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Lactic acid fermentation of apricot juice by mono- and mixed cultures of probiotic *Lactobacillus* and *Bifidobacterium* strains

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Abstract Apricot is a popular fruit in the world with rich in carbohydrates, vitamins and elements as well as has high antioxidant capacity. In this study, fermentation of this juice by mono- and mixed cultures was investigated. All tested strains exhibited good growth properties on apricot juice without any nutrient supplementation. In monoculture fermentation, 7.2, 7.25, 7.06 and 7.16 log (cfu/mL h) cell yields were observed for *Bifidobacterium lactis* Bb-12, *Bifidobacterium longum* Bb-46, *Lactobacillus casei* 01 and *Lactobacillus acidophilus* La-5 strains, respectively, and higher cell yields were obtained in the mixed culture fermentation. The antioxidant capacity increased slightly during fermentation. The concentration of acetic acid (27–48 mM) were about doubled in cases of the mixed culture fermentations than of monoculture fermentations (18–30 mM), while the levels of lactic acid were similar (70–90 mM). The relatively high values of these properties offer the potential for development of novel probiotic apricot juice.

Keywords Apricot · Bifidobacteria · Lactic acid bacteria · Fermented juice · Probiotics

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Introduction

Nowadays, claims of consumers to special juice-based products containing functional components such as probiotics and/or prebiotics increase worldwide. Fermentation by lactic acid bacteria and bifidobacteria is known as process to increase nutritional values of foods as well as to provide protection against some diseases. During fermentation, probiotic bacteria produce some beneficial components such as vitamins and antioxidants, but also have metabolic activity that convert high-calorie into low-calorie sugars and that can degrade lipids and cholesterol [1]. Furthermore, health-promoting properties of probiotic bacteria are the following: inhibition of pathogens and harmful bacteria that colonize and/or infect the gut mucosa; regulation of intestinal microbial homeostasis; repression of pro-carcinogenic enzymatic activities within the microbiota; modulation of local and systemic immune responses; bioconversion of a number of dietary compounds into bioactive molecules; and the prevention of cancer [2, 3].

Strains of two bacteria genus *Lactobacillus* and *Bifidobacterium* are commonly used as probiotics in production of functional foods. Recently, most of commercial probiotics are dairy-based products that cannot be consumed by humans who are allergic to milk proteins or have severe lactose intolerance. Fruit juices are rich sources of functional food components, minerals, vitamins, dietary fibres, antioxidant and phytochemicals for the human diet [4]. Additionally, fruits do not contain any allergic components [5]. The World Health Organization (WHO) as well as the Food and Agriculture Organization (FAO) also recommended intake of a specific dose of vegetables and fruits in daily food to prevent chronic pathologies such as

hypertension, coronary heart problems and risk of strokes [6].

Apricot (*Prunus armeniaca*) is a popular fruit in the world and rich in carbohydrate content. This also contains high amounts of essential minerals such as potassium (226–296 mg/100 g), phosphorus (20 mg/100 g), calcium (13.8 mg/100 g), magnesium (8–14 mg/100 g), iron (0.3–0.54 mg/100 g), zinc (0.16–0.26 mg/100 g). Similarly, the vitamins found in apricot are pro-vitamin A (0.2–1.56 mg/100 g), vitamins C (5–10 mg/100 g), K, E (0.5–0.88 mg/100 g), thiamin (0.02–0.06 mg/100 g), riboflavin (0.02–0.05 mg/100 g), niacin, pyridoxine, folic acid and pantothenic acid. Overall, apricot is especially rich in numerous phenolic components including hydroxycinnamic acid derivatives (caffeic, p-coumaric and ferulic acids) and their esters [7]. Phenolics have been shown to have antioxidative, antimutagenic and anticarcinogenic effects, and have protective roles against cardiovascular diseases and cataract [8]. Of course, the chemical composition of the apricot juice is different and depending on soil, climate, ripeness and processing method. Owing to their contents of several nutritious and health-promoting bioactive and nutraceutical compounds, the apricot is considered as an ideal substrate for carrying probiotic cultures which also has a beneficial effect on consumer health and prevention of diseases. Moreover, the organoleptic and nutritional quality of the fermented fruits and vegetables as well as retaining of their nutrients and coloured pigments can be enhanced by lactic acid fermentation [9], thus fermented probiotic apricot drink may serve as a good alternative product to replace the milk-based probiotics [10–12].

In this study, changes of viable cell count as well as some biochemical compounds and antioxidant capacity during the mono- and mixed culture fermentation of the apricot juice with probiotic bifidobacteria and lactobacilli were investigated.

Materials and methods

Fruit juices

The apricot fruit juices were purchased commercially from the local market. The initial pH of the culture medium was adjusted to pH 6.5 with 4 N NaOH before fermentation.

Media

Trypticase–Phytone–Yeast medium (TPY) contained (per litre) trypticase (BBL) 10 g, phytone (BBL) 5 g, glucose 5 g, yeast extract (Difco) 2.5 g, Tween 80 1 mL, L-cysteine HCl 0.5 g, K_2HPO_4 2 g, $MgCl_2{\cdot}6H_2O$ 0.5 g, $ZnSO_4{\cdot}7H_2O$ 0.25 g, $CaCl_2$ 0.15 g, $FeCl_3$ 0.03 g. TPY agar is the TPY medium supplemented by agar–agar in a concentration of 15 g/L. Its pH was around 6.0.

Beeren's agar medium contained (per litre) Columbia agar (Oxoid CM331) 44 g, glucose 5 g, L-cysteine HCl 0.5 g, agar–agar 5 g and propionic acid 5 mL. The propionic acid was added to the medium after sterilization, and the final pH was adjusted to pH 5.0 with 1 N NaOH.

MRS agar was prepared based on recipe given by de Man et al. [13].

Micro-organisms and their maintenance

Two probiotic *Bifidobacterium* (*Bifidobacterium lactis* Bb-12 and *Bifidobacterium longum* Bb-46) and two probiotic *Lactobacillus* strains (*Lactobacillus casei* 01 and *Lactobacillus acidophilus* La-5) were applied in this study. All strains were purchased from Chr. Hansen A/S (Hřrsholm, Denmark). Bifidobacteria were pre-cultured anaerobically (in Bugbox anaerobic chamber, Ruskin Technology, USA) in the TPY medium at 37 °C for 24 h. Lactobacilli were cultivated in the MRS medium at 37 °C for 24 h.

Determination of colony forming units

Samples from the fermented juices were diluted by 10-fold with sterile 0.85% sodium chloride solution and then the dilution was used for microbial enumeration by pour plate methods. The aliquots of dilution were transferred into Petri dishes, mixed with the appropriate medium. The Beeren's agar was used as a selective medium for bifidobacteria, while the TPY agar was used to determine the cell number of bifidobacteria. The plates were incubated under anaerobic conditions in Anaerobe Jar + GasPak System or in Bugbox anaerobic chamber at 37 °C in case of bifidobacteria. The colonies were counted after 72 h or 96 h of incubation. Lactic acid bacteria were counted after plating on the MRS agar (Scharlau, Spain) and incubated at 37 °C for 48–72 h at aerobic conditions.

Fermentation process

Fruit juices (50 mL medium/100 mL flask) was inoculated with *Bifidobacterium* and/or *Lactobacillus* strain and kept under anaerobic conditions at 37 °C using Anaerobe Jar + GasPak System (OXOID) or a Bugbox anaerobic chamber. Samples were taken at regular time intervals and the colony-forming unit (cfu) of bifidobacteria and lactobacilli were counted. In addition, the pH was measured. Also, the carbohydrate, lactic and acetic acid content and antioxidant capacity of the samples were determined.

Analysis of carbohydrates and organic acids

The amounts of sugars and organic acids in cell-free culture supernatant (centrifuged at 14.000 rpm for 10 min) of fruit juices were determined by HPLC methods. The Surveyor HPLC system (Thermo Scientific Corporation, USA) consisted of quadruple pump, autosampler, refractive index (RI) and photodiode array (PDA) detectors, column oven equipped with the Agilent HiPlexH column (7.7 mm × 300 mm, Agilent, USA) was applied. The mobile phase was 5 mM H_2SO_4. Isochromatic elution mode with 0.6 mL/min flow rate was applied. Temperatures of the column was kept at 45 °C in column oven. The data acquisition and integration were performed using the ChromQuest 5.0 software package (Thermo Scientific Corporation, USA). Both internal and external standards of sugars (glucose, fructose, maltose as disaccharides) and organic acids (acetic, lactic, oxalic, butyric, citric, succinic and malic acids) were applied to qualify and quantify the sugars and short chain fatty acids, respectively.

Analysis of antioxidant capacity

The total antioxidant capacity of the fermented apricot juice obtained through ferric reducing antioxidant power (FRAP) assay described by Benzie and Strain [14]. The FRAP assay measures the change in absorbance at 593 nm due to the formation of a blue coloured ferrous-tripyridyltriazine complex from colourless oxidized ferric form by the action of electron donating antioxidants.

Statistical analysis

All fermentations were carried out in triplicate and samples were twice analysed (total of six analyses for each sample). All data are presented as the mean and standard deviation (SD). One-way analysis of variance (ANOVA), unpaired and paired Student's t-tests were done using Statistica v9.0 software package (StatSoft, USA). Generally, only $p < 0.05$ was accepted as the statistical significance level.

Results and discussion

Fermentation with monoculture

Apricot juice was fermented in monoculture mode by two *Lactobacillus* and two *Bifidobacterium* strains with initial cell numbers about 10^6 cfu/mL. The pH and antioxidant capacity of apricot juice were pH 6.1 and 2.15 mM, respectively. The juice was also rich in fermentable sugars like fructose (6 g/100 mL), glucose (3.4 g/100 mL) and disaccharides (1.2 g/100 mL). Cell yield, pH, antioxidant capacity and content of some sugars after 24 h of fermentation were collected in Table 1.

All investigated *Bifidobacterium* and *Lactobacillus* strains were able to grow well in the apricot juice without supplement of any nutrients meaning this matrix in itself was suitable medium for propagation of probiotic bacteria. In all cases the cell numbers at 24 h of fermentation were higher than 10^8 cfu/mL, and cell yields varied from 1.15×10^{10} cfu/L h to 1.78×10^{10} cfu/L h (Table 1). These values were significantly higher than ones (2.7×10^9 cfu/L.h and 1.0×10^{10} cfu/L.h for *B. lactis* Bb-12 and *B. longum* Bb-46, respectively) reported by Havas et al. [15] when they propagated these bacteria on soymilk. Our results are in agree with data published by Fonteles et al. [11] in the case of fermentation of the cantaloupe juice with *Lactobacillus casei* NRRL B-442. About 8.5 log (cfu/mL) viable cell counts were determined after 1 day fermentation with *L. casei* on the cashew apple juice [12]. Volumetric productivities of *B. lactis* Bb-12, *B. bifidum* B7.1 and *B. bifidum* B3.2 were 2.16×10^{10} cfu/L h, 4.65×10^{10} cfu/L h, and 3.85×10^{10} cfu/L h, respectively, were reported by Kun et al. [16] when they carried out fermentation of the carrot juice. Both genera *Lactobacillus* and *Bifidobacterium* were reported to have high requirements of free amino acids, peptides, vitamins and fermentable carbohydrates for growth [17] due to lack proteolytic activity [2]. The apricot is one of the fruits that is abundant in free amino acids with about 1.5 mg/100 g [18], thus it provided enough nitrogen source for growth of probiotic bacteria.

Due to the metabolic activity of probiotic bacteria, short chain fatty acids (SCFA) were produced increasing acidity of media. *Bifidobacterium lactis* Bb-12 and *Bifidobacterium longum* Bb-46 produced a bit higher amount of acids than two *Lactobacillus* cultures and reduced the pH of the apricot juice from the initial value of pH 6.1 to pH 4.8 after 24 h of fermentation (Table 1). During fermentation of the apricot juice, while the content of disaccharide decreased at similar rates in all cases, whereas the concentration of glucose and fructose did not change considerably. In general, the order of sugar utilization by *L. acidophilus* strains were glucose ≥ fructose > sucrose ≥ lactose > galactose. Since *L. acidophilus* is a homofermentative organism, it utilizes glucose through the Embden Meyerhof Parnas (EMP) pathway [19]. It can be revealed that in medium containing different sugars *L. casei* preferred lactose as a carbon source for its growth and lactic acid production, followed by glucose and maltose, while sucrose was poorly utilized [20]. Our results showed that the two probiotic *Bifidobacteria* and *Lactobacillus* in monoculture prefer doing disaccharides to monosaccharides. Kun et al. [16] concluded that during the carrot juice fermentation by *Bifidobacterium lactis* Bb-12,

Table 1 Cell yield and change of pH, antioxidant capacity and content of carbohydrate during apricot juice fermentation (24 h)

Strains	pH	Cell yield log (cfu/mL h)	Antioxidant capacity (mM)	Disaccharides (g/100 mL)	Glucose (g/100 mL)	Fructose (g/100 mL)
B. lactis Bb-12	4.8 ± 0.1	7.20 ± 0.27	2.55 ± 0.08	0.71 ± 0.02	3.59 ± 0.07	5.49 ± 0.12
B. longum Bb-46	4.8 ± 0.1	7.25 ± 0.13	2.35 ± 0.07	0.76 ± 0.02	3.49 ± 0.05	5.81 ± 0.17
L. casei 01	4.9 ± 0.1	7.06 ± 0.29	2.18 ± 0.08	0.63 ± 0.05	3.25 ± 0.09	5.41 ± 0.09
L. acidophilus La-5	5.1 ± 0.2	7.16 ± 0.23	2.39 ± 0.08	0.63 ± 0.04	3.33 ± 0.09	5.61 ± 0.13

the amounts of glucose and sucrose decreased significantly, meanwhile the fructose concentration did not change. *Lactobacillus* sp. showed similar affinity towards mono- and disaccharide substrates, which were homofermentatively converted mostly to L-(+)-lactic acid [21].

The antioxidant capacity of juice increased slightly from 2.15 mM to 2.55, 2.35 and 2.39 mM in the cases of *B. lactis* Bb-12, *B. longum* Bb-46 and *L. acidophilus* La-5 strains, respectively, as well as did not show any significant changes in the case of *L. casei* 01 strain. Martin and Matar [22] also registered the increase in antioxidant activity of blueberry juice during fermentation with a novel bacterium from the fruit microflora *Serratia raccinii*. Probiotics can produce various metabolites with antioxidant activity such as glutathione, butyrate, folate etc. [23]. Amaretti et al. [24] carried out the comprehensive series of in vitro experiments (thirty-four strains of lactic acid bacteria 7 *Bifidobacterium*, 11 *Lactobacillus*, 6 *Lactococcus* and 10 *Streptococcus thermophilus*) for investigation of antioxidant activity. They found that the strains *Bifidobacterium animalis* subsp. *lactis* DSMZ 23032, *Lactobacillus acidophilus* DSMZ 23033 and *Lactobacillus brevis* DSMZ 23034 exhibited among the highest ascorbic and linoleic acid oxidation, trolox-equivalent antioxidant capacity and intracellular glutathione values. It means that probiotic bacteria convert some compounds in medium to structurally related products. Generally accepted opinion is that the antioxidative properties of probiotic bacteria are specific features of individual strains.

Organic acids are natural compounds in fruits and have an important effect on the organoleptic properties and stability of fruit juices. The apricot juice contains citric acid, malic acid and succinic acid in concentration of 17, 48 and 22 mM, respectively (Table 2). During fermentation on the apricot juice, the change of organic acid content in case of *L. casei* 01 was contrary to other strains. While the initial malic acid concentration reduced by half in case of *B. longum* Bb-46, *B. lactis* Bb-12 and *L. acidophilus* La-5, whereas in the apricot juice fermented by *L. casei* 01 the concentration of malic acid was only 16 mM at the end. Similar trend has been observed in case of citric acid that was utilised by the *L. casei* 01 strain dropped from the initial concentration of 17 to 10 mM at 24 h of the fermentation. Lactic acid bacteria are also known to metabolize citric acid, producing lactic acid, diacetyl, acetoin and acetic acid [25]. Moreover, citric acid and L-malic acid are important compounds in the pathway of utilisation of D-glucose, thus their concentration can decrease during the fermentation of the juice. This phenomenon was also observed by several authors when doing fermentation of must (grapes) [26]. Roses et al. [26] reported that organic (citric and L-malic) acids were metabolised by lactic acid bacteria in wine before D-glucose depending on growth conditions.

The end of the apricot fermentation, the concentration of acetic acid produced by bifidobacteria were 18–20 mM, while by lactic acid bacteria were 25–30 mM. Concentration of lactic acid were determined to be in the range from 74 up to 87 mM and the values in the cases of bifidobacteria strains were higher than in the cases of lactobacilli. It well known that *Bifidobacteria* ferment glucose via so called "bifidus" pathway, which is different from genus of *Lactobacillus*. The key reaction in this pathway appeared to be a phosphoketolase that cleavages fructose-6-phosphate into acetyl-phosphate and erythrose-4-phosphate. Theoretically, through the bifidus pathway, 1 molecule lactic acid and 1.5 molecule acetic acid are generated from the fermentation of 1 molecule glucose [27]. While presence of lactic acid in fermented product may give its savouriness taste, whereas acetic acid causes the odour taste that make product to be unacceptable. Excessive growth of *Bifidobacteria* may yield products with vinegar-like taste and aroma, which were obviously not accepted by consumers [28]. In our cases, the results observed are quite favourable. In the all fermentations, the contents of lactic acid were significantly higher than acetic acid, and the molar ratios of acetic acid to lactic acid in the apricot juice fermented by *Bifidobacterium* and *Lactobacillus* were 1:4.42 and 1:4.33 as well as 1:2.98 and 1:2.67, respectively. From this point of view, our fermented juices may be more acceptable by

Table 2 Change of organic acid content during apricot juice fermentation

Strains	Acetic acid Concentration (mM)	Lactic acid	Citric acid	Malic acid	Succinic acid	Molar ratio of lactic acid to acetic acid
0 h	–	–	17 ± 1.05	48 ± 1.81	22 ± 1.13	–
B. lactis Bb-12	18 ± 1.08	81 ± 3.22	17 ± 0.89	24 ± 1.01	12 ± 0.82	4.42
B. longum Bb-46	20 ± 1.31	87 ± 4.06	17 ± 0.91	25 ± 1.01	13 ± 0.64	4.33
L. casei 01	25 ± 1.84	74 ± 3.18	10 ± 0.68	16 ± 0.87	8 ± 0.17	2.98
L. acidophilus La-5	30 ± 1.78	80 ± 4.28	17 ± 0.95	26 ± 0.98	14 ± 0.34	2.67

consumers organoleptically. The ratios of acetic acid to lactic acid depend on substrate, media, fermentation time and oxygenation [16, 29]. Zalán et al. [30] investigated the production of organic acids by ten strains of *Lactobacillus* cultured in different media and they confirmed that some strains can change their fermentative profile from homofermentative to mix-acid fermentation depending on the composition of media. Moreover, the correlation between the production of lactic acid and content of nitrogenous components was observed by Nancib et al. [31] when they reported that yeast extract clearly showed the greatest enhancing effect on lactic acid production by *L. casei* in date juice. Our favourable results can be explained by high nitrogenous content in the apricot juice.

Fermentation with mixed cultures

The use of combined cultures of bifidobacteria and lactobacilli may lead to gain many advantages in growth as well as in changes of chemical composition and sensorial properties of the fermented fruit juice. Moreover, in some cases the symbiosis and/or synergist effects of different cultures are also exploitable. In our study, the apricot juice was inoculated with 1:1 combinations of bifidobacteria and lactic acid bacteria (Table 3). The initial cell counts for each strain were adjusted to be about 10^6 cfu/mL.

After 24 h of fermentation of apricot juice with all combinations, the cell counts were at higher levels than in the cases of monocultures, reaching about 10^8–10^9 cfu/mL (data are not shown) for individual strains. Different commercial mixed cultures (ROSELLAC, Agmaster Alfalfa Silage, COOP SILE) were used for fermentation of vegetable juice medium (mixture of carrots, cabbages, beets and anions) and total colony forming units after 3 days were counted to be 3.16×10^8 cfu/mL [31]. Di Cagno et al. [33] reported significant differences in growth properties (about 1 log cfu/mL after 1 day) of autochthonous and allochthonous mixed starter cultures of lactic acid bacteria when doing fermentation of different vegetable juice media (carrots, French beans and marrows). The yield of cell varied from 2.29×10^9 cfu/L h to 4.17×10^{10} cfu/L h (Table 3). The best combination should be mixing *B. lactis* Bb-12 strain with *L. casei* 01 strain resulted volumetric productivities of 4.17×10^{10} cfu/L h and 2.63×10^{10} cfu/L h for *B. lactis* Bb-12 and *L. casei* 01, respectively. These values were three-two times higher than ones obtained in monoculture fermentation. Also, good results of cell yield in the case of combination of *B. lactis* Bb-12 strain and *L. acidophilus* La-5 strain were observed after 24 h of fermentation of the apricot juice. These results led to conclude that both probiotic bifidobacteria strains (*B. lactis* Bb-12 and *B. longum* Bb-46) performed better growth dynamic properties in presence of lactic acid bacteria than absence of them meaning that two types of bacteria may be in symbiosis with each other.

The antioxidant capacity of the apricot juice did not change significantly during fermentation in the cases of combinations of *L. casei* 01 with bifidobacteria, increased slightly from 2.15 mM to 2.38 mM and 2.29 mM in the cases of combinations #3 and #4 (with *L. acidophilus* La-5), respectively (Table 4). From the lactic acid bacteria point of view, these results completely agree with ones in monoculture fermentation where *L. casei* 01 was not able to produce antioxidant agents during fermentation of the apricot juice. The decrease in scavenging effects was reported by Tien et al. [34] in all cases of mono- and mixed starter fermentation of the apple puree and juice with *L. delbrueckii* subsp. *lactis* ATCC 7830, *L. paracasei* subsp. *paracasei* ATCC 25598 and *L. casei* subsp. *casei* ATCC 393 strains. It reconfirms that antioxidant capacity (production of antioxidative metabolites and/or metal ion chelating ability etc.) of probiotic micro-organisms is strain-depending and may not be affected synergistically. More studies are needed to understand this mechanism.

The pH of the juice dropped from pH 6.6 to about pH 4.6–4.9 after the fermentation with combinations indicating intensive growth and metabolic activity of probiotic bacteria. These values are completely in agreement with results reported by Di Cagno et al. [32] with both cases autochthonous and allochthonous mixed cultures. Minimal decreases in glucose and fructose were observed, while the

Table 3 Cell yield and change of pH, antioxidant capacity during apricot juice fermentation

Mixed cultures	pH		Cell yield (cfu/L h)	Antioxidant capacity (mM)	
	0 h	24 h	24 h	0 h	24 h
(1) *B. lactis* Bb-12 and *L. casei* 01		4.6 ± 0.2	$(4.17 \pm 0.12) \times 10^{10}$ $(2.63 \pm 0.08) \times 10^{10}$		2.21 ± 0.15
(2) *B. longum* Bb-46 and *L. casei* 01	6.6 ± 0.4	4.7 ± 0.3	$(2.57 \pm 0.06) \times 10^{10}$ $(1.51 \pm 0.06) \times 10^{10}$	2.15 ± 0.18	2.09 ± 0.18
(3) *B. lactis* Bb-12 and *L. acidophilus* La-5		4.9 ± 0.3	$(2.69 \pm 0.06) \times 10^{10}$ $(2.24 \pm 0.08) \times 10^{10}$		2.38 ± 0.13
(4) *B. longum* Bb-46 and *L. acidophilus* La-5		4.8 ± 0.3	$(2.0 \pm 0.07) \times 10^{10}$ $(2.29 \pm 0.11) \times 10^{9}$		2.29 ± 0.17

Table 4 Change of organic acid and carbohydrate content during apricot juice fermentation (24 h)

Mixed cultures	Disaccharides (g/100 mL)	Glucose (g/100 mL)	Fructose (g/100 mL)	Acetic acid (mM)	Lactic acid (mM)	Molar ratio of lactic acid to acetic acid
(1) *B. lactis* Bb-12 and *L. casei* 01	0.48 ± 0.02	3.15 ± 0.12	5.55 ± 0.22	48 ± 2.3	89 ± 4.1	1.84
(2) *B. longum* Bb-46 and *L. casei* 01	0.29 ± 0.01	2.99 ± 0.10	5.37 ± 0.28	27 ± 1.2	72 ± 2.8	2.71
(3) *B. lactis* Bb-12 and *L. acidophilus* La-5	0.46 ± 0.02	3.08 ± 0.14	5.37 ± 0.20	38 ± 3.1	86 ± 3.5	2.23
(4) *B. longum* Bb-46 and *L. acidophilus* La-5	0.25 ± 0.01	2.74 ± 0.14	4.84 ± 0.20	35 ± 1.9	70 ± 3.4	2.00

content of disaccharides was fallen to one-third or one-fourth of the initial concentration in juice. Concentration of disaccharides considerably decreased in the cases of combinations containing *B. longum* Bb-46. Despite of the lowest cell yield of mixed cultures of *B. longum* Bb-46 and *L. acidophilus* La-5, the highest rate of consumption of all carbohydrate was observed. Minimal consumption of fructose as well as utilisation of glucose were observed by Di Cagno et al. [32], while Gardner et al. [31] reported the increase in glucose and fructose content in vegetable juice

medium, when fermentation with ROSELLAC and COOP SILE commercial starter cultures.

In fermentations with the mixed cultures, the concentration of acetic acid (27–48 mM) were about double than in the cases of monoculture fermentations (18–30 mM). This phenomenon can be explained by intensive growth of bifidobacteria applied. In all combinations, probiotic bifidobacteria strains performed better in cell yield and due to utilisation of sugars through bifidus pathway, higher amount of acetic acid was produced. Di Cagno et al. [32] used mixed autochthonous starter or mixed allochthonous starter of lactic acid bacteria to ferment carrot, French bean and marrow juices and they also observed the production of acetic acid (about 3–6 mM after 1 day), thus formation of this organic compound may also depend on the quality and composition of medium used. It is worth to carry out experiments to clarify this hypothesis.

The levels of lactic acid (70–89 mM) after 24 h of fermentation with mixed cultures were similar as levels at the monoculture (74–87 mM). Our results showed similarity to ones published by Gardner et al. [31], but significantly higher than ones (20–30 mM) reported by Di Cagno et al. [32]. The molar ratios of lactic acid to acetic acid decreased from about 4–2 in the cases of the fermentation with mixed cultures. This may lead to fall off organoleptic properties of the fermented juice. Interestingly, Omoya and Akharaiyi [35] did fermentation of tropical fruits (watermelon, banana and pineapple) and they concluded that the monoculture fermented beverages were of better characteristics than ones fermented by the mixed cultures. This phenomenon may be due to the consumption rate of nitrogen source by different probiotic bacteria, thus it is worth to check the effect of ratio of carbon to nitrogen content in fermentation medium to enhance production of lactic acid. This topic is still in progress in our laboratory.

In conclusion, the apricot juice can be used as substrate for growth of probiotic bifidobacteria and lactobacilli without any nutrient supplementation. This can also serve as good alternative matrix for carrying probiotic bacteria because it is rich in carbohydrates, vitamins and elements and no preservatives are required. The high level of antioxidants in the fermented product is a promising characteristic deserving further exploration in terms of the apricot juice potentially being a food with special function. Probiotic strains which are capable to limit excessive amounts of reactive radicals in vivo may contribute to prevent and control several diseases associated with oxidative stress.

While the volumetric productivity of probiotic bacteria cells can be enhanced by doing fermentation with mixed cultures, whereas the molar ratios of lactic acid to acetic acid will turn onto unfavourable trend (decrease). Based on the present results, a fermentation technology can be developed for the production of probiotic drink with high nutrient values and antioxidant capacity using bifidobacteria and lactic acid bacteria. This product will serve the persons who are unable to consume probiotic dairy products due to severe lactose intolerance and/or milk protein allergy. Moreover, the process may be of substantial help to preserve apricots in the form of a probiotic beverage and thus reduce wastage in areas, where it is produced in maximum.

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Compliance with ethical standards

Conflict of interest Authors declare that they have not any conflicts of interest.

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