Biotech Health Sci. 2014 November; 1(3): e24683.
Published online 2014 November 13.
Research Article

Producing Probiotic Peach Juice

Babak Pakbin [1]; Seyyed Hadi Razavi [1]; Razzagh Mahmoudi [2,*]; Payman Gajarbeygi [3]

[1]Department of Food Science and Technology, Faculty of Biosystems Engineering, Campus of Agriculture and Natural Resources, University of Tehran, Karaj, IR Iran
[2]Department of Food Hygiene and Aquatics, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, IR Iran
[3]Department of Food Health and Safety, School of Public Health, Qazvin University of Medical Sciences, Qazvin, IR Iran

*Corresponding author: Razzagh Mahmoudi, Department of Food Hygiene and Aquatics, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, IR Iran. Tel: +98-9127868571, E-mail: r.mahmodi@yahoo.com

Received: October 20, 2014; **Accepted:** November 29, 2014

Background: Probiotics have been used for dairy products such as yogurt and yogurt drinks, however cholesterol content and lactose intolerance are important drawbacks. Recently, consumption of non-dairy probiotic food especially for probiotic drink products has been intensified.
Objectives: This research was conducted to determine the suitability of peach as a raw material for producing probiotic peach juice by lactic acid bacteria.
Materials and Methods: Peach juice was inoculated with a 24-hour-old lactic acid bacteria culture and incubated at 30°C. Changes in the pH, titratable acidity, sugar content, and viable cell counts during fermentation under controlled conditions were monitored. Viability of lactic acid bacteria cultures in fermented peach juice was also measured during four weeks of cold storage at 4°C.
Results: *Lactobacillus delbrueckii* grew well in peach juice, reached nearly 10×10^9 CFU/mL, after 48 hours of fermentation at 30 °C and was capable of more sugar consumption, pH inclination and production of lactic acid during fermentation. After four weeks of cold storage at 4 °C, the viable cell counts of *L. delbrueckii* were 1.72×10^7 CFU/mL, in fermented peach juice. *Lactobacillus casei* could not survive in fermented fruit juice after the cold storage.
Conclusions: In conclusion, *L. delbrueckii* in peach juice was appropriate to produce a probiotic beverage. Therefore, this juice can serve as a healthy beverage for vegetarians and lactose-allergic consumers.

*Keywords:*Fermentation; Probiotic; Cultures

1. Background

Probiotic foods are products containing live microorganisms with healthy properties for consumers (1). The concept of probiotic was first explained by Metchnikoff (Russian scientist) in 1907. Accordingly, consumption of large amounts of fermentative dairy products contributes to health effects through balancing intestinal micro-flora. This was the first time that a scientific investigation on functional effects of lactic acid bacteria available in fermented dairy products was performed. More than 90% of probiotic food supplements contain different varieties of *Lactobacilli* and *Bifidobacteria*. In addition to the discovery of the health effects of probiotic bacteria, many studies have been implemented on the process and production of probiotic products fermented with probiotic organisms (2, 3). The low viability of probiotic organisms is amongst important problems in processing and production of probiotic food supplements because of their sensitivity to difficult conditions such as low pH in food and powerful enzymes of stomach. As reported by the Food and Agriculture Organization (FAO), a standardized probiotic food must contain a minimum amount of 10^6 CFU/g active and live organisms (probiotic) at the time of consumption (4). However, the most important part of research in this field is the production of food products that provide a more appropriate media for maintenance and survival of probiotic microorganisms for longer durations. Fruits and vegetables are rich in functional food components such as minerals, vitamins, dietary fibers and antioxidants (phytochemicals). Furthermore, fruits and vegetables do not contain any dairy allergens that might prevent usage by certain segments of the population (5). Fruit and vegetable juices have an established market sector as a functional drink including calcium and vitamin-fortified juices. These drinks are consumed regularly, which is essential if the full benefits and advantages attributed to probiotics are to be experienced (6). It has been suggested that fruit and vegetable juices could serve as a suitable media for cultivating probiotic bacteria (7). Probiotic stability in fruit and vegetable juice products is difficult to maintain during cold storage however probiotic encapsulation might solve this problem (8). Different studies have been implemented to investigate the suitability of fruit juices such as tomato, orange, grape, carrot, pomegranate, beet, potato and cabbage juices as raw vegetables and fruits for probiotic drink production. *Lactobacillus plantarum*, *L. acidophilus*, *L. casei* and *L. paracasei* have been used as probiotic cultures. Results have shown that all the strains of probiotic bacteria are capable of growth in the mentioned juices. The microbial population increases significantly after 48 hours of fermentation. Moreover, *L. plantarum*, *L. acidofilus* and *L. delbrueckii* have shown to be resistant to high acidic and low pH conditions during storage periods at 4°C for four weeks (3, 8-10). The study by King et al. showed that immobilized cells of probiotic lactic acid bacteria retained more during the cold storage period of ten weeks in fermented tomato juice compared with free cells because the immobilized cells were protected from oxygen, high concentrations of substrate and products, and inappropriate conditions such as low pH and high acidity (8). Peaches (*Prunus persica*) are rich in minerals, vitamin C and contain a lot of sugar (11). They are high in phytochemicals, dietary fibers and polyphenols that lead to health developments in consumers (12).

2. Objectives

The objective of this study was to determine the suitability of peach juice as a raw material for production of probiotic juice by lactic acid bacteria and to evaluate the viability of this product in cold storage conditions.

3. Materials and Methods

3.1. Strains and Cultures

Probiotic lactic acid bacteria (*Lactobacillus plantarum* DSMZ 20179, *L. delbrueckii* DSMZ 15996, *L. casei* DSMZ 20011) were supplied by the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany. All bacterial cultures were stored at -20°C in MRS medium (Merck, Germany) containing 20% glycerol. The probiotic cultures were grown for 24 hours at 30°C in MRS broth (Merck, Germany) for reactivation.

3.2. Fermentation of Probiotic Peach Juice

Commercial peach (*P. persica*) juice was purchased from a local store (Takdaneh group company, Tehran) and kept at 4°C prior to use. Peach juice as suggested by Mousavi et al. was pasteurized for five minutes at 80°C before fermentation (9). Fermentation experiments were performed in test tube containers (25 × 200 mm). Each tube was filled separately with 40 mL of sterile peach juice. All samples were incubated with a 24-hour-old probiotic culture (> 10^5 CFU/mL) at 30°C for 72 hours. For chemical and microbiological analysis, samples were taken at 24-hour intervals.

3.3. Effect of Cold Storage Time on Cell Viability of Fermented Peach Juice

The fermented juice samples were stored at 4°C for four weeks after 72 hours of fermentation at 30°C. Viabilities of probiotic cultures in fermented peach juice were investigated and expressed as colony forming units (CFU/mL). All samples were examined at weekly intervals.

3.4. Microbiological and Chemical Analysis During the Fermentation of Peach Juice

The pH value of samples was measured separately by a digital pH meter (Metrohm 744, the Netherlands). Total acidity, expressed as percentage of lactic acid, was determined by titrating with 0.02 N NaOH to pH = 8.2. Reducing sugar content was analyzed as glucose equivalents by the 3, 5-dinitrosalicylic acid (DNS) method of Miller (13). Finally, viable cell counts of all samples were analyzed by the standard plate count (SPC) method with MRS medium (Merck, Germany) at 30°C after 48 hours of incubation.

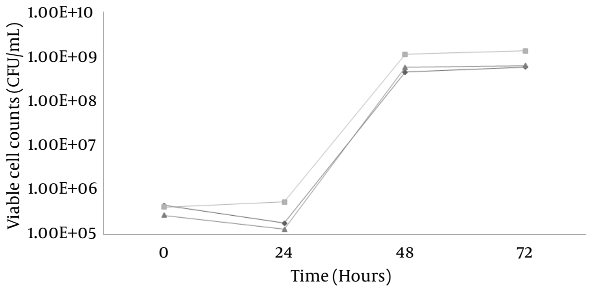
3.5. Statistical Analysis Method

All experimental measurements were carried out in triplicate. The statistical analysis was conducted using the SPSS software version 18.0 (Chicago, IL, USA). Analysis of variance (ANOVA) was performed using the ANOVA procedure. Significant differences ($P < 0.05$) between means were determined by Duncan`s multiple range test.

4. Results

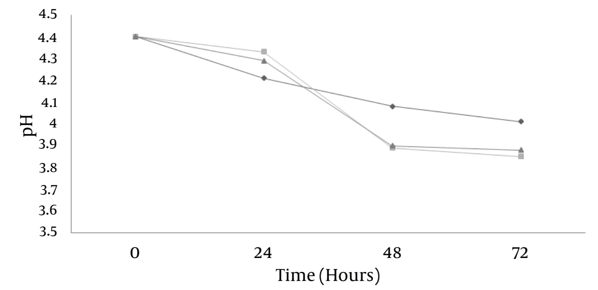
All species of lactic acid bacteria (*L. casei*, *L. delbrueckii* and *L. plantarum*) were found to be capable to grow well and appropriately ferment the peach juice. The kinetics of lactic acid growth in peach juice with *L. casei*, *L. delbrueckii* and *L. plantarum* are illustrated in Figure 1. *L. casei*, *L. plantarum* and *L. delbrueckii* grew well on peach juice and reached 1.12×10^9 CFU/mL after 48 hours at 30°C. The changes in pH value of peach juice have been shown in Figure 2. Also, titratable acidity changes of peach juice have been shown in Figure 3 during fermentation with lactic acid bacteria. The initial value for pH in peach juice was 4.4 and titratable acidity was 0.26% (14). As demonstrated in Figure 4, sugar is somewhat consumed by lactic acid bacteria in peach juice during probiotic fermentation. Initial sugar content was 20.2 g/L in peach juice before consumption by lactic acid bacteria. *Lactobacillus delbrueckii* consumed the greatest amount of sugar content of peach juice during fermentation when compared with the other strains and decreased the initial sugar content of 20.2 g/L to 12.9 g/L. The effects of cold storage on the viability of lactic acid bacteria species in fermented peach juice is presented in Table 1. The viable cell counts of *L. plantarum* and *L. delbrueckii* in fermented peach juice were still 7.2×10^5 and 1.7×10^7 CFU/mL after four weeks of storage at 4°C. However, *L. casei* was unable to survive the low pH and high acidity conditions in all fermented peach juice samples at 4°C and lost its cell viability completely after one week of cold storage.

Figure 1. Growth Kinetics of *L. plantarum*, *L. delbruekii* and *L. casei* During Fermentation of Peach Juice at 30°C With Initial pH of 4.4



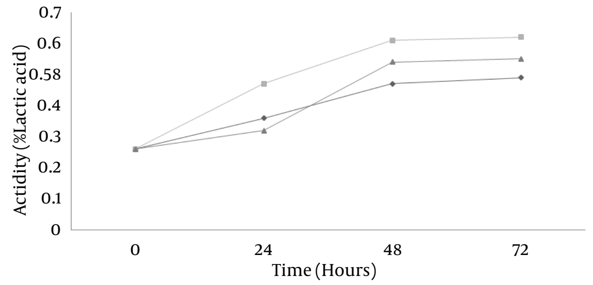
Filled square = *L. delbruckii*, filled triangle = *L. plantarum* and filled diamond = *L. casei*.

Figure 2. Changes in pH During Fermentation by *L. plantarum*, *L. delbruckii* and *L. casei* in Peach Juice



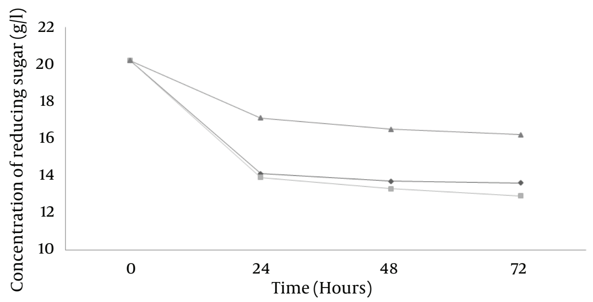
Initial pH = 4.4; filled square = *L. delbruckii*, filled triangle = *L. plantarum* and filled diamond = *L. casei*.

Figure 3. Changes in Titratable Acidity During Fermentation by *L. plantarum*, *L. delbruckii* and *L. casei* in peach juice



Initial acidity = 0.26%; filled square = *L. delbruckii*, filled triangle = *L. plantarum* and filled diamond = *L. casei*.

Figure 4. Changes in Sugar Content During Fermentation by *L. plantarum, L. delbrueckii* and *L. casei* in Peach Juice



Initial concentration of sugar content in the medium = 20.2. Filled square = *L. delbruckii*, filled triangle = *L. plantarum* and filled diamond = *L. casei*.

Table 1. Effect of Cold Storage (4°C) During Four Weeks on the Viability of *L. plantarum*, *L. delbruckii* and *L. casei* in Fermented Peach Juice [a, b]

Time, Weeks	CFU/mL		
	Lactobacillus plantarum	*Lactobacillus casei*	*Lactobacillus delbrueckii*
0	$6.1 \times 10^8 \pm 0.0 \times 10^8$	$5.7 \times 10^8 \pm 0.1 \times 10^8$	$1.3 \times 10^9 \pm 0.1 \times 10^9$
1	$4.8 \times 10^8 \pm 0.3 \times 10^8$	$9.8 \times 10^4 \pm 0.3 \times 10^4$	$7.4 \times 10^8 \pm 0.1 \times 10^8$
2	$2.3 \times 10^7 \pm 0.3 \times 10^7$	ND	$1.7 \times 10^8 \pm 0.1 \times 10^8$
3	$9.2 \times 10^6 \pm 0.3 \times 10^6$	ND	$7.9 \times 10^7 \pm 0.3 \times 10^7$
4	$7.2 \times 10^5 \pm 0.6 \times 10^5$	ND	$1.7 \times 10^7 \pm 0.8 \times 10^7$

[a] Abbreviation: ND, not detected.
[b] 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.

5. Discussion

5.1. Fermentation of Peach Juice With Lactic Acid Bacteria

As indicated by Figure 1, extension of fermentation time beyond 48 hours did not result in a significant increase in viable cell counts of lactic acid bacteria. This phenomenon could be due to low pH and high acidity (11) in fermented fruit juice. Also, oxygen tension and water activity are two important characteristics of fruit juices that lead to weak growth ability of probiotic bacteria (7). However, Rakin et al. and Aeschlimann and Von Stockar showed that addition of autolysate positively influenced the cell count of lactic acid bacteria during fermentation and the time of fermentation was reduced by increasing amino acids, vitamins, minerals and antioxidants content of juices (14, 15). Low pH of media contributes to a decline in the growth rate and an extension in length of the lag phase (14). Also, the extended lag phase was considerable for all strains during which the bacterial cells adapted with the new conditions such as pH, acidity (natural and metabolized acidity) and carbon sources (carbohydrates) (3). Growth of probiotic organisms in an appropriate medium contributes to the release of organic acid (lactic acid) and consequently decreases the pH value of the substrate. The stress induced because of variations between the pre-culture and the fermentation medium leads to a decline in growth rate during the lag phase of the fermentation process. Furthermore, MRS broth culture (as the pre-culture) has a pH of about 5.6 but the initial pH of the fermentation medium (peach juice) was significantly lower than this amount (about 4.4 in peach juice). As expressed by some authors (9), acid tolerance is an important probiotic trait for survival during fermentation in food medium. Natural and acidic conditions in fruit juice media limit the growth rate of probiotic bacteria (16); however, Tuorila and Cardello suggested that fruit and vegetable juices could serve as a good medium for probiotic bacteria growth because of their vitamin, sugar, mineral and antioxidant contents (17). Ding and Shah reported that the microencapsulation method protected probiotic bacteria from acidic and low pH conditions in fruit juice media during fermentation (18). Among all strains, *L. delbrueckii* produced a greater amount of lactic acid during fermentation of all peach juice samples and thus there was a greater decrease in the pH value of samples containing *L. delbrueckii*. It could be observed in Figure 1 that *L. delbrueckii* had a better growth rate and caused enhancement in titratable acidity in peach juice from 0.26% to 0.62%; titratable acidity produced by *L. casei* and *L. plantarum* in peach juice were lower than *L. delbrueckii* as indicated by Figure 3. Amongst all examined strains, pH value was more decreased by *L. delbrueckii* due to the rapid growth of this bacteria and greater production of lactic acid during fermentation of fruit juice, decreasing the pH from 4.4 to 3.85. According to Figure 4, greater growth of *L. delbrueckii* has led to higher consumption of sugar, metabolism of lactic acid and decline in pH value during fermentation (3). Yoon et al. during 2004 and 2005 found that *L. delbrueckii* has better growth rate, consequently lactic acid production, pH value inclination and sugar consumption, when compared to other strains used in probiotic fermentation of tomato and cabbage juices, respectively (11). Mousavi et al. also found that the growth rate of *L. delbrueckii* and *L. plantarum* were better than other strains in probiotic fermentation of pomegranate juice (9).

5.2. Effect of cold Storage on Cell Viability

According to Figure 1 initial microbial population of each microorganism before storage at 4°C could affect the final survival of bacteria. *Lactobacillus plantarum* with a Lowest population in fermented peach juice was not able to survive after 72 hours of fermentation cell count of probiotic bacteria should be 10^6 CFU/mL for probiotic purposes) after four weeks at 4°C yet *L. delbrueckii* was able to survive at a cell count of 10^7 CFU/mL after four weeks of cold storage. As Heenan et al. and Granato et al. have reported, low pH fruit juice, with a range of pH typically between 2.5 and 3.7, causes an increase in bacterial sensitivity in stressful conditions, and in cold storage conditions this affect is intensified (6, 19). Survival of lactic acid bacteria is an important factor for choosing an appropriate microorganism to produce probiotic fruit juice that can resist cold storage conditions after production. As it can be seen in Table 1, *L. delbrueckii* and *L. plantarum* in fermented peach juice were capable of surviving cold storage conditions. For maximum health benefits, the minimum number of probiotic organisms in a food product should be 10^7 CFU/mL; also, the viability of the lactic cultures is the most important factor during refrigeration or frozen storage (7), thus *L. delbrueckii* was suitable to produce probiotic fruit juice, as indicated by our study. The viability of probiotic organisms is dependent on the level of oxygen in products, oxygen permeation of the package, fermentation time and storage temperature (18). The viability of probiotic organisms is also influenced by inhibitory components such as lactic acid during fermentation and cold storage. Some other Conditions such as increased acidity resulting from the fermentation process can reduce the survival and viability of probiotic bacteria (16, 20-22). As mentioned above, we found that *L. delbrueckii* and *L. plantarum* could survive the high acidity and low pH in fermented peach juice. Fruit and vegetable juices are not a suitable media for the growth of *L. casei* and production of probiotic beverages (23). *Lactobacillus delbrueckii* and *L. plantarum* are stronger strains than *L. casei* to survive in pomegranate juice after four weeks in cold storage and high acidity conditions (24). Yoon et al. found *L. casei* is weak and does not survive in cabbage juice after 72 hours of fermentation for four weeks of cold storage (11). However, *L. casei* Zhang are stronger and survive in fruit and vegetable juices as probiotic bacteria (25). Microencapsulated probiotic bacteria could survive in fermented low pH and high acidity fruit and vegetable juices such as orange and apple juices compared with cell free bacteria in the same conditions of storage and temperature because of the protection effects of the microencapsulation method from high acidity and low pH (18). In addition to the functional properties of raw fruit juices, probiotic fruit juices have greater health benefits for consumers.

Three strains of lactic acid bacteria including *L. plantarum*, *L. casei* and *L. delbrueckii* were investigated for their ability to be fermented in peach fruit juice, and perform cell synthesis and lactic acid production without any nutrient supplements, to produce a probiotic fruit juice. These strains grew in peach juice at 30°C and finally reached 10×10^9 CFU/mL after 48 hours of fermentation.

Each strain that was capable to grow more, consumed a greater amount of sugar content, decreased the pH value more and produced a higher amount of lactic acid (as titratable acidity) during fermentation. *Lactobacillus plantarum* and *L. delbrueckii* were capable of surviving the conditions of low pH and high acidity in fermented peach juice during cold storage (four weeks) at 4°C. In contrast, *L. casei* was not able to survive the low pH and high acidic conditions and consequently lost cell viability completely after only one week of cold storage. From the results of this research, it is concluded that *L. delbrueckii*, according to properties required for probiotic beverage production (10^6 CFU/mL cell count), could be used as the culture for production of healthy beverages from peach, for vegetarians or consumers who are allergic to lactose present in probiotic dairy products.

Acknowledgements

The authors would like to acknowledge the support provided by "the Research Council of the University of Tehran (Tehran, Iran)" and "the Research Council of the College of Agriculture and Natural Resources of the University of Tehran".

Authors' Contributions

Design and conduct of the study: Babak Pakbin and Seyyed Hadi Razavi. Management: Babak Pakbin and Seyyed Hadi Razavi. Analysis of the data: Babak Pakbin, Seyyed Hadi Razavi and Razzagh Mahmoudi. Preparation, review and approval of the manuscript: Babak Pakbin, Seyyed Hadi Razavi, Razzagh Mahmoudi and Payman Gajarbeygi.

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