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ORIGINAL ARTICLE

Probiotic Viability, Physicochemical Characterization and Sensory Properties of Cornelian Cherry (*Cornus mas* L.) Juice Supplemented with *Lactobacillus acidophilus* and *Lactobacillus delbrueckii*

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KEYWORDS

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ABSTRACT: A large variety of fruits and vegetables and a large number of *Lactobacillus* strains provide a great opportunity for the development and industrialization of non-dairy beverages. In this study, *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* were used for cornelian cherry probiotic juice. Physicochemical factors such as the survival of probiotic bacteria, acidity and the total amount of reducing carbohydrates in probiotic juice were studied. After four weeks, the survival of *Lactobacillus delbrueckii* was significantly higher than other treatments (P<0.05). After four weeks, no *Lactobacillus acidophilus* bacteria had survived. In terms of sensory evaluation, there was a significant difference between two treatments of *Lactobacillus acidophilus* and *Lactobacillus delbrueckii*, while the treatment with *Lactobacillus acidophilus* probiotic bacteria was more acceptable than *Lactobacillus delbrueckii* (P<0.05). Due to the sensory characteristics, the viability of bacteria, and other physicochemical properties, it can be concluded that probiotic juice can be useful for human health if the probiotic bacteria can survive.

INTRODUCTION

A large variety of fruits and vegetables and a large number of *Lactobacillus* strains provide a great opportunity for the development and industrialization of non-dairy fermented beverages. Considering the biological and nutritional potential of the extracts of vegetables and fruits and since most of juices and vegetables have no allergenic compounds, it seems that the use of their extracts is very useful and these products are consumed by a large number of people [1]. Currently, some non-dairy probiotic beverages are commercially available and their consumption is increasing in the world [2, 3].

Functional food refers to a kind of food which has a health-consciousness for the consumer in addition to a nutritional characteristic. In other words, it has also a medicinal value beyond nutritional value [4]. Probiotic bacteria are the living microorganisms which can improve intestinal microbial balance for increasing their usefulness and, inhibiting the activity of non-useful microorganisms and pathogen by their activity,. Probiotic is also used as a food trait containing these bacteria. The use of probiotic supplements provides useful colonies which can help the human body, while providing the natural bacterial of the intestine to repair and rebuild

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itself. Then, these colonies will gradually be replaced by a natural intestinal bacterial that has reconstructed itself. Therefore, probiotic bacteria are also called biological restorers [5, 6].

Obviously, all foods consumed on a daily basis contain different amounts of microorganisms, while almost none of these foods are probiotic because there is a difference between the microorganisms present in these foods with probiotic products [7]. In 2001, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) came to a common definition that probiotic bacteria are the living microorganisms whose administration of sufficient amounts would have beneficial effects on host health [8]. The health effects of probiotic bacteria depend on their concentration in food products, as well as the ability to survive the bacteria in an inappropriate gastrointestinal condition [9, 10]. It should be at least 10^7 cfu/ml in the final product and at the end of product shelf life [11].

Cornelian cherry with the scientific name *Cornus mas* L., belonging to the *Cornaceae* family, has fruits in pink, yellow, red, dark red or even black and olive-shape like having sour and sweet flavors [12]. Cornelian cherry juice contains anthocyanins, vitamins C, tannins and organic acids and has anti-inflammatory and anti-oxidant effects and is used in traditional medicines to improve liver and kidney function and relieve pain. Therefore, given the many health benefits it contains, this fruit has the potential to be used as a food supplement, including the production of probiotic beverages [13].

Although juices contain many nutritious ingredients such as minerals, vitamins, food fibers and antioxidants, there are some important factors which can limit probiotic bacterial survival in fruit juices. Tripathi and Giri (2014) categorized the effective factors in probiotic bacterial survival in fruit juices; Factors such as pH, titratable acidity, molecular oxygen, water activity, the presence of salts, sugar and chemical compounds such as hydrogen peroxide, bacteriocin, dyeing agents and artificial flavors, can be mentioned. Process parameters also include heat treatment, temperature of incineration, packaging materials, storage methods and oxygen levels. Microbiological parameters are also probiotic strains, rate and inoculation ratio [9]. Based on the characteristics of cornelian cherry and probiotic properties of probiotic bacteria, this study was carried out.

MATERIALS AND METHODS

Preparation of bacteria

Probiotic bacteria *Lactobacillus delbrueckii DSMZ 15996* and *Lactobacillus acidophilus 946744* were prepared from National Iranian Standard Institute (http://www.isiri.com).

Probiotic juice preparing

In order to produce cornelian cherry juice, the cornelian cherry concentrate was diluted with distilled water and were pasteurized for 5 minutes at 80°C before fermentation. 40 ml pasteurized cornelian cherry juice was transferred to sterilized tubes. One sample (first treatment) was considered as a control sample. To the other tubes, the activated probiotic bacteria were inoculated to a concentration of about 10^6 cfu/ml. Determination of biomass was performed by spectrophotometer at 590 nm. For this purpose, the dilution of juice with a weight ratio of 1 to 10 with distilled water was performed and the biomass was determined by calibration curve. Firstly, the treatments were evaluated on day 0 in terms of physicochemical properties (pH, total acidity, total sugar content, reducing carbohydrates, ash, soluble solids) and probiotics survival. Then, all of these tubes were incubated for 72 hours at 30°C and incubation at 24, 48 and 72 hour intervals was investigated in terms of properties. After 72 hours of incubation, all fermentation samples were transferred to refrigerated temperature and evaluated each week until the fourth week in terms of their properties.

pH measurement

The measurement of pH in probiotic cornelian cherry juice was carried out based on Iran's National Standard No. 2685 (http://www.isiri.com). The samples were taken by digital pH meter at 20°C. Before the experiment, the pH meter was calibrated using buffer solutions with pH=7 and pH=4.

Acidity measurement

The measurement of acidity in probiotic cornelian cherry juice was carried out based on Iran's National Standard No. 2685 (http://www.isiri.com). Total acidity was measured using pH meter and potentiometric method. 90 ml of distilled water was boiled twice, transferred to a beaker, added 10 g of sample, and titrated on a magnetic stirrer to pH = 3.8 with a 0.1 N Sodium hydroxide and the acidity was calculated using this ratio:

$A = 0.9\ v\ m^{-1}$

A: Total acidity in lactic acid (grams per 100 gram),

v: Sodium hydroxide 0.1 N solution volume (ml),

m: Sample weight (g).

Determination of total sugar and reducing carbohydrates

This measurement was performed with Lane-Eynon method, according to Iran's National Standard No. 2685 (http://www.isiri.com).

Ash measurement

Ash measurement was conducted based on Iran's National Standard No. 2685 (http://www.isiri.com). The w / w method was used to calculate the ash. In this method, electric furnace (550 °C) was used.

Survival cell counting

Live cell counting was determined by standard plate method on MRS agar culture media. The decimal dilutions of samples with sterile serum solution were prepared (10^1-10^{10}) and cultured in MRS agar media, and then 100 µl of each dilution was removed on a culture media and incubated for 48 hours at 35°C. After incubation, the colonies were identified and counted.

Sensory evaluation

The samples were prepared and evaluated in terms of sensory properties in the second and weeks. Before the sensory evaluation, the content of this study was described to the evaluators and a moral satisfaction questionnaire was completed. The sensory acceptance test (questionnaire) was used to evaluate the sensory characteristics of probiotic supplementation. The sensory assessment was trained by a group of 10 sensory assessors using a 9-point Hedonic method. Thus, the scoring was performed by sensors from 1 to 9 based on the designed forms [14].

Statistical analysis

In order to investigate the quantitative characteristics of the data, three different treatments and three replications were used for one-way analysis of variance and for comparing the mean of data. The Duncan test was used at a significant level of 0.05 to evaluate the results. Statistical analysis was performed by SPSS version 22 and Excel software was used for drawing graphs.

RESULTS AND DISCUSSION

pH is one of the most significant factors affecting the survival of probiotic bacteria. The results of pH measurements are shown in Table 1. The results of this study showed no significant difference in the pH of the samples in the time intervals of 0 and 24 hours ($P<0.05$). In the 48-hour period, the highest pH was observed in the control sample and its lowest level was found in sample containing *Lactobacillus delbrueckii* ($P<0.05$). In the 72-hour interval, the highest pH belonged to the control sample and no significant difference was found in the pH of other treatments ($P<0.05$). In general, it can be stated that the pH of cornelian cherry juice containing probiotic bacteria decreased significantly during fermentation period.

The results of total acidity changes are indicated in Table 2. The results revealed that there was no significant difference in the acidity of the samples in the time intervals of 0 and 24 hours ($P<0.05$). In the 48 hour and 72 hour storage period, the highest acidity was observed in juice containing *Lactobacillus delbrueckii* and its lowest level in the control sample ($P<0.05$).

The results of changes in the amount of reducing carbohydrates are shown in Table 3. The results of the mean comparison of samples indicated no significant difference in the amount of reducing carbohydrates in the time interval of 0 hour (P<0.05). In the 24-hour storage period, the highest amount of reducing carbohydrates was observed in the control sample and its lowest level was observed in the cornelian cherry juice containing *Lactobacillus acidophilus* (P<0.05). At 48 and 72 hours, the highest level of reducing carbohydrates belonged to the control sample and its lowest level was observed in *Lactobacillus delbrueckii* bacteria in juice (P<0.05). In general, it can be concluded that the amount of reducing carbohydrates in fermented juice significantly decreased during fermentation period. Sugar consumption also declined as the probiotic bacteria lowered to the end of the maintenance period in the refrigerator.

The results of changes in total solids are shown in Table 4. The results of the mean comparison of samples indicated no significant difference in total solids content in the time interval of 0 hours (P<0.05). In the 24 and 72-hour storage period, the total solids content of the whole control sample was significantly higher than other treatments (P<0.05), and no significant difference was found in other samples (P<0.05). At 48- hour intervals, the highest amount of total solids belonged to the control sample and the lowest amount was observed in cornelian cherry juice containing *Lactobacillus delbrueckii* (P<0.05).

The results of ash changes are indicated in Table 5. The results of the mean comparison of samples showed no significant difference in the ash content of the samples at time intervals of 0 and 24 hours (P<0.05). In the 48-hour storage period, the amount of ash in cornelian cherry containing *Lactobacillus acidophilus* was significantly lower than the control sample (P<0.05). In the intervals of 72 hours, the content of ash in cornelian cherry juice content of *Lactobacillus delbruckii* was significantly lower than the control sample (P<0.05). Furthermore, the results showed that there was no statistically significant difference in ash content over time (P<0.05).

The results of the comparison of the mean microbial population of all samples are presented in Table 6. The results of the mean comparison of samples showed that the bacterial population in cornelian cherry juice containing *Lactobacillus delbrueckii* was significantly higher than other treatments in all time intervals (P<0.05). With time, the population of bacteria decreased significantly (P<0.05).

The results of the sensory evaluation are shown in Figures 1, 2 and 3. After four weeks, the odor and taste of Lactobacillus acidophilus treatment was significantly (P<0.05) more acceptable than *Lactobacillus delbrueckii* treatment and control group. However, no difference was observed between *Lactobacillus delbrueckii* and *Lactobacillus acidophilus* treatments in terms of color.

pH is one of the most significant factors affecting the survival of probiotic bacteria. Fruits contain high levels of organic acids which reduce pH. *Lactobacillus* is often resistant to pH changes in juices (pH=3.6). In other words, *Bifidobacteria* have lower acid resistance and lower pH, and pH = 4.6 is harmful to their survival [9]. In general, it can be stated that the pH of cornelian cherry juice cultures in this study significantly decreased during fermentation. In addition, the results showed that bacterial cells, even during storage period, had fertile activity and during the consecutive weeks a significant decrease was observed in pH. Based on a study conducted by Malganji et al. (2014), the initial pH of the grape juice was 4.6, which after 72 hours of fermentation by *Lactobacillus delbrueckii* decreased to 3.6 [15]. Ding and Shah (2008) reported that the pH of the orange and apple juice containing *Lactobacillus acidophilus* in six weeks decreased from the initial value of 2.81 and 2.95 to the final value of 2.57 and 2.4, respectively [16]. The difference in results can be due to the high buffering capacity of these products, which makes the pH adjustable. This decrease in pH leads to antimicrobial activity of *Lactobacillus* [17] and improves the organoleptic properties of fermented foods and prevents the growth of unwanted microorganisms [18]. Based on the results of Nematollahi et al., pH levels affect the survival of probiotic bacteria, while pH is only one of the most effective parameters on survival. Other factors, for example, are the amount of phenolic compounds in juice [13].

The high acidity of probiotic products protects them against the development of microbial degradation and increases their shelf life [19]. Acidity is one of the critical factors in the production of a probiotic product, because the reduction of pH during the storage period is accompanied by an increase in the production of acid by bacteria and the highest acid produced is lactic acid. If the amount of this acid is too high, it will affect the taste of the product and creates adverse conditions for the product [20]. In the present study, it can be generally stated that the acidity of cornelian cherry juice increased significantly during fermentation and the highest variation was observed in the range of 24-48 h. In a study, a probiotic pineapple beverage based on whey containing *Lactobacillus acidophilus* was produced at a ratio of 65:35, and its physical and chemical properties were evaluated. The results of acidity changes showed that the titratable acidity (in terms of lactic acid) ranged from 0.546% to 0.890% during the 28 days of storage at 5°C [21].

Many studies confirmed the use of carbohydrates by probiotic bacteria [16]. In general, it can be concluded that in this study the amount of reducing carbohydrates in fermentable cornelian cherry juice significantly decreased during fermentation. With the approaching end of the maintenance period, the consumption of sugars also decreased due to the decrease in the number of probiotic bacteria. The reduction in sugars in this study was in line with the report published by Malganji et al. study [15].

In addition, the results of the study indicated no statistically significant difference in the ash content of the samples over time (P<0.05). Furthemore, the population of bacteria decreased significantly over time (P<0.05). AdebayoTayo et al. (2016) studied the physicochemical properties, survival and sensory evaluation of pineapple juice containing probiotics *Pediococcus pentosaceus LaG1*, *Lactobacillus rhamnosus GG* and *Pediococcus pentosaceus LBF2*. The viability of probiotics was reported 1.05-1.5×10^9 cfu/ml during the maintenance of period. Moreover, no significant change was observed in the taste, smell, color and appearance of the samples [22].

Considering the results of the present study on the physicochemical properties and the study of the sensory and apparent properties of cornelian cherry probiotic juice supplemented with *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* comparing it with the control sample, it is concluded that the production of probiotic juice under the name of a functional foods can have very beneficial properties for human's health. The results indicated that the product had an acceptable shelf life and high quality. Based on the results, juice with *Lactobacillus acidophilus* had better sensory characteristics than *Lactobacillus delbrueckii*. It is critical to increase the viability of probiotic bacteria in the juice to increase the juice health beneficial and the shelf-life of the product.

Table 1. Results of pH during storage period

Testing sample	0 hour	24 hour	48 hour	72 hour
1	3.80±0.00^{aA}	3.80±0.00^{aA}	3.80±0.00^{aA}	3.80±0.00^{aA}
2	3.80±0.10^{aA}	3.70±0.20^{aB}	3.20±0.00^{cC}	3.10±0.10^{bD}
3	3.80±0.10^{aA}	3.80±0.00^{aA}	3.40±0.10^{bB}	3.20±0.10^{bC}

* The different small letters have a significant difference in the column (P<0.05)
** The different capital letters have a significant difference in the line (p<0.05)
*** Samples: Code (1): juice without probiotic bacteria, Code (2): juice containing *Lactobacillus delbrueckii,* Code (3): juice containing *Lactobacillus acidophilus*.

Table 2. Results of acidity of samples during storage period

Testing sample	0 hour	24 hour	48 hour	72 hour
1	1.24±0.00^{aA}	1.24±0.00^{aA}	1.24±0.00^{cA}	1.24±0.00^{cA}
2	1.24±0.02^{aB}	1.27±0.05^{aB}	1.68±0.02^{aA}	1.73±0.01^{aA}
3	1.24±0.02^{aC}	1.29±0.01^{aB}	1.47±0.03^{bA}	1.49±0.03^{bA}

* The different small letters have a significant difference in the column (P<0.05)
** The different capital letters have a significant difference in the line (p<0.05)
*** Samples: Code (1): juice without probiotic bacteria, Code (2): juice containing *Lactobacillus delbrueckii*, Code (3): juice containing *Lactobacillus acidophilus*.

Table 3. Results of reducing carbohydrates of samples during storage period

Testing sample	0 hour	24 hour	48 hour	72 hour
1	8.83±0.00^{aA}	8.83±0.00^{aA}	8.83±0.00^{aA}	8.83±0.00^{aA}
2	8.83±0.05^{aA}	8.56±0.04^{bB}	6.48±0.04^{cC}	6.23±0.10^{cD}
3	8.83±0.05^{aA}	8.50±0.03^{cB}	7.78±0.07^{bC}	7.12±0.12^{bD}

* The different small letters have a significant difference in the column (P<0.05)
** The different capital letters have a significant difference in the line (p<0.05)
*** Samples: Code (1): juice without probiotic bacteria, Code (2): juice containing *Lactobacillus delbrueckii*, Code (3): juice containing *Lactobacillus acidophilus*.

Table 4. Results of solids of all samples during storage period

Testing sample	0 hour	24 hour	48 hour	72 hour
1	17.61±0.00^{aA}	17.61±0.00^{aA}	17.61±0.00^{aA}	17.61±0.00^{aA}
2	17.61±0.06^{aA}	17.24±0.04^{bB}	17.18±0.01^{cB}	16.80±0.07^{bC}
3	17.61±0.06^{aA}	17.20±0.02^{bB}	17.12±0.03^{bB}	16.85±0.14^{bC}

* The different small letters have a significant difference in the column (P<0.05)
** The different capital letters have a significant difference in the line (p<0.05)
*** Samples: Code (1): juice without probiotic bacteria, Code (2): juice containing *Lactobacillus delbrueckii*, Code (3): juice containing *Lactobacillus acidophilus*.

Table 5. Results of ashes of samples during storage period

Testing sample	0 hour	24 hour	48 hour	72 hour
1	0.71±0.01^{aA}	0.69±0.02^{aA}	0.71±0.01^{aA}	0.71±0.00^{aA}
2	0.71±0.01^{aA}	0.71±0.02^{aA}	0.70±0.02^{abA}	0.68±0.02^{bA}
3	0.71±0.00^{aA}	0.71±0.00^{aA}	0.69±0.00^{bA}	0.69±0.01^{abA}

* The different small letters have a significant difference in the column (P<0.05)
** The different capital letters have a significant difference in the line (p<0.05)
*** Samples: Code (1): juice without probiotic bacteria, Code (2): juice containing *Lactobacillus delbrueckii*, Code (3): juice containing *Lactobacillus acidophilus*.

Table 6. Results of survival of probiotic bacteria in samples during storage period.

Testing sample	0	First week	Second week	Third week	Forth week
1	0.00±0.00^{cA}	0.00±0.00^{cA}	0.00±0.00^{bA}	0.00±0.00^{bA}	0.00±0.00^{bA}
2	8.90±0.08^{aA}	8.46±0.11^{aB}	8.48±0.23^{aC}	7.63±0.23^{aC}	7.41±0.06^{aC}
3	8.38±0.19^{bA}	7.62±0.07^{bB}	0.00±0.00^{bC}	0.00±0.00^{bC}	0.00±0.00^{bC}

* The different small letters have a significant difference in the column (P<0.05)
** The different capital letters have a significant difference in the line (p<0.05)
*** Samples: Code (1): juice without probiotic bacteria, Code (2): juice containing *Lactobacillus delbrueckii, Code* (3): juice containing *Lactobacillus acidophilus*.

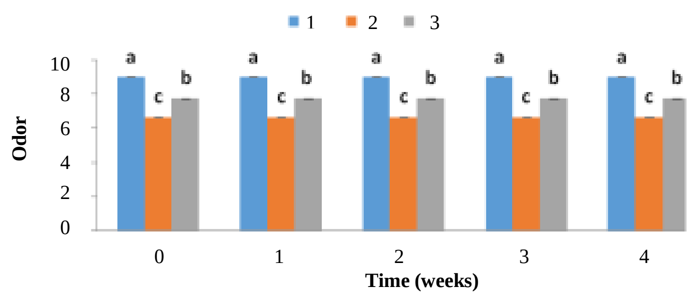

Figure 1. Results of sensory evaluation (odor) during storage period
* The different small letters have a significant difference (P <0.05)
** Samples: Code (1): juice without probiotic bacteria, Code (2): juice containing *Lactobacillus delbrueckii, Code* (3): juice containing *Lactobacillus acidophilus*.

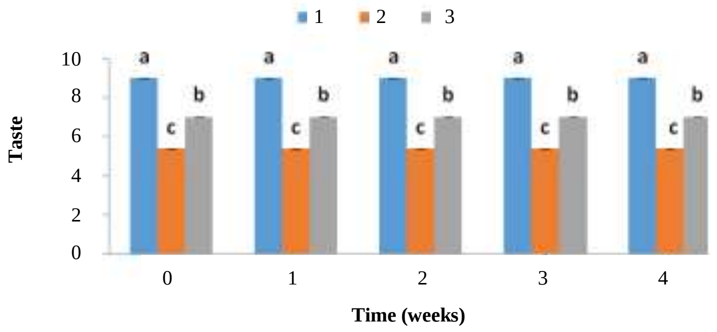

Figure 2. Results of sensory evaluation (taste) during storage period
* The different small letters have a significant difference (P <0.05)
** Samples: Code (1): juice without probiotic bacteria, Code (2): juice containing *Lactobacillus delbrueckii, Code* (3): juice containing *Lactobacillus acidophilus*.

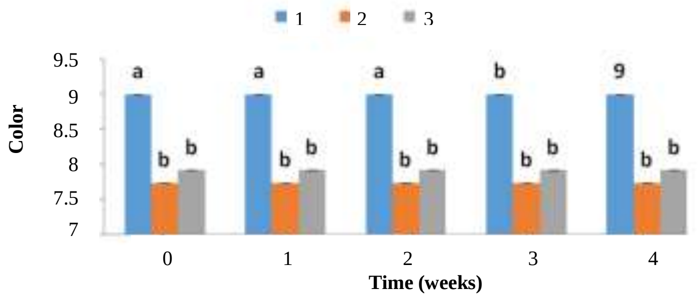

Figure 3. Results of sensory evaluation (color) during storage period
* The different small letters have a significant difference (P <0.05)
** Samples: Code (1): juice without probiotic bacteria, Code (2): juice containing *Lactobacillus delbrueckii, Code* (3): juice containing *Lactobacillus acidophilus*.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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