PRODUCTION OF PROBIOTIC JUICE IN FRUIT MATRIX

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ABSTRACT

Probiotics are organisms that contribute to the intestinal and digestive systems. Microorganisms that are considered commercial probiotics, most often belong to the genus *Lactobacillus*. Examples of probiotic *Lactobacillus* species are *Lactobacillus acidophilus*, *L. rhamnosus, L. casei, L. plantarum, L. bulgaricus, L. Delbrueckii,* and *L. helveticus*. *Lactobacillus* species are generally recognized as safe (GRAS) organisms. Lactic acid bacteria (LAB) are widely used in the production of fermented foods and beverages because they produce the desired properties in food and beverages thanks to their fermentation properties. They play an important role in ensuring food safety and extending the shelf life of food by producing inhibitory metabolites against other undesirable microorganisms while developing in food. In recent years, non-dairy products, and carriers of probiotics have become more and more used. The main reasons for this are lactose intolerance, vegetarianism, and the fact that people want to move away from animal foods. Juices are non-dairy matrices for probiotics and are compatible with popular dietary options.

Keywords: *Probiotic, Fruit juice, Lactic acid bacteria (LAB).*

INTRODUCTION

The intestinal microbiota in humans plays a specific role in the physiology and metabolism of the host **[1]**. Probiotics, on the other hand, are living microorganisms that have a positive effect on human health by being present in the digestive tract of the body. Probiotics are defined as; "live microorganisms which when administered in adequate amounts confer a

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health benefit on the host." **[2]**. But in addition to the fact that probiotics are consumed regularly and their number is sufficient, these bacteria can be found in difficult environmental conditions in the digestive tract (gastric acidity, bile salts, enzymes, etc.) they must be able to maintain their viability and reach and colonize the appropriate number of intestines **[3]**.

Probiotics have usually been used in fermented dairy products such as kefir, yoğurt, and so on. But for many reasons, such as lactose intolerance, and vegetarianism, which have appeared in recent years, there is an increasing interest in non-dairy probiotic products. Being the main source of water-soluble vitamins, minerals, dietary fibers, and phytochemicals, fruits play an important role in the human diet **[4].** Juices are useful food products for health and are regularly consumed by many people. Due to the rich content of juices, the absence of allergenic effects, and the fact that they can be consumed by everyone, they are quite suitable for the use of probiotic cultures **[5]**. In addition, the fruits have the appropriate matrix for probiotic adhesion. Because of this, it is thought that fruits with high fiber content, such as apples, will have a protective effect on probiotic microorganisms **[6]**. In accordance with this information; various studies have been conducted on the survival of probiotic LAB in fruit juices such as apple juice **[7],** grape juice **[8, 9],** pomegranate juice **[10],** as well as in juices. In different studies, it has been stated that fruits and vegetables are a good food matrix for probiotic bacteria **[11, 12].**

Probiotics in Fruit Matrix

The main purpose of probiotic foods that have positive effects on health is for microorganisms to stay in the body longer by being taken into the body with different matrices and kept in the intestinal microflora **[13].** Most of the probiotic microorganisms belong to the genera *Lactobacillus, Enterococcus, and Bifidobacterium*, which are lactic acid bacteria (LAB). Some species of *Saccharomyces, Streptococcus, Enterecossuc, Pediococcus, Lactococcus, Leunocostoc, Bacillus,* and *Clostridium* can also be used as yeast and other species of bacteria **[14-18].** LAB are a group of microorganisms that produce lactic acid by fermenting Gram-positive, immobile, non-spore-forming, rod-shaped or coke-shaped, catalase-negative, aerotolerant, acid-resistant, carbohydrates, and high alcohols **[19]**. The largest group in the LAB is the genus *Lactobacillus* **[20, 21].** As for the species that are widely studied probiotics, they usually belong to the genus *Lacto-*

bacillus. Probiotic strains belonging to the genus *Lactobacillus*, which are naturally found in the human gastrointestinal tract, are considered GRAS (generally recognized as safe) **[22]**.

The most important factors affecting the number of living cells contained in probiotics are the fermentation time of the product, the storage temperature, and the oxygen content **[23]**. A microorganism that will be used as a probiotic must remain alive during its passage through the digestive tract. For this reason, it must be resistant to enzymes such as lysozyme, pepsin, and pancreatin and survive in the gastric environment of the stomach (pH 1.5-3.0). Probiotic microorganisms, thanks to their resistance to enzymes, can reach the intestine without being adversely affected by gastric acidity **[24]**. Compared to dairy products, the survival of probiotics in the fruit matrix is more complicated. Because of the acidic structure of the fruits, LAB needs to protect themselves from the high acidity in this environment **[25]**. Bile salts are synthesized from cholesterol in the liver and secreted from the gallbladder into the duodenum (500-700 ml/day) **[26].** These acids then undergo chemical modifications (dehydrogenation, deconjugation, deglucuronidation, and dehydroxylation) due to microbial activity in the colon **[27-29].** All conjugated and unconjugated bile salts have an antibacterial effect for strains of *Escherichia coli*, *Klebsiella* and *Enterococcus* species **[26, 30, 31].** Unconjugated bile salts have higher antimicrobial activity. The sensitivity of Gram-positive bacteria to bile salts is higher than that of Gram-negative bacteria **[32, 33].** In vitro resistance tests have shown that bifidobacteria are more resistant to bile salts than lactobacilli **[34, 35].**

By adding prebiotic components to probiotic juices, improvement in sensory and physicochemical properties and an increase in probiotic survival occur **[5, 36].** But it is impossible not to mention the definite positive effect of the addition of prebiotics on probiotic survival. Because while some studies **[7]** support the positive effect of prebiotics on the development of probiotics, some studies **[5, 38, 39]** have stated that there is no positive effect**.** In addition, it has been stated that processing techniques such as peeling and small-piece separation applied to fruits and vegetables will have a positive effect on probiotic beverage production by releasing cellular content in terms of minerals, sugars, vitamins, and other nutrients and therefore creating a richer substrate environment for probiotics **[40, 41]**. Table 1 shows the probiotic juice studies produced using different fruit matrices and microorganisms.

Table 1. Probiotic application in fruit juices

Plant matrix	Microorganisms	References
Apple	*Lactobacillus casei*	**[42]**
Apple	*L. paracasei*	**[7]**
Cashew apple	*L. casei*, *Bifidobacterium breve*, *B. infantis*	**[43]**
Grape	*L. paracasei*	**[44]**
White grape	*L. paracasei*, *L. casei*-01	**[9]**
Grape	*L. plantarum* PTCC, *L. delbruecki* PTCC 1737i, *L. rhamnosus* PTCC 1657	**[45]**
Citrus	*L. pentosus* MU-1, *L. plantarum* SI-1	**[46]**
Orange	*Pediococcus acidilactici*, *L. monocytogenes*	**[47]**
Orange	*L. rhamnosus*	**[48]**
Orange	*L. paracasei*	**[5]**
Orange	*L. case*i-01	**[49]**
Pineapple	*P. pentosaceus* LaG1, *L. rhamnosus* GG, *P. pentosaceus* LBF2	**[50]**
Pineapple	*Lactobacillus* and *Bifidobacterium* spp.	**[51]**
Mango	*L. acidophilus*	**[52]**
Mango	*L. plantarum, L. casei, L. acidophilus, L. delbrueckii*	**[53]**
Mango	*L. bulgaricus* S1, L. *plantarum* Lp-115, *Streptococus thermophilus* 606*3*	**[54]**
Pomegranate	*L. plantarum, , L. paracasei, L. delbruekii, L. acidophilus*	**[55]**
Pomegranate	*L. plantarum*	**[56]**
Pomegranate	*L. plantarum* PTCC 1745, *L. delbruecki*i: PTCC 1333	**[10]**
Watermelon	*L. helveticus, L. acidophilus*, *L. plantarum*	**[57]**
Melon	*L. casei* B-442	**[58]**
Cornelian cherry	*L. plantarum* ATCC 14917	**[59]**

Cornelian cherry	*L. casei* T4	**[13]**
Blueberry and blackberry	*L. plantarum, Streptococcus thermophilus*, *B. bifidum*	**[60]**
Blueberry	*L. plantarum* J26	**[61]**
Prickly pears	*L. fermentum*	**[62]**
Peach	*L. plantarum*, *L. delbrueckii*, *L. casei*	**[63]**
Noni	*L. casei, L. plantarum*, *B. longum*	**[64]**
Carrot, beet and apple	*L. casei*	**[65]**
Cupuassu	*L. casei*	**[66]**

In the study of Pereira et al. **[42],** optimizing the living standards of *L. casei* NRRL B-442 in cashew apple juice, determining the appropriate amount of inoculum and the fermentation time were investigated. As a result of the analysis, they observed that there was no loss of vitality of *L. casei* in cold storage for 42 days in apple juice and that the color, which is one of the most important factors after flavor, was preserved in the juice. For this reason, it has been reported that cashew apple juice fermented with *L. casei* is a healthy alternative to functional foods containing probiotics.

In the study of Pimentel et al. **[7],** the effects of using oligofructose or sucralose instead of sugar on probiotic apple juice were investigated by adding *L. paracasei* ssp. Apple juice containing oligofructose showed less sweetness than that containing sucrose. It has been observed that sucralose apple juices have a light color compared to sucrose apple juice. The addition of probiotics has increased the turbidity of apple juice. The increase in its turbidity did not affect the general acceptability of apple juice. It has been reported that with *L. paracasei*, synbiotic apple juice can be created that creates a sensory profile (excluding turbidity and particle presence) and acceptance level close to the sucrose-added apple juice.

In the study of Leite et al. **[43],** cashew apple juice containing gluco-oligosaccharides, dextran, and tagatose has been confirmed to exhibit prebiotic properties after in vitro digestion. These components have reached the digestive tract and are used as a source of carbon by probiotic bacteria (*L. casei* NRRL B-442, *B. breve* NRRL B-41408, and *B. infantis* NRRL B-41661). For all the bacteria studied, a different growth was observed against substrates with prebiotic properties (oligosaccharides, dextran, and tagatose). It has been reported that the substrates used support probiotic microbial growth.

In the study of Silva & Ferrari **[44]**, the effect of inulin on the viability of microorganisms was observed in grape juice produced using *L. paracasei.* In order to observe the effect of inulin, 3 formulations were compared: grape juice without probiotics and inulin, grape juice with *L. paracasei* (7.5%), and grape juice with *L. paracasei* (7.5%, v/v) / inulin (10%, w/v), which did not participate in inulin, and grape juice with *L. paracasei* (7.5%, v/v). The probiotic grape juices produced were stored at 4ºC for 28 days and subjected to physical and microbiological analysis. Although the pH and total acidity values of dec grape juices ranged between 3.28-3.31 and 0.193-0.209 g/ml, respectively, no statistically significant difference was observed. It has been determined that the amount of soluble solids in the juice of probiotics containing inulin is greater. Grape juice has proved to be a promising plant-based matrix for the production of a probiotic drink, as it is able to ensure the viability of *L. paracasei* above 6 log CFU/ml by the end of storage. The results of the study showed that *L. paracasei* is able to survive in grape juice even without the addition of a prebiotic component.

In the study of Okina et al. **[9],** probiotic white grape juice was made using lyophilized *L. paracasei* culture (2% v/v), and the culture was compared with non-culture-added juice. It has been determined that the white grape juice produced by the added probiotic culture has a darker color and a lower total phenolic amount. As a result, it was emphasized that probiotic white grape juice prepared using *L. paracasei* is an alternative for individuals who do not consume/can not consume dairy products and can resist the probiotic effect (21 days at 4°C) and gastrointestinal conditions (28 days at 4°C). The study also stated that white grape juice is a good carrier of probiotics for *L. paracasei*.

In the study of Manganji vd. **[45],** by adding *L. delbrueckii, L. Plantarum,* and *L. rhamnosus* to pasteurized grape juice, its change in microbiological and sensory properties was studied for 4 weeks at 4°C. According to the study, the resistance of *L. rhamnosus* and *L. delbrueckii* to refrigerator conditions was found to be better compared to *L. plantarum*. A decrease in probiotic levels was observed starting from the 1st to the 28th day, but the least decrease was observed in *L. rhamnosus*. In addition, the sensory properties of grape juice added to L rhamnosus and kept in refrigerator conditions for 4 weeks were found to be at a higher level of acceptability compared to others. According to the findings obtained as a result of the study, it is recommended to produce probiotic grape juice with the addition of *L. rhamnosus*, whose vitality is higher and organoleptic properties are more desired.

In the study of Yuasa et al. **[46],** *L. plantarum* SI-1 and *L. pentosus* MU-1 were inoculated into three different citrus juices and fermented. It has been reported that there is a decrease in the number of living bacteria in citrus juices, but their taste and other sensory properties have not changed.

In the study of de Oliveira Vieira et al. **[47],** it has been confirmed that oranges can be used as an ideal food matrix for a probiotic drink. It has been reported that *P. acidilactici* CE51 is suitable for the production of probiotic orange juice. It has also been reported that *P. acidilactici* can be used to provide control against *L. monocytogenes*.

In the study of Sengun et al. **[48],** it has been reported that orange-based juices are suitable substrates for the development of *L. rhamnosus*, and these products can be considered probiotic products. In the same study, they reported that the addition of stinging nettle (*Urtica dioica* L.) together with *L. rhamnosus* did not significantly increase the viability of *L. rhamnosus* in cold-stored orange juice, but increased the total content of phenolic substances and slowed the rate of decline of antioxidant activity during storage. As a result of the study, it was reported that the production of probiotic orange juice using *L. rhamnosus* and stinging nettle was successfully completed.

In the study of Costa et al. **[5],** the effect of the use of oligofructose or ascorbic acid on the viability of *L. paracasei* has been investigated. Probiotic juices produced high probiotic culture viability during the 28-day cold storage period. However, oligofructose or ascorbic acid did not have a protective effect on probiotic strains. As a result, it has been reported that orange juice is a suitable substrate for the development of *L. paracasei*.

In the study of Miranda et al. **[49],** the effect of the addition of *L. casei* to orange juice on the quality of the juice was studied. The cultures used in the study were added to orange juice directly, activated, and encapsulated. As a result of the direct addition of commercial cultures directly, it was found that the probiotic drink is more similar to pure fruit juice in terms of its physical, chemical, and sensory properties. At the same time, it was the drink that had the most suitable products in terms of volatile compounds. As a result of the addition of activated cultures, an increase in the content of organic acids (acetic, lactic, and citric) was observed, while the volatile components decreased and were less appreciated sensually. By adding encapsulated cultures, products with a high consistency index were obtained, while lower organic acid and sensory results were determined.

In the study of AdebayoTayo & Akpeji **[50],** probiotic LAB (*P. pentosaceus* LaG1, *L. rhamnosus* GG, and *P. pentosaceus* LBF2) of fermented pineapple juice produced by using single and multiple the viability of LAB, lactic acid production, vitamin C has been reported to increase the growth and antagonistic potential. It has been reported that the probiotic fermented pineapple juice produced in this way can be a beneficial drink for human health.

In the study of Nguyen et al. **[51],** it has been reported that pineapple juice is a good substrate for the development of probiotic bacterial strains such as *Lactobacillus* and *Bifidobacterium*, however, it is more appropriate to use *L. plantarum* for probiotic pineapple juice.

In the study of Ryan et al. **[52],** the effect of mango juice and milk-based fermented beverage on the viability of probiotic strains has been investigated. In the study, *L. acidophilus* strain was detected in different concentrations of mango juice in cow's milk (formulations containing 0%, 10%, 20%, 30%, and 40% mango juice (w/w)) were added and kept at 4 °C for five weeks. It has been determined that the concentration of 10% mango juice increases the viability of probiotic cultures. Similarly, it was determined that 10% mango juice increases the probiotic tolerance of gastrointestinal digestion under artificial conditions. It has been reported that increasing the concentration of mango juice from 20% to 40% increases sensory scores and therefore is more appreciated.

In the study of Reddy et al., **[53],** it has been observed that four LAB such as *L. plantarum, L. delbrueckii, L. casei* and *L. acidophilus* can use mango juice for cell synthesis and lactic acid production without pH adjustment.

All of the LAB used in the study survived under conditions of low pH and high acidity during 4 weeks of cold storage at 4 °C. It has been reported that mango juice can be used as one of the non-dairy raw materials for LAB, and the product can be used as a probiotic drink for vegetarians.

In the study of Wang et al. **[54],** ultraviolet-assisted ultrasonic pretreatment for probiotic mango juice containing *L. bulgaricus* S1, 6063 *S. Thermophilus,* and *L. plantarum* Lp-115 strains have been applied as pre-processing. It has been reported that probiotic mango juices produced using probiotic strains have positive effects such as better storage stability and longer shelf life compared to probiotic mango juice produced without pretreatment.

In the study of Mousavi et al. **[55]**, the suitability of pomegranate juice produced by probiotic LAB for use as a probiotic beverage has been studied. Pomegranate juice was fermented at 30°C for 72 hours. *L. delbruekii* and *L. plantarum* were able to survive the first 2 weeks of storage at 4°C, while *L. acidophilus* and *L. paracasei* were observed to lose their viability after the second week in the same conditions. It has been reported that fermented pomegranate juice is a suitable medium for probiotic cultures to increase.

In the study of Mantzourani et al. **[56]**, It was tried to obtain a low-alcohol functional pomegranate juice drink by adding *L. plantarum*. Fermented pomegranate juice has had a high total phenolic content and antioxidant activity during the storage period compared to pomegranate juice which has not undergone a fermentation process. *L. plantarum* has maintained its vitality and increased in fermented pomegranate juice. As a result of these studies, it was reported that *L. plantarum* can be used for the development and production of a new functional drink.

In the study of Oruç & Çakır **[57]**, for the fermentation of watermelon juice, *L. helveticus, L. Acidophilus,* and *L. plantarum* were used. Watermelon juice was fermented at 37°C for 18 hours. During the fermentation process, total LAB count, titration acidity, pH, and water-soluble dry matter analyses of watermelon juices were performed. It was determined that the LAB numbers increased significantly in all watermelon juice samples. It has been reported that watermelon juice is a favorable environment for the development of LAB, and with fermentation, there is an increase in the values of phenolic substances and antioxidant activity of watermelon juice.

In the study of Fonteles et al. **[58],** melon juice fermented with *L. casei* was subjected to ultrasonic processing. During the 42-day storage period in fermentation without the use of preservatives and heat treatment, it was not observed that the bacterium did not lose its viability and that there was no deterioration in the juice. It has been reported that melon juice fermented with the support of ultrasonic processing can be considered a low-calorie drink, which is a potential new source of probiotic bacteria.

In the study of Mantzourani et al. **[59]**, *L. plantarum* ATCC 14917 was used in cornelian cherry juice fermentation. Cornelian cherry juice was fermented for 24 hours and kept at 4 °C for 4 weeks. As a result, it has been shown that *L. plantarum* immobilized on the wheat bran carrier used can be used in the production of a low-alcohol grade cranberry beverage. As a result of sensory evaluations, it was determined that there is no unpleasant/undesirable taste in fermented cranberry juice. It has been reported that fermented cornelian cherry juice is a suitable medium for probiotic cultures to increase.

In the study of Nematollahi et al. **[12],** cornelian cherry juice with a pH of 2.6 is harmful to probiotic cultures, and probiotic species that are resistant to even the worst conditions have been found to disappear completely after a maximum of 7 days of cold storage. The viability of *L. casei* T4, which was applied by adjusting to pH 3.5, was even greater after 28 days than at the beginning. As a result, it has been reported that *L. casei* T4 will be a strong and resistant strain for production in beverages with harsh conditions, such as fruit juice and beer.

In the study of Wu et al. **[60]**, it has been revealed that potential probiotic strains (*L. plantarum, B. Bifidum, and S. thermophilus*) have effective values on the metabolism of phenolic acids, biotransformation of organic acids, and sensory properties in blueberry and blackberry juices. It has been reported that the study conducted can help produce potential non-dairy probiotic drinks.

In the study of Zhang et al. **[61]**, blueberry juice has been fermented using *L. plantarum* J26 as the fermentative strain for the investigation of its functional properties. Functional ingredients fermented blueberry juice (cyanide, chloride, petunidin, and pelargonidin peonid), and beneficial activities (α-glucosidase and α-amylases inhibition) observed an increase in

LAB can produce a beverage with functional and also used blueberry has been reported.

In the study of Panda et al. **[62]**, total LAB count, titration acidity, pH and water-soluble dry matter analyses were performed by fermenting prickly pear juice with *L. fermentum* at 28°C for 48 hours. As a result of the study, it was confirmed that it is acceptable research to prevent prickly pears from losing their properties and the value of pear juice. It has also been reported that prickly pear juice is a new functional drink that contains antioxidants and phytochemicals that are beneficial to health.

In the study of Pakbin et al. **[63],** probiotic peach juice production was carried out by fermentation at 30 °C for 24 hours using *L. plantarum* DSMZ 20179, *L. delbrueckii* DSMZ 15996 and *L. casei* DSMZ 20011 strains. Changes in pH, titratable acidity, sugar content, and the number of living cells were studied during fermentation. As a result of the study, *L, casei* was not considered suitable for cold storage, but it was found that *L. delbrueckii* is suitable for the production of probiotic beverages. Therefore, it has been reported that peach juice can be a healthy beverage alternative for vegetarians and consumers with food allergies.

In the study of Wang et al. **[64]**, *L. casei, L. Plantarum,* and *B. longum* were used to produce probiotic noni (*Morinda citrifolia*) juice. It has been observed that *L. casei* produces less lactic acid than *B. longum* and *L. plantarum*. It has been observed that *L. plantarum* and *B. longum* survive at low pH in cold storage at 4° C for 4 weeks, and cell viability has not been observed in *L. casei* after 3 weeks. As a result of the study, it was reported that *L. plantarum* and *B. longum* are optimal probiotics for fermentation with noni juice.

In the study of Zandi et al. **[65]**, a mixture of carrot, beet, and apple juices was infused with *L. casei* to produce a fermented beverage. A mixture of carrot, beet, and apple juices and a suspension of *L. casei* was prepared and added to the mixture of juices by 20%, 30%, and 40%, respectively. In all applications, during the fermentation process, the number of probiotic bacteria increased with the use of sugars and nutrients contained in the juice, while the values of sugar and brix decreased. It has been reported that the sample with a concentration of 40% and $1.5x10^6$ CFU/ml *L. casei* has maximum cell viability during 4 weeks of storage at 4° C and is suitable for use as a probiotic beverage.

In the study of Pereira et al. **[66],** cupuassu (tropical fruit native to the Brazilian Amazon) was fermented for 18 hours at 30 °C, pH 5.8 by adding *L. casei* to the juice. During fermentation, the antioxidant activity and the number of *L. casei* increased. After fermentation, the organic acids contained in cupuassu water increased. As a result, the components of cupuassu water (natural sugars and organic acids) were found to be suitable for the development of probiotic bacteria. It has been reported that the probiotic cupuassu drink is considered an alternative functional food.

CONCLUSION

Probiotics are organisms that contribute to the intestinal and digestive systems. Probiotics, which are usually used in yogurt, kefir, and similar fermented products, have also started to be used in fruits and vegetables that are considered to be the different probiotic matrices. In different studies, it has been shown that many fruits are a suitable environment for probiotics. The rich content of fruits, the absence of allergenic effects, and the fact that they can be consumed by everyone are quite suitable for the use of probiotic cultures. It will be useful to investigate the production of probiotic functional juice in different types of fruits and with different microorganisms.

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