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Study of Production Possibility of Synbiotic Beverage Containing Red Cabbage, Carrots, Turnip Juices and Oligofructose

***M. Jafari*[a], *M. Hashemiravan*[b*], *R. Pourahmad*[c]**

[a] PhD Student of the Department of Food Science and Technology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran.
[b*] Assistant Professor of the Department of Food Science and Technology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran.
[c] Professor of the Department of Food Science and Technology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran.

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ABSTRACT: Enriching beverages with useful compounds such as probiotics and prebiotics is of particular importance in the food industry due to the lack of consumption restrictions by certain people due to the lack of lactose and cholesterol, as well as (vitamins, antioxidants and minerals). In this study, first appropriate amounts of oligofructose were selected as prebiotic material and then a mixture of vegetable water produced in proportions of 20, 30 and 40% was prepared and inoculation of *Lactobacillus casei* strain by half McFarland method at two levels of 10^6 and 10^7 *cfu/ml* were performed. All physicochemical tests such as pH, acidity, Brix and microbial count were evaluated before fermentation, after 72 hours of fermentation at 37°C and during four weeks of storage at 4°C. Reduced glucose and total glucose were evaluated before fermentation, after 72 and 4 weeks of fermentation. During fermentation and storage, with increasing bacterial density and storage time, pH, Brix, reducing sugar and total sugar of probiotic beverages decreased and acidity increased significantly. The highest survival rate of probiotic bacteria during 28 days of storage related to A_3B_2 treatment (with bacterial density of 10^7 *CFU/ml*) was selected as the best treatment according to the results.

Keywords: *Lactobacillus casei*, Oligofructose, Probiotic, Peribiotic, Vegetables Juice.

Introduction

Today, interest in functional foods and beverages has increased among consumers due to increased awareness of good nutrition and health, as well as increasing scientific evidence about their effectiveness. Functional or extra-beneficial foods are defined as food products that have additional or advanced benefits that are greater than their original nutritional value (Santeramo *et al*., 2018). Three groups of important compounds known in Functional food are: probiotics, prebiotics and synbiotics (Jenkins & Mason, 2022). Probiotics as food supplements attract a lot of attention due to their ability to increase and improve the diversity and activity of intestinal flora (Jafari *et al*., 2017). They are living microorganisms that have beneficial effects on human health by maintaining or improving intestinal microbial balance (Souri, 2019) The most important quality indicator of a probiotic product is the viability rate or the number of live probiotic cells per gram or milliliter of the product at the time of consumption. The number of probiotic bacteria in the product should be sufficient to ensure that it reaches the standard number of live probiotic cells (10^7 cfu/ml) in the intestinal

[*] Corresponding Author: m_hashemiravan@yahoo.com

tract after consumption (Jafari *et al*., 2017). The term prebiotic was expressed for the first time in 1961, when it was determined that compounds strengthen and stimulate the growth of probiotic bacteria (Eva & Socaciu, 2008). Prebiotics are able to reach the large intestine and are consumed by beneficial bacteria such as *lactobacillus* and *bifidobacteria* without being digested and absorbed in the upper part of the digestive tract (Valdes *et al*., 2018). Usually, prebiotics are defined as compounds with selective fermentability that cause special changes in the composition or activity of the gut microbiome and the well-being and health of the host. The most studied and used prebiotics include inulin, fructo-oligosaccharides and galactoligosaccharides (Golestani & Pourahmad, 2017). Oligofructose is one of the fructooligosaccharides. This compound is a dietary fiber and prebiotic substance, and it is sweet and has a pleasant taste, and it can be used for food enrichment without negative effects and help to improve the taste and aroma, texture and sweetness of the food. This substance is qualitatively similar to sugar or glucose syrup, and it is used as a substitute for sugar or fat and a calorie reducer in dairy products (Jafari *et al*., 2016). Improving diarrhea, reducing the risk of osteoporosis, reducing the risk of heart diseases by reducing the synthesis of triglycerides and fatty acids in the liver and blood serum, significantly reducing blood cholesterol levels (Boyle *et al*., 2008). Stimulating the body's immune system, balancing the level of insulin and glucagon hormones, improving intestinal function and increasing the absorption of isoflavones are among the most important advantages of using oligofructose as a prebiotic or dietary fiber (Jafari *et al*., 2016).

Regarding the prebiotic effect of oligofructose on probiotic bacteria in food products, researches have been conducted, including the studies conducted by (Aghajani *et al*., 2011; Broughani *et al*., 2018; Karegar *et al*., 2021; Momtaheni *et al*., 2015) Cited.

A food that simultaneously contains probiotic microorganisms and prebiotic compounds is called synbiotic, which are used in synbiotic products, probiotics and prebiotics in order to achieve a synergistic effect. Synbiotic foods make up a significant part of Functional foods (Golestani & Pourahmad, 2017).

Since fruits and vegetables have useful substances such as minerals, antioxidants, dietary fibers and vitamins and are free of allergenic substances found in milk, they can be considered a suitable environment for the production of non-dairy probiotic Beverages (Dogahe *et al*., 2015). Carrots are rich in active ingredients such as vitamins (A ،D ،B ،E ،C and K) and minerals (calcium, potassium, phosphorus, sodium and iron) and it is reported that 100 grams of carrots contain about 6 to 15 milligrams of carotenoids (Rafiq *et al*., 2016). Turnip contains carbohydrates and minerals such as iron, potassium, magnesium, calcium, phosphorus and various vitamins B, C and E (Campbell et al., 2012). Red cabbage is rich in group vitamins (A, B, C, E, K), antioxidants, Minerals (calcium, iron, manganese, magnesium and potassium) and lack of protein, cholesterol and saturated fats (Chen *et al*., 2018).

In recent years, several studies have been conducted on the use of fruit and vegetable juice as a base medium for lactic fermentation (Mamaghani *et al*., 2021; Rahimabadi *et al*., 2018; S, 2019; Sepahi *et al*., 2023; Shalaby & Mohamed, 2022; Zandi *et al*., 2016) Considering the biological and nutritional potential of vegetable and fruit extracts, it seems that the use of their extracts is very beneficial and these products are consumed by a large part of the people. Therefore, according to the studies and research done by other researchers, the chemical, microbial and sensory properties of probiotic fermented beverage containing a mixture of red cabbage, carrot, turnip and oligofructose juice were investigated.

Materials and Methods

- *Consumable raw materials*

Consumable vegetables (red cabbage, carrots and turnips) were purchased from the local fruit and vegetable market in Tehran. *Lactobacillus casei* 1608 PTCC strain was obtained in lyophilized form from Iranian Research Organization for Science and Technology. Chemical materials including buffer 4 and 7, sodium 0.1 normal, lead acetate, activated carbon, dipotassium oxalate, hydrochloric acid, phenolphthalein, methylene blue, Ringer's tablets were obtained from Merck, Germany. Also, MRS Agar culture medium was prepared from Liofilchem-Italy and MRS Broth culture medium was prepared from Merck-Germany.

- *Vegetable extract mixture including red cabbage, carrot, turnip*

Inorder to produce the synbiotic product, red cabbage, carrots and turnips were purchased from the local fruit market. After washing the vegetables and homemade juicer, the juicing process was carried out in the aseptic environment of the laboratory in order to reduce the microbial load, and then the vegetable extract was taken and stored at 4 degrees Celsius. The preparation of vegetable juice mixture including red cabbage, carrot and turnip was done with proportions of 40%, 40% and 20% respectively (concentration is fixed in percent), which formed the vegetable juice extract in the present research.

- *Pasteurization before bacterial inoculation*

Inorder to pasteurize the vegetable juice mixture, a temperature of 80°C was used for 5 minutes. The samples were poured into the containers and their lids were closed and placed in the bain-marie machine, then the desired temperature and time were set. After the samples reached the desired temperature and passed the necessary time, the samples were immediately cooled with cold water to complete the pasteurization process (Mousavi *et al*., 2011).

- *Preparation of the strain and inoculation of the microorganism to the samples*

MRS Broth culture medium was prepared according to the brochure. It is prepared according to the activation instructions of the received strain. For microbial inoculation, McFarland's half method was used, which was a method to determine the amount of bacteria at two levels of 1.5 x 10^7 cfu/ml and 1.5 x 10^6 cfu/ml. The said culture was inoculated with an initial concentration of 10^6 cfu/ml (microbial suspension 2) and 10^7 cfu/ml (microbial suspension 1) to vegetable extract with an initial pH of 4 and placed in an incubator at 30°C for 72 hours in order to perform fermentation. When *Lactobacillus casei* strain was inoculated, 2% of oligofructose was also added to the treatments.

- *Preparation of carrot, red cabbage and turnip extract mixture*

Vegetable juice extract is 40 cc of fresh carrot juice, 40 cc of fresh red cabbage juice and 20 cc of fresh turnip juice. Preparation of treatments is as follows (according to Table 1):

Table 1. Research Treatments

Treatments	Concentration of mixed vegetable juice (red cabbage, carrot and turnip)	Distilled Water	Microbial Suspension	Density of *Lactobacillus casei bacteria*
A_1B_1	20 cc	79 cc	1 cc	10^6 cfu/ml
A_2B_1	30 cc	69 cc	1 cc	10^6 cfu/ml
A_3B_1	40 cc	59 cc	1 cc	10^6 cfu/ml
A_1B_2	20 cc	79 cc	1 cc	10^7 cfu/ml
A_2B_2	30 cc	69 cc	1 cc	10^7 cfu/ml
A_3B_2	40 cc	59 cc	1 cc	10^7 cfu/ml
T	30 cc	70 cc	-	-

- ***Tests***

- ***Bacterial counting***

In order to count live microbial cells, decimal dilution method and porplate according to SPC (Standard Plate Counts) method were used. Dissolve two Ringer's tablets in 1000 milliliters of distilled water, this process is carried out by the Ben-Marie method. In this way, Ringer's solution was prepared for dilution. 9 cc of Ringer's solution was transferred into the test tube and then sterilized in an autoclave at 121°C for 15 minutes. After cooling the solutions, 1 cc of the sample containing microorganisms was added to Ringer's solution and mixed well with a shaker. The resulting dilution is 10^{-1}. Then, 1 milliliter of microbial suspension was added to the second tube containing 9 milliliters of sterile Ringer's solution, and thus a 10^{-2} dilution was prepared. Again, after vortexing, the resulting microbial suspension was used to prepare the next dilutions, this process continued until the 10^{-8}th dilution was prepared. Final dilutions were used to count the number of microorganisms. From the selected dilutions, 1 ml of microbial suspension was transferred into a sterile plate and a sufficient amount of sterile MRS culture medium was added to it. After the culture medium was closed, the plates were moved into the jar and anaerobized by an anaerobic device. Then the plates were kept for 72 hours in an incubator with a temperature of 37°C. The number of grown colonies was counted after 72 hours.

- ***pH measurement***

Inorder to measure the pH, the company's pH meter (Mettler Toledo model MP220, Germany) was used. Before use, the device was first calibrated with buffer solution 7 and then with buffer solution 4. Then some sample was poured into the beaker and the electrode of the device was placed in the solution and waited until the number was fixed on the screen of the device and the fixed number was reported as pH (Iran National Standards Organization, 1997).

- ***Total acidity measurement***

Acidity was measured with respect to lactic acid using the titration method against 0.1 normal sodium hydroxide solution and using phenolphthalein solution as a reagent (Mridula & Sharma, 2015; Sepahi *et al*., 2023).

- ***Measurement of total sugar and reducing sugars***

The amount of total sugar and reducing sugars was measured by Linn-Ionon method, mentioned in the Iranian National Standard No. 2685 (Iran National Standards Organization, 2007).

- ***Water soluble solids (brix)***

It was made using a refractometer device (Optek, Germany). First, the device was calibrated with distilled water, then a few drops of the fermented sample, which was separated from the desired Erlenmeyer near the flame and under the hood, were poured onto the prism of the refractometer in such a way that it completely covers it, after removing the light scattering and creating two equal light and dark parts on the display screen, the concentration of dissolved solids in water was read in terms of Brix at a temperature of 20°C. The desired number unit is percentage, which shows the amount of dissolved solids in 100 grams of the sample (Iran National Standards Organization, 2007).

- ***Sensory evaluation***

After the fermentation of the beverage to perform the panel test, the test was kept in the first and fourth week and was taken from five permanent people and experts in the field of food industry. The studied characteristics of the samples, the level of acceptability was asked by the consumer in the sensory factors of fermented beverages based on the 9-level method, and an average was considered for each level, which ranges from 9 for the highest level to 1 for the lowest level. The evaluators first tasted the fermented beverage, and then scored the factors in question based on their level of interest (Luckow *et al*., 2006).

- ***Statistical Analysis***

All the experiments were conducted in a completely random design, the experiment was done factorially, where factor A (concentration of vegetable juice) was 20, 30 and 40% in 3 levels, factor B (density of bacteria) was 10^6 and 10^7 cfu/ml in 2 levels. Finally, there were 6 treatments along with 1 control treatment (T) to check the traits, and the characteristics of the treatments are given in Table 1. It should be noted that all experiments were repeated 3 times. To analyze the variance of the results, SPSS 22 software was used, the averages were compared using Duncan's multi-range test at the 95% level, and Excel software was used to draw the graph.

Results and Discussion

- ***The survival results of probiotics before fermentation, after 72 hours of fermentation at a temperature of 37°C and during 4 weeks of storage at a temperature of 4°C***

According to the results mentioned in Table (2), it was found that the density of bacteria, the concentration of vegetable juice and the storage time had a significant effect on the count of probiotic bacteria in the produced beverage ($P<0.01$). With the increase in bacterial density, the count of probiotic bacteria in the beverage produced based on the mixture of red cabbage, carrot, and turnip juice increased significantly ($P<0.05$). By increasing the storage time from the moment of production until after 72 hours of fermentation to the second week, the probiotic bacteria count of the produced beverage increased significantly ($P\leq0.05$). From the third week to the end of the process in the fourth week, the trend decreased. Since microorganisms need a carbon source to grow and consume sugars

Table 2. Comparison of the average count of probiotic bacteria in the beverage (log cfu.ml) during the storage period (mean ± standard deviation)

Treatment	Production moment	After 72 hours of fermentation	first week	second week	Third week	fourth week	Average treatment in time
A_1B_1	6.26±0.11^{dDE}	6.89±0.12^{dC}	7.62±0.08^{fB}	11.76±0.10^{dA}	6.34±0.10^{fD}	6.09±0.10^{fE}	7.49±0.11^{e}
A_2B_1	6.52±0.15^{cF}	7.43±0.11^{cD}	10.81±0.05^{dB}	14.87±0.14^{aA}	8.43±0.10^{dC}	7.78±0.01^{cD}	9.31±0.15^{c}
A_3B_1	6.43±0.12^{Ecd}	6.95±0.08^{Dd}	10.04±0.19^{Be}	14.06±0.10^{Ac}	7.75±0.10^{Ce}	6.44±0.10^{Ee}	8.61±0.12^{d}
A_1B_2	7.63±0.07^{Fa}	9.37±0.05^{Db}	14.24±0.10^{Bb}	14.64±0.10^{Ab}	10.00±0.11^{Cb}	8.84±0.05^{Eb}	10.79±0.10^{b}
A_2B_2	7.10±0.07^{Eb}	7.34±0.06^{Dc}	11.02±0.07^{Bc}	14.24±0.10^{Ac}	8.72±0.15^{Cc}	6.900±0.04^{Fd}	9.22±0.14^{c}
A_3B_2	7.82±0.14^{Fa}	9.77±0.11^{Da}	14.76±0.05^{Ba}	14.95±0.07^{Aa}	10.83±0.06^{Ca}	9.58±0.09^{Ea}	11.29±0.17^{a}
(T)	0.00±0.00^{Ae}	0.00±0.00^{Ae}	0.00±0.00^{Ag}	0.00±0.00^{Ae}	0.00±0.00^{Ag}	0.00±0.00^{Ag}	0.00±0.00^{Af}

- Latin lowercase letters in each column and Latin capital letters in each row indicate a significant difference at the 5% level (P<0.05).

as one of the best sources of carbon, that's why in the first two weeks we saw the growth of microorganisms due to proper feeding of sugary substances. But in the last two weeks, in order to reduce the amount of sugar and nutrients in the samples, we saw a decrease in the amount of *Lactobacillus casei* microorganism in the samples. During the storage time, the highest count of probiotic bacteria (average treatment time) with the number of 11.29 log cfu/ml was related to the A3B2 treatment containing 40% mixture of vegetable juice and *Lactobacillus casei* with a density of 10^7 cfu/ml which had a significant difference with other treatments ($P \leq 0.05$). It is obvious that the lowest mean count of probiotic bacteria belonged to the treatments without *Lactobacillus casei* bacteria inoculation.

Mamaghani *et al*. (2021) researched Production and Evaluation of Some Physical, chemical and Sensory Properties of Fermented Carrot Juice Using *Lactobacillus casei* and *Lactobacillus plantarum* and Their Shelf Life. they stated that at all times, among the prepared carrot juice samples, the sample containing the combination of two types of probiotic bacteria had a significantly higher number of bacteria, and with the passage of time, the number of counted bacteria decreased. The least changes in comparing the first day and the 45th day were related to the carrot juice sample containing *Lactobacillus casei* and the most changes were related to the carrot juice sample containing two bacteria which was consistent with the results obtained from the research.

Daliri *et al*., (2020) Investigated the possibility of producing probiotic juice based on a mixture of cherry, blueberry and apple by *Lactobacillus acidophilus* and *Lactobacillus casei*. The effect of treatment on the number of bacteria was significant at all times ($P \leq 0.05$). In all treatments, except for the control, there were significant changes over time, therefore the number of bacteria increased until the second week, but then decreased until the fourth week ($P<0.05$). In this case, it was consistent with the results obtained from the present research.

- ***The results of pH and acidity before fermentation, after 72 hours of fermentation at 37°C and during 4 weeks of storage at 4°C***

According to the results mentioned in Table 3, it was found that the density of bacteria and the concentration of vegetable juice had a significant effect on the pH value of the probiotic beverage ($P<0.01$). Since the growth of bacteria has led to a decrease in pH during fermentation and storage. The main reason for this is related to the consumption of sugars and the production of organic acids. With increasing bacterial density and storage time, pH of probiotic beverage decreased significantly (*P*<0.05). During the storage time, the highest pH value (3.85) belonged to the control treatment T (containing 30% vegetable juice mixture). The lowest pH value (3.15) belonged to A1B2 treatment (containing 20% vegetable juice mixture with *Lactobacillus casei* with a density of 10^7 cfu/ml).

According to the results mentioned in Table 4, it was found that the density of bacteria and the concentration of vegetable juice had a significant effect on the acidity of the probiotic beverage (*P*<0.01). With increasing bacterial density and storage time, the acidity of probiotic beverage increased significantly (*P*≤0.05). During the storage time, the highest acidity value (0.67 g/ml100) was related to A3B2 treatment (containing 40% vegetable juice mixture with *Lactobacillus casei* with a density of 10^7 cfu/ml). The lowest acidity value (0.52/g ml100) was related to the control treatment T (containing 30% vegetable juice mixture).

Jaddi *et al*., (2020) after Production of functional beverage of grape juice and lemon skin distilment contained Bacillus coagulans, they expressed, that the pH value decreased significantly during 90 days of storage in all treatments, which was consistent with the obtained results.

Ghaempanah *et al*. (2020) in a new study on Production of Probiotic Fermented Beverage from Turnip (*Brassica rapa*) and Purple Carrots (*Daucus carota*) Using *Lactobacillus plantarum*, *Lactobacillus* fermentum and *Lactobacillus paracasei* and Saccharomyces cerevisiae. reported that the pH decreased over time, which was similar to the results of the present study.

Table 3. Comparison of the average pH of the beverage during the storage period (mean ± standard deviation)

Treatment	Production moment	After 72 hours of fermentation	first week	second week	Third week	fourth week	Average treatment in time
A_1B_1	4.1 ± 0.10^{Aab}	3.96 ± 0.01^{Be}	3.11 ± 0.01^{Cb}	3.10 ± 0.00^{Cb}	2.90 ± 0.00^{Cb}	2.80 ± 0.00^{Cd}	3.32 ± 0.55^{d}
A_2B_1	4.2 ± 0.00^{Aa}	4.11 ± 0.01^{Bab}	2.85 ± 0.00^{Cc}	2.90 ± 0.20^{Cc}	2.90 ± 0.00^{Cb}	3.00 ± 0.00^{Cc}	3.32 ± 0.64^{e}
A_3B_1	4.0 ± 0.00^{Ab}	3.95 ± 0.00^{Be}	3.20 ± 0.02^{Cb}	3.15 ± 0.00^{Cb}	3.10 ± 0.00^{Ca}	3.20 ± 0.10^{Cb}	3.43 ± 0.42^{b}
A_1B_2	4.1 ± 0.10^{Aab}	4.05 ± 0.00^{Bc}	2.81 ± 0.00^{Ccd}	2.65 ± 0.02^{Cd}	2.70 ± 0.10^{Cc}	2.60 ± 0.20^{Ce}	3.15 ± 0.71^{g}
A_2B_2	4.0 ± 0.00^{Ab}	3.98 ± 0.02^{Bd}	3.10 ± 0.00^{Cb}	3.10 ± 0.00^{Cb}	3.10 ± 0.00^{Ca}	3.20 ± 0.00^{Cb}	3.41 ± 0.44^{c}
A_3B_2	4.2 ± 0.10^{Aa}	4.10 ± 0.00^{Bb}	2.70 ± 0.02^{Cd}	2.70 ± 0.10^{Cd}	2.90 ± 0.10^{Cb}	3.00 ± 0.00^{Cc}	3.26 ± 0.69^{f}
(T)	4.1 ± 0.00^{Aab}	4.12 ± 0.00^{Ba}	4.00 ± 0.00^{Ca}	4.20 ± 0.00^{Ca}	2.90 ± 0.00^{Eb}	3.80 ± 0.00^{Da}	3.85 ± 0.48^{a}

- Latin lowercase letters in each column and Latin capital letters in each row indicate a significant difference at the 5% level (P<0.05).

Table 4. Comparison of the average acidity (in terms of lactic acid) of the beverage during the storage period (mean ± standard deviation)

Treatment	Production moment	After 72 hours of fermentation	first week	second week	Third week	fourth week	Average treatment in time
A_1B_1	0.51 ± 0.01^{Dcd}	0.53 ± 0.01^{Cc}	0.62 ± 0.01^{Be}	0.64 ± 0.02^{Ac}	0.61 ± 0.01^{Bd}	0.62 ± 0.01^{Be}	0.58 ± 0.05^{f}
A_2B_1	0.50 ± 0.02^{Ed}	0.52 ± 0.01^{Dc}	0.68 ± 0.01^{Cc}	0.72 ± 0.01^{Ab}	0.70 ± 0.01^{Bbc}	0.72 ± 0.00^{Bab}	0.64 ± 0.10^{d}
A_3B_1	0.54 ± 0.01^{Dab}	0.56 ± 0.01^{Cb}	0.65 ± 0.02^{Bd}	0.72 ± 0.00^{Ab}	0.68 ± 0.00^{Bc}	0.65 ± 0.00^{Bd}	0.63 ± 0.06^{e}
A_1B_2	0.53 ± 0.01^{Dabc}	0.55 ± 0.01^{Cb}	0.70 ± 0.00^{Bb}	0.73 ± 0.00^{Aab}	0.72 ± 0.00^{Bab}	0.70 ± 0.00^{Bc}	0.65 ± 0.09^{c}
A_2B_2	0.55 ± 0.01^{Ea}	0.58 ± 0.01^{Da}	0.67 ± 0.00^{Cc}	0.75 ± 0.00^{Aa}	0.71 ± 0.00^{Bab}	0.73 ± 0.01^{Ba}	0.66 ± 0.08^{b}
A_3B_2	0.54 ± 0.01^{Dab}	0.59 ± 0.01^{Ca}	0.73 ± 0.00^{Ba}	0.75 ± 0.01^{Aa}	0.73 ± 0.03^{Ba}	0.71 ± 0.00^{Bbc}	0.67 ± 0.08^{a}
) T(	0.52 ± 0.01^{Bbcd}	0.53 ± 0.00^{Bc}	0.50 ± 0.00^{Bf}	0.52 ± 0.02^{Bd}	0.50 ± 0.00^{Be}	0.55 ± 0.01^{Af}	0.52 ± 0.01^{g}

- Latin lowercase letters in each column and Latin capital letters in each row indicate a significant difference at the 5% level (P<0.05).

Amin Moghadasi, (2019) investigated the possibility of probiotic production base on Celery and Blackberry beverages by using *Lactobacillus plantarum*, The results showed that with the passage of time and the growth of the target bacteria in the blackberry and celery beverage, the pH has decreased significantly, which is attributed to the consumption of sugar, the fermentation process and the production of acid by *Lactobacillus plantarum* bacteria.

Łopusiewicz *et al*., (2022) in another study used natural gum from a flaxseed byproduct as a potential stabilizing and thickening agent for acid whey-fermented beverages. The results of the researchers' findings showed that the addition of flaxseed gum had a significant effect ($P≤0.05$) on the pH of the beverage, which increased the pH of fermented beverages made from acidic whey.

- ***Brix results before fermentation, after 72 hours of fermentation at 37°C and during 4 weeks of storage at 4°C***

According to the results mentioned in Table 5, it was found that the concentration of bacteria and the concentration of vegetable juice had a significant effect on the Brix content of the probiotic beverage (P<0.01). With the increase in bacterial density, Brix of probiotic beverage produced based on a mixture of red cabbage, carrot, and turnip juice increased significantly ($P≤0.05$). By increasing the storage time from the moment of production to after 72 hours of fermentation, Brix of the produced probiotic beverage decreased significantly ($P≤0.05$). Since microorganisms need a carbon source for growth, and they consume sugars as one of the best sources of carbon. As a result, the growth of bacteria has led to a decrease in Brix during fermentation and storage. The main reason for this is related to the consumption of sugary substances and the production of organic acids.

Zandi *et al*., (2016), investigated the production of a fermented probiotic mixture from carrot, beet and apple juice. It was stated that, during fermentation, in all treatments, the amount of Brix decreased, which was consistent with the results obtained from the present study.

Asadzadeh *et al*., (2021), reported the production of a functional oat bran probiotic beverage using Bifidobacterium lactis. During 21 days of storage, the Brix level decreased, which was consistent with the research results.

Table 5. Comparison of the average Brix of the beverage during the storage period (mean ± standard deviation)

Treatment	Production moment	After 72 hours of fermentation	first week	second week	Third week	fourth week	Average treatment in time
A_1B_1	$3.70±0.10^{Ac}$	$3.70±0.10^{Ac}$	$3.25±0.04^{Cd}$	$3.30±0.01^{Be}$	$3.20±0.02^{Df}$	$3.30±0.01^{Be}$	$3.40±0.22^{f}$
A_2B_1	$3.73±0.02^{Ac}$	$3.70±0.02^{Ac}$	$3.11±0.04^{Ce}$	$3.10±0.02^{Cf}$	$3.30±0.03^{Be}$	$3.20±0.03^{Bf}$	$3.35±0.28^{g}$
A_3B_1	$4.70±0.10^{Ab}$	$4.65±0.04^{Bb}$	$4.30±0.03^{Dc}$	$4.30±0.03^{Dd}$	$4.40±0.02^{Cc}$	$4.50±0.05^{Cb}$	$4.47±0.17^{d}$
A_1B_2	$4.75±0.02^{Ab}$	$4.70±0.06^{Bb}$	$4.32±0.01^{Cc}$	$4.30±0.02^{Cd}$	$4.20±0.03^{Dd}$	$4.20±0.02^{Dd}$	$4.41±0.24^{e}$
A_2B_2	$5.40±0.04^{Aa}$	$5.36±0.03^{Ba}$	$5.10±0.02^{Da}$	$5.20±0.03^{Ca}$	$5.40±0.01^{Ba}$	$5.40±0.04^{Ba}$	$5.31±0.12^{a}$
A_3B_2	$5.44±0.02^{Aa}$	$5.40±0.04^{Ba}$	$5.11±0.06^{Da}$	$5.10±0.01^{Db}$	$5.40±0.02^{Ba}$	$5.35±0.02^{Ca}$	$5.30±0.15^{b}$
(T)	$4.72±0.02^{Ab}$	$4.70±0.07^{Ab}$	$4.70±0.07^{Ab}$	$4.75±0.01^{Ac}$	$4.60±0.02^{Bb}$	$4.40±0.04^{Cc}$	$4.64±0.13^{c}$

- Latin lowercase letters in each column and Latin capital letters in each row indicate a significant difference at the 5% level (P<0.05).

- ***The results of reduced sugar and total sugar before fermentation, after 72 hours of fermentation at 37°C and the fourth week of storage at 4°C***

According to Table 6, it was found that the density of bacteria, the concentration of vegetable juice and the storage time had a significant effect on the reducing sugar content of the probiotic beverage produced (P<0.01). With the increase in bacterial density, the reducing sugar of the probiotic beverage produced based on a mixture of red cabbage, carrot, and turnip juice increased significantly ($P \leq 0.05$). With increasing storage time, there was no significant difference between the average reducing sugar of probiotic beverage from the moment of production to after 72 hours of fermentation ($P>0.05$). With the increase of storage time, until the 28th day of storage, reducing sugar decreased significantly ($P \leq 0.05$). According to the obtained results, with the growth of microorganisms, the amount of reducing sugars has decreased, because these microorganisms need a source of carbon to grow and consume sugars as one of the best sources of carbon.

According to Table 7, it was found that bacterial density, vegetable juice concentration and storage time had a significant effect on the total sugar content of the produced probiotic beverage ($P<0.01$). With the increase in bacterial density, the total sugar of probiotic beverage produced based on a mixture of red cabbage, carrot, and turnip juice increased significantly ($P \leq 0.05$). By increasing the storage time from the moment of production to after 72 hours of fermentation, the total sugar of the probiotic beverage decreased, but this decrease was not significant ($P>0.05$). The process of reducing total sugar continued until the 28th day of storage. If the lowest amount of total sugar was observed on this day. The total sugar of the probiotic beverage on the 28th day of storage had a significant difference with the moment of production and 72 hours after fermentation ($P \leq 0.05$). According to the obtained results, it was found that the amount of probiotic bacteria increased during fermentation, which increased the amount of sugar consumed by the bacteria, and for this reason, the amount of total sugar decreased significantly.

Rahimabadi *et al*. (2018) investigated the production of synbiotic malt beverage was investigated using inulin and different probiotic strains of *Lactobacillus* bacteria. These researchers stated that with the growth of microorganisms, the amount of total sugar and reducing sugars decreased and this decrease had a direct relationship with the intensity of the growth of microorganisms.

Daliri *et al*. (2020), also stated that with the passage of time, reducing sugar and total sugar decreased significantly ($P<0.05$). The results obtained from this research were consistent with the results of other researchers.

– ***Sensory evaluation results in the first and fourth weeks of storage***

According to the results mentioned in Figure 1, there is a significant relationship between vegetable juice concentration, bacterial density and storage time with the sensory evaluation score of flavor, aroma, sweetness, sourness, color and appearance, concentration and overall acceptance ($P<0.01$).

(Souri, 2019) concerning the sensory properties, the effect of green tea leaf extract on probiotic bacteria of watermelon juice was significantly different from the control sample, and in all treatments, the score of taste factor decreased significantly with the passage of time. It was consistent with the results

Table 6. Comparison of the average Reducing sugar of the beverage during the storage period (mean ± standard deviation)

Treatment	Production moment	After 72 hours of fermentation	fourth week	Average treatment in time
A_1B_1	1.40±0.00^{Ad}	1.40±0.10^{Ac}	1.10±0.00^{Bd}	1.30±0.00^{f}
A_2B_1	1.40±0.00^{Ad}	1.30±0.00^{Ac}	1.00±0.00^{Bd}	1.23±0.00^{g}
A_3B_1	2.30±0.20^{Ac}	2.20±0.00^{Ab}	1.70±0.00^{Bc}	2.06±0.00^{d}
A_1B_2	2.35±0.40^{Ac}	2.30±0.10^{Ab}	1.60±0.10^{Bc}	2.08±0.00^{c}
A_2B_2	3.10±0.00^{Ab}	3.30±0.00^{Aa}	2.80±0.00^{Ba}	3.06±0.00^{b}
A_3B_2	3.30±0.10^{Aa}	3.40±0.00^{Aa}	2.60±0.10^{Bb}	3.10±0.00^{a}
(T)	2.30±0.00^{Ac}	2.20±0.10^{Ab}	1.60±0.10^{Bc}	2.03±0.00^{e}

- Latin lowercase letters in each column and Latin capital letters in each row indicate a significant difference at the 5% level ($P<0.05$).

Table 7. Comparison of the average total sugar of the beverage during the storage period (mean ± standard deviation)

Treatment	Production moment	After 72 hours of fermentation	fourth week	Average treatment in time
A_1B_1	3.20±0.10^{Ae}	3.20±0.02^{Af}	2.10±0.00^{Be}	2.83±0.63^{g}
A_2B_1	3.20±0.00^{Ae}	3.30±0.00^{Ae}	2.30±0.00^{Bd}	2.93±0.55^{f}
A_3B_1	4.15±0.00^{Ad}	4.20±0.00^{Ac}	3.25±0.00^{Bc}	3.86±0.53^{c}
A_1B_2	4.26±0.05^{Ac}	4.00±0.00^{Ad}	3.20±0.00^{Bc}	3.82±0.55^{e}
A_2B_2	4.80±0.00^{Ab}	4.90±0.10^{Aa}	3.80±0.00^{Ba}	4.5±0.60^{a}
A_3B_2	4.90±0.00^{Aa}	4.80±0.00^{Ab}	3.60±0.00^{Bb}	4.43±0.72^{b}
(T)	4.23±0.02^{Ac}	4.15±0.03^{Ac}	3.20±0.00^{Bc}	3.86±0.57^{d}

- Latin lowercase letters in each column and Latin capital letters in each row indicate a significant difference at the 5% level ($P<0.05$).

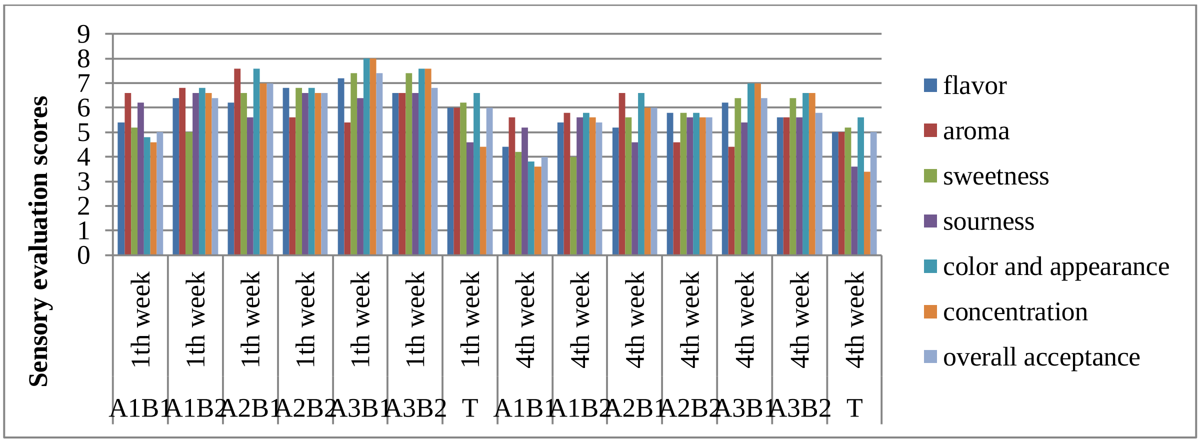

Fig. 1. Sensory evaluation of probiotic beverages during the 1th and 4th week of storage.

obtained from the present research.

(Islam *et al.*, 2021) Work on Development of probiotic beverage using whey and pineapple (Ananas comosus) juice: Sensory and physico-chemical properties and probiotic survivability during in-vitro gastrointestinal digestion. The results showed that the beverage with 25% whey and 75% pineapple juice (fermented for 5 hours at 37°C) had the highest acceptance rate. It is evident from the results that the flavor score of the selected beverage has increased up to 28th days and then little declined with the advancement of the storage period ($p = 0.030$). this might be accredited to oxidation and enzymatic activity in the beverage. Data on overall acceptability indicated that the values significantly decreased ($p = 0.021$). The scores showed that the WBB could be preserved up to 42 days with greater consumer acceptability (>4, very good).

Conclusion

The current research conducted with the aim of investigating the possibility of producing a synbiotic beverages containing red cabbage, carrot, turnip juice and oligofructose. The results showed that the density of bacteria and the concentration of vegetable juice had a significant effect on pH, acidity, Brix, reducing sugar, total sugar and the amount of *Lactobacillus casei* bacteria. Thus, in the first two weeks of the process, the growth of bacteria increased significantly, which can be attributed to the use of suitable sugar compounds in the samples. at the last two weeks, we saw a decrease in microorganisms, which was attributed to a decrease in pH, an increase in acidity, and a decrease in useful compounds such as sugar in the samples. Also, they had a significant effect on sensory evaluation (flavor, aroma, sweetness, sourness, color and appearance, concentration and general acceptability) of probiotic beverages ($P<0.01$). By increasing bacterial density and storage time, the pH and Brix of the beverages decreased significantly ($P\leq0.05$) and the acidity of the beverages increased significantly ($P\leq0.05$). By increasing the storage time from the moment of production to after fermentation and one week of storage, the amount of *Lactobacillus casei* bacteria in the beverages increased significantly ($P\leq0.05$) and decreased significantly from the second to the fourth week of storage ($P\leq0.05$).

According to the obtained results, it was found that *Lactobacillus casei* can be used as a probiotic microbial culture to produce healthy beverages for vegetarians, but in terms of sensory evaluation, the resulting beveragess need to be modified by other fruit juices or vegetable juices. Finally, the highest survival of probiotic bacteria up to four weeks of storage is recommended for A_3B_2 treatment containing 40% plant extract with a bacterial density of 10^7 with a number of Lactobacillus casei bacteria of 11.29 *log cfu/ml* for industrial production.

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