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Evaluation of Fruit Juices as Probiotic Delivery Systems: Challenges, Current Strategies and Health Benefits

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A B S T R A C T

There is an increasing trend for development of alternatives to deliver probiotics with non-dairy products. Fruit juices have become one of main food products for delivery of probiotics. The availability of different fruit juice types, their fresh and healthy perception from the consumer's side and demand for plant-based products increase attention to fortification of fruit juices with probiotics. Yet, development of probiotic fruit juices is still an emerging area for the functional food concept. Probiotic juices can be developed by using both probiotic *Lactobacillus* and *Bifidobacterium* and their viability can be strain specific as well dependent on the utilized fruits. The transformation of the fruit components can play roles for the improvement of the potential health promoting functions of fruit juices which should be well-characterized. The insufficient viability of probiotic strains during shelf-life of fruit juices is one of the main challenges and efficient and relatively cheap encapsulation techniques should be developed to ensure their viability. In this study, recent achievements and developments to produce probiotic fruit juices have been summarized. Also, potential role of probiotic fortification for the health promoting functions of fruit juices related to probiotic metabolism has been discussed. Finally, strategies to increase the viability of distinct probiotics have been discussed.

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Introduction

In recent years, the functional beverage market is a rapidly growing area of the food industry due to the increasing interests of modern, health-conscious consumers for these products that can be effective to reduce disease risks and increase their quality of life (Corbo et al., 2014; Gupta et al., 2015). The demand for fermented plant-based beverages produced with different raw materials (fruits, vegetables, cereals etc.) is also increasing due to consumer's perception in the relationship between daily diet and healthy life as well as an increase in the number of individuals with a vegan preference. Fortification of the plant-based beverages with probiotics and conducting the fermentation processes with probiotic microorganisms especially with probiotic lactic acid bacteria (LAB) have become the method of choice for the development of functional plant-based products. These non-dairy raw materials are both cheaper and rich in phytochemicals, and they do not pose a health problem for individuals with lactose intolerance or cholesterol-restricted diets (Pereira and Rodrigues, 2018). In addition, vegetarian nutrition, and the desire to avoid allergic reactions caused by milk consumption have increased the demand for non-dairy probiotic products (Mojikon et al., 2022).

Lactiplantibacillus plantarum, Lactobacillus acidophilus, Lacticaseibacillus paracasei, and *L. brevis* are commonly used LAB species in fruit and vegetable fermentations. The probiotic functions of these species increase the attention to these strains in fruit and vegetable fermentations as they have positive effects on human metabolism with the consumption of these beverages (Guan et al., 2021). Probiotics are defined as "live microorganisms that, when administered in adequate quantities, confer a health benefit on the host by the United Nations Food and Agriculture Organization (FAO) and World Health Organization (WHO) experts in 2001. Several health benefits are associated with the probiotic consumption such as reduction in the intestinal pH, improvement of the intestinal microflora, helping to restore the natural microflora especially after antibiotic treatments, lowering cholesterol levels, reduction of ammonia and other toxic compounds, consumption of lactose, improvement of the digestion as well as the stimulation of immune system. However, for probiotic microorganisms to show these benefits, at least 6 log CFU (colony forming unit) ml^{-1} of live microorganism must be taken at the time of consumption and probiotic strains should be able to maintain their viability, colonize and multiply in the host gastrointestinal tract (GIT). Many factors such as the food matrix, the properties of the probiotic strain, pH or storage conditions can have an impact on viability (Istrati et al., 2018; Andrade et al., 2019). If sufficient amounts of probiotic bacteria cannot reach the target area, the probiotic product is not expected to be beneficial (Aspri et al., 2020).

Fruits and vegetables are rich in vitamins (vitamin C and B-complex vitamins, provitamin A), minerals, aromatic compounds, carbohydrates, dietary fibers, phytochemicals, and polyphenols, as indicated in worldwide nutritional studies. The composition of these raw materials varies depending on the degree of ripening, the preparation stages of the fruits and vegetables for the process. While their water content varies between 70%-90%, they contain low amounts of oil in the pulp and shell parts, as well as in the core parts that are mostly not consumed. The protein content of fruits is variable but very low. Fruit juices help maintain blood pressure with their low potassium and sodium content. In addition, their low-fat content makes them suitable for consumption to maintain a healthy cardiovascular system (Rodríguez et al., 2021; Mojikon et al., 2022). As a result, the bioactive components of fruits have shown that they can reduce the risk of certain chronic and metabolic diseases when they are consumed regularly (Charlton et al., 2014).

It should be noted that there are also some problematic issues for fruits and vegetables that need to be solved to increase their consumption rates. Economic reasons (e.g., high cost-low affordability), changes in consumer behavior, scarcity of fresh fruit supply in some regions, short lifespan of fruits, seasonality and difficulty in storage are factors that affect fruit intake in public nutrition. These obstacles can be overcome by investments in research and development and the creation of appropriate regulations (Gerritsen et al., 2019; Rodríguez et al., 2021).

Fruits are sensitive to microbial spoilage due to their nutritional values and water activities and their shelf life is short. They are prone to spoilage by yeast and mold species, not by such bacteria, due to their low pH values. For this reason, fungi and LABs that can grow at high acidity values dominate the autochthonous microbiota of fruits. The safety of such products can be ensured by inhibiting spoilage species by pasteurization, cooking and chemical preservation methods. However, applied food processes can cause undesirable changes in the physicochemical and nutritional properties of the final product. One of the most used preservation methods is the addition of some chemical preservatives (potassium sorbate and sodium benzoate etc.). However, the presence of these preservatives does not meet the expectations of some consumers who want to consume food products with green labels (Plessas, 2021). To prevent these disadvantages, non-thermal food preservation methods (high hydrostatic pressure processing, pulsed electric field and ionizing radiation), smart packaging systems or natural antimicrobial preservatives can be used (Swain et al., 2014; Rodríguez et al., 2021). Development of plant-based fermented food products with LABs with GRAS status is a good alternative as a healthy preservation method accepted by consumers and has been used for centuries (Di Cagno et al., 2013). With the developing fermentation techniques, different microorganisms and substrates are used, and various end products are formed (Melini et al., 2019). These fermentation techniques enable the production of functional beverages with various properties by fermenting fruits and vegetables in recent years. By fermentation, various desirable aromatic compounds such as amino acids, organic acids can be produced, while sources of undesirable flavor such as olefinic in vegetables can be reduced, nutritional values as well as organoleptic quality of the products can be improved (Guan et al., 2021). In addition, metabolites such as organic acids, carbon dioxide, ethanol, hydrogen peroxide, bacteriocins and some fatty acids produced help to provide biological protection (Di Cagno et al., 2013). In a study, antioxidant, carotenoids, and phenolic contents of fermented orange juice by different strains of *L. brevis* and *L. plantarum* were determined. Compared to the unfermented sample, it was observed that the fermented beverage had higher antioxidant capacity, while it was observed that different microorganisms used had different effects on the amount of carotenoid and phenolic compounds formed (de la Fuente et al., 2021). In a study conducted by Yang et al. (2018), the antioxidant capacity and physicochemical properties of apple, pear, carrot mixture fermented by two *L. plantarum* strains were tested and it was concluded that the antioxidant capacity in the fermented product was high, and the product was suitable for consumption.

Microorganisms alter the food matrix and produce various bioactive components during the fermentation of fruits and vegetables which results in the development of the nutritional and sensory properties of the final product (Garcia et al., 2020). In addition to the pleasant taste and smell of fruit and vegetable drinks, organic acids, phenolics and vitamins produced as a result of fermentation also increase their preference in terms of consumption (Ghosh et al., 2015). Some fruit juices with probiotic properties produced with fermented systems are already on the market (Table 1). Fruit juices are important because of their fresh food appearance and being suitable carrier environments where probiotics can show beneficial effects on health. In this system, it is promising to reach a wide consumer mass with a well-designed product (Aspri et al., 2020). With this study, it was aimed to reveal the important points in the production of fermented fruit juices by explaining the relationship between the environment provided by the fruit matrix and the selected microorganisms. In this context, mostly studied fruits and microbial species were brought together and methods that would ensure the continuity of the viability of these species in the final product were reported.

Lactic Acid Metabolism and Its Importance in Fermented Beverage Production

Fruit juices are often preferred by people of all age groups with their nice refreshing tastes. Fruits are rich food sources in terms of important nutritional components (antioxidants, minerals, and vitamins), and due to the natural sugars in their structure, they can allow probiotic LAB to thrive in these environments. Probiotic microorganisms grow by using carbon sources present in plant-based raw materials such as glucose, fructose, galactose, sucrose, maltose, mannitol and raffinose for their metabolic activities. Fruit juices may be easier to digest in the stomach environment than dairy products. Thus, less time spent in the stomach environment with high acidity, especially in fermented products where a probiotic effect is expected, can increase the number of live microorganisms that can reach the digestive tract (Pereira and Rodrigues, 2018; Mojikon et al., 2022). Fermentation of fruits and vegetables is a widely preferred method, but they are less favorable environments for microbial activity than meat and dairy products. These environments are less favorable especially due to their acidity levels, indigestible components, and some bioactive components (Di Cagno et al., 2013; Balthazar et al., 2018; Li et al., 2021a).

Table 1. Fermented fruit juices in commercial production

Kind of fruit	Probiotic microorganisms	Commercial origin and name
Orange-red grapefruit Orange-mango Strawberry-lime-mint Pear-apple-matcha Pineapple-apple-ginger	*L. plantarum* 299v	ProViva-Sweden
Blueberry acai Pomegranate- blackberry Mango Strawberry-banana Raspberry-blackberry Mango-orange Orange	*L. plantarum* 299v	GoodBelly Probiotics-USA
Orange-mango Apple-pear	*L. rhamnosus* GG	Biola-Norway
Sparkling lemon-apple juice Blackberry-apple-lemon Peach-apple-lemon Apple cider vinegar-red beet Apple cider vinegar-turmeric-ginger Apple cider vinegar-kale lemon Apple cider vinegar-cinnamon Apple cider vinegar-chili-ginger-lime	Water Kefir Culture *Bacillus coagulans* Gbi-30 6086	Kevika-USA
Apple-ginger Pineapple-carrot Orange-peach Apple-grape Orange-grape-mango-peach-passion fruit Orange-grape-sea buckthorn-carrot Blueberry-raspberry Pineapple-coconut Orange-mango Mango-apple-orange-banana-passion fruit	*L. rhamnosus* GG	Valio Geofilus-Finland
Apple-mango	*L. paracasei* 8700:2 *L. plantarum* HEAL 9	Healty life-Australia
Grape-orange	*L. paracasei*	Malee probiotic juices, Thailand
Apple-mango-pineapple-banana Mango lemonade Peach-passion fruit	*Bifidobacterium lactis* HN019	Tropicana - USA
Lemon-strawberry-dragon fruit	*B. coagulans*	Press- UK
Mango-passion fruit Strawberry-watermelon	*L. casei* *Bifidobacterium*	PERKii probiotics-Australia
Strawberry-raspberry-lemon-tart cherry juices Coconut-pineapple-ginger-turmeric-orange-lime Juices Pineapple-cucumber-spinach-celery-ginger-kale-collard green-lemon juices	*B. coagulans* Gbi-30 6086	Suja Organic-USA

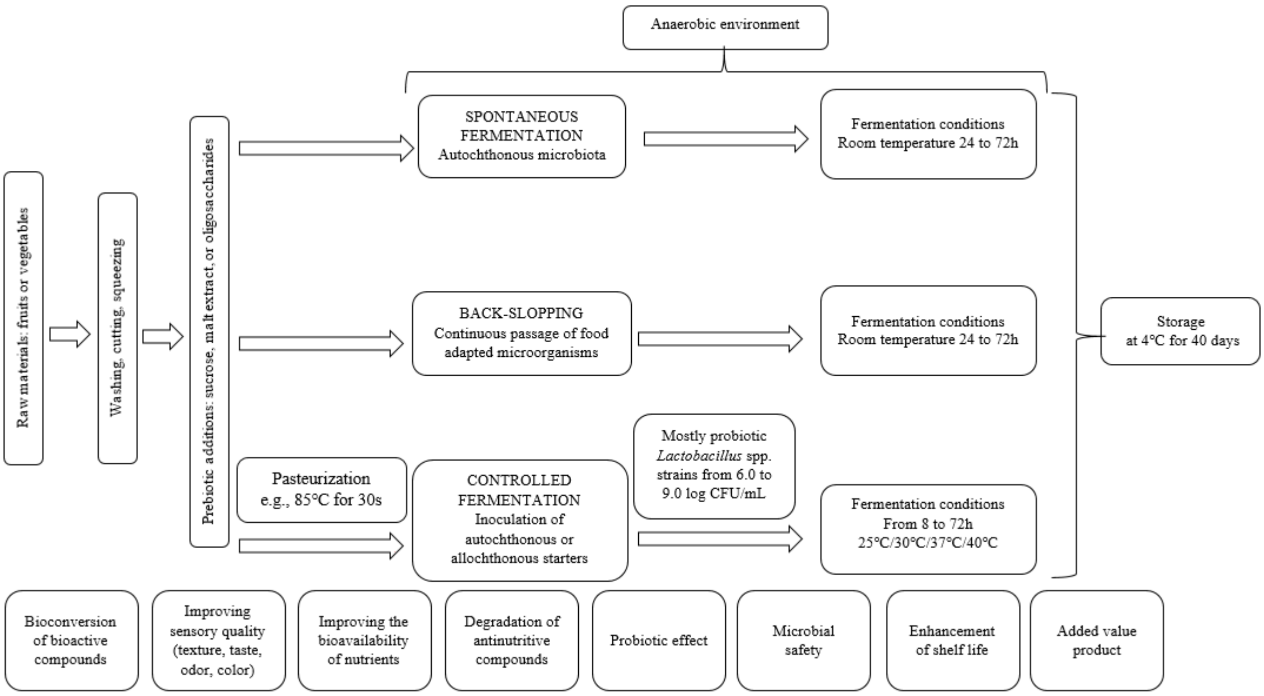

Figure 1. Fermented fruit juice production flow chart (Szutowska, 2020)

Fruit fermentation can occur spontaneously with autochthonous LAB under suitable environmental conditions. As spontaneous species, *Lactobacillus* spp., *Leuconostoc* spp., *Pediococcus* spp., *Weissella* spp., *Fructobacillus* spp., and *Enterococcus* spp. are common. Another method in which the fermentation of the raw material is carried out by inoculating it with a part of the previously fermented product is the back-slopping method. This method prevents unsuccessful fermentation in the face of changing microbiota and improves the quality of the product. The continuous use of the fermented substrate allows the selection of the strain that is best adapted to the environment and the implementation of a starter culture-like application. Although these two methods are preferred in the production of traditional fermented products, it is more reliable to use defined starter cultures with potential probiotic functions (Ilango and Antony, 2021). For this reason, controlled fermentation is preferred for a reliable, reproducible, and standard quality product. The preferred starter cultures in this process are LABs, e.g., *L. plantarum*, *L. rhamnosus, L. gasseri* and *L. acidophilus* (Rodríguez et al., 2021). The most important criterion for effective fermentation is the microbiota of the fruit. It is necessary to investigate suitable microorganisms that can be effective in the food matrix. In particular, the fermentative properties of microbial strains isolated from the fruit's own microbiota responsible for spontaneous fermentation are higher (Li et al., 2021a).

The only difference from the traditional method in obtaining a probiotic juice is the inoculation of the probiotic culture. The juice processing process begins with the cleaning, sterilization, and classification of the raw material. Afterwards, the fruit flesh is broken down and the juice yield is increased. Depending on the raw material, the juice mass can be cloudy, viscous, and dark in color, containing colloidal components due to polysaccharide compounds such as pectin or starch. With the clarification process, this situation is adjusted according to the desired quality criteria. This process includes removal of solid particles by filtration, depectinization with pectinolytic enzymes or degradation of starch with amylase. The solid particles formed after the process are removed by conventional methods or ultrafiltration. Finally, gelatin, bentonite thinners are used and diatomaceous earth or kieselguhr filters are used to make the clarification process more active. Membrane processes such as microfiltration or ultrafiltration have been preferred recently (Bhattacharjee et al., 2017; Urošević et al., 2017). After this stage, heat treatment is applied to the raw fruit juice. Although it is classified according to the applied heat treatment temperature, pasteurization (<100 °C) is mostly preferred for fruit juices. By heat treatment, inactivation of the flora from the microbiota of the fruit and unwanted enzymes (polyphenol oxidase, peroxidase, pectin esterase, polygalacturonate) can be achieved. To ensure a controlled fermentation in fermented juice production, the main mass temperature is cooled to the appropriate temperature for the development of the selected culture. At the end of the incubation period, fruit juices are stored at cooling temperature (4–10°C) (Petruzzi et al., 2017; Pimentel et al., 2019). Figure 1 demonstrates the production of the fermented juices with the methods explained above and the potential technological and functional roles of the probiotic inoculation for the juice production (Szutowska, 2020).

Lactic acid fermentation is used as a preferred method to preserve the safety, nutritional and sensory properties of fruits and vegetables. It is known that LAB increases the flavor of fermented products with various compounds which form as a result of their metabolism (Fan and Hansen, 2012; Zheng et al., 2020). Homofermentative LAB produces lactic acid as the main end product of carbohydrate fermentation, while heterofermentative LAB produces various products such as acetic acid, carbon dioxide, ethanol, acetoin, and diacetyl (Leroy and De Vuyst, 2004; Gänzle, 2015). Various volatile compounds are produced as a result of fermentation utilized by LABs that can provide specific flavors and aromas to the final product.

Microbial strain, juice composition (acidity, carbohydrate content, nitrogen sources, mineral substances) and possible interactions between microorganisms and food components can be counted as the challenging factors affecting the viability of probiotics. In this case, we can categorize various factors affecting the viability of probiotics under four headings. First, the factors in the environment that occur in relation to the fruit itself; fruit, pH, titratable acidity, molecular oxygen, water activity, presence of components such as salt and sugar, artificial flavoring and coloring agents. The second group includes microbiological parameters; characteristics and inoculation rate of selected probiotic strains. Finally, environmental factors related to food processing parameters; heat treatment, incubation temperature, cooling rate, selection of packaging material, storage methods and conditions and finally presence of oxygen (Tripathi and Giri, 2014; Perricone et al., 2015), and the last one is sensory properties (Perricone et al., 2015, Lebaka et al., 2018).

Challenging factors affecting the viability of probiotics

Properties of the raw material

The first effective factor in ensuring probiotic vitality is the selection of fruit itself. Fruits and vegetables are naturally rich in water-soluble vitamins, minerals, carbohydrates, and dietary fiber. In addition to these nutritional components, they are also valuable in nutraceutical terms because they contain phytochemicals and polyphenols such as anthocyanins or carotenoids (Shah and Singhal, 2017; Istrati et al., 2018). The chemical composition of fruits can directly affect the viability of the probiotic culture. While protein and dietary fibers in the structure can protect bacterial cells from acidic stress, citric and malic acid might affect its adversary. The presence of phenolic compounds affects probiotic viability. It has been reported that the presence of high concentrations of phenolic compounds may cause loss of viability (Perricone et al., 2015). Benzoic acid, an important phenolic acid found in fruits, is used as a preservative in many foods. Previously it was suggested that, due to the very low pH and high benzoic acid content of cranberry juice, probiotic viability might decrease during shelf life (Shori, 2016; Pimentel et al., 2019). On the contrary, in a study in which passion fruit was used in the production of probiotic fruit juice and green tea was added to this fruit juice, the increase in the amount of bioactive compounds positively affected the growth of *L. gasseri* (Lima et al., 2022).

High levels of acids such as malic and citric acids (11 mg/L) and fibers (2.8 g/L) in the structure of orange juice are important in terms of being metabolized by some cultures, so orange juice can provide a suitable environment for the continuation of probiotic vitality. In a study conducted with orange juices in which *L. plantarum, L. rhamnosus, L. paracasei* and *L. brevis* species were used as probiotic strains, all strains were able to maintain their vitality during storage by converting malic acid to lactic acid by malolactic fermentation (Multari et al., 2020). Other LAB strains such as *Pediococcus acidilactici* were also shown to survive in fruit juices as reported for strain CE51 in concentrated orange juice with a number of survivals around 8.5 log CFU mL^{-1} at the end of the storage period (de Oliveira et al., 2021). In another study, *L. rhamnosus* was used as a probiotic strain in orange juice and 6 log CFU mL^{-1} microbial count was obtained after 28 days of storage at 4 °C (Sengun et al., 2020). As a result of fermentation of pomegranate juice with *L. plantarum* ATCC 14917, it was reported that the viability reached as high as 10 log CFU mL^{-1} in the first three weeks and this value was 8.83 log CFU mL^{-1} in the last week of storage. This situation has been associated with lactic acid fermentation with increased bioaccessibility of phenolic compounds that can act as prebiotics (Mantzourani et al., 2019).

The pH value is one of the main factors affecting the microbial viability and sustainability of fermented fruit juices. Fruits naturally contain high levels of organic acids in their structure. The low pH value of the medium increases the concentration of their undissociated forms. Consequently, it is hypothesized that the combined effect of acidic conditions and the intrinsic antimicrobial activity of acids affect probiotic viability. Among LABs, *Lactobacillus* are generally more resistant and can survive at pH values of 3.7-4.3, while *Bifidobacterium*, another important group, are less tolerant and even around pH 4.6 may be unfavorable in some cases (Tripathi and Giri, 2014; Perricone et al. 2015; Patel, 2017). The chemical composition of the fruit and its juice is important in maintaining microbial viability. In particular, protein and dietary fiber in the structure have a protective effect on bacteria against acidic stress. Protein, which causes turbidity in the production of clear fruit juice, is removed from the environment by using components such as gelatin and bentonite. For this reason, especially in clear fruit juices, microbial vitality can be lost to a great extent due to protein deficiency. It has been reported that the minimum protein concentration should be 0.3% for the preservation of viability (Nualkaekul and Charalampopoulos , 2011; Pimentel et al., 2019).

Probiotic strains in the fermented system

Fruit matrices are suitable environments for the growth of probiotic bacteria, but for microorganisms to maintain their viability, they must first protect themselves from the natural acidity conditions of the fruit, and also need to adhere to the fruit matrix and compete with pathogenic and spoilage microorganisms (Di Cagno et al., 2013; Rodríguez et al., 2021). Possible reasons that prevent the growth of probiotic LAB in these environments: first, high acidity, presence of oxygen in the system, lack of free amino acids, short chain peptides and oligosaccharides can be counted. For these reasons, the selection of strains that can maintain their stability, vitality and functionality in the system is important for fruits and vegetables, which have more challenging environments than other common fermented products (Žuntar et al., 2020). Microbial flora is an important factor in the development of fermentation and quality in the product, therefore, it is necessary to investigate suitable microorganisms for the biological transformation of the target food matrix, which is the environment where fermentation will take place (Pereira and Rodrigues, 2018).

The carbon sources used by LAB in metabolic activity differ between strains. In general, this metabolism may depend on the composition of the food and the fermentation conditions. Fruits are a good source of fermentable sugars (glucose, fructose, galactose, and sucrose etc.). The main energy source for many bacteria is glucose, however, fructophilic lactic acid bacteria (FLAB), a subgroup of lactic acid bacteria, use fructose as their main energy source. These bacteria, which belong to the genus *Fructobacillus* spp, have poor glucose metabolizing abilities, but grow well in fructose-rich environments (Garcia et al., 2020; Mojikon et al., 2022). In a study where *L. casei* was used in a local fruit fermentation unique to Brazil and the fermentation conditions were examined, it was reported that fructose was the most metabolized sugar (Pereira et al., 2017).

Two methods that can be preferred to carry out a controlled lactic acid fermentation in vegetables or fruits; it is the use of autochthonous starters isolated from the same raw material matrix, or the use of allochthonous bacteria isolated from a particular raw material and used to ferment various products (Di Cagno et al., 2013). For the fermentation of fruits and vegetables, non-autochthonous mostly animal-derived distant species are used, and the viability of these microorganisms decreases during the storage process. For the allochthone/commercial starter cultures, high performance strains are needed to guarantee fermentation on an industrial scale as they have limitations such as not considering other properties other than accelerating acidification, poor adaptation to the main sensory and functional properties of the food matrix, being metabolically inflexible and not reflecting the ecosystem in which they are found. Microorganisms to be isolated from environments obtained by spontaneous fermentation of fruits and vegetables are important as they can guarantee a longer shelf life as autochthonous cultures and have adaptive advantages that can provide nutritional, sensorial, and rheological enhanced properties (Di Cagno et al., 2013, Rodríguez et al., 2021). Table 2 shows the preferred fruits and bacterial strains in fermented juices. To produce a probiotic fruit juice, either an acid-tolerant microbial strain is added to the fruit juice, or it is fermented with probiotic microorganisms.

The fermentation process is advantageous over the addition of microbial strains because, depending on the development of the added strain during fermentation, a product with a lower sugar content and the development of an adapted strain that can maintain its viability are provided. In addition, metabolites such as bacteriocins synthesized by selected species improve product quality during storage (Pereira and Rodrigues, 2018).

The method and the level of addition of probiotic cultures affect their survival. Generally, the preferred method starts with the activation of lyophilized probiotic cultures in appropriate media. Afterwards, the biomass obtained by centrifugation is washed three times in sterile saline solution and dissolved in this solution again and inoculated into the fermented system at a certain rate (~2%). Direct addition of cultures with currently used aseptic dosing technologies does not require the propagation process, and it provides an advantage in terms of time, while the use of high concentrations to reach the minimum viability creates a cost disadvantage. In addition, it is important to ensure the stability of the environment for minimum viability. Addition of a high concentration of culture may also cause physicochemical and sensory changes in proportion to the amount used (Istari et al., 2018; Pimentel et al., 2019).

Environmental factors affecting the survival of probiotics in the fermented system

The viability of probiotic microorganisms in fruit and vegetable juices depends on many factors such as oxygen level, pH, antimicrobial components, strain type, substances in the nutrient medium and temperature. However, survival of probiotics in fermented fruit and vegetable juices is more difficult during storage compared to fermented milk products due to the food matrix (Lillo-Pérez et al., 2021).

Probiotic viability and potency of fermented fruit juices are affected by various factors. Some of the suggested methods for incorporating probiotic cultures into the food matrix to ensure their continuity until consumption and passage to the gastrointestinal tract are fortification with prebiotics, adaptation and induction of resistance, storage under refrigeration and use of antioxidants as well as microencapsulation (Lillo-Pérez et al., 2021). One of the most effective ways to ensure probiotic stability in fruit juice is to enrich the environment with prebiotics or protective components (Perricone et al., 2015). Prebiotics are defined as non-digestible components that are selectively used by certain bacteria in the colon to support their growth and improve host health by providing health benefits. The two most preferred approaches for providing prebiotic activity in fruit juices are addition of the prebiotic carbohydrate directly to the food matrix and synthesis of the prebiotic carbohydrate in fruit juice. The direct addition of purified oligosaccharides as prebiotics can increase production costs. If it is synthesized directly in the environment, it suppresses the purification steps and uses the natural sugars of the fruit juice. Converting simple sugars to prebiotics can reduce the sugar content of the product. However, it is of great importance that the prebiotic compounds should be stable in the food matrix and during the applied processes (temperature, low pH etc.). In addition, if prebiotics are reducing sugars, they can reduce the prebiotic activity of carbohydrates by supporting Maillard reactions (Charalampopoulos & Rastal, 2012; Fonteles & Rodrigues, 2018). Khezri et al. (2018) reported that addition of inulin to the fig juice resulted in an increment of the numbers of *L. delbrueckii* as well as increased antioxidant capacity and organoleptic properties for the inulin added juice sample was observed. Saarela et al. (2006) stated that oat flour and β-glucan added to apple juice preserved probiotic species during storage.

It has been determined that exposing probiotic bacteria to a non-lethal stress will enable them to gain some kind of resistance and that their response to stress can be induced (Gobbetti et al., 2010; Perricone et al., 2015). For instance, Perricone et al. (2014) aimed to reduce the vitality loss of *L. reuteri* DSM 20016 in different fruit juices against low pH and phenolic compounds according to this approach. For this purpose, strains were cultivated by preparing media with different properties (pH and phenolic acid variables) in the laboratory environment. As a result, they showed that they were able to extend the viability of *L. reuteri* DSM 20016 by 5 days against phenol stress and 11 days against acid stress.

Table 2. Microorganism species and fruits used in fermented systems

Fruit Juice	Microorganism	Reference
Blueberry juice	*L. plantarum*	Zhang et al., 2021
Citrus juice	*L. plantarum* SI-1; *L. pentosus* MU-1	Yuasa et al., 2021
Pineapple juice	*B. lactis* Bb12*; L. plantarum* 299V; *L. acidophilus* La5	Nguyen et al., 2019
Kuntze Fruit *(Elaeagnus angustifolia var. orientalis* (L.)	*B. animalis* subsp. *lactis* HN-3	Wang et al., 2022a
Blueberry pomace juice	*L. rhamnosus* GG; *L. plantarum*-1; *L. plantarum*-2	Yan et al., 2019
Broccoli juices	*Pediococcus pentosaceus*	Xu et al., 2021
Jujube juice	*L. plantarum* CICC20265; *Bifidobacterium breve* CICC6184; *Streptococcus thermophilus* CICC6220	Xu et al., 2019
Blueberry and blackberry juices	*L. plantarum* BNCC 337796 *Streptococcus thermophilus* CGMCC 1.8748; *Bifidobacterium bifidum* CGMCC 1.5090	Wu et al., 2021
Kiwifruit juice	*L. acidophilus* 85 (La85)*; L. helveticus* 76 (Lh76) *L. plantarum* 90 (Lp90)	Wang et al., 2022b
Ginkgo kernel juice	*L. acidophilus* BNCC 185342*; L. plantarum* BNCC 337796*; L. casei* ATCC 393	Wang et al., 2019
Carrot juice	*L. plantarum* NUC116	Wan et al., 2019
Grape juice	*O. oeni* MS9 *and* MS46	Del Valle et al., 2022
Sohiong juice	*L. plantarum* MCC 2974	Vivek et al., 2019
Carrot-orange juice	*L. acidophilus* CECT 903 (ATCC4356)	Valero-Cases & Frutos, 2017
Blueberry juices	*L. plantarum; L. fermentum* *L. plantarum* BNCC337796	Li et al., 2021a
Melon Juice	*L. delbrueckii* subsp. *Bulgaricus; L. paracasei* subsp. *paracasei* 34; *L. rhamnosus; L. lactis* subsp. *cremoris* 660; *L. lactis* subsp. Lactis*; S. salivarius* subsp. *thermophilus*	Rúa et al., 2018
Apple juice	*L. plantarum* NCIMB 8826	Roberts et al., 2018
	L. paracasei subsp. *paracasei*	Pimentel et al., 2015
	L. acidophilus TISTR 1338; *L. casei* TISTR 390 *L. plantarum* TISTR 543	Kaprasob et al., 2017
Mediterranean fruit; Juices (Apple- quince- grape- kiwifruit-prickly pear-pomegranate)	*L. fermentum; L. kefiran; L. lactis; L. mesenteroides; Saccharomyces cerevisiae*	Randazzo et al., 2016
Pineapple (*Ananas comosus* L. Merril) and Jussara Fruit	*L. rhamnosus* GG	de Andrade Pires et al., 2020
Cupuassu Fruit *(Theobroma grandiflorum)*	*L. casei* NRRL B-442	Pereira et al., 2017
Apple Juice	*L. acidophilus; L. plantarum; L. fermentum*	Peng et al., 2021
Prickly pears	*L. fermentum* ATCC 9338	Panda et al., 2017
Pomegranate (Punica granatum)	*L. casei* subsp. *casei (*NRRL B-1922*)*	Mustafa et al., 2020
Sweet Orange (Citrus sinensis) juices	*L. plantarum* B42; *L. rhamnosus* B68 *L. paracasei* B37; *L. brevis* DSM32386	Multari et al., 2020
Sweet melon (Cantaloupe)	*L. plantarum* FBS05	Muhialdin et al., 2021a
Watermelon	*L. plantarum* DSM 9843 *(Lp299v®)*	Kanafusa et al., 2021
Jackfruit Juice	*L. casei* ATCC334	Muhialdin et al.,2021b
Dragon fruit juice	*L. plantarum* FBS05	Muhialdin et al., 2020
Mango juice	*L. rhamnosus* GG	Moreira et al., 2017
Orange juice	*L. casei*	Miranda et al., 2019
Black Chokeberry and Sea buckthorn juices	*L. plantarum (*DSM 16365, DSM 20174, DSM 10492, DSM 100813), *O. oeni* strains LAB6, LAA1 and B2013	Markkinen et al., 2019
Cornelian cherry	*L. paracasei* K5	Mantzourani et al., 2019
Goji berry juice	*L. plantarum* strains (LP 39, Lp goji and C8-1*)* *L. acidophilus* strains (NCFM, 6081 6075) *L. helveticus* strains (6024 and LH, LH10)	Liu et al., 2022a
Sea buckthorn juice	*L. acidophilus* La-26 *L. delbrueckii subsp. bulgaricus* Lb-57 *L. casei* Lc-630; *L. plantarum* Lp-6	Liu et al., 2022b
Jujube juice	*L. acidophilus* 85; *L. casei* 37; *L. helveticus* 76; *L. plantarum* 90	Li et al., 2021b
Mulberry juice	*L. plantarum* Lp-115™ (ATCC SD5209) *L. acidophilus* La-14™ (ATCC SD5212) *L. paracasei* Lpc-37™ (ATCC SD5275)	Kwaw et al., 2018
Coconut water	*L. plantarum* DW12	Kantachote et al., 2017
Cherimoya Juice	*L. brevis* CRL 2050; *L. brevis* CRL 2051 *L. plantarum* CRL 2030; *L. rhamnosus* CRL 2049; *F. tropaeoli* CRL 2039	Isas et al., 2020
Peach juice	*L. acidophilus* PTCC 1643; *L. fermentum* PTCC 1744	Hashemi et al., 2021

LABs are very sensitive to fluctuations in storage temperatures. While high temperature values change the microbial activities and growth of probiotics, low temperatures may reduce their metabolic activities and cause inhibition of cellular development (Lillo-Pérez et al., 2021). In this process, cooling may provide longer survival, while thermal stress may cause detrimental effects and cause probiotic species to lose their viability (Patel, 2017).

Probiotic strains preferred in fermented systems are generally anaerobic or microaerophilic in terms of oxygen needs. For this reason, changes in the oxygen level during storage may affect their viability and functionality. In addition, the presence of reactive oxygen species (ROS) such as oxygen, H_2O_2 or superoxide ions can cause oxidative damage. *Bifidobacterium* are often more sensitive to such conditions than LAB. In this context, the effect of food packaging is important, and although glass packages are more advantageous due to their low oxygen permeability levels, they can be used in a limited way due to their high cost and fragility. When packaging materials other than glass material are preferred, it is recommended to use probiotic strains that are more resistant to oxygen, to include antioxidants in the packaging material, to change the atmosphere in the product by increasing the CO_2 content of the headspace in the packaging, and to develop multi-layer packaging designs with selective permeability (Patel, 2017; Pimentel et al., 2019).

Sensory Properties

Probiotics fruit juices have limitations in terms of their overall acceptability and sensory attributes. Additionally, sensory evaluation is also commercially important. Therefore, when developing probiotic juice, it is important to consider acceptability in terms of appearance, flavour, texture and taste (Naseem et al., 2023). The sensory qualities of probiotic foods that are non-dairy can be influenced by interactions between various probiotic strains and food substrates, where textures, flavors, aromas, and colors can be enhanced or aggravated by the production of different metabolic compounds, such as lactic acid and other metabolites, during processing and storage (Gomes et al., 2021). Some studies have shown that the addition of probiotics does not negatively affect the sensory acceptability of fruit juice (de Souza Neves Ellendersen et al., 2012; Ryan et al., 2020; Kardooni et al., 2023). The results of a descriptive sensory analysis conducted in a study of orange juice containing probiotics and prebiotics showed that the functional juices were described as "dairy" aromas, and "dirty," "medicinal," "artificial," and "earthy" flavors, distinguishing them from the conventional juices (Luckow & Delahunty, 2004). In another study conducted, mango juice produced using different probiotic strains. While the sensory results of mango juices produced with some of these strains were positive, mango juices produced separately with *L. plantarum* and *L. rhamnosus* were described as 'bitter', 'sour', 'aftertaste', and 'off-flavor (Mandha et al., 2022). In a study with passion fruit juice, fermented fruit juice was mainly correlated with the terminologies "salty, acidic and bitter tastes" and "sweetener aftertaste" (Fonseca et al., 2022). Reducing undesirable taste and odor in non-dairy probiotic products can be masked by the addition of desired taste or volatile molecules. The addition of tropical fruit juices such as pineapple, mango and passion fruit can significantly affect the taste and aroma of the final product (Luckow et al., 2006; Naseem et al., 2023). In addition, Aziz et al. (2023) added 8% sucrose to fermented pineapple juice in order to reduce the sour taste and this increases the overall acceptability of juice (Aziz et al., 2023).

Current strategies to ensure the probiotic viability

Although the viability of probiotics is affected by many other factors, the strain used is important and the most suitable strain that can adapt to environmental conditions by meeting the requirements should be selected. Environmental factors such as high acidity and low temperature can also affect the metabolism and growth of the probiotic in the matrix. The presence of certain substances in probiotic fruit juice or the external addition of these substances can contribute to vitality. Fermentable sugars, phenolic compounds, antioxidants, proteins, and prebiotics found naturally in fruit juice or added later can contribute to maintaining vitality. These substances can protect probiotics from damage caused by stomach acidity (Patel, 2017; Mojikon et al., 2022; Plessas, 2021). In a study in which passion fruit was used in the production of probiotic fruit juice and green tea was added to the fruit juice, the amount of phytochemicals in the environment increased, and as a result of this increase, the development and viability of *L. gasseri* was supported (Lima et al., 2022). The addition of prebiotics also increases probiotic activity and viability. Ascorbic acid, on the other hand, can contribute to vitality by providing the environment desired by probiotic bacteria by showing antioxidant properties and preventing oxidation (Tang et al., 2023).

Andrade et al. (2019) conducted a study in which they evaluated the survival rate of a probiotic strain, *L. rhamnosus* ATCC 7469, in guava juices with simulated gastrointestinal conditions during refrigerated storage. In this study, they reported that the initial survival rates of bacteria were higher in unfermented media in fruit juices fermented by adding inulin and stevia, but there was a lower decrease in viability and survival in fermented juices after 28 days of storage. This showed that fermentation can improve stability in storage. In another study, in which *L. rhamnosus* was preferred, fermented and unfermented passion fruit was used, and they reported higher bacterial survival rate in fermented juice in simulated gastrointestinal environment (Farias et al., 2016). Nguyen et al. (2019) modelled the survival of probiotics in fermented pineapple juice and found that the probiotics showed 10 times higher survival against stress factors, such as after treatment with 0.3% pepsin and 0.6% bile salt in comparison to the unfermented sample. The increase in bacterial survival is associated with the final pH after fermentation. Bacteria that can survive at low pH have increased their ability to survive in the gastrointestinal environment, as they encounter pH pre-adaptation (Mathipa & Thantsha, 2015). The fermentation process can eliminate potential anti-nutritional factors present in foods by producing flavors, aromas, and desired textures, as well as increasing bacterial survival time (Swain et al., 2014).

Various strategies have been developed to increase and maintain probiotic viability. These include emulsification, encapsulation, ultrasound, pre-adaptation applications, use of high acid fruit juice, use of more than one fruit juice. The use of high-acid fruits in the production of probiotic juice may reduce the negative effects that the probiotic bacteria may encounter in the host by providing a pre-stress. In probiotic fruit juices produced using more than one fruit juice, a fruit juice with a high pH value is added to the juice with a low pH value in order to increase the viability of the probiotic. Thus, by obtaining a relatively higher pH value, the viability of the probiotic strain is increased. In this respect, carrot juice is a fruit juice to be preferred due to its high pH value (Plessas, 2021).

Encapsulation is an application that protects bioactive components from all kinds of harmful factors in the environment. The use of microencapsulation technology to protect probiotic species from the damage they are exposed to in the external environment has provided successful results. Studies have shown that this method is more suitable for providing an anaerobic environment, while creating a physical barrier against harsh environmental conditions (temperature, pH, gastric acid, bile salt etc.), especially for sensitive probiotics (Perricone et al., 2015). By using various encapsulation agents (alginate, κ-carrageenan, resistant starch, inulin etc), vitality is maintained both in the food matrix and in the digestive tract until it reaches the host gut. Thus, the shelf life of the probiotic juice produced can be increased. For this purpose, methods such as spray drying, lyophilization and emulsification are used. Spray drying is preferred especially in probiotic fruit juice production due to its low cost, high capacity, and efficiency method (Plessas, 2021; Tang et al., 2023; Vivek et al., 2023).

In a mixed fruit juice enriched with probiotic *L. rhamnosus* LPAA 01, *L. casei* LPAA 02 and *L. plantarum* LPAA 03 strains microencapsulated by spray drying using maltodextrin, microcapsules were evaluated for their physicochemical properties and microbial viability and stability. As a result, it was reported that the number of viable cells were > 6 log CFU g^{-1} up to 20 days at 5 °C, and the physicochemical properties were within acceptable limits for 14 days at 25 °C (Souza et al., 2021). da Silva et al. (2021) produced microcapsules containing probiotic *L. acidophilus* LA-02 with a complex preservation and then cross-linking method with transglutaminase, and investigated the effects and viability of these added fruit juices on fruit juice during storage. They reported that after 63 days of storage at 4 °C, orange juice was the most suitable medium and the protective effect of microcapsules on the probiotic strain was promising. In a study using a different approach, Mantzourani et al. (2018) added probiotic *L. plantarum* to Cornelian cherry fruit juice as wheat bran immobilized cells and in free form. As a result, they found that the viability (9.95 log cfu mL^{-1} at the 4^{th} week) and total phenolic content (214–264 mg GAE 100 mL^{-1}) were higher in fruit juice containing immobilized cells at 4 ℃ for 4 weeks. In a study by Ding et al. (2008), the effects of storage time and microencapsulation methods on the viability of probiotic bacteria in apple and orange juices were investigated. The effect of using probiotics on physical changes in fruit juice was compared with the control groups. As a result of the study, it was observed that the microencapsulated probiotics showed higher viability than the free probiotics, and in general, an increase in pH and a decrease in °Brix concentration during the storage of probiotic juices was observed.

One of the current strategies ensure the probiotic viability is ultrasound application. Ultrasound is expanding technologies, particularly high-intensity ultrasound (HIUS), which has been thoroughly researched for food processing, primarily with regard to liquid foods (Guimarães et al., 2019). Ultrasound effectively activates waste metabolism by creating temporary holes in cells and affecting cell permeability. As a result, microorganisms receive oxygen and nutrients faster. Thus, microbial viability can be increased by applying ultrasound at low levels (Rahman et al., 2023). In probiotic strawberry juice produced by applying HIUS, it was stated that 2.5 minutes of HIUS application shortened the fermentation time (3 hours), probiotic viability was better in simulated gastrointestinal conditions, probiotic viability was higher during storage, and also improved other properties of strawberry juice (Mizuta et al., 2023).

Increment of health promoting effects of fermented fruits by probiotic fortification

The survival of probiotics during transit through the upper GIT, their colonization, and proliferation mainly in the human colon are key determinants of their health effects. Although initially the concentrations of probiotics in foods seem sufficient to benefit the host, some factors throughout the digestive system cause the probiotics to lose their effectiveness. These factors are low pH, bile salts and gastric enzymes. Gastric juice contains hydrochloric acid and pepsin which ensure a very low pH and the digestion of proteins, respectively. While this gastric juice is beneficial in inhibiting pathogenic microorganisms, it can be an obstacle for the preservation of probiotic viability. The reduction of probiotic survival during processing, storage, and after digestion is frequently mentioned in evaluations of probiotics (Shori, 2016). Because the health benefits of probiotic food products depend on the number of viable cells present at the time of consumption, maintaining the viability of the probiotic strain is the main challenge for the effectiveness of a probiotic food product. This is a prerequisite for achieving health benefits (Aspri et al., 2020). Figure 2 summarizes the potential health promoting functions of probiotic juices with respect to their effects as well as effects originating from their roles as probiotic delivery systems (Garcia et al., 2020).

Fruits and vegetables naturally contain high amounts of minerals, vitamins, carbohydrates, polyphenols, and are very important for human health with their high antioxidant capacity. Many studies have revealed the antioxidant properties of natural products used to treat various diseases such as hypertension, cancer, Alzheimer's disease, diabetes, and Parkinson's disease (Guan et al., 2021). These components, which are naturally present in the structure with fermentation, undergo various biochemical changes, improving the properties of foods and increasing their nutritional value (Tresserra-Rimbau et al., 2019). With their mechanism of action, LABs, microbial enzymes and activated endogenous plant enzymes change the structural properties and provide the release of phytochemicals (hydrolyzed polyphenols and glucosinolates, peptides, secondary metabolites, short chain fatty acids and exopolysaccharides) known for their antioxidant, anti-inflammatory and anticancer properties. They make the consumption of fermented products more attractive. During fermentation, the activity of phytase enzyme may increase with the increase of acidity in the medium. Thus, the bioavailability of phytic acid, which is effective on the absorption of minerals, can be inhibited and its bioavailability can be increased (Leitzmann, 2016; Septembre-Malaterre et al., 2018; Rastogi et al., 2022). Consumption of probiotics is considered to have beneficial effects in the prevention of various diseases (Kandylis et al., 2016). Previous studies suggested that single or multi-strain use of probiotics in fermented beverage production might reduce blood pressure and hypertension as well as blood cholesterol levels (Khalesi et al., 2014; Sun & Buys, 2015) and can be good to fight against gastrointestinal disorders (de Oliveira et al., 2021) and to strengthen the immunity against COVID-19 virus (Singh & Rao, 2021).

Some probiotic LABs produce exopolysaccharide (EPS) and GABA (γ-aminobutyric acid) with various functions in the organism. EPS is a bacterial polysaccharide with potential effects to provide stability to food products. EPSs increase the viscosity in fruit juices and stabilize the structure, while helping probiotics to colonize in the gastrointestinal tract. It is also known to have antioxidant, antibacterial and anticancer properties (Korcz & Varga, 2021). GABA (γ-aminobutyric acid), which acts as a neurotransmitter in the central nervous system, is an amino acid group compound that controls hormone secretion, has potential antidepressant properties and calming effects (Garcia et al., 2020; Szutowska, 2020). By producing these key health promoting metabolites during fermentation as well as their delivery period, probiotics can act as key components of the juice systems which also favors their consumption from the consumer's side. The role of probiotics might rely on specific LAB species. For instance, many health benefits of *L. casei* and *L. plantarum*, which are frequently used in fruit and vegetable fermentation, are known to have probiotic properties. *L. casei* has various probiotic properties, including lipid-lowering, immunomodulatory and antioxidant properties, while *L. plantarum* colonizes the intestine effectively and improves the intestinal flora. It also has high probiotic properties in terms of regulating cholesterol and blood lipids (Khalesi et al., 2014).

Conclusion

Nowadays, plant-based food products especially fruits are at the forefront with their evaluation as fermented systems due to their valuable nutritional profiles. Fortification of these products with probiotics has become a strategy to develop fermented juices containing physiologically active numbers of probiotic cells during their shelf life. Both strain specific and intrinsic factors originating from the raw materials of the juices as well as process conditions are determinant factors for the successful development of fermented juices and delivery of the probiotics. Probiotics can also trigger the transformation of various phytochemicals during fermentation and delivery, they also produce certain metabolites such as EPS and GABA which can both improve the potential health promoting functions of these products. The survival of the probiotics in the juice matrix can be challenging and adaptation of the probiotics to the juice environment as well as development of certain encapsulation techniques should be applied. Future works should focus on the assessment of probiotic characteristics of specific LAB and FLAB strains from fruit environments to meet the increased demand of the food industry for functional probiotic juices.

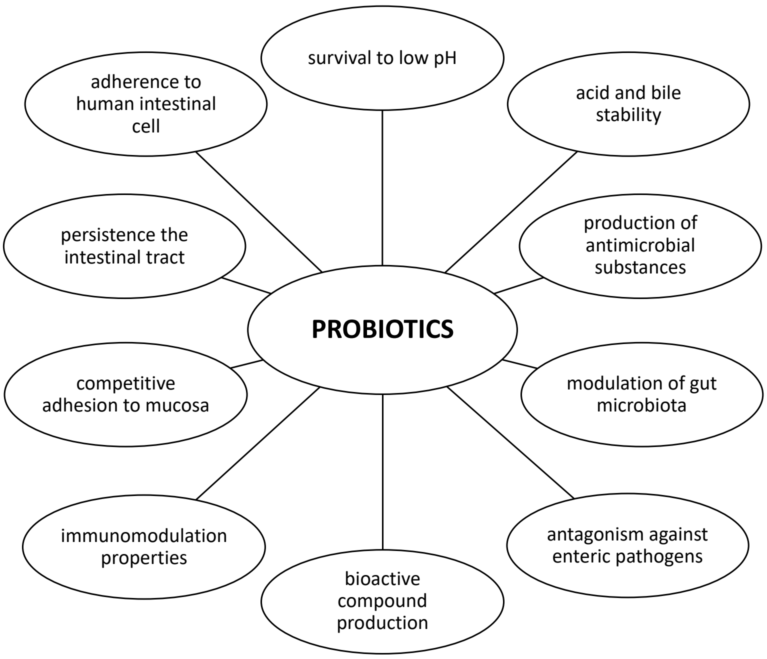

Figure 2. Health-promoting probiotic effect of fermented fruit juices and their roles as probiotic delivery systems (Garcia et al., 2020)

Declaration of competing interest

All authors declare no other competing interests.

Data availability

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

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