Pharmaceutical Sciences
December 2016, 22, 260-266
doi: 10.15171/PS.2016.40
http://journals.tbzmed.ac.ir/PHARM

Research Article

Fig Juice Fermented with Lactic Acid Bacteria as a Nutraceutical Product

Sima Khezri[1], Parvin Dehghan[2*], Razzagh Mahmoudi[3], Mahdi jafarlou[4]

[1]*Department of Food Science and Technology, Faculty of Nutrition and Food Sciences, Student Research Committee, Talented Student Center, Tabriz University of Medical Sciences, Tabriz, Iran.*

[2]*Department of Food Science and Technology, Faculty of Nutrition and Food Sciences, Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*

[3]*Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran.*

[4]*Department of Human Anatomy, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Selangor Darul Ehsan, Malaysia.*

Article Info

Article History:
Received: 11 June 2016
Accepted: 14 August 2016
ePublished: 30 December 2016

Keywords:
-Fig juice
-Probiotic
-Lactobacillus
-Nutraceutical
-Survival

ABSTRACT

Background: Probiotics are live microorganisms bringing useful effects to the host through balancing intestine microbiota. This research was undertaken to determine the suitability of fig juice as raw material for production of probiotic juice by three species of lactic acid bacteria *(Lactobacillus casei*, *Lactobacillus plantarum* and *Lactobacillus delbrueckii).*
Methods: Heat treated fig juices were inoculated (6 log CFU/ml) by three species inocula separately and incubated at 30 °C for 72 h. Changes in the pH, acidity, reducing sugar content and viable cell counts during the fermentation were monitored. Sensory characteristics of probiotic fig juice were also evaluated.
Results: *L. delbrueckii* grew well on fig juice; reached nearly 9 log CFU/ml after 48 h of fermentation at 30 °C. After 4 weeks of cold storage at 4 °C, the viable cell counts of *L. delbrueckii and L. plantarum* were still 6 and 5 log CFU/ml, respectively, in fermented fig juice; but *L. casei* was just survived until 2th week of cold storage time, reduced from 9 to 3 log CFU/ml. The results of the sensory evaluation showed that fermented fig juice samples were significantly different ($P<0.05$) from the control sample in taste, odor, consistency and overall acceptability. *L. casei* was more acceptable comparing to the others.
Conclusion: *L. delbrueckii* was the most suitable strain from the point of survivability among other species at the consumption time. Therefore, probiotic fig juice can serve as healthy beverage for vegetarians and consumers with lactose-allergy.

Introduction

Probiotics are defined as live microorganisms that, when administered in adequate amounts, could confer a health benefit on the host.[1] Prebiotics are as non-digestible fiber compounds that induce the growth or activity of probiotics. They do it by modulating gut microbiota.[1,2] Several reports indicated that their health promoting aspects like alleviation of infections, improvement of lactose intolerance, antimicrobial and antiviral activity, serum cholesterol and blood pressure reduction, antimutagenic and anticarcinogenic properties, better mineral absorption, antidiarrheal and anticonstipation characteristics, immune system modulation and improvement in inflammatory bowel disease could be obtained by addition of selected right strains of probiotics and prebiotic compounds to the food products.[2-5]

Traditionally probiotics have been incorporated into fermented dairy products but consumers with lactose intolerance, milk allergy or high cholesterol levels and also vegetarians have limitation to consume these products.[6] So, healthy fruit juices could be a suitable alternative.[7]

Factors like acidity content, fermentation medium and optimum temperature are critical in lactobacilli activities.[8] The genus Lactobacillus can survive in the wide range of pH and temperature.[9] As, almost fruit juice drinks have a pH value of below 5, addition of these products by resistant probiotic

***Corresponding Author:** Parvin Dehghan, E-mail: dehghan.nut@gmail.com

lactobacilli is possible.

Fig (*Ficus carica)* is a kind of deciduous plant which belongs to the *Moraceae* family. Its fruit is generally referred as figs which have been used as food and medicine for several centuries.[10] Figs are a great source of calcium, potassium, vitamin A, ascorbic acid, dietary fiber, some fatty acids and many phenolic compounds.[11] Moreover, they contain cholesterol-lowering phytosterols like stigmasterol.[12] The main polyphenolic constituents in figs are flavones (apigenin, campherol, astragaline and rutin), catechins (epicatechin), flavonones, anthocyanins, gallic acid, chlorogenic acid and syringic acid.[13] It has been proved that these compounds can protect plasma lipoproteins from oxidation and significantly elevate plasma antioxidant capacity.[14] Nowadays nutraceuticals, compounds in foods that are not nutrients but have beneficial effects, are so favored in supplementation of fermented products.[15] Due to their biological characteristics, polyphenols and prebiotics may be appropriate nutraceuticals or as supplementary treatments for different disease like diabetes mellitus.[16,17] Polyphenols are phenolic acids, flavonoids, stilbenes, lignans and polymeric lignans which are secondary metabolites of the plants that play an important role as a defense against ultraviolet radiation, oxidants and pathogens.[17] Bioavailability of these functional molecules are dependent on food preparation processes, gastrointestinal digestion, absorption and fermentation.[18] So, probiotic fig juice which is plentiful in bioactive compounds could be considered as a noteworthy nutraceutical product.

The objective of this study was to monitor survival of *L. plantarum, L. casei and L. delbrueckii* in fermented fig juice during cold storage time and to evaluate its physic-chemical and sensory properties.

Materials and Methods

Strains and cultures

Used strains (*L. plantarum* DSMZ 20179, *L. delbrueckii* DSMZ 15996, *L. casei* DSMZ 20011) were obtained from the Deutsche Sammlung von Microorganismen und Zellkulturen GmbH, Germany. All bacterial cultures were kept at -20 °C in MRS medium (Merck, Germany) containing 20% glycerol. The inoculum was prepared by activation of pre-culture in MRS broth at 30˚C for 24 h.[19]

Preparation of probiotic fig juice

Commercial concentrated fig was supplied from Tin Tin Co. (Shiraz, Iran) and stored at 4˚C prior to use. Firstly, the fig juice concentrate with 70° Brix was diluted to 13° Brix by deionized water and then was carefully heat treated at 80 °C for 5 minutes. Fermentation experiments were done in test tubes (25 × 200 mm), each containing 40 mL of prepared fig juice. Consequently samples were inoculated with three species of probiotics (6 log CFU/ml) separately and incubated aerobically at 30 °C for 72 hours. Finally, samples were taken at 24 h intervals and all experiments were done at 0, 24, 48, and 72 hours.[20]

Effect of cold storage on cell viability

After 72 hours of incubation at 30 °C, the fermented samples were stored at 4 °C for 4 weeks. Samples were taken at weekly intervals, and then viability of probiotic cultures in probiotic fig juice was determined and reported as colony forming units (log CFU/ml). Viable cell counts were enumerated by the standard plate method with Lactobacilli MRS-Agar medium after 48 h of incubation at 30 °C.[19]

Chemical analysis

The pH value of prepared probiotic juice was evaluated by a digital pH meter (Metrohm 744, Netherland). Total acidity, expressed as percent lactic acid, was conducted by titrating with 0.02 N NaOH to pH 8.2. Reducing sugar content was measured as glucose equivalents by the DNS method using a UV–visible spectrophotometer.[21]

Sensory evaluation

The sensory characteristics were evaluated by a panel of ten judges consisting of the quality control students whom were informed of fermented beverage characteristics and different quality expectations. Each panelist evaluated the samples by rating them using a 9-point scale, where 9=like extremely and 1=dislike extremely, for various criteria such as taste, odor, consistency and overall acceptability.[22] Mean scores for each attribute were calculated for comparison of the samples.

Statistical analysis

The statistical analysis was conducted with SPSS 18.0 software (Chicago, IL, USA). All experiments were carried out in triplicate. Data analysis was done by ANOVA followed by Duncan's post hoc test, when a homogenous variance was observed, according to the Levene test. Non-homogenous data were analyzed using non-parametric analysis of variance, multiple comparisons based on Kruskal–Wallis test. $p<0.05$ was considered as statistically significant.

Results

Growth kinetics of probiotics

Investigated species including *L. casei*, *L. delbrueckii*, and *L. plantarum*, were found capable of growing well on fig juice without any specific nutrient supplementation.

The kinetics of lactic acid fermentation of fig juice with *L. casei*, *L. plantarum*, and *L. delbrueckii* were depicted in Figure 1. All species grew rapidly in fig juice and reached 8 log CFU/ml after 48 h of fermentation at 30 °C. Extended fermentation time over 48 hours did not lead to an increment in proliferation of lactic acid bacteria.

The changes in pH value and titrable acidity of fig juice have been shown in Figure 2 and Figure 3, respectively, during fermentation with lactic acid bacteria. The initial value of pH in fig juice was 4.52 and titrable acidity was 0.25% as natural acidity. Citric acid as dominant organic acid in fruits and initial acidity, expressed as the natural acidity of fruit juices, but developed acidity were expressed by lactic acid.[23] Among all the strains, *L. delbrueckii* produced more lactic acid than other strains in samples. It could be observed that *L. delbrueckii* had fast growth rate than other strains and caused an enhancement in titrable acidity in fig juice to increase from initial content of 0.25% as natural acidity to after fermentation content of 0.61%. Accordingly, lowest pH value of *L. delbrueckii* was because of producing more lactic acid and enhanced proliferation among other strains during the fermentation of fruit juice and it was decreased in fig juice from initial pH value of 4.52 to final pH of 3.92. As it has been shown in Figure 4, sugar was converted by lactic acid bacteria in fig juice during probiotic fermentation. *L. delbrueckii* converted more sugar content of fig juice during fermentation among other strains which decreased it from initial content of 32.1 mg/ml to 18.2 mg/ml.

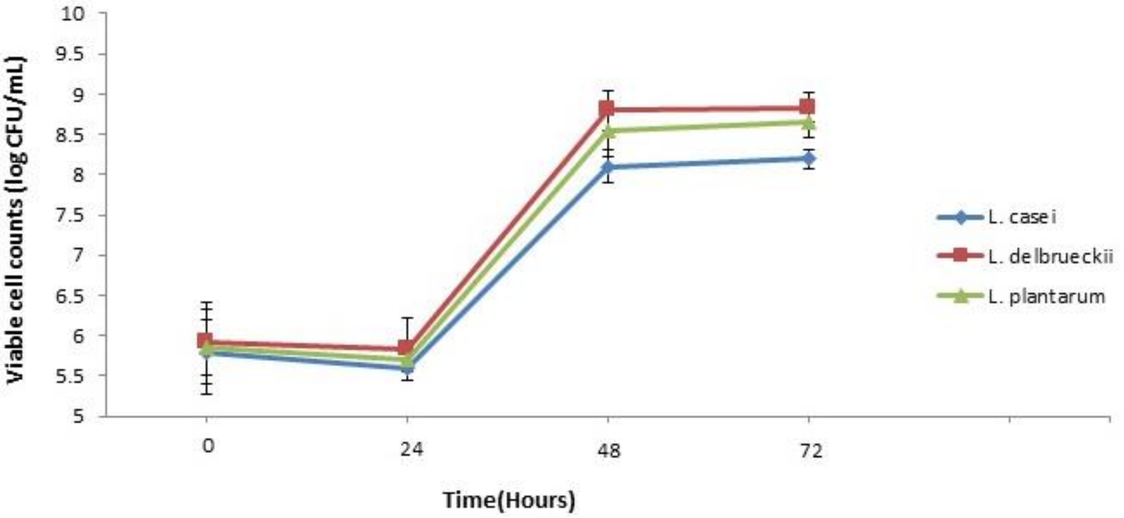

Figure 1. Growth kinetic of *L. plantarum*, *L. delbrueckii* and *L. casei* during fermentation of fig juice at 30 °C with initial pH = 4.52, Error bars represent the standard deviation.

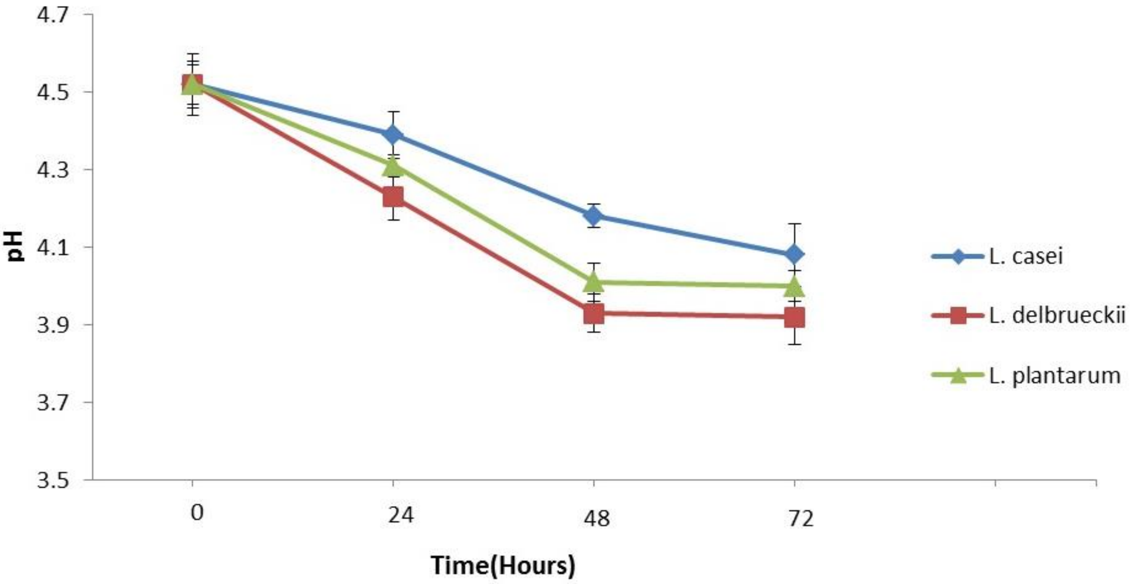

Figure 2. Changes in pH during fermentation by *L. plantarum*, *L. delbrueckii* and *L. casei* in fig juice (Initial pH = 4.52), Error bars represent the standard deviation.

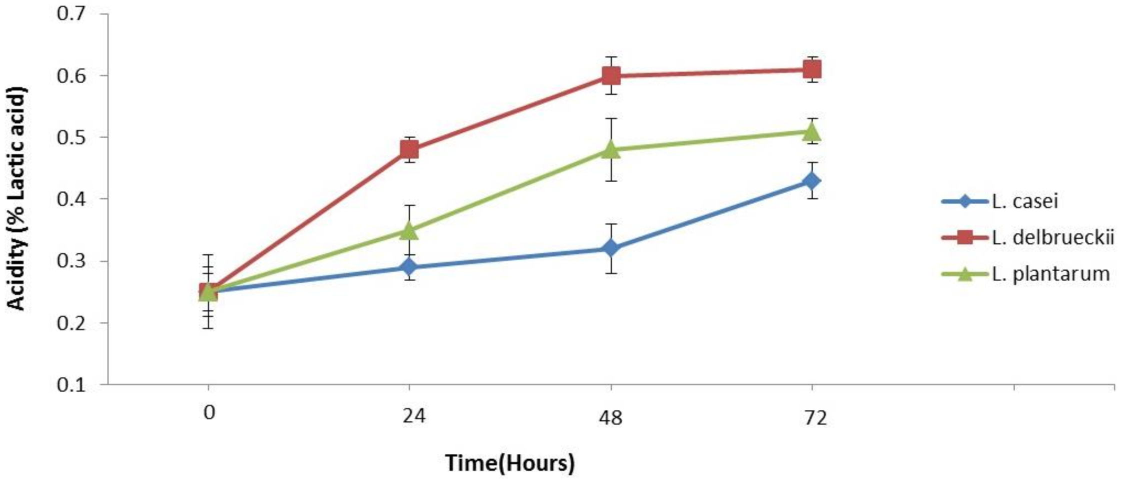

Figure 3. Changes in titrable acidity during fermentation by *L. plantarum*, *L. delbrueckii* and *L. casei* in fig juice (Initial acidity = 0.25%), Error bars represent the standard deviation.

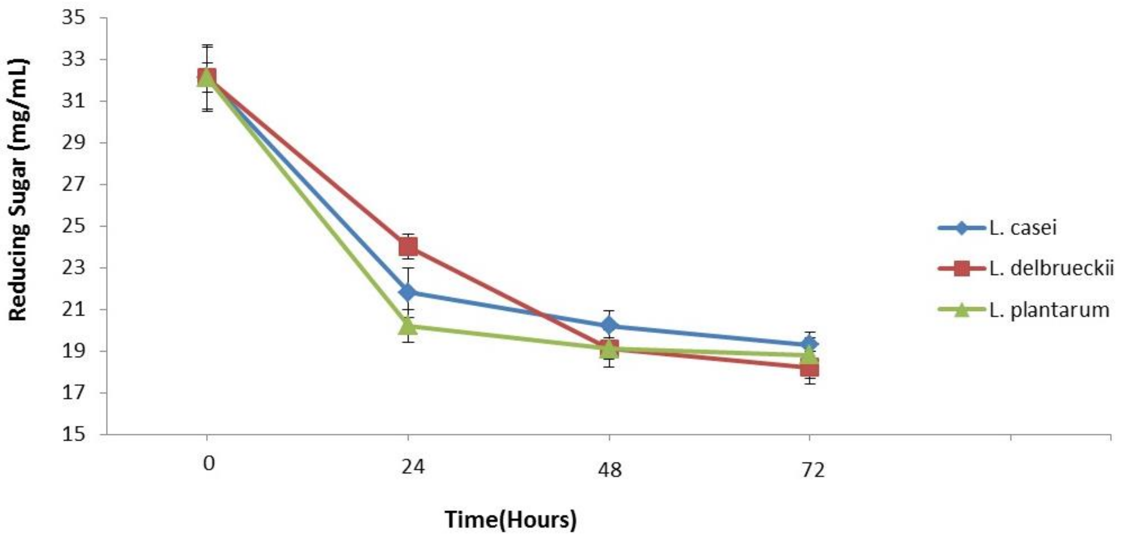

Figure 4. Changes in reducing sugar contents during fermentation by *L. plantarum*, *L. delbrueckii* and *L. casei* in fig juice (Initial concentration of reducing sugar content in the medium = 32.1), Error bars represent the standard deviation.

Table 1. Effect of cold storage (4°C) during 4 weeks on the viability of *L. plantarum*, *L. delbrueckii* and *L. casei* in fermented fig juice.

Time (Weeks)	Log CFU/mL		
	Lactobacillus plantarum	***Lactobacillus casei***	***Lactobacillus delbrueckii***
day1	8.77±0.1[a]	9.14±0.1[a]	8.83±0.2[a]
1	8.23±0.1[b]	5.53±0.2[b]	8.14±0.3[b]
2	7.11±0.8[c]	3.32±0.2[b]	7.50±0.2[c]
3	5.76±0.4[c]	ND	6.91±0.6[c]
4	5.34±0.9[c]	ND	6.27±0.1[c]

ND: not detected.
Means and standard deviations for n = 3. The experimental values within rows that have no common superscript are significantly different (p < 0.05) according to Duncan`s multiple test range.

Effect of cold storage on survival of probiotics

The represented data in Table 1 have been illustrated the effect of cold storage on the viability of three species of lactic acid bacteria in fermented fig juice. The viable counts of *L. plantarum* and *L. delbrueckii* after 4 weeks of storage at 4 °C were still 5 and 6 log CFU/ml, respectively. However, *L. casei* was just survived until 2th week of cold storage time.

Organoleptic properties

The mean acceptability scores of the samples were shown in Figure 5. Fermented fig juice samples were significantly different (P<0.05) from the control in taste, odor, consistency and overall acceptance. Significant difference was observed for *L. plantarum* and *L. delbrueckii* compared to *L. casei* (p<0.05). Mean scores for *L. casei* was the highest for taste (6.5), consistency (7.2) and overall acceptability (7). There was no significant difference observed between *L. plantarum* and *L. delbrueckii.* No significant difference was found for odor among the fermented juice samples.

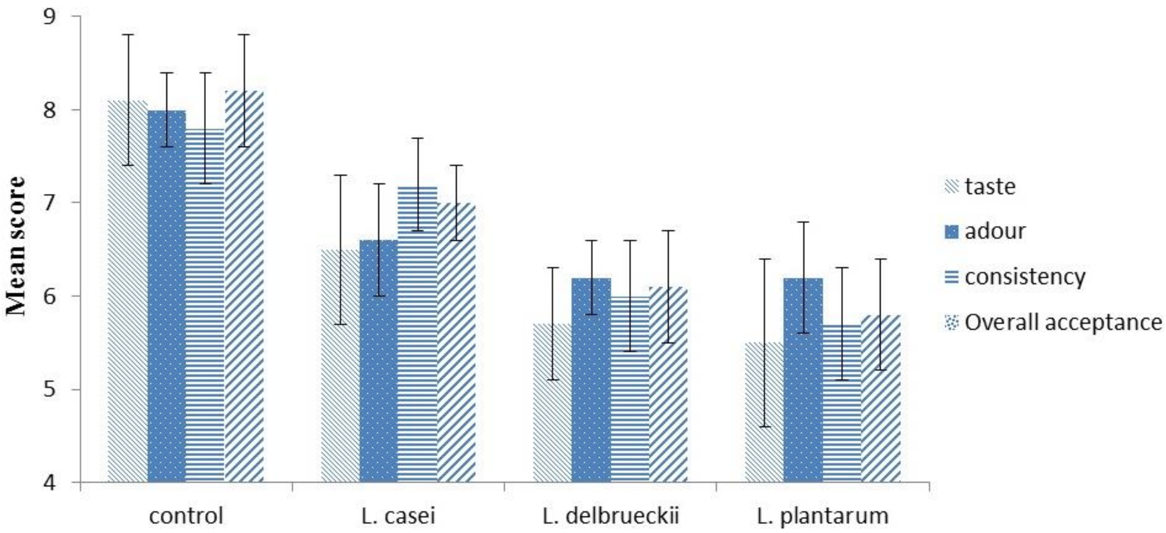

Figure 5. Sensory properties of fig juice samples fermented by *L. plantarum*, *L. delbrueckii*, *L. casei* and control. Error bars represent the standard deviation.

Discussion

As fig juice is a potent source of minerals, healthy fibers and antioxidants but it must be kept in mind that intrinsic factors like pH, titrable acidity, molecular oxygen and water activity could restrict probiotic viability.[24] Also low pH condition in fruit juices lead to undissocaited forms of organic acids and retards probiotic proliferation.[25] Ranadheera *et al*[26] predicted that the incorporation of lactic acid bacteria into low pH fruit juices may cause the resistance of bacteria to subsequent stressful acidic conditions, like gastrointestinal tract. So, it can be concluded that growth rate differences between pre-culture and fermentation medium could be related to stress mechanism. MRS broth, as the pre-culture, has a pH of about 5.6 but the initial pH of the fig juice was lower (about 4.52). It is noteworthy that acid tolerance is critical feature in the survival of probiotics in fermentation mediums.[20] Growth of probiotic bacteria in a suitable medium lead to acid production (usually lactic acid) and consequently reduction in the pH value of medium. Natural acidic conditions in fruit juice media limited the growth rate of probiotic bacteria.[27] Ding & Shah[28] have shown that microencapsulation process protect probiotics against acidic conditions of fruit juice media during the fermentation, so in this case, growth rate of probiotic bacteria was improved in comparison with free cells. Yoon *et al* in years 2004 and 2006 found that *L. delbrueckii* has the growth rate, acid production and sugar fermentation better than other strains used in probiotic tomato and cabbage juices, respectively[29,19] which are in agreement with our results. Mousavi *et al*[20] also showed that *L. delbrueckii* and *L. plantarum* were appropriate strains for fermentation of pomegranate juice. According to the Figure 1 initial microbial population of each micro-organism before storage at 4°C could affect the final surviving of bacteria. *L. casei* with less population in fermented fig juice after 72 hours fermentation was not capable to survive (according to Shah,[30] cell count of probiotic bacteria should be 6 log CFU/ml in probiotic definition) after 4 weeks at 4°C but *L. delbrueckii* was capable to survive 6 log CFU/ml after 4 weeks of cold storage. Surviving of lactic acid bacteria is a substantial factor to choose a proper micro-organism to produce probiotic fruit juices that can resist to the cold storage condition after the production.

As mentioned above, we found that *L. delbrueckii* and *L. plantarum* could survive in high acidity and low pH in the fermented fig juice. Costa *et al*[31] reported that fruit and vegetable juices were not suitable media for *L. casei* to grow in and produce probiotic beverage.

According to Figure 5, fermented fig juice samples were significantly different ($P<0.05$) from the control in taste, odor, consistency and overall acceptability. The highest acceptance was obtained by *L. casei* and the lowest was achieved by *L. plantarum* among the fermented fig juice samples. According to Cruz et al,[32] the aroma and taste of the product may be affected negatively by components produced by metabolism of the probiotic culture (probiotic off-flavor). But health information and flavor-masking techniques can have a positive effect on the hedonic qualities of probiotic juices.

Conclusion

From the results of this research, it can be concluded that *L. delbrueckii* which had the highest viability would be used as suitable cultures for production of probiotic fig juice for vegetarians or consumers who are allergic to lactose present in probiotic dairy products.

Acknowledgements

The authors would like to acknowledge the financial support provided by the Nutrition and food sciences faculty, Nutrition Research Center and the Vice Chancellor of Research of Tabriz University of Medical Sciences, Iran. This article was written on the basis of data obtained from M.Sc. thesis of Food Science and Technology (quality control).

Conflict of interests

The authors claim that there is no conflict of interest.

References

1. Champagne CP, Ross RP, Saarela M, Hansen KF, Charalampopoulos D. Recommendations for the viability assessment of probiotics as concentrated cultures and in food matrices. Int J Food Microbiol. 2011;149(3):185-93. doi:10.1016/j.ijfoodmicro.2011.07.005
2. Aliasgharzadeh A, Khalili M, Mirtaheri E, Gargari BP, Tavakoli F, Farhangi MA, et al. A combination of prebiotic inulin and oligofructose improve some of cardiovascular disease risk factors in women with type 2 diabetes: a randomized controlled clinical trial. Adv Pharm Bull. 2015;5(4):507-14. doi:10.15171/apb.2015.069
3. Rad AH, Mehrabany EV, Alipoor B, Mehrabany LV, Javadi M. Do probiotics act more efficiently in foods than in supplements?. Nutrition. 2012;28(7-8):733-6. doi:10.1016/j.nut.2012.01.012
4. Aliasgharzadeh A, Dehghan P, Gargari BP, Asghari-Jafarabadi M. Resistant dextrin, as a prebiotic, improves insulin resistance and inflammation in women with type 2 diabetes: a randomised controlled clinical trial. Br J Nutr. 2015;113(02):321-30. doi:10.1017/S0007114514003675
5. Karimi P, Farhangi MA, Sarmadi B, Gargari BP, Zare Javid A, Pouraghaei M, et al. The therapeutic potential of resistant starch in modulation of insulin resistance, endotoxemia, oxidative stress and antioxidant biomarkers in women with type 2 diabetes: a randomized controlled clinical trial. Ann Nutr Metab. 2015;68(2):85-93. doi:10.1159/000441683
6. Homayouni A, Vaghef Mehrabany E, Alipoor B, Vaghef Mehrabany L. The comparison of food and supplement as probiotic delivery vehicles. Crit Rev Food Sci Nutr. 2016;56(6):896-909. doi:10.1080/10408398.2012.733894
7. Kun S, Rezessy-Szabó JM, Nguyen QD, Hoschke Á. Changes of microbial population and some components in carrot juice during fermentation with selected Bifidobacterium strains. Process Biochem. 2008;43(8): 816-21. doi:10.1016/j.procbio.2008.03.008
8. Homayouni A, Azizi A, Javadi M, Mahdipour S, Ejtahed H. Factors Influencing Probiotic Survival in Ice Cream: A Review. Int J Dairy Sci. 2012;7(1):1-10. doi:10.3923/ijds.2012.1.10
9. Dicks LMT, Endo A. Taxonomic status of lactic acid bacteria in wine and key characteristics to differentiate species. S Afr J Enol Vitic. 2009;30(1):72–90.
10. Lansky EP, Paavilainen HM, Pawlus AD, Newman RA. Ficus spp. (fig): Ethnobotany and potential as anticancer and anti-inflammatory agents. J Ethnopharmacol. 2008;119(2):195-213. doi:10.1016/j.jep.2008.06.025
11. Hatano KI, Kubota K, Tanokura M. Investigation of chemical structure of nonprotein proteinase inhibitors from dried figs. Food Chem. 2008;107(1):305-11. doi:10.1016/j.foodchem.2007.08.029
12. Del Caro A, Piga A. Polyphenol composition of peel and pulp of two Italian fresh fig fruits cultivars (Ficus carica L.). Eur Food Res Technol. 2008;226(4):715-9. doi:10.1007/s00217-007-0581-4
13. Nakilcioğlu E, Hışıl Y. Research on the phenolic compounds in Sarilop (Ficus carica L.) fig variety. GIDA. 2013;38(5):267-74.
14. Vinson JA, Zubik L, Bose P, Samman N, Proch J. Dried fruits: excellent in vitro and in vivo antioxidants. J Am Coll Nutr. 2005;24(1):44-50. doi:10.1080/07315724.2005.10719442
15. Hurst WJ. Methods of analysis for functional foods and nutraceuticals. CRC Press; 2008.
16. Gargari BP, Namazi N, Khalili M, Sarmadi B, Jafarabadi MA, Dehghan P. Is there any place for resistant starch, as alimentary prebiotic, for patients with type 2 diabetes?. Complement Ther Med. 2015;23(6):810-5. doi:10.1016/j.ctim.2015.09.005
17. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009;2(5):270-8. doi:10.4161/oxim.2.5.9498
18. Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. J Nutr. 2000; 130(8):2073-85.
19. Yoon KY, Woodams EE, Hang YD. Production of probiotic cabbage juice by lactic acid bacteria. Bioresour Technol. 2006;97(12):1427-30. doi:10.1016/j.biortech.2005.06.018
20. Mousavi ZE, Mousavi SM, Razavi SH, Emam-Djomeh Z, Kiani H. Fermentation of pomegranate juice by probiotic lactic acid bacteria. World J Microbiol Biotechnol. 2011;27(1):123-8. doi:10.1007/s11274-010-0436-1
21. Kim NJ, Jang HL, Yoon KY. Potato juice fermented with Lactobacillus casei as a

probiotic functional beverage. Food Sci Biotechnol. 2012;21(5):1301-7. doi:10.1007/s10068-012-0171-5

22. Meilgaard M, Civille GV, Carr BT. Descriptive analysis techniques. Sensory evaluation techniques. Boca Raton:CRC Press. 1999; 3:161-170.
23. Yenez R, Marques S, Gírio FM, Roseiro JC. The effect of acid stress on lactate production and growth kinetics in Lactobacillus rhamnosus cultures. Process Biochem. 2008;43(4):356-61. doi:10.1016/j.procbio.2007.12.014
24. Tripathi MK, Giri SK. Probiotic functional foods: Survival of probiotics during processing and storage. J Funct Foods. 2014;9:225-41. doi:10.1016/j.jff.2014.04.030
25. Perricone M, Bevilacqua A, Altieri C, Sinigaglia M, Corbo MR. Challenges for the Production of Probiotic Fruit Juices. Beverages. 2015;1(2):95-103. doi:10.3390/beverages1020095
26. Ranadheera RDCS, Baines SK, Adams MC. Importance of food in probiotic efficacy. Food Res Int. 2010;43(1):1-7. doi:10.1016/j.foodres.2009.09.009
27. Granato D, Branco GF, Nazzaro F, Cruz AG, Faria JA. Functional foods and nondairy probiotic food development: trends, concepts, and products. Compr Rev Food Sci Food Saf. 2010;9(3):292-302. doi:10.1111/j.1541-4337.2010.00110.x
28. Ding WK, Shah NP. Survival of Free and Microencapsulated Probiotic Bacteria in Orange and Apple Juices. Int Food Res J. 2008;15(2):219-32.
29. Yoon KY, Woodams EE, Hang YD. Probiotication of tomato juice by lactic acid bacteria. J Microbial. 2004;42(4):315-8.
30. Shah NP. Functional foods from probiotics and prebiotics. Food Technol. 2001;55(11):46.
31. Costa MGM, Fonteles TV, de Jesus AL, Rodrigues S. Sonicated pineapple juice as substrate for L. casei cultivation for probiotic beverage development: Process optimisation and product stability. Food Chem. 2013;139(1-4):261-6. doi:10.1016/j.foodchem.2013.01.059
32. Cruz AG, Cadena RS, Walter EH, Mortazavian AM, Granato D, Faria JA, et al. Sensory analysis: relevance for prebiotic, probiotic, and synbiotic product development. Compr Rev Food Sci Food Saf. 2010;9(4):358-73. doi:10.1111/j.1541-4337.2010.00115.x