

Effect of banana puree on the survival of *Lactobacillus casei* in banana and apple juice cocktail during storage

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ABSTRACT

Probiotic juice is one of the great innovations in various kinds of healthy drink businesses. The aim of this study was to investigate the effect of banana puree on the survival of *Lactobacillus casei* and physicochemical and sensory properties of cocktail containing banana and apple juice during storage.

For the purpose of the study, 7% and 15% banana puree were added to apple juice in three phases. After adding banana puree, the sugar content of fruit juice was estimated as 13 °Bx. The produced fruit juice was pasteurized at 90 °C for 2 min. Then, depending on the treatment, *L. casei* was added to some samples. The samples were examined under four conservation periods (i.e., 1, 20, 40, and 60 days). The results of the study showed that the level of *L. casei* decreased significantly during storage time in all treatments; however, it was significantly less than the number of those of control treatments containing banana puree. The enhancement of banana puree was accompanied with the elevation of pH and significant reduction of acidity and sugar content. The treatment groups containing probiotic showed a higher increase in pH and decrease in acidity level and sugar content, compared to the groups without probiotic. The results obtained from sensory evaluation indicated that with the enhancement of banana puree to 15%, the flavor and color levels decreased significantly. As the findings indicated, the use of probiotic showed no significant effect on flavor and color levels in juice. Therefore, it is recommended to use 7% banana puree with a storage duration of 20 days to produce cocktail juice containing apple juice and banana puree with *L. casei*.

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Introduction

Today, there is a tendency towards the consumption of specific foods containing valuable medicinal benefits in addition to basic nutritional values. Consumption of functional food results in the improvement of the balance of intestinal microbial population and health status. Some studies investigated the use of probiotic bacteria in dairy products in Iran. However, the consideration of some factors, such as significant amounts of fat in dairy products and lactose intolerance in a wide range of people, have paved the way for setting up experiments on non-dairy products.

In this regard, the production of probiotic juices has been recently considered. Fruit and vegetable juices contain useful nutrients, such as minerals, vitamins, fiber, and antioxidants, causing no allergies unlike dairy products. Therefore, they are ideal options for probiotic culture and have a high potentiality for turning to probiotic products (1). The tendency to consume natural beverages and use them in daily diet is due to the presence of such nutrients as vitamins, minerals, and amino acids (2, 3).

Probiotics are living microorganisms that sustain the optimum balance in the microbial flora of the host. Many

studies have shown that the consumption of probiotic foods lead to the survival and maintenance of normal intestinal flora; therefore, they entail great benefits for human health.

The viability and activation of probiotic microorganisms in the final product are often determined by counting the number of live and active probiotic cells in the culture medium. According to the probiotic function, it is thought to be durable and available in order to exert beneficial effects. Moreover, the concentration of probiotics should be at least 10^8 - 10^9 cfu per gram of product to enable these microorganisms to survive in the gastric juice and reach to the small and large intestines (4).

Juices are rich in nutrients and does not contain any microbial starter that is competing for food with probiotics. Juices consist of a high amount of sugar that can enhance probiotic growth; therefore, they can be used as carriers for probiotics. Unlike dairy products, little information is available on the factors affecting probiotic survival in juices. Based on the evidence, the most important and effective factors influencing the survival of probiotics are the amount of organic acids, dietary fibers, pH, protein, phenolic compounds, oxygen, used microbial species, temperature, and storage time

(5). Probiotic bacteria have a more complicated survival process in juices is than in dairy products because these bacteria usually need to be protected against the acidic conditions in juices (6). Probiotic juices not only supply minerals and vitamins, but also transmit useful and alive bacteria to the body, thereby improving health status. These microorganisms have anti-cancer, anti-mutation, and disinfective properties; moreover, they result in cholesterol reduction, immune stimulation, diarrhea treatment, and improvement of gastrointestinal and urinary tract infections (6, 7). In a study performed by Pimentel et al. (8), the chemical components of probiotic apple juice were reported as moisture ($85.59 \pm 0.88\%$), protein ($0.042 \pm 0.002\%$), ash ($0.210 \pm 0.05\%$), fat ($0.133 \pm 0.01\%$), and carbohydrate ($14.03 \pm 0.86\%$). Malganji et al. showed that *Lactobacillus rhamnosus* and *L. delbrueckii* in grape drinks survived longer than *L. plantarum* during cold storage (at 40 °C) for 30 days (9). With this background in mind, the aim of this study was to investigate the effect of banana puree on survival of *L. casei* and physicochemical and sensory properties of cocktail juice containing banana and apple juice during storage.

Materials and Methods

Apple concentrates were procured from Shahd Company in Mahabad, Iran. The sugar content of the concentrates was measured as 70 °Bx, and titratable acidity was calculated as 0.33% based on citric acid with a pH of 4; furthermore, color and transparency were 0.24 and 94.5, respectively. Banana puree was obtained from the Farm Fresh Company in India. In this puree, the sugar content and titratable acidity (based on citric acid with a pH of 4.77) were obtained as 21°Bx and 0.27%, respectively. Furthermore, the probiotic *L. casei* (LAFTI-L26DSL) was purchased from the DSM Company in Australia.

Preparation of probiotic juice

The banana puree was added to apple juice at two levels (i.e., 7% and 15%) with the sugar content of 13 °Bx. Moreover, it was pasteurized at 90°C for 2 min, and then cooled to 4°C. Subsequently, depending on the type of treatment, probiotic *L. casei* was added to the samples. For this purpose, the lyophilized powder of *L. casei* was dissolved in sterile apple juice (according to the manufacturer's brochure) and added to the juice samples in sterile conditions. The sterilized containers were filled with treatments, and then sealed (5). The samples were stored at 5°C (refrigerator temperature) for 60 days and tested every 20 day. In this research, six treatments were performed in three replicates; in this regard, 7% and 15% banana puree were tested with and without *L. casei*.

Lactobacillus casei counting

The preparation of dilution was performed by adding 1 mL of the sample to 9 mL sterile ringer's solution. The

samples were cultured in Man-Rogosa-Sharpe agar by pour plate method in different dilutions. Then, the plates were incubated at 37 °C for 72 h under anaerobic conditions using a Gas-pack system (Merck, Germany) (10).

Microbial counting

Mold and yeast culture was performed by pour plate method in Dichloran Rose Bengal Chlortetracycline agar medium. The plates were placed in a refrigerated incubator at 25 °C for 72 h. The acidophilic bacteria were cultured on orange serum agar medium at 35-32 °C for 48 h (11).

Compositional analysis

The pH values of the samples were measured using a digital pH-meter (Metrohm 691, Swiss) after calibration with fresh pH 4.0 and 7.0 standard buffers at +20 °C. The titratable acidity (based on citric acid) was measured by the titration of 5 mL of samples against 0.1 N NaOH using phenolphthalein as an indicator, which turned into a light pink color. Sugar content was also measured using a manual refractometer (Garlzeiss Jena, Germany) (12).

Sensory evaluation

Sensory properties (i.e., color and flavor) were determined by 10 sensory evaluators using the tendency of consumer test and 9-point Hedonic method. For this purpose, the scores of 9 and 1 were representative of desirable and undesirable quality levels, respectively (13).

Statistical analysis

The results were analyzed by factorial analysis with three factors and three replications and Minitab 16 software. The comparison of the averages was performed using Tukey's test.

Results

Lactobacillus casei level variations in samples during storage time

The results of statistical analysis showed that the interaction of banana puree and storage time on *L. casei* population was significant ($P < 0.05$). Figure 1 illustrates the interaction of banana puree on *L. casei* population changes during storage time for 60 days at 5 ± 1 °C in mixed apple and banana treatments. The reduction of *L. casei* level in the treatment groups containing banana was significantly lower than that of the control group.

Changes in acidity, pH, and sugar content

As indicated in table 1, the enhancement of banana puree was accompanied with the significant elevation of pH and reduction of acidity ($P \leq 0.05$). Table 2 depicts the significant drop of pH and increase of acidity during storage time. The reduction of pH and acidity increase were higher in the probiotic treatment groups than those

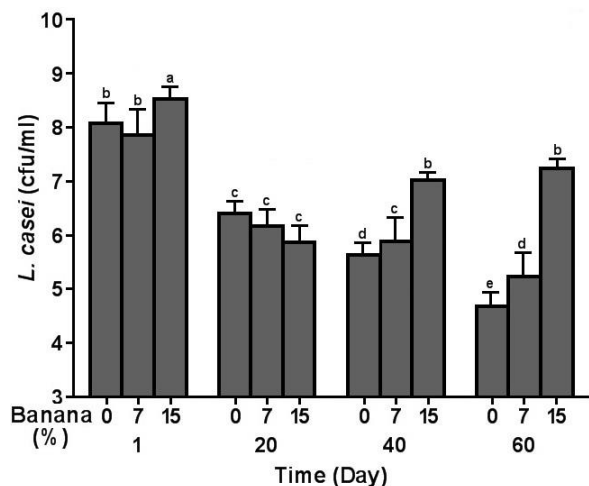


Figure 1 Effect of banana puree on the variation of *Lactobacillus casei* level in apple and banana cocktail

Table 1 Effects of banana puree on sugar content, acidity, pH, and color intensity of apple and banana cocktail samples

Banana Puree (%)	Sugar content	Acidity (%)	pH	Color intensity
0	13.25±0.005 ^a	0.18±0.0003 ^a	4.18±0.00 ^c	0.16±0.038 ^c
7	13.25±0.009 ^a	0.16±0.0007 ^b	4.37±0.003 ^b	0.66±0.08 ^b
15	13.23±0.008 ^b	0.16±0.0002 ^b	4.54±0.009 ^a	1.33±0.26 ^a

The experimental groups with the symbol "a" has a significant difference with groups "b", and "c" (P<0.05).

The experimental groups with the symbol "b" has a significant difference with groups "a", and "c" (P<0.05).

The experimental groups with the symbol "c" has a significant difference with groups "a", and "b" (P<0.05).

Table 2 Effect of treatment type and storage time on the changes of pH, acidity, and sugar content

Experiments	Treatments	Storage time (day)			
		1	20	40	60
pH	Without Probiotics	4.4 ^a	4.4 ^a	4.4 ^a	4.37 ^b
	With Probiotics	4.39 ^b	4.36 ^b	4.36 ^b	4.24 ^c
Acidity (%)	Without Probiotics	0.16 ^d	0.163 ^d	0.161 ^d	0.163 ^d
	With Probiotics	0.163 ^d	0.168 ^c	0.172 ^b	0.18 ^a
Sugar content	Without Probiotics	13.3 ^a	13.28 ^b	13.24 ^b	13.23 ^b
	With Probiotics	13.29 ^a	13.25 ^b	13.22 ^b	13.13 ^c

The experimental groups with the symbol "a" has a significant difference with groups "b", "c", and "d" (P<0.05).
 The experimental groups with the symbol "b" has a significant difference with groups "a", "c", and "d" (P<0.05).
 The experimental groups with the symbol "c" has a significant difference with groups "a", "b", and "d" (P<0.05).
 The experimental groups with the symbol "d" has a significant difference with groups "a", "b", and "c" (P<0.05).

of non-probiotic treatment group (P≤0.05). Furthermore, the sugar content decreased in the treatment groups during the storage time, which was significantly higher in the *L. casei* treatment groups (P≤0.05; Table 2).

Sensory properties

As table 3 demonstrates, the use of probiotics had no significant effects on the color and flavor of fruit juice. However, the increase of banana purees up to 7% did not exert any significant effects on the color and flavor score. Nonetheless, the sensory and color sensitivity significantly decreased following a further increase of this puree (P < 0.05; Table 4). The color and flavor score of the fruit juices decreased during the storage time. This decrease was higher in the treatment groups containing bananas (P < 0.05).

Table 3 Effect of probiotics on the score of sensory properties

Sample	Color	Flavor
Without Probiotics	5.04	5.68
With Probiotics	5.00	5.58

Microbial counting

The results of microbial tests showed that the counting of acidophilic bacteria, mold, and yeast was negative in all samples during the storage. Accordingly, it can be concluded that the conditions of pasteurization process and packaging had been done correctly and appropriately.

Discussion

As the findings indicated, the level of *L. casei* decreased significantly in all treatment groups of fruit juices during the storage time. The cells consume high energy when

Table 4 Effect of banana puree and storage duration on the score of sensory properties

Banana puree (%)	Storage time (day)							
	1		20		40		60	
	Flavor	Color	Flavor	Color	Flavor	Color	Flavor	Color
0	7 ^a	7.2 ^a	6.3 ^b	7.1 ^a	6.9 ^{ab}	7.4 ^a	6.2 ^b	7.4 ^a
7	7.6 ^a	6.9 ^a	5.9 ^{bc}	6.5 ^{ab}	5.1 ^c	6.7 ^{ab}	5.0 ^{bc}	6.6 ^{ab}
15	5.0 ^b	5.0 ^b	4.8 ^b	4.8 ^b	4.0 ^c	4.0 ^b	4.0 ^c	3.7 ^b

The experimental groups with the symbol "a" has a significant difference with groups "b", and "c" (P<0.05).

The experimental groups with the symbol "b" has a significant difference with groups "a", and "c" (P<0.05).

The experimental groups with the symbol "c" has a significant difference with groups "a", and "b" (P<0.05).

placed in an environment with low pH to maintain their intracellular pH. Therefore, other basic cell functions are affected by adenosine 5'-triphosphate deficiency stress, which threatens the cells' survival (14). There are a number of reports on the reduction of the number of probiotics in various fruit juices. In this regard, Vinderola *et al.* demonstrated that the level of *L. acidophilus* was significantly reduced during the storage time of pineapple and kiwi juices (15). Furthermore, they reported a significant decrease in the level of *L. lactis* in green apple juice. Sheikh Ghasemi and Zomorodi also showed that the number of *L. acidophilus* decreased in apple juice during the storage time, which is consistent with the results of the present study (5). In the current study, the reduction of *L. casei* level in the treatment groups containing banana was significantly lower than that in the control group. This can be due to the prebiotic effect of banana, which leads to an increase in the level of probiotics.

The existence of a large amount of sugar (absorbing sugar) in banana is one of the main sources of energy in this fruit. Banana also contains oligosaccharides, inulin, and oligofructose, as well as a significant amount of amylase, starch, protein, vitamins (i.e., C, carotene, thiamine, riboflavin, and niacin), minerals (i.e., calcium, potassium, and phosphorus), and dietary fiber (16, 17).

Therefore, the presence of these compounds stimulates the growth of *L. casei* and increases their viability. The presence of prebiotic compounds is one of the most important reasons for the survival of bacteria due to the stimulation of the growth and activity of probiotics. It has been reported that the lactulose-inulin compound stimulates the growth and survival of *L. casei* and *L. acidophilus* (18).

The level of *L. casei* was sufficient to provide health effects (>10⁷ cfu/g) in the control group, as well as the treatment groups containing 7% and 15% banana up to the 20th, 40th, and 60th days, respectively (4). Therefore, large amount of banana puree should be consumed for improving the efficacy regarding the growth of probiotics.

In the present study, enhancement of banana puree volume was accompanied with a significant increase in pH and decrease in acidity, which can be due to the high pH and low acidity of banana puree.

Sheikh Ghasemi and Zomorodi reported an elevation in the percentage of titratable acidity and a drop in pH in all apple juice treatment groups during the storage time (5). In this regard, the percentage of titratable acidity in apple juice with probiotic increased by 0.017% and 0.15% at the refrigerator and ambient temperatures, respectively. Yoon *et al.* also found that the pH in fermented beet juice, containing *L. plantarum* and *L. delbrueckii*, decreased from 4.28 to 3.79 during 40 days (19). In a similar study, Nualkaekul and Charalampopoulos (3) reported that the concentrations of lactic and acetic acids increased by using sugar in orange, grapefruit, pineapple, pomegranate, and lemon juices, containing *L. plantarum*, during the 6-week storage at 4°C. In addition, Shah *et al.* showed that pH decreased from 3.8 to 3.3 in probiotic juices, containing *L. rhamnosus*, *L. paracasei*, and *L. lactis*, during the 6-week storage (20).

In the present study, sugar content decreased in the treatment groups during the storage period. The level of this substance was significantly higher in the *L. casei* treatment groups, which may be due to the consumption of sugar by probiotics. The results reported by Sheikh Ghasemi and Zomorodi also indicated that the sugar content of apple juice, containing *L. acidophilus*, decreased during the storage time (13). Shah *et al.* also demonstrated that the sugar content of *L. rhamnosus*, *Bifidobacterium lactis*, and *L. paracasei* decreased from 11.8 to 9.1 °Bx after 6 weeks of storage at 4°C, which is in line with the results of the present research (20). The investigation and recognition of the factors affecting sensory properties are mandatory to prevent the development of undesirable properties. Sheikh Ghasemi and Zomorodi reported that the addition of *L. acidophilus* to apple juice did not have a negative effect on sensory properties (5). Similar results have been obtained by Luckow and Delahunty (21),

which are consistent with the results of the current study. These researchers showed that the awareness of consumers regarding the healthy effects of probiotics would increase their preference for using these products (21).

Conclusion

According to the results of this study, with the increase of banana puree in apple and banana cocktail, the survival of *L. casei* enhanced during the storage time. Furthermore, according to the sensory evaluation results, the color and flavor scores showed a significant reduction by increasing the volume of banana puree up to 15%. Therefore, it is recommended to use 7% banana puree in the preparation of probiotic apple and banana cocktail with *L. casei* during the storage time of 20 days.

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Ethics approval and consent to participate

None.

Conflicts of interests

The authors declare that they have no conflicts of interest.

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