactic acid (LA), which has antibacterial characteristics and transforms the end product to have a tangy and sour taste, fermentation utilizing probiotic strains might improve the aroma and taste profile along with increasing the shelf-life [2]. Based on this, this review aimed to summarize the literature on the potential application of probiotics in fruit-based drinks and value-added products. It discusses the concept of probiotics, fruit as prebiotics, the comparative fermentation mechanism of dairy-based and non-dairy based probiotics, and physiochemical and sensory changes on completion of fermentation.

2. Applications of probiotics

A probiotic is a viable or inviable microbial cell (vegetative or spore;

whole or ruptured) that can potentially benefit the host [6]. Probiotic has negative effects of antibiotics and antibacterial agents on the intestinal microbiota [7]. The word “probiotics” has undergone several changes over time due to a study into how they work and clinical trials in various human and animal models. Probiotics is “live microorganisms that, when administered in adequate amounts, confer a beneficial health effect on the host”. The majority of probiotic supplements are produced using LAB as well as some other yeast genera. Some of the bacterial strains used in probiotics beverages are *Lactobacilli*, *Bifidobacteria*, *Streptococci*, *Bacillus*, *Escherichia*, and *Propionibacterium*. As shown in (Fig. 1). Several *Lactobacilli* species and strains, such as *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, and *Lactobacillus helveticus*, have been thoroughly investigated in the prevention of human and animal illnesses [7]. A significant portion of the intestinal microbiota, including many probiotic bacteria, has been added to meals to promote gut health by preserving the microbial balance in the gastrointestinal tract [8]. Probiotics are being utilized more frequently for their health advantages to treat various illnesses and disorders, such as bacterial vaginosis, depression, obesity, allergies, and even cancer [9]. The different types of fruit probiotics, their substrate, and the microorganism used are presented in Table 1.

Probiotics are first exposed to saliva in the mouth, which contains immunologic and nonimmunologic components that defend teeth and mucosal surfaces. Saliva promotes the development of non-cariogenic microbiota and appears to have little effect on the survival rates of probiotics [10]. Later, probiotics enter the stomach, where they are exposed to the stomach's acidic contents after passing via the oesophagus. Most bacteria, especially those that are not acid-resistant, are very toxic in an acidic environment, which can lower the pH of the bacteria's cytoplasm. The probiotics' ability to survive under low pH is due to a decrease in the activity of the proton pump F1F0-ATPase. Other conditions like ionic strength, enzyme activity, and mechanical movement affect probiotics' viability [11]. In the small intestine, pancreatic juice and bile influence probiotic viability by damaging cell membranes and DNA [12]. Fig. 1 depicts the mechanism of action of probiotics, which

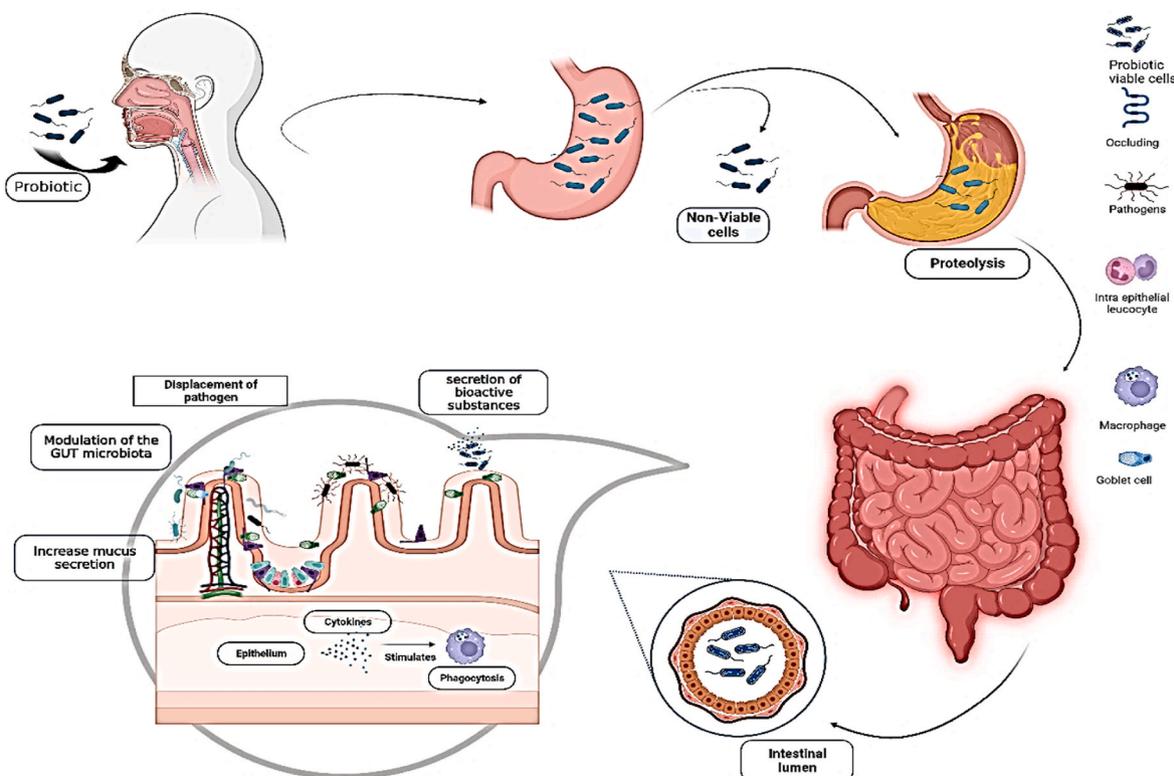


Fig. 1. The Mechanism of Probiotics in human digestive system.

Table 1
Different types of fruit probiotics their substrate and microorganism used.

Sr No.	Substrate	Microorganism	Reference
1	Pineapple juice	<i>Lactobacillus casei</i> , <i>Lactobacillus rhamnosus</i>	[37]
2	Citrus Puree	<i>Lactobacillus paracasei</i> , <i>Bifidobacterium infantis</i>	[39].
3	Apple and star fruit juice	<i>L. plantarum</i> , <i>L. fermentum</i>	[32]
4	Grapefruit juice	<i>L. plantarum</i> 01, <i>L. rhamnosus</i> B01725, <i>L. fermentum</i> D13, and <i>B. bifidum</i> B7.5	[33]
5	Blueberry and blackberry juice	<i>Streptococcus thermophilus</i> , <i>Bifidobacterium bifidum</i> , and <i>Lactobacillus plantarum</i>	(Wu et al., 2021)
6	Cornelian cherry juice	<i>Lactobacillus plantarum</i>	[41]
7	Kiwifruit juice	Yeast and lactic acid bacteria	[34]
8	Dragon fruit Juice	<i>Lactobacillus plantarum</i> FBS05	(Muhialdin et al., 2020)
9	Blueberry juice	Lactic acid bacteria	[40]
10	Cashew apple juice	<i>Lactobacillus plantarum</i>	[36]
11	Jackfruit juice	<i>Lactobacillus casei</i>	[38]

may be summarised as follows: ingestion of probiotics raises the mucus production of goblet cells, mobilizes intraepithelial leucocytes, and tightens tight junctions in order to inhibit pathogen invasion. The enhancement of the gut microbiota and increased mucus secretion promote competitive displacement and reduce pathogen adherence to the surface of the gut epithelium. Moreover, bioactive molecules like lysozyme and cytokines enhance macrophage phagocytosis [13].

The mechanisms include colonization and normalization of disturbed intestinal microbial communities, competitive exclusion of pathogens, and bacteriocin production, modulation of enzymatic activities related to metabolism of a number of carcinogens and other toxic substances, and production of volatile fatty acids, specifically SCFAs (short-chain fatty acids) and BCFAs (Branch chain fatty acids), which play a role in the maintenance of energy homeostasis and regulation of functionality in peripheral tissues [14]. The mechanism of probiotics in the human digestive system is presented in Figure (1).

3. Fruits as prebiotics

For example, mango, apple, and banana peels have been extensively used as by-products as functional food ingredients in various industrial formulations, including baking and confectionery goods. The primary components of fruit fibres include cellulose, hemicellulose, polysaccharides, and oligosaccharides. The health advantages of fibres are well documented. According to reports, increasing dietary fibre consumption can lower the risk of becoming obese and developing some cancers, avoid diabetes, and promote healthy intestinal function [15]. In the human diet, fruits are a good source of carbohydrates, acids, minerals, polyphenols, water-soluble vitamins (including vitamins C and B complex), provitamin A, amino acids, aromatic compounds, carotenoids, fibres, phytosterols, and other bioactive components. Fruits contain between 70 and 90% water [16]. Incorporation of fruits and its products into daily diets reduces the risk of degenerative diseases [17]. It may directly improve human health by decreasing cholesterol, preventing obesity, curing constipation, regulating blood sugar, inhibiting germs, carcinogens and they enhance calcium, and magnesium absorption while improving immunological and digestive systems [18].

Prebiotics are nondigestible food components that positively impact the host by selectively promoting the activity of one of a select group of bacteria in the colon. As a result, they can enhance the gut microbiome by promoting the fermentation of a particular type of beneficial bacteria in the colon [1]. Inulin, fructo-oligosaccharides (FOSs), and

galacto-oligosaccharides (GOS) are examples of nondigestible oligosaccharides that have been the focus of prebiotic research. Fruits contain FOSs, which can also be produced through the enzymatic breakdown of inulin or the transfructosylation of sucrose [19]. Polysaccharides, polyphenols, dietary fibres, and flavouring agents have all been found to be useful and functional components in fruit that act as prebiotics by different modes of action.

Prebiotics can improve the beneficial gut microbiota, which in turn can improve host health [20]. Due to the absence of hydrolysing enzymes in the human digestive system, prebiotics can evade small-intestinal digestion and pass through the colon intact, where they are fermented by healthy bacteria like *Lactobacilli* and *Bifidobacteria* [21]. Complex polysaccharides are converted by microorganisms into monosaccharides via a variety of metabolic processes that are mediated by microbial enzymatic activity. SCFAs (short chain fatty acids), specifically acetate, propionate, and butyrate, are the primary complex carbohydrates produced by bacteria during fermentation [22]. Certain microorganisms' metabolism of prebiotics indirectly promotes the growth of others, while the by-products of fermentation serve as a substrate for the development of further bacteria. For example, the major fructan consumers *Bifidobacteria* and *Lactobacilli* produce lactate and acetate as by-products of their fermentation, which can then be used as an energy source by other bacteria like *Eubacterium*, *Roseburia*, and *Faecalibacterium*, which in turn make butyrate [21]. Butyrate is the main SCFA that colonocytes and enterocytes use for energy. Propionate can also be used locally by intestinal gluconeogenesis, which converts it into glucose, or it can diffuse into the portal vein, where it can be used as a substrate for hepatic gluconeogenesis. Between 90 and 99% of SCFAs are metabolized by the microbiota or absorbed in the gut. The peripheral circulation does contain a modest quantity of SCFAs, namely propionate and acetate [22]. SCFAs influence toll-like receptor-4 signalling and the generation of pro-inflammatory cytokines due to their immunomodulatory characteristics. Prebiotics can raise the lymphocyte and leukocyte counts in GALTs (gut-associated lymphoid tissues) and peripheral blood. The IgA secretions are then increased by GALTs, directly stimulating the phagocytic activity of intra-inflammatory macrophages.

Prebiotics act as nourishment for healthy bacteria, which makes it harder for infection causing microbes or pathogens to adhere to the epithelium [23]. Reactive oxygen species can be indirectly removed by inulin-type fructan. Immune function is modulated by GOS mixture. *Bifidobacteria* increased after lactulose treatment. Certain prebiotics, like FOS, can control the formation of mucin [24].

Fruits are beneficial prebiotics that have intrinsic qualities such as buffering capacity, FOS, inulin, fibre, and antinutritional factors [25]. In a study *L. plantarum* CECT220, *L. acidophilus* CECT903, *B. longum* subsp. *infantes* CECT4551, and *B. bifidum* CECT870 all thrived in fermented pomegranate juices due to the biotransformation of phenolic compounds during fermentation and gastrointestinal digestion, indicating a prebiotic effect [1].

According to a study, dragon fruit oligosaccharides significantly increased IgG and IgA proliferation in rats at a dosage of 4 g/kg per day within two weeks. They also produced short-chain fatty acids in the three-stage continuous colon system and demonstrated prebiotic properties that can modulate the gut microbiota [26]. The prebiotic impact of pomegranate ellagitannins is caused by the breakdown of ellagic acid by the gut flora [1]. Not only fruit juices but its peel also has shown prebiotic effects.

In a study done by Gupta, et al., the prebiotic effects of apple peel extract and sweet lime peel extract were examined using three strains: *L. casei*, *L. rhamnosus*, and *L. plantarum*. The findings show that both extracts improved probiotic culture development. Apple peel had a higher prebiotic index for *Lactobacillus plantarum* than sweet-lime peel. Sweet-lime peel had a higher prebiotic index for *Lactobacillus rhamnosus* than apple peel. For *Lactobacillus casei*, the prebiotic index for apple peel and sweet lime peel was almost comparable [27]. Fruits are an excellent source of vitamins, minerals, carbohydrates, and other phytonutrients,

which makes them perfect for microbial development and a prebiotic.

4. Fermentation in fruit-based probiotic

Juices containing probiotics can be made by either directly adding the probiotic strain to the juice or by fermenting the juice with the probiotic bacteria. The latter results in a low-sugar product and a better adapted microbial strain, which might increase its survival rates, so it is preferable to the former over direct addition. Moreover, microbial metabolites like bacteriocins may be created during the fermentation of the juice with the probiotic bacteria, which may aid in enhancing the product's quality and lengthen its shelf life when stored [28].

Fermentation in probiotics includes the conversion of sugars to organic acids. LA is produced during the fermentation of lactose by dairy-based probiotics. The process of fermenting lactose is happening as glycolysis or the glycolytic pathway. Firstly, lactose is converted to glucose and galactose, then further galactose gets converted to glucose, by gaining one phosphate, it gets converted to galactose-1-P and later on to glucose-1-P, which leads to formation of pyruvate by the process of glycolysis after some conversions and interconversion. This pyruvate is subsequently reduced to LA using the reducing energy that was previously generated in the cycle in the form of NADH [29].

Fruit sugar such as glucose, sucrose and fructose are converted into pyruvate and LA. The d- or l-lactate dehydrogenase enzyme (aldolases enzymes) is a component of the metabolic pathway that converts 1 mol of glucose into 2 mol of LA and 2 mol of ATP [30].

Homo-fermentation is when lactic acid is primarily produced from glucose up to 85% of the time; therefore, one molecule of glucose fermented produces up to two molecules of LA, leading to the production of two molecules of adenosine triphosphate for every molecule of glucose metabolized and hetero-fermentation is when LA generation is roughly 50% in this fermentation process.

One molecule of glucose is converted into one molecule of adenosine triphosphate, one molecule of LA, one molecule of ethanol, and one molecule of carbon dioxide throughout the fermentation process [31]. The Embden- Meyerhof route catabolizes C6 carbohydrates, such as glucose, into LA. The various fruit probiotics available in the market are presented in Table 2.

5. Changes after fermentation

Fermentation is generally considered as the conversion of complex bio-molecules to complex ones. However, most of the constituents, including macromolecules and micro-molecules, undergoes some changes during fermentation. The major changes are happening as the increase in microbial cell count, increase in acid concentration, and subsequent decrease in soluble sugars. These changes occurring in fruit-based probiotic beverages are detailed as follows.

5.1. pH

As the bacteria population grows and generates more acid over time, the pH content of probiotic drinks gradually lowers [32]. Lowering pH during probiotic product fermentation is crucial for determining the timing of fermentation and preserving product quality [33]. A rapid rise in acidity was caused by the LAB in lactic acid fermented samples, which reproduced and converted glucose or lactose into LA under anaerobic conditions [34]. Different strains of *Lactobacillus* and *Bifidobacterium* (*L. plantarum* 01, *L. rhamnosus* B01725, *L. fermentum* D13, and *B. bifidum* B7.5) were added to grapefruit juice. Short chain fatty acids were formed during fermentation, which resulted in pH values that were lower. In the current study, the final pH varied from 4.4 to 4.7 [33]. Three probiotic juice samples were created for each apple and star fruit juice by inoculating with *L. plantarum*, *L. fermentum*, and a combination of the two. After 21 days of storage, the pH of the apple juice sample that served as the control (4.743) was reduced to 4.263, 4.337, and 4.140,

Table 2
Fruit probiotics available in market.

Sr. No.	Commodity	Microorganism	Product	References
1.	Beverage (Mango)	<i>Lactobacillus plantarum</i> 299V	Juice drink	https://goobelly.com/probiotic-drinks/s
2.	Beverage (purified water, lemon, cayenne, maqui berry, vegan probiotics and stevia)	<i>Bacillus coagulans</i> GBI-30 6086	Juice drink	https://www.sujaorganic.com/products/juice/refreshing/wild-probiotic/
3.	Beverage (orange guava)	<i>Bacillus coagulans</i> GBI-30 6086	Juice drink	https://sogoodsyou.com/products/happy
4.	Beverage	<i>Lactobacillus acidophilus</i> , <i>Lactobacillus delbreukii</i>	Cocobiotic	https://shop.bodyepinecology.com/collections/all
5.	Gummies	<i>Lactobacillus</i>	Probiotic amla gummies	https://www.kapiva.in/
6.	Beverage (blueberry)	<i>Bifidobacterium</i>	Juice smoothie	https://www.nakedjuice.com/our-products/
7.	Beverage (strawberry, passion fruit, lemon, rose water)	<i>Bacillus coagulans</i> GBI-30 6086	Juice drink	https://www.kroger.com/p/simple-truth-organic-roseberry-mist-probiotic-juice-drink/0001111005861
8.	Beverage (pomegranate)	<i>Lactobacillus casei</i>	Juice drink	https://ihr.res.in/probiotic-pomegranate-juice
9.	Beverage (mango mangue)	<i>Bacillus coagulans</i>	Juice smoothie drink	https://www.foobdev.com/news/agropur-makes-probiotic-juices-the-latest-addition-to-iogo-brand/
10.	Beverage (Pineapple, mango)	<i>Bifidobacterium lactis</i> HN019	Juice drink	https://www.tropicana.com/products/tropicana-essentials/pineapple-mango-tep
11.	Beverage (raspberry, lemon, ginger)	<i>Bacillus Coagulans</i> GBI-30 6086	Kombucha	https://www.walmart.com/ip/GT-S-Synergy-Trilogy-Kombucha-Drink-Organic-Raw/51259247
12.	Beverage (Peach, mint)		Juice drink	https://www.foodbusinessnews.net/articles/16470-chobani-unveils-two-new-product-platforms
13.	Beverage (orange, pineapple, ginger)	<i>Bacillus coagulans</i>	Juice drink	https://www.unclematts.com/products/organic-orange-turmeric-with-living-probiotics/
14.	Beverage (blackberry hops)	<i>Bacillus coagulans</i> MTCC 5856	Master brew kombucha drink	https://www.kevita.com/blackberry-hops/
15.	Beverage (mixed fruit)	<i>Lactobacillus sporogens</i>	Probiotic health drink	https://www.zotazo.com/in/reviews/health-potion-organic-probiotic-

(continued on next page)

Table 2 (continued)

Sr. No.	Commodity	Microorganism	Product	References
				drink-mixed-fruit-enzymes-500-servings/

respectively, by the inoculation of *L. plantarum*, *L. fermentum*, and mixed cultures. The pH of the control sample (3.345) in star fruit juice was decreased by *L. plantarum*, *L. fermentum*, and mixed isolates to 2.950, 2.937, and 2.937, respectively [32].

5.2. Total soluble solids (TSS)

The TSS remained nearly the same throughout initial fermentation, but as the retention time increased, the value declined. That is thought to be the result of solid matter decomposition and LAB using these materials during fermentation [35]. Probiotic bacterial growth is anticipated to be accelerated by total accessible carbohydrates. Bacteria utilize these nutrients as a source of energy without additional nutrients as storage times increase [32]. A study investigated the change of bioactive compounds in yeast and LAB fermented kiwifruit extract. In the first ten days of fermentation, the soluble solids content in samples declined quickly; however, in the last days of fermentation, it tended to stabilize around 10–15 °Brix [34]. In the study of lactobacilli strains inoculated in apple and star fruit juices, TSS value decreased from 13° B to 10.33° B for apple juice and from 5° B to 3.50° B for star fruit juice after a period of 21 days after inoculation. TSS content was the same for all samples at day 0, but the value dropped as storage time progressed [32].

5.3. Acidity

Acidity is closely correlated with pH values of the probiotic drink [36]. A rapid rise in acidity was caused by the LAB in LAB fermented samples, which reproduced and converted glucose or lactose into LA under anaerobic conditions [34]. By breaking down oligofructose, or simple sugars, the bacteria increased acidity by producing a modest amount of organic acids [37]. A study investigated the potential for lactic acid bacteria (LAB) strains to ferment blueberry juice as well as the impact of microbial metabolism on juice composition. Malic acid content dropped from 511.47 ± 10.50 mg/L to below 146.38 ± 3.79 mg/L after 48 h of fermentation, whereas LA content rise from 0 mg/L to over 2184.90 ± 335.80 mg/L [36].

5.4. Cell count

It is observed that cell count increases initially and later declines after a period of time. It can be caused by the oxygen present or by trace levels of nitrogen molecules. When the pH of the lactic acid bacterium cell wall drops, more energy is required to maintain the intracellular pH, which reduces ATP and ultimately causes cell death due to the disintegration of the cell wall. Additionally, because probiotics lack catalase and the electron transport chain, the presence of oxygen may cause harmful compounds to build up in the cells, which could lead to oxidative damage and cell death [37]. *Lactobacillus plantarum*-fermented cashew apple juice (CAJ) and 11.4 °Bx concentrated cashew apple juice (CCAJ) was studied for changes in physicochemical characteristics, astringency chemicals, antioxidant activities, and taste volatile components. After 72 h of fermentation, the population of *L. plantarum* in CAJ and 11.4 °Bx CCAJ dropped from 9.18 Log₁₀ CFU/mL to 5.12 Log₁₀ CFU/mL and 4.95 Log₁₀ CFU/mL, respectively [36]. In a study, the potential of lacto-fermenting jackfruit juice using *Lactobacillus casei* ATCC334 to produce probiotic beverages was studied. After 24 h of fermentation at 37 °C, the viable cell count dramatically grew to 8.176

Log₁₀ CFU/mL from 6 Log₁₀ CFU/mL, and it then significantly fell to 7.672 Log₁₀ CFU/mL after 3 weeks at 8 °C. The composition of the raw materials, the fermentation conditions, and the storage conditions all have a significant impact on the cells count density. Probiotics have a strong ability to ferment fruit juices and significantly boost the number of cells under ideal development conditions [38].

5.5. Total flavonoids content (TFC) and total phenol content (TPC)

Citrus puree was fermented using *Lactobacillus paracasei* JLPF-176 (LP) and *Bifidobacterium infantis* JYBI-373 (BI) as starters separately. Enzymatic hydrolysis raised TPC and TFC, while fermentation further improved them. When subjected to BI treatment, the contents of TPC and TFC were both noticeably elevated. After 28 days of storage, phenol content increased with LP treatment from 0.97 g GAE/kg to 1.26 g GAE/kg and with BI treatment from 1.08 g GAE/kg to 1.24 g GAE/kg. These outcomes could be explained by probiotic-produced chemicals or by phenolics that were isolated from citrus tissues. The formation of phenolics is also thought to be a result of a reaction by plant cells to heal physiochemical damage. Additionally, after 28 days of storage, a slight decrease in TFC was seen during fermentation with LP, going from 0.46 g RT/kg to 0.44 g RT/kg, and fermentation with BI, going from 0.59 g RT/kg to 0.47 g RT/kg. This was most likely brought on by the combination of phenolics with either sugar or amino acids. TFC was seen to rise in the two treatments at intervals of 7 days–14 days of storage and 21 days–28 days of storage, probably as a result of the (poly)-phenol oxidase in citrus puree releasing dietary flavonoids as water-soluble flavonoid glycosides and rising TFC throughout storage [39].

A study was conducted by Ref. [40] on 4 strains of *Lactobacillus plantarum* and 5 strains of *Lactobacillus fermentum* fermented blueberry juice, it was observed that the phenolic content of juice was significantly influenced by these strain's metabolism. The final total phenolic contents in all the fermented samples were significantly higher than the initial total phenolic contents in blueberry juices, which were 866.15 ± 19.23 mg/L. When *Lactobacillus plantarum* LSJ-TY-HYB-T9 and *Lactobacillus fermentum* LSJ-TY-HYB-H33 were used to ferment samples, their respective total phenolic contents increased by 41.8% and 57.9%.

The total phenolic content (TPC) of fermented Cornelian cherry juice by free cells (FC) and immobilized cells (IC) was also assessed after 24 h of fermentation and after each week of cold storage. Freshly made Cornelian cherry juice initially contained about 175.15 ± 10.19 mg GAE/100 mL of total phenolics. Higher TPC values were obtained when *L. plantarum* ATCC 14917 was used for the fermentation of Cornelian cherry in free or immobilized form. In the first week of cold storage (257.20 mg GAE/100 mL), as well as over the next 3 weeks (264.71 mg GAE/100 mL at the second, 258.84 mg GAE/100 mL at the third, and 249.61 mg GAE/100 mL at the fourth), the TPC of Cornelian cherry juice fermented by IC achieved its highest values. The LA fermentation is likely responsible for the observed improvement in TPC. It appears that LA fermentation promotes both the depolymerization of high molecular weight phenolic compounds and the conversion of simple phenolic compounds [41]. The process of ATP synthesis in a probiotic microbial cell is presented in Fig. (2).

Various evidence-based perspective of probiotic safety, focusing on probiotic fruit beverages and nutraceuticals have been studied and reported recently. A study was conducted to investigate if daily treatment of adults with *Lactobacillus reuteri* DSM 17938 (LR) for 2 months is safe and can be tolerated, where 40 healthy adults were randomized to dose of 5×10^8 CFU of LR ($n = 30$) daily for 2 months. Also, if LR treatment has immune effects as determined by regulatory T cell percentages, expression of toll-like receptors (TLR)-2 and -4 on circulating peripheral blood mononuclear cells (PMBCs), cytokine expression by stimulated PBMC and intestinal inflammation. Participants completed a daily diary card and were observed during treatment on alternate days (7 clinic visits). It was observed that there were no severe adverse events (SAEs) and no significant differences in adverse events. There were no

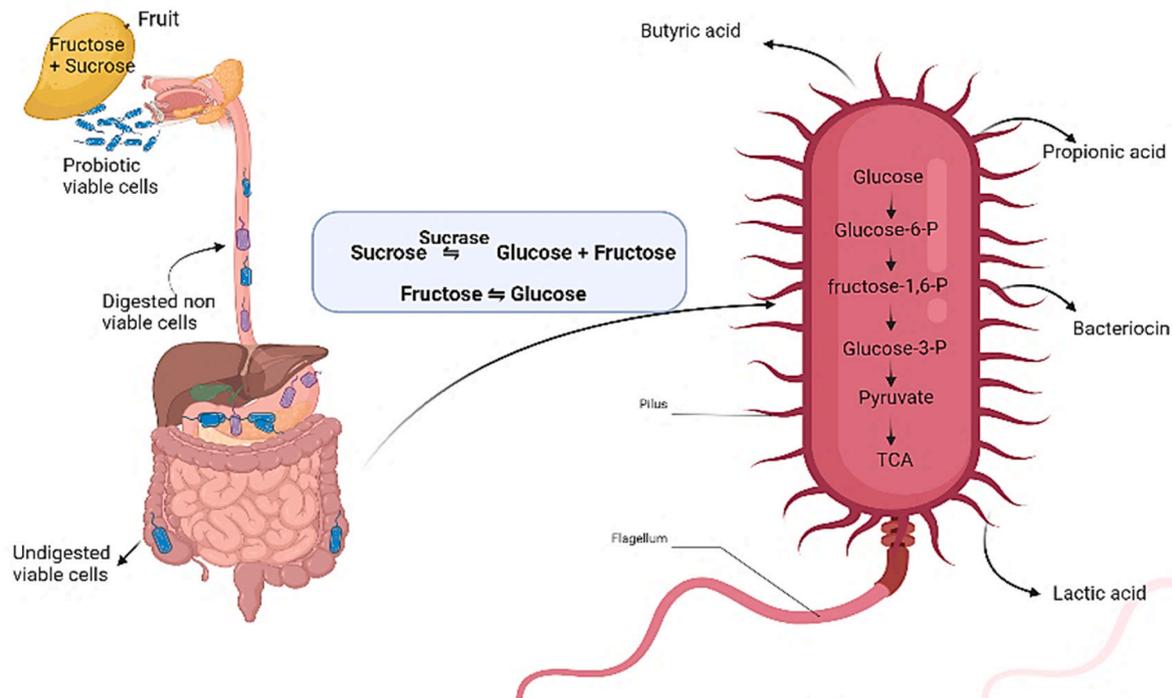


Fig. 2. ATP synthesis in a probiotic microbial cell.

differences in PBMC subclasses, TLRs, or cytokine expression after the treatment period. The probiotic-treated group had a significantly higher faecal calprotectin level than the placebo group after 2 months of treatment [42]. Another study was done, where the potential effect of *Bifidobacterium longum* CECT 7347 in children with newly diagnosed coeliac disease (CD) was observed. A double-blind, randomized, placebo-controlled trial was carried out on 33 children who were fed with capsule containing either *B. longum* CECT 7347 (10^9 colony-forming units) or placebo (excipients) for 3 months on daily basis along with gluten-free diet (GFD). It was observed that the incorporation of *B. longum* CECT 7347 decreased the number of *Bacteroides fragilis* group and faecal secretory IgA (sIgA) content in stools as compared to incorporation of placebo. It has been suggested that *B. longum* CECT 7347 can enhance the health status of CD patients who tend to show variation in gut microbiota and biased immune response even on GFD [43].

6. Conclusion and future perspective

Probiotics are food products containing viable or inviable microbial cell that potentially attach to intestinal linings of the host giving numerous health benefits. The majority of probiotic supplements are created using LAB, including *Lactobacilli*, *Bifidobacteria*, and *Streptococci*. Probiotics help to cure various diseases such as inflammation, relieve gastrointestinal pain and diarrhoea, and enhance the immunity of the body. There must be a minimum number of probiotics in the juice greater than 10^6 to 10^7 cfu/ml to influence the human body. Two basic types of probiotics are dairy based and non-dairy based. However, the consumption of milk-based probiotics hindered due to milk protein allergy, hypercholesterolemic, veganism, and lactose intolerance, dairy-based probiotics are not consumed by many people. Fruit based probiotic are good alternatives for dairy based probiotics as they are good source of nutrients, including vitamins, minerals, dietary fibres, sugars, bioactive compounds, and antioxidants. These fruit-based probiotics are good combination of food and health. Many physiochemical changes are observed after fermentation, including decrease in pH, increase in acidity, reduction in TSS, reduction in cell viability and lower

carbohydrate content. During fermentation, organic acids are produced from sugar present in juice, and this takes place with the help of glycolytic pathway where sugar is converted to pyruvate and then reduced to lactic acid. Minimal changes are observed in sensory analysis after fermentation. Indian being one of the most diversified regions for the growth of exotic fruits, due to unavailability of dedicated processing protocols and utilization techniques, most of these fruits remain underutilized leading to qualitative and quantitative losses. As an alternative utilization approach incorporating underutilized fruit-based probiotics will help develop beneficial beverages with therapeutic effects such as anti-inflammatory, treat diarrhoea, constipation etc. along with generating elevated income for the growers as an additional benefit. This fruit based probiotic beverages will also be a good value-added product of fruits which will help to reduce fruit wastage due to certain problems. It can help to prevent post-harvest losses and make fruit available for longer duration in different forms. The research needs to be undertaken design and development of fruit-based probiotics and their comparison to dairy-based probiotics.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

- [1] E. Valero-Cases, D. Cerdá-Bernad, J.-J. Pastor, M.-J. Frutos, Non-dairy fermented beverages as potential carriers to ensure probiotics, prebiotics, and bioactive compounds arrival to the gut and their health benefits, *Nutrients* 12 (6) (2020) 1666.
- [2] F.D. Mojikon, M.E. Kasimin, A.M. Molujin, J.A. Gansau, R. Jawan, Probiotication of nutritious fruit and vegetable juices: an alternative to dairy-based probiotic functional products, *Nutrients* 14 (17) (2022), <https://doi.org/10.3390/nu14173457>.

- [3] L. Abenavoli, E. Scarpellini, C. Colica, L. Boccutto, B. Salehi, J. Sharifi-Rad, V. Aiello, B. Romano, A. De Lorenzo, A.A. Izzo, R. Capasso, Gut microbiota and obesity: a role for probiotics, *Nutrients* 11 (11) (2019) 1–27, <https://doi.org/10.3390/nu11112690>.
- [4] I. Syiemlieh, S. Morya, Dairy and non-dairy based probiotics: a review, *Pharma Innov.* 11 (6S) (2022) 2956–2964, <https://doi.org/10.22271/tpi.2022.v11.i6sak.13568>.
- [5] I.A. Gomes, A. Venâncio, J.P. Lima, O. Freitas-Silva, Fruit-based non-dairy beverage: a new approach for probiotics, *Adv. Biol. Chem.* 11 (6) (2021) 302–330, <https://doi.org/10.4236/abc.2021.116021>.
- [6] F. Zendeboodi, N. Khorshidian, A.M. Mortazavian, A.G. da Cruz, Probiotic: conceptualization from a new approach, *Curr. Opin. Food Sci.* 32 (2020) 103–123, <https://doi.org/10.1016/j.cofs.2020.03.009>.
- [7] M.A.K. Azad, M. Sarker, T. Li, J. Yin, Probiotic species in the modulation of gut microbiota: an overview, *BioMed. Res. Int.* (2018), <https://doi.org/10.1155/2018/9478630>, 2018.
- [8] C. Maldonado Galdeano, S.I. Cazorla, J.M. Lemme Dumit, E. Vélez, G. Perdígón, Beneficial effects of probiotic consumption on the immune system, *Ann. Nutr. Metabol.* 74 (2) (2019) 115–124, <https://doi.org/10.1159/000496426>.
- [9] J. Zucko, A. Starcevic, J. Diminic, D. Oros, A.M. Mortazavian, P. Putnik, Probiotic – friend or foe? *Curr. Opin. Food Sci.* 32 (Figure 1) (2020) 45–49, <https://doi.org/10.1016/j.cofs.2020.01.007>.
- [10] S. Han, Y. Lu, J. Xie, Y. Fei, G. Zheng, Z. Wang, J. Liu, L. Lv, Z. Ling, B. Berglund, M. Yao, L. Li, Probiotic gastrointestinal transit and colonization after oral administration: a long journey, *Front. Cell. Infect. Microbiol.* 11 (March) (2021) 1–12, <https://doi.org/10.3389/fcimb.2021.609722>.
- [11] M. Yao, J. Xie, H. Du, D.J. McClements, H. Xiao, L. Li, Progress in microencapsulation of probiotics: a review, *Compr. Rev. Food Sci. Food Saf.* 19 (2) (2020) 857–874, <https://doi.org/10.1111/1541-4337.12532>.
- [12] M. Yao, B. Li, H. Ye, W. Huang, Q. Luo, H. Xiao, D.J. McClements, L. Li, Enhanced viability of probiotics (*Pediococcus pentosaceus* Li05) by encapsulation in microgels doped with inorganic nanoparticles, *Food Hydrocolloids* 83 (April) (2018) 246–252, <https://doi.org/10.1016/j.foodhyd.2018.05.024>.
- [13] I.D. Kwoji, O.A. Aiyegoro, M. Okpeku, M.A. Adeleke, Multi-strain probiotics: synergy among isolates enhances biological activities, *Biology* 10 (4) (2021) 1–20, <https://doi.org/10.3390/biology10040322>.
- [14] J. Plaza-Diaz, F.J. Ruiz-Ojeda, M. Gil-Campos, A. Gil, Mechanisms of action of probiotics, *Adv. Nutr.* 10 (2019) S49–S66, <https://doi.org/10.1093/advances/nmy063>.
- [15] H.F. Zahid, C.S. Ranadheera, Z. Fang, S. Ajlouni, Utilization of mango, apple and banana fruit peels as prebiotics and functional ingredients, *Agriculture (Switzerland)* 11 (7) (2021), <https://doi.org/10.3390/agriculture11070584>.
- [16] L.G.R. Rodriguez, V. Manuel, Z. Gasga, M. Pescuma, C. Van Nieuwenhove, F. Mozzi, J. Alberto, S. Burgos, Fruits and fruit by-products as sources of bioactive compounds. Benefits and trends of lactic acid fermentation in the development of novel fruit-based functional beverages, *Food Res. Int.* (2020), 109854, <https://doi.org/10.1016/j.foodres.2020.109854>.
- [17] J. Yi, H. Toy, Y. Lu, D. Huang, K. Matsumura, Enzymatic treatment, unfermented and fermented fruit-based products: current state of knowledge, *Crit. Rev. Food Sci. Nutr.* 0 (0) (2020) 1–22, <https://doi.org/10.1080/10408398.2020.1848788>.
- [18] D. Mohanty, S. Misra, S. Mohapatra, P.S. Sahu, Probiotics and synbiotics: recent concepts in nutrition, *Food Biosci.* 26 (2018) 152–160, <https://doi.org/10.1016/j.food.2018.10.008>.
- [19] S. Lockyer, S. Stanner, Probiotics – an added benefit of some fibre types, *Nutr. Bull.* 44 (1) (2019) 74–91, <https://doi.org/10.1111/nbu.12366>.
- [20] J.H. Meurman, I.V. Stamatova, Probiotics: evidence of oral health implications, *Folia Med.* 60 (1) (2018) 21–29, <https://doi.org/10.1515/folmed-2017-0080>.
- [21] M.P.L. Guarino, A. Altomare, S. Emerenziani, C. Di Rosa, M. Ribolsi, P. Balestrieri, P. Iovino, G. Rocchi, M. Cicala, Mechanisms of action of prebiotics and their effects on gastro-intestinal disorders in adults, *Nutrients* 12 (4) (2020) 1–24, <https://doi.org/10.3390/nu12041037>.
- [22] H.D. Holscher, Dietary fiber and prebiotics and the gastrointestinal microbiota, *Gut Microb.* 8 (2) (2017) 172–184, <https://doi.org/10.1080/19490976.2017.1290756>.
- [23] I. Khangwal, P. Shukla, Prospecting prebiotics, innovative evaluation methods, and their health applications: a review, *3 Biotech* 9 (5) (2019) 1–7, <https://doi.org/10.1007/s13205-019-1716-6>.
- [24] R. Alkasir, J. Li, X. Li, M. Jin, B. Zhu, Human gut microbiota: the links with dementia development, *Protein Cell* 8 (2) (2017) 90–102, <https://doi.org/10.1007/s13238-016-0338-6>.
- [25] A. Septembre-malaterre, F. Remize, P. Pouchet, PT. Food research international, <https://doi.org/10.1016/j.foodres.2017.09.031>, 2017.
- [26] N. Pansai, K. Chakree, C. Takahashi Yupanqui, P. Raungrut, N. Yanyiam, S. Wichienchot, Gut microbiota modulation and immune boosting properties of prebiotic dragon fruit oligosaccharides, *Int. J. Food Sci. Technol.* 55 (1) (2020) 55–64, <https://doi.org/10.1111/ijfs.14230>.
- [27] E. Gupta, N. Mishra, P. Mishra, A. Shiekh, K. Gupta, P. Singh, Fruit peels: a strong natural source of antioxidant and prebiotics, *Carpathian J. Food Sci. Technol.* 12 (5) (2020) 134–143, <https://doi.org/10.34302/CRPJEST/2020.12.5.10>.
- [28] M. Aspri, P. Papademas, D. Tsaltas, Review on non-dairy probiotics and their use in non-dairy based products, *Fermentation* 6 (1) (2020) 1–20, <https://doi.org/10.3390/fermentation6010030>.
- [29] T. Bintsis, Lactic acid bacteria as starter cultures: an update in their metabolism and genetics, *AIMS Microbiol.* 4 (4) (2018) 665–684, <https://doi.org/10.3934/microbiol.2018.4.665>.
- [30] T. Raj, K. Chandrasekhar, A.N. Kumar, S.H. Kim, Recent biotechnological trends in lactic acid bacterial fermentation for food processing industries, *Syst. Microbiol. Biomanuf.* 2 (1) (2022) 14–40, <https://doi.org/10.1007/s43393-021-00044-w>.
- [31] S.A.A. Rawoof, P.S. Kumar, D.V.N. Vo, K. Devaraj, Y. Mani, T. Devaraj, S. Subramanian, Production of optically pure lactic acid by microbial fermentation: a review, *Environ. Chem. Lett.* 19 (1) (2021) 539–556, <https://doi.org/10.1007/s10311-020-01083-w>.
- [32] S. Study, M.A. Hossain, R. Das, Potentials Of Two Lactobacilli In Probiotic Fruit Juice Development And Evaluation Of Their Biochemical And Organoleptic Stability Potentials Of Two Lactobacilli In Probiotic Fruit Juice Development And Evaluation Of Their Biochemical And Organoleptic Sta, 2022. June.
- [33] A.M. Tran, T.B. Nguyen, V.D. Nguyen, E. Bujna, M.S. Dam, Q.D. Nguyen, Changes in bitterness, antioxidant activity and total phenolic content of grapefruit juice fermented by *Lactobacillus* and *Bifidobacterium* strains, *Acta Aliment.* 49 (1) (2020) 103–110, <https://doi.org/10.1556/066.2020.49.1.13>.
- [34] L. Cai, W. Wang, J. Tong, L. Fang, X. He, Q. Xue, Y. Li, Changes of bioactive substances in lactic acid bacteria and yeasts fermented kiwifruit extract during the fermentation, *Lwt* 164 (June) (2022), 113629, <https://doi.org/10.1016/j.lwt.2022.113629>.
- [35] X. Jin, W. Chen, H. Chen, W. Chen, Q. Zhong, Comparative evaluation of the antioxidant capacities and organic acid and volatile contents of mango slurries fermented with six different probiotic microorganisms, *J. Food Sci.* 83 (12) (2018) 3059–3068, <https://doi.org/10.1111/1750-3841.14373>.
- [36] R. Kaprasob, O. Kerdechueuen, N. Laohakunjit, B. Thumthanaruk, K. Shetty, Changes in physico-chemical, astringency, volatile compounds and antioxidant activity of fresh and concentrated cashew apple juice fermented with *Lactobacillus plantarum*, *J. Food Sci. Technol.* 55 (10) (2018) 3979–3990, <https://doi.org/10.1007/s13197-018-3323-7>.
- [37] S. Ghafari, S. Ansari, Microbial viability, physico-chemical properties and sensory evaluation of pineapple juice enriched with *Lactobacillus casei*, *Lactobacillus rhamnosus* and inulin during refrigerated storage, *J. Food Meas. Char.* 12 (4) (2018) 2927–2935, <https://doi.org/10.1007/s11694-018-9908-z>.
- [38] B.J. Muhiyaldin, A.S. Meor Hussin, H. Kadum, A. Abdul Hamid, A.H. Jaafar, Metabolomic changes and biological activities during the lacto-fermentation of jackfruit juice using *Lactobacillus casei* ATCC334, *LWT-Food Sci. Technol.* 141 (2021), 110940, <https://doi.org/10.1016/j.lwt.2021.110940>.
- [39] R. Tao, Q. Chen, Y. Li, L. Guo, Z. Zhou, Physicochemical, nutritional, and phytochemical profile changes of fermented citrus puree from enzymatically hydrolyzed whole fruit under cold storage, *Lwt* 169 (August) (2022), <https://doi.org/10.1016/j.lwt.2022.114009>.
- [40] S. Li, Y. Tao, D. Li, G. Wen, J. Zhou, S. Manickam, Y. Han, W.S. Chai, Fermentation of blueberry juices using autochthonous lactic acid bacteria isolated from fruit environment: fermentation characteristics and evolution of phenolic profiles, *Chemosphere* 276 (2021), 130090, <https://doi.org/10.1016/j.chemosphere.2021.130090>.
- [41] I. Mantzourani, C. Nouska, A. Terpou, A. Alexopoulos, E. Bezirtoglou, M. I. Panayiotidis, A. Galanis, S. Plessas, Production of a novel functional fruit beverage consisting of cornelian cherry juice and probiotic bacteria, *Antioxidants* 7 (11) (2018), <https://doi.org/10.3390/antiox7110163>.
- [42] N. Mangalat, Y. Liu, N.Y. Fatheree, M.J. Ferris, M.R. Van Arsdall, Z. Chen, J. M. Rhoads, Safety and tolerability of *Lactobacillus reuteri* DSM 17938 and effects on biomarkers in healthy adults: results from a randomized masked trial, *PLoS One* 7 (9) (2012), e43910.
- [43] M. Olivares, G. Castillejo, V. Varea, Y. Sanz, Double-blind, randomised, placebo-controlled intervention trial to evaluate the effects of *Bifidobacterium longum* CECT 7347 in children with newly diagnosed coeliac disease, *Br. J. Nutr.* 112 (1) (2014) 30–40.