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CHANGES IN BITTERNESS, ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF GRAPEFRUIT JUICE FERMENTED BY *LACTOBACILLUS* AND *BIFIDOBACTERIUM* STRAINS

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Four strains of *Lactobacillus* and *Bifidobacterium* including *L. plantarum* 01, *L. fermentum* D13, *L. rhamnosus* B01725, and *B. bifidum* B7.5 exhibiting naringinase production were applied in grapefruit juice fermentation. All investigated strains grew well in grapefruit juice without nutrition supplementation. In all cases, cell counts were 10^8–10^9 CFU ml^{-1} after 24 hours of fermentation. The highest lactic acid and acetic acid productions were observed in the case of strain *L. plantarum 01*. The *L. plantarum* 01 and *L. fermentum* D13 strains prefer glucose over fructose and sucrose, whereas fructose was the most favoured sugar for *L. rhamnosus* B01725 and *B. bifidum* B7.5. At the end of the fermentation process, antioxidant activity and total polyphenol content of grapefruit juice decreased in all cases, but the changes were not significant. Significant decrease of naringin was observed in the case of *L. plantarum* 01, 28% naringin in grapefruit juice was removed after fermentation. This result is promising for development of technology for production of probiotic grapefruit juice.

Keywords: grapefruit juice, naringin, bitterness, lactic acid fermentation, *Lactobacillus*, *Bifidobacterium*

Nowadays, fruit juice is consumed frequently worldwide because of its freshness and health promoting effects. Citrus family fruits such as grapefruit, orange, lemon, tangerine, etc. are rich in bioactive compounds such as minerals, vitamins, fibres, and antioxidants (Zhang, 2007). Unfortunately, bitterness makes these fruit juices undesirable and unacceptable by consumers (Ribeiro & Ribeiro, 2008).

Bitterness in citrus fruits is primarily related to two compounds – naringin and limonin, and naringin is the main bitter component. Grapefruit juices contain more than 300–400 μg ml^{-1} naringin (Soares & Hotchkiss, 1998). Some techniques have been reported for debittering, including adsorption technique (Kola et al., 2010) or use of β-cyclodextrin (Mongkolkul et al., 2006). These methods have some drawbacks affecting juice acidity, flavour, sweetness, and turbidity as well as low efficiency (Ribeiro & Ribeiro, 2008). The application of enzyme in reduction of naringin concentration is a promising technique, because it improves the quality of citrus juices while maintaining health properties.

Naringinase is an enzyme complex consisting of α-L-rhamnosidase (EC.3.2.1.40) and β-D-glucosidase (EC.3.2.1.21). While α-L-rhamnosidase hydrolyses naringin to prunin and rhamnose, β-D-glucosidase hydrolyses prunin into non-bitter naringenin and glucose. These products have great potential, especially in food and pharmaceutical industries (Puri, 2012). Production of naringinase from fungi has been well documented (Chen et al., 2013; Zhu et al., 2017), although very few studies are found in the literature regarding naringinase activity

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from bacteria. Despite some studies on bacterial naringinase, especially naringinase from probiotic bacteria were published (Avila et al., 2009; Beekwilder et al., 2009; Zhang et al., 2015), the debittering capacity of these bacteria is still less understood. In this study, naringinase from probiotics as well as simultaneous debittering and fermentation of grapefruit juice were studied.

1.1. Materials and methods

1.1. Grapefruit juice

The 100% grapefruit juice was purchased from the local market. The initial pH of the juice was adjusted to pH 6.3 with 4N NaOH before fermentation.

1.2. Strains and cultures

Bacteria (*Lactobacillus plantarum* 01, *Lactobacillus rhamnosus* B01725, *Lactobacillus fermentum* D13, and *Bifidobacterium* B7.5) were from the strain collection of the Department of Brewing and Distilling, Szent István University, Budapest, Hungary.

All strains of *Lactobacillus* were cultured in MRS medium at 37 °C for 24 h. *B. bifidum* B7.5 was cultivated in TPY medium at 37 °C for 24 h under anaerobic conditions (in Bugbox anaerobic chamber, Ruskin Technology, USA).

1.3. Fermentation of grapefruit juice

Grapefruit juice in 100 ml flask was inoculated with bacteria cultures and kept under aerobic conditions (in the case of lactobacilli) or anaerobic conditions (in the case of *B. bifidum* B7.5) at 37 °C for 24 hours. Samples were taken at given time intervals and analysed. TPY and MRS agar media were used to determine the cell number of bifidobacteria and lactobacilli, respectively (Bujna et al., 2018).

1.4. Analysis of carbohydrates and organic acids

The samples of fermented grapefruit juice were centrifuged at 14 000 r.p.m. for 10 min. The cell-free supernatant of grapefruit juice was used to determine the concentration of sugars and organic acids by HPLC method (Bujna et al., 2018).

1.5. Analysis of antioxidant capacity

Ferric-reducing power (FRAP) assay was used to measure the total antioxidant capacity of the fermented grapefruit juices (Nguyen et al., 2019).

1.6. Analysis of total polyphenol content (TPC)

Total phenolic content was determined using the method described previously (Nguyen et al., 2019).

1.7. Determination of naringin concentration

Naringin in fermented grapefruit juices were evaluated by HPLC method (Ribeiro and Ribeiro, 2008). The Surveyor HPLC system (Thermal Scientific Corporation, USA) consisted of a quadruple pump, an autosampler, a photodiode array (PDA) detector, and a column of

Supelcosil™ LC-18 (250×4.6 mm) was applied. The mobile phase consisted of acetonitrile (A) and water (B). Separation was performed using a gradient program: 0–8 min 23% A; 8–15 min 23–65% A linear; 15–20 min 65–70% A linear; 20–21 min 70–23% A linear; 21–22 min 23% A, and components were detected at 280 nm. The standard solution of naringin (0.1%) was prepared in a mixture of absolute ethanol:sodium acetate buffer 0.02M, pH 4.0 (1:1 ratio in volume). Both internal and external standards were prepared and injected.

1.8. Statistical analysis

All data were analysed by one-way ANOVA as well as unpaired and paired *t*-tests using Statistica v9.0 software package (StatSoft, USA). Generally, $P<0.05$ was accepted as statistical significance level. The results were presented as mean and standard deviation (SD).

2. Results and discussion

2.1. Changes of cell counts, pH, carbohydrates, and organic acids in grapefruit juice

Grapefruit juice was inoculated with different strains of *Lactobacillus* and *Bifidobacterium* (*L. plantarum* 01, *L. rhamnosus* B01725, *L. fermentum* D13, and *B. bifidum* B7.5) with about 10^6 CFU ml^{-1} of initial cell concentration. The results are presented in Figure 1. All investigated lactic acid bacteria grew well in grapefruit juice without nutrition supplementation. After 24 h of fermentation, almost all strains reached cell counts of 10^9 CFU ml^{-1} except *L. rhamnosus* B01725, where the population was 5.3×10^8 CFU ml^{-1}. Wang and co-workers (2009) produced probiotic noni juice with lactic acid bacteria and bifidobacteria, and they reported that all *L. casei*, *L. plantarum, B. longum* strains reached about 1×10^9 CFU ml^{-1} after 48 h of fermentation at 30 °C. In another study, *L. rhamnosus* was used to ferment carrot juice (Nazzaro et al., 2008). The cell count of *L. rhamnosus* was higher than our data in the case of *L. rhamnosus* B01725 (about 5×10^9 CFU ml^{-1}). However, this cell count was reported after 48 h of fermentation at 37 °C. Our results were significantly higher than those published by Bujna and co-workers (2018), when apricot juice was fermented by mono and mixed cultures of probiotic *Lactobacillus* and *Bifidobacterium* strains; when carrot juice was fermented by *Bifidobacterium* strains (Kun et al., 2008), or in the case of mulberry (Zheng et al., 2014).

During fermentation, short chain fatty acids were produced decreasing the pH values (Fig. 2), and at the end of the process, the final pH ranged from 4.4 to 4.7. The reduction of pH during fermentation of probiotic products is of great importance for deciding the time of fermentation as well as maintaining the quality of product. Lactic acid and acetic acid were produced in the ranges of 9.6–186 mM and 8.9–1074 mM, respectively (Table 1). These results were higher than those conducted by Champagne and co-workers (2009), when they cultured mono and mixed starters of probiotic strains in milk and laboratory soy beverage. In the case of grapefruit juice fermented by *Lactobacillus* spp., greater acetic acid production was observed. The presence of citric acid in the grapefruit juice with the initial concentration of 123 mM can explain the formation of acetic acid by *Lactobacillus* species through the citric cycle (Krebs cycle) leading to decreasing concentration of citric acid and increasing acetic acid content (Torino et al., 2005). Citric acid could not be detected in grapefruit juice after fermentation by *Lactobacillus*, while the amount of citric acid decreased by *B. bifidum* B7.5 fermentation (Table 1). Our results are in agreement with data reported by Mousavi and co-workers (2010), where the initial concentration of citric acid in pomegranate juice

decreased significantly during the fermentation by lactic acid bacteria (*L. plantarum*, *L. delbruekii*, *L. acidophilus,* and *L. paracasei*). The production of organic acids in lactic fermentation in different media including fruit and vegetable juices could depend on the quality of media and strains (Zalán et al. 2009; Havas et al., 2014).

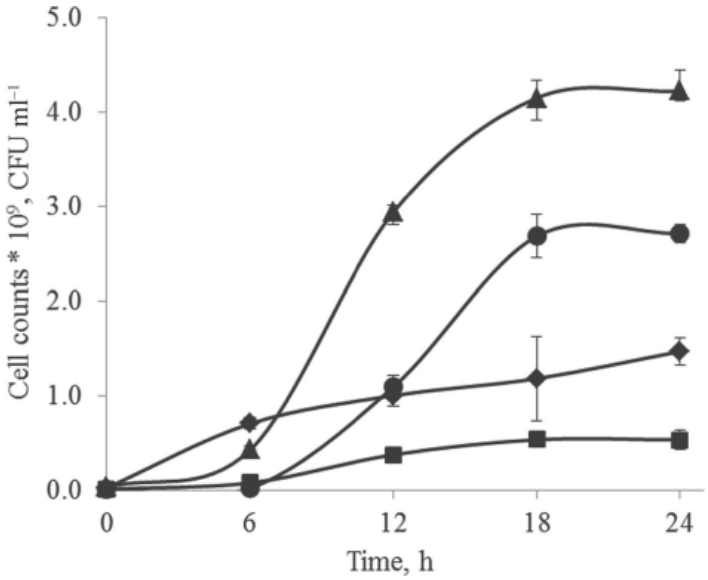

Fig. 1. Changes of microbial population in grapefruit juice during fermentation by *B. bifidum* B7.5 (●); *L. plantarum* 01 (♦); *L. rhamnosus* B01725 (■); and *L. fermentum* D13 (▲)

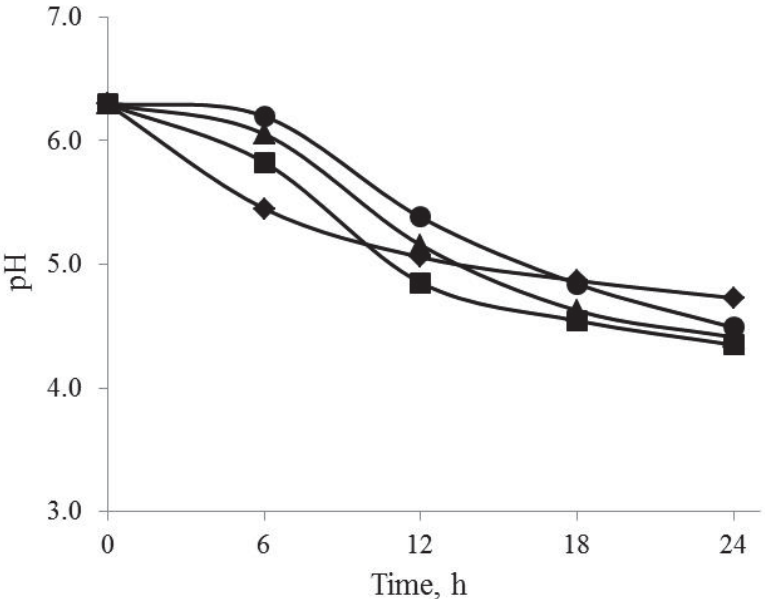

Fig. 2. Changes of pH of grapefruit juice during fermentation by *B. bifidum* B7.5 (●); *L. plantarum* 01 (♦); *L. rhamnosus* B01725 (■); and *L. fermentum* D13 (▲)

Table 1. Organic acid concentrations of grapefruit juice after fermentation

Strain	Lactic acid	Acetic acid	Citric acid	Oxalic acid	Malic acid
	Concentration (mM)				
0 h	2.17^{a}±0.42	4.53^{a}±0.8	123^{c}±5.3	2.84±0.99	0.09^{c}±0.002
L. plantarum 01	186^{c}±3.5	1074^{c}±28	–	10.7^{c}±0.32	0.05^{c}±0.001
L. rhamnosus B01725	172^{c}±5.2	645^{c}±30	–	9.8^{b}±0.76	0.04^{c}±0.001
L. fermentum D13	161^{c}±6.4	539^{b}±51	–	7.1^{c}±0.21	–
B. bifidum B7.5	9.6^{b}±0.82	8.9^{a}±1.6	77.7^{c}±3.4	3.8^{c}±0.11	0.02^{b}±0.002

[a]: Significant at 80% level; [b]: significant at 90% level; [c]: significant at 95% level

During the fermentation of grapefruit juice, the concentration of sucrose decreased in all cases, and there was no significant difference of sucrose content after fermentation ($p < 0.05$) among strains (Table 2). The result of sucrose consumption was in agreement with the study of Bujna and co-workers (2018), where they carried out lactic fermentation of apricot juice by mono and mixed cultures of probiotic *Lactobacillus* and *Bifidobacterium* strains. The contents of glucose and fructose were utilized at different rates. In general, the orders of sugars consumption were fructose > glucose > sucrose in cases of *L. rhamnosus* B01725 and *B. bifidum* B7.5, and glucose > fructose > sucrose in cases of *L. plantarum* 01 and *L. fermentum* D13 strains. The sugars can be fermented by probiotic bacteria via the Embden-Meyerhof pathway (EMP) or the phosphoketolase pathway (PKP), leading to homolactic and heterolactic fermentation profiles, respectively.

Table 2. Concentration of carbohydrates in grapefruit juice after fermentation

Strain	Sucrose	Glucose	Fructose
		(g/100 ml)	
0 h	2.5^{c}±0.02	3.6^{c}±0.03	3.1^{c}±0.04
L. plantarum 01	2.01^{c}±0.03	2.52^{c}±0.04	2.61^{c}±0.04
L. rhamnosus B01725	2.17^{b}±0.11	3.01^{a}±0.16	2.03^{b}±0.1
L. fermentum D13	2.07^{c}±0.02	2.31^{b}±0.02	2.7^{c}±0.02
B. bifidum B7.5	2.17^{a}±0.17	2.8^{a}±0.21	2.29^{a}±0.17

[a]: Significant at 90% level, [b]: significant at 95% level, [c]: significant at 99% level

2.2. Changes of antioxidant capacity and total polyphenol content of grapefruit juice

Fermented fruit juice is considered "functional food" because of bioactive compounds such as fibre, oligosaccharides, and bacteria that promote the equilibrium of intestinal bacterial strains (Perricone et al., 2015). The highest TPC and antioxidant activity were observed in juice fermented by *B. bifidum* B7.5 strain (Table 3). Significant reduction of antioxidant activity was observed in all other cases, especially when grapefruit juice was fermented by *L. rhamnosus* B01725 strain (7.72 mM $FeSO_4$). Due to anaerobic fermentation, fermented

grapefruit juice did not get exposed to oxygen, leading to minimum decrease in TPC as well as antioxidant activity. An increase in antioxidant capacity was obtained in carrot juice fermented by *L. bulgaricus* and *L. rhamnosus* (Nazzaro et al., 2008), and in noni juice by *B. longum*, *L. casei*, and *L. plantarum* (Wang et al., 2009). It confirms that antioxidant activity varies with starter microorganism and cannot be affected synergistically (Bujna et al., 2018).

Table 3. Changes of TPC and antioxidant capacity in fermented grapefruit juice

Strains	TPC ($\mu g\ ml^{-1}$ gallic acid)	Antioxidant activity (mM $FeSO_4$)
0 h	$1036^{b}\pm16$	$8.57^{c}\pm0.09$
L. plantarum 01	$997^{b}\pm16$	$7.9^{b}\pm0.27$
L. rhamnosus B01725	$1006^{b}\pm12$	$7.72^{b}\pm0.25$
L. fermentum D13	$991^{b}\pm34$	$8.05^{b}\pm0.25$
B. bifidum B7.5	$1029^{b}\pm20$	$8.23^{b}\pm0.18$

[a]: Significant at 90% level, [b]: significant at 95% level, [c]: significant at 99% level

2.3. Change of naringin concentration

The initial naringin content in the grapefruit juice was about 2.5 g l^{-1}. In all cases, the maximum decrease of naringin (about 28%) was obtained after 24 h of fermentation by mono starter *L. plantarum* 01 strain (Fig. 3). This result can be explained by the plant-related origin of the *L. plantarum* species. In view of the frequent occurrence of lactobacilli on decaying plant material and fermented vegetable substrates, it is expected that their genomes carry one or more genes encoding enzymes capable of utilizing rhamnosilated compounds (Beekwilder et al., 2009).

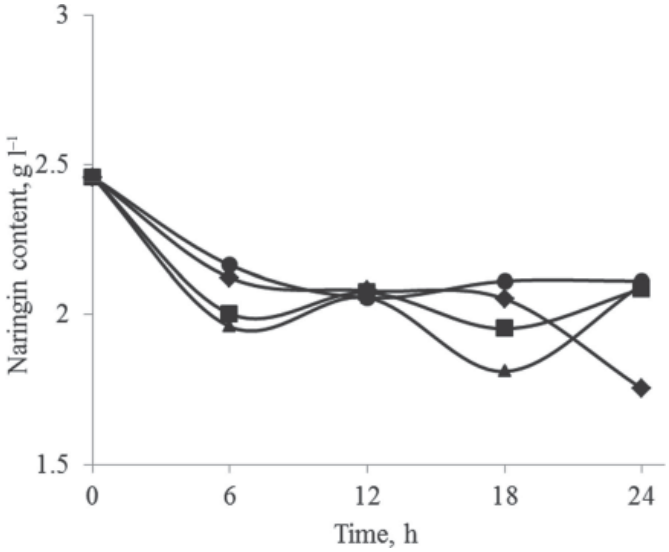

Fig. 3. Changes of naringin concentration of grapefruit juice during fermentation by *B. bifidum* B7.5 (●); *L. plantarum* 01 (♦); *L. rhamnosus* B01725 (■); and *L. fermentum* D13 (▲)

Zhu and co-workers (2017) purified naringinase from *Aspergillus oryzae* 1125 and used to debitter orange juice. The naringin concentration decreased to below 30 μg ml^{-1}, meaning that the bitterness can be efficiently lowered by naringinase to below the threshold of taste. Naringinase from *Cryptococcus albidus* can reduce up to 84% naringin at 40 °C and 100% at 60 °C after 60 min of incubation (Borzova et al., 2018). Pandove and co-workers (2017) reported that the biotechnological potential of naringinase producing yeast, *Clavispora lusitaniae,* has been exploited for the processing of kinnow and lemon in the form of low-alcoholic naturally carbonated debittered fermented beverage. After three months of storage at refrigerated temperature, the naringin content in fermented kinnow-lemon beverage decreased from 443.2 ppm to 176.4 ppm.

The efficiency of naringinase in our study is not as high as of other naringinase sources, however, it is the first report on simultaneous fermentation and reducing of naringin content in grapefruit juice.

3. Conclusions

Grapefruit juice can be used as substrate for growth of probiotic bacteria without any nutrient supplementation. All investigated *Lactobacillus* and *Bifidobacterium* strains have the ability to reduce naringin concentration, the main component causing the bitterness in grapefruit, while only minimal changes in the levels of antioxidants and TPC were observed. Our results are very promising and can form the basis of technology development for the production of less bitter probiotic citrus juices rich in nutrients.

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