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ORIGINAL ARTICLE

Chemical and sensorial properties of probiotic beverage based on rice bran extract and honey

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Abstract
Probiotic beverage is one of the most recent developments in drink industry. The non-dairy drinks are popular all over the world. In this regard, the beverage containing *Lactobacillus casei*–containing rice bran extract and honey was examined. The effects of rice bran extract (0, 5, 10, and 15%) on the microbial, chemical, and sensory properties of probiotic beverage were investigated. The pH, acidity value, soluble solids content, cell viability, and sensory properties of samples were evaluated in 0, 7, 14, and 21 days of storage. The bacterial bioavailability decreased during refrigerator storage. The pH and soluble solid content of treatments decreased and the acidity value level increased significantly (P <0.05) during the storage. The results of sensory evaluation showed that the sample with 10% rice bran extract was more acceptable than others. Our findings considered using rice bran extract and honey for the production of beverage containing *Lactobacillus casei* as a valuable drink. Rice bran extract and honey along with probiotics would be an appropriate combination for making a unique drink in beverage industry.

Keywords Probiotic · Rice bran · Honey · Beverage

1 Introduction

Nowadays people are more aware of the food and drink and their nutritional values. The probiotic foods and beverages are increasing all around the world [1, 2]. Probiotics and prebiotics have been used by consumers for years as functional food. Prebiotics are non-digestible food materials with healthful effects on the host by helping the activity of useful bacteria and therefore increasing the hosts' health [3, 4].

Honey is a natural prebiotic composed of sugars, amino acids, enzymes, vitamins, and minerals [5]. Monosaccharides, glucose and fructose, are the major sugars (nearly 75%) and disaccharides, sucrose, maltose, turanose, isomaltose, and maltulose, are in the small amount in honey [6]. Some researches have been reported some active components in honey that are responsible for the antibacterial activity like flavonoids and phenolic compounds [7]. It has antibacterial activity against *Salmonella*, *Escherichia coli*, and *Shigella* [6].

The probiotics are live microorganism which shows health benefits for their consumers after consumption [8, 9]. It is reported that probiotic foods are about 70% of the total functional foods' market [10, 11]. These valuable bacteria have many health benefits such as better digestion, improved immune system, lowering blood cholesterol, and anticancer [12, 13].

Rice is one of the main food crops consumed by a huge global population [14]. The by-products of rice after milling are germs, broken rice, and bran. Bran is the most favorable by-products of rice and wheat [15, 16]. Rice bran is a source of fiber with the balance level of soluble and insoluble dietary fiber, high amount of minerals, and vitamin. Rice bran is rich in nutrients such as protein (15%), fat (20%), and fiber (10%) [17, 18]. It also provides a rich source of vitamins and minerals such as thiamin, niacin, aluminum, chlorine, iron, magnesium, phosphorus, potassium, and sodium [19]. Also rice bran has some antioxidants such as ferrulic acid, tocopherol, oryzanol, and tocotrienol. It contains lysine nearly 4 times higher than rice due to having the out layer of rice [20].

There was a study on oat-based drink with *Lactobacillus plantarum* and honey. The optimized viable cell count (16.9 log CFU/mL) was observed in 8% of oat flour [21]. The effect

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of fructooligosaccharides on the characteristics of a beverage containing a mixture of corn and rice was also investigated. The results showed that the sample containing 50 g/L of fructooligosaccharide had 10^7 CFU/mL of probiotic bacteria after 28 days of storage [22].

The grains, fruits, and vegetables contain minerals, antioxidants, dietary fiber, and vitamins so they are valuable base for non-dairy probiotic drinks. Beverages are among the most acceptable ready to use foods. They are also suitable for transporting of various nutrients to the body. Functional beverages are usually dairy, cereal, and fruit based. The aim of this study is to produce a non-alcoholic probiotic beverage based on rice bran extract and honey and evaluating the survival of probiotic bacteria as well as chemical and sensory characteristics of the desirable drink during storage time.

2 Materials and methods

2.1 Microorganism preparation

Lactobacillus casei PTCC NO: 1301 was prepared from Iranian Research Organization for Science and Technology, Tehran, Iran. The bacteria were activated applying de Man, Rogosa agar (MRS; Merck, Germany) and incubated at 37 °C for 48 h. The colony count method was used to determine cell viability [23].

2.2 Chemicals

The used chemicals were Sigma-Aldrich (USA) and Merck (Germany). Honey was purchased from a local farm in Sabalan, northwest of Iran, from the mountain flowers.

2.3 Rice bran extract

Rice bran was obtained from a local market (Rasht, Iran). First it was dry steamed in hydrothermal autoclave (Germany) at 160 °C for 1 h. Then, the buffer solution (pH = 5), 200 mL of distilled water was added along with the selected enzyme (Phytase) 5% (w/v) and heated at 37 °C for 60 s (the optimal conditions the enzyme activity). After that, liquid phase was separated from the solid phase by a filter paper and the extract was separated by a centrifuge (Hettich, Germany) at 6000*g* for 15 min [24, 25].

2.4 Preparation of probiotic beverage

As Fig. 1 shows, to prepare the sample beverage, first rice bran extract (5, 10, and 15%) was mixed with honey (7.5%), pectin (0.5%), citric acid (0.12%), and drinking water (Table 1) and pasteurized for 10 min. Then, *L. casei* (10% v/v) was inoculated into each container and incubated at 37°C for 72 h, then the samples were kept at 4°C for the microbial, chemical, and sensory analysis during storage.

2.5 pH and acidity value measurement

pH was measured by using a pH meter (Swiss, Metrohm 632). The acidity value tests were carried out as lactic acid concentration (g/L) with a titration method using 0.1 N Sodium hydroxide and phenolphthalein. pH and acidity value were estimated by standard AOAC Methods (AOAC, 2014) [26].

2.6 Soluble solid content

Soluble solid content of the samples was measured by standard AOAC Methods (AOAC, 2014) using a refractometer DR 6000 (Germany) [26].

2.7 Bacterial count

For the viability of *L. casei*, the pour plate method was used. First the samples were diluted (10^{-4} to10^{-6}) with sterile saline (0.9% w/v). Then the plates were incubated at 37°C for 48 h and the colonies were counted. Viable cell counts were calculated as colonies (CFU/mL) [27].

2.8 Sensory evaluation

The sensory evaluation (color, flavor, aroma, and overall acceptance) of the beverage samples was performed by 10 experienced panelists who were trained in this issue before. The samples served in 100-mL glasses at room temperature during the storage. All sensory properties were scored by a 5-point hedonic scale from one to five: strongly dislike (1) to strongly like (5) [28].

2.9 Statistical analysis

All data were analyzed by using analysis of variance (ANOVA) to show the significant differences. The cell counts, pH values, acidity value, and soluble solid content expressed as mean ± standard deviation of 3 replicates by using SPSS (version 20.0).

Table 1 The different combinations of beverage treatments

T1	Control sample (without any rice bran extract) + honey (7.5%), pectin (0.5%), citric acid (0.12%), and drinking water
T2	5% rice bran extract + control sample
T3	10% rice bran extract + control sample
T4	15% rice bran extract + control sample

Fig. 1 A flow diagram of samples' production

Rice bran extract (5, 10 and 15%) + honey (7.5%), pectin (0.5%), citric acid (0.12%)
+
Drinking water
↓
Pasteurized for 10 minutes
↓
Adding *L. casei* (10% v/v)
↓
keeping at 4°C

3 Results and discussion

3.1 Probiotic viability

Based on the results, the effect of different concentrations of rice bran extract (5, 10, and 15%) on the viability of probiotic bacteria was significant (P<0.05). The viability of the bacteria first increased and then decreased during storage and it reached to the lowest level in the third week. The rice bran beverage sample containing 15% of the extract (T4) and the control sample (T1) showed the highest and lowest survival of *L. casei*, respectively, as shown in Fig. 2.

According to the results, the viability of probiotic first increased and then decreased during storage. This ascending trend until the 14th day could be attributed to the nutritional richness of the samples that is suitable for the bacteria to survive [29]. Despite the reduction of the number of bacterial cells, they were not less than the minimum acceptable level for probiotic products (≥ 10^6 log CFU/mL). The viability of probiotics in food samples depends on some factors such as pH, temperature and refrigeration period, and the presence of micronutrients [30]. On the other hand, the bacteria's activity cause increasing acidity value and decreasing pH, as well as the producing of hydrogen peroxide which can gradually reduce the number of bacterial cells [29].

The reason for the significant decrease in lactobacillus in the last week could be due to the inhibitory effect of organic acids that produced during storage [29, 31].

The inhibitory effect of organic acids varies according to pH, the type and concentration of acid, and bacterial strain [31]. Lind et al. [32] stated that propionic acid has more inhibitory properties than lactic and acetic acid and also, the inhibitory effect of organic acids decreases significantly with decreasing pH. The results of our study are in accordance with the previous studies.

3.2 Changes in pH and acidity value

Based on the results, the effect of different concentrations of rice bran extract on pH changes and acidity value of beverage samples was significant (P <0.05). As shown in Fig. 3, pH of the samples decreased during storage and it reached to the lowest level in the third week. The samples containing 15% of rice bran extract (T4) and the control sample (T1) showed the lowest and highest pH changes, respectively.

Also, according to Fig. 4, the acidity value of the samples increased and reached to the highest level in the third week of storage. The sample containing 15% of rice bran extract (T4) and the control sample (T1) showed the highest and lowest acidity value changes after 3 weeks, respectively. The acidity value of beverage samples changed significantly (P<0.05) after 21 days of storage so, shelf life is significant in the pH and acidity value changes of the beverage samples.

The results of this study showed a decrease in pH and an increase in acidity value levels of beverage samples during storage. Wzorek and Koskowsk [33] evaluated the pH

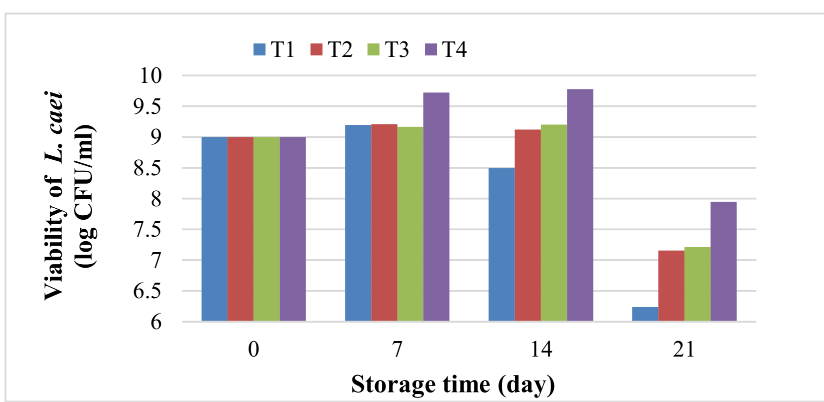

Fig. 2 The effect of different concentrations of rice bran extract on the viability of *L. casei* during storage

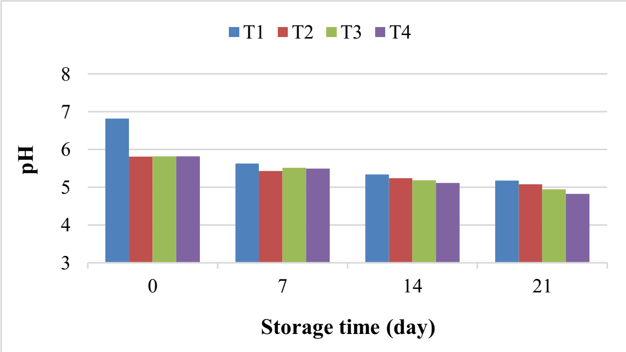

Fig. 3 The effect of different concentrations of rice bran extract on the pH of the samples during storage

changes in malt-based probiotic beverages at 4°C for 8 weeks. They stated that pH decreased from 3.97 to 3.46 and the acidity value increased from 0.14 to 0.27 (g/100 mL), and this trend was attributed to the sugar fermentation in the drink. Some researchers have suggested that pH drop during storage is the result of enzymes produced by starters during fermentation. They attributed the decrease in pH to the acid production as a result of the activity of the beta-galactosidase enzyme and the increase in fermentable sugars during storage [34].

Our results are in accordance with the study of Cyzowska et al. [35] that studied the red beet juice containing *L. plantarum* and *L. casei* strains; pH was decreased from 5 to 3.5. Also Yoon et al. [36] used *L. casei* to in red beet juice and observed slightly higher pH decrease (from 6.0 to 3.3) and acidity value increase (from 4.4 to 7.8 g/L) after 72 h. The main cause of the pH decrease was synthesis of organic acids (mainly lactate) by lactic acid bacteria [37].

3.3 Changes in total solid content

According to the results, the effect of different concentrations of rice bran extract on the soluble solid content in beverage samples was significant (P <0.05). As shown in Fig. 5, the soluble solids content of the samples decreased during the storage and the lowest value was observed in the control sample.

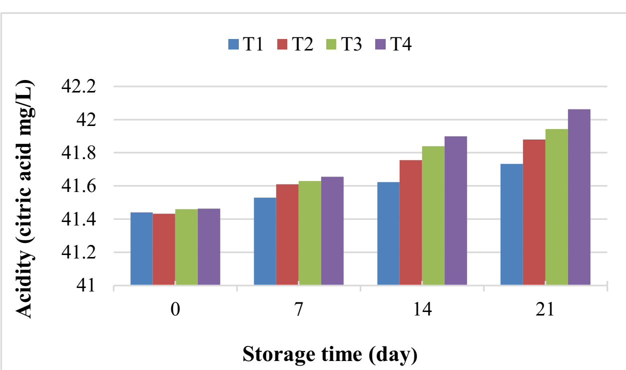

Fig. 4 The effect of different concentrations of rice bran extract on the acidity of the samples during storage

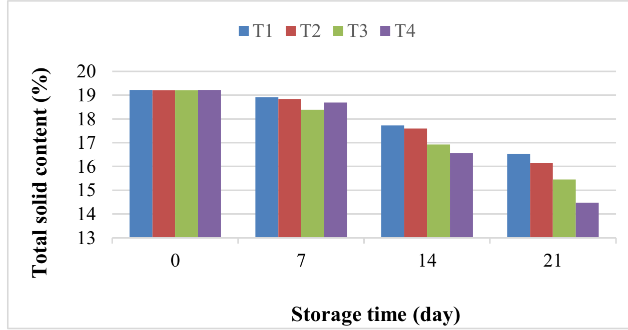

Fig. 5 The effect of different concentrations of rice bran extract on the total solid content of the samples during storage

In this study, the total solid content of samples were evaluated in 21 days of storage. The results showed that there was a significant difference in the amount of soluble solid content (P <0.05).

Baccouche et al. [38] observed a significant change in the amounts of soluble solid content and brix of whey-based prickly pear drink after 40 days of storage. In another study, there was a significant difference in total solid content of blackcurrant nectar after 2 months of storage [39]. Lupien-Meilleura et al. [40] evaluated the effect of probiotic bacteria on the maple sap beverage during refrigerated storage. The results also showed that the total solid content decreased during storage.

The total solid content of the beverages contains water-soluble solids and sugars. Lactic acid bacteria have the ability to use the sugars and make lactic acid and a series of volatile compounds. Therefore, to a large extent, the reason for the decrease of total solid content would be due to the consumption of sugar in the drinks by probiotics (Lupien-Meilleura et al., 2016).

3.4 Sensory evaluation

The results of this study showed that the effect of different concentrations of rice bran extract on the sensory characteristics of the beverages was significantly different (P <0.05). As

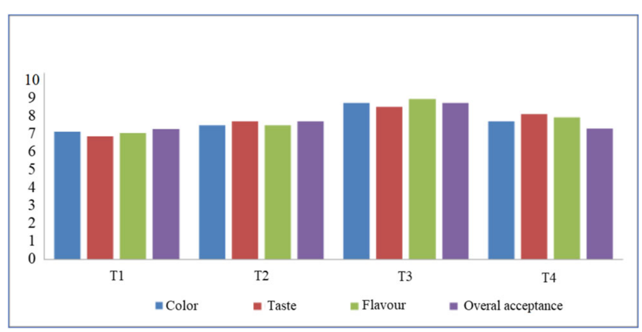

Fig. 6 The effect of different concentrations of rice bran extract on the sensory evaluation of the samples during storage

shown in Fig. 6, T3 has the highest sensory evaluations score followed by T2, T4, and T1. The highest color score is in T3 (8.80) and lowest in T1 (7.00). In case of taste, the highest score belonged to T3 (8.50) and the lowest to T1 (6.80). In respect of flavor, the highest score was for T3 (9.00) and lowest in T1 (6.90). About the overall acceptance, the highest score was observed in T3 (8.80) and the lowest in T1 (7.30). In general, the results of sensory evaluation showed that the sample with 10% of rice bran extract obtained the best sensorial acceptability.

4 Conclusion

Cereals provide more than 60% of the world's food production. They are important part of our diet due to having fiber, protein, minerals, and vitamins which mainly present in their bran. Nowadays, grains and their bran are used to produce various functional foods. In this regard, the effect of different concentrations of rice bran extract on probiotic beverage was investigated in this study. The results showed that pH and total solid content of the sample beverage decreased and the acidity value increased during the storage time. The viable probiotic bacteria in all samples were more than the standard value ($\geq 10^6$) by the end of storage time. Also, by adding rice bran extract, all the sensory properties of the drinks were acceptable and better than the control sample. The results of sensory evaluation showed that the sample with 10% rice bran extract was more acceptable than others. Therefore, the results of this study represent that using rice bran extract along with honey as a natural sweetener provides a beneficial, desirable, and unique beverage.

Declarations

Conflict of interest The authors declare no competing interests.

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