

Journal of Food Science and Technology (Iran)

Homepage: www.fsct.modares.ir



Production of low-calorie shortening from high internal phase emulsion gel and its application in the food system

Anis Talebi¹, Leila Rafiei^{2*}

1-PhD candidate in Food Science and Technology, Agriculture Faculty, Urmia university, Urmia, Iran.

2-PhD candidate in Food Science and Technology, Agriculture Faculty, Urmia university, Urmia, Iran.

ABSTRACT

Nowadays, with the change of life culture and the use of unsuitable diet, the need to develop of safe, non-allergenic, enriched with nutrients and bioactive compounds foods in order to optimize the immune system, prevent and reduce diseases has increased; Therefore, the aim of this research was to prepare a gluten-free functional beverage based on oat containing ginger extract and stevia sweetener. For this purpose, the effect of ginger extract at the levels of 1.5, 3 and 4.5% and the replacement of stevia with sucrose at the levels of 25, 50 and 75% in the form of a factorial experiment based on a completely randomized design in the formulation of a functional gluten-free beverage based on oat containing *Lactobacillus plantarium* probiotic bacteria was evaluated in order to investigate its physicochemical, sensory and microbial properties during the storage (21 days). The results showed that during the storage, in all the investigated treatments, the amount of Brix, total solid, pH and antioxidant capacity decreased significantly and the acidity of the samples increased ($P < 0.05$), and on the other hand, with the addition of ginger extract the amount of Brix, total solid and pH of the produced beverage samples decreased and the antioxidant capacity and acidity of the samples increased ($P < 0.05$); Also, the replacement of stevia with sucrose led to a decrease in Brix, total solid, acidity, but the pH and antioxidant capacity increased significantly. The results of the survival study of *Plantarium* probiotic bacteria showed that in all samples, the growth of bacteria decreased during the storage, but despite the decrease in bacteria, the amount of live bacteria was maintained within the standard range, and the addition of ginger and stevia extract had a positive effect on the survival of bacteria. Based on the sensory evaluation results, the sample containing 3% ginger extract and 25% stevia received a higher score from the panalists. Finally, based on all the results, it can be said that the addition of ginger extract and stevia sweetener in gluten-free functional beverage led to the improvement the properties of beverage and it can be introduced as a new functional beverage with favorable nutritional properties.

ARTICLE INFO

Article History:

Received: 2023/5/31

Accepted: 2023/9/30

Keywords:

Gluten-free beverage,
probiotic,
ginger extract.
stevia.

DOI: 10.22034/FSCT.20.143.46

DOR: 20.1001.1.20088787.1402.20.143.4.6

*Corresponding Author E-Mail:
anis.ta1994@gmail.com
an.talebi@urmia.ac.ir

1- Introduction

Beverages are one of the most attractive and desirable categories of food due to their suitable functional properties and their ability to meet the demands of consumers in terms of contents, size, shape, appearance, as well as easier distribution and storage [1]; Among the types of drinks, the practical and useful drinks section is due to its desirable sensory properties, richness in terms of nutrients and bioactive compounds, including vitamins, minerals, polyphenols, plant pigments, extracts, essential oils, phytosterols, fatty acids, fiber, prebiotics and probiotics with antioxidant, anti-inflammatory, anti-tumor, anti-cholesterol, antimicrobial properties [2] and potential health benefits (preventing diseases such as blood pressure, diabetes mellitus, digestive disorders, improving mental health, etc.) in the years Recently, they have registered a significant growth, and after that, consumers' desire for carbonated and high-calorie drinks has decreased [3, 4]. One of the most important subgroups of functional drinks that have received much attention in recent years, we can mention fermented drinks containing probiotic bacteria; That consumption of a certain amount (Normal minimum level is 6-7 log cfu/ml) of them stimulating growth, strengthening the activity of various types of bacteria in the intestine, creating therapeutic and health-giving properties beyond nutritional value, improving lactose digestion, improving calcium absorption, synthesis of vitamins and proteins, stimulating and improving the body's immune system, reducing cholesterol levels, Reducing allergies, preventing all types of cancer, especially colon cancer, improving intestinal microbial balance, preventing the growth and activity of pathogenic microorganisms, and increasing nutritional value. It also improves the taste and texture of food [5]. There are different microbial species that are used as probiotics [6]; that the most common bacteria used as probiotics in business are bifidobacteria and lactic acid bacteria such as lactobacilli, lactococci and streptococci [7]; Among the mentioned bacteria, *Lactobacillus plantarum* (*Lactiplantibacillus plants*) One of the most important Species in the fermentation of different plant and subspecies products It is lactic acid bacteria. *Lactobacillus plantarum* It has a

high ability to adapt to different environments. Among the proven health effects of consuming food products containing *Lactobacillus plantarum*, we can mention the reduction of the risk of inflammation of the veins, infection of the digestive tract and the stimulating effects of the immune system. Studies have shown that *Lactobacillus plantarum* bacterium as a natural inhibitor in bio-processed foods prevents the growth of pathogenic bacteria and spoilage microorganisms during storage and increases the shelf life of the product. and also *Plantarium* strains are sold as probiotics in the market [8, 9]; which is one of the conventional ways to make probiotic microorganisms available in the gut through the consumption of fermented items based on dairy products, which usually use milk as a raw material; However, the consumption of such products is not suitable for groups of the population who are allergic to lactose and milk protein, follow a vegetarian diet, or are concerned about cholesterol content and gastroesophageal reflux disease [10]; Therefore, the use of non-dairy probiotic drinks based on cereals and pseudo-cereals has attracted the attention of researchers due to avoiding allergens and intolerance to dairy products, reducing cholesterol and saturated fatty acids consumption, and supporting the lifestyle of vegetarians. But on the other hand, in patients with celiac disease, which is one of the most common food sensitivities and due to immune responses to gliadin, it causes an inflammatory reaction, mainly in the upper part of the small intestine; It prevents the absorption of nutrients such as iron, folic acid, calcium, and fat-soluble vitamins in the body and leads to constipation, anemia, abdominal pain, bloating, fatigue, infertility, and osteoporosis, so gluten-containing foods should be removed from the diet of such people. be removed [11, 12]; For this purpose, one of the pseudo-cereals without gluten and with high nutritional value is used instead of cereals containing gluten *Avena sativa* L.) is. Judosar is one of the food and grain products with a long history of consumption, which is cultivated and consumed all over the world and has become more popular due to its nutritional and bioactive compounds. This is

an annual crop that has been cultivated for more than 2000 years in different parts of the world. It is an important source of carbohydrates (59-70%), dietary fiber (12%), protein (15-17%), lipids (4.5%), various phenolic compounds, vitamins, unsaturated fatty acids, sterols and minerals. [13, 14]. Oat β -glucan (2-6%), one of the main components of soluble fiber, is a viscous polysaccharide made of a branched linear chain of D-glucose monosaccharides, which is linked by β (1 \rightarrow 3) and β (1 \rightarrow 4) are connected. This main active compound in juduser reduces cholesterol, anti-diabetes, strengthens the immune system and improves intestinal microbiota for health, prevention of diseases such as arteriosclerosis, dermatitis and some types of cancer [15]. Plant extracts contain many biologically active components such as: phenolic substances, carotenoids, nutrients, minerals, vitamins and dietary fibers, which makes it suitable substances for enhancing the growth of bacteria. Ginger (*Zingiber officinale*) a popular family spice *Zingiberaceae* It is used in traditional diets all over the world. Ginger contains many nutrients including: vitamins, starch, gingerol, shogaol and amino acids. 6-Gingerol is one of the main biologically active ingredients of ginger, which has analgesic and anti-inflammatory, anti-cancer, anti-inflammatory, antioxidant, cardiac and blood pressure lowering activities. Also, ginger consumption leads to the treatment of diabetes, arthritis, rheumatoid arthritis, fatty liver, reducing nausea during pregnancy and chemotherapy due to its antioxidant activity [16, 17]. on the other hand Due to the change in the culture of life, the decrease in physical activity of people, the excessive consumption of sugar in the diet, which lead to the occurrence of problems such as obesity and blood pressure, the spread of cardiovascular diseases, as well as other metabolic disorders; Therefore, it is necessary to pay attention to the production of low-calorie food formulations [18]. *stevia* (*Stevia rebaudiana*) Perennial herbaceous plant of the family *Asteraceae* is that It was grown for the first time in some areas of South America (Paraguay and Brazil). And today it grows in Brazil, Paraguay, Central America, Thailand, Korea and China and grows well in Iran in the suitable climate of Gilan [19]. The dry leaves of stevia plant contain

glycosides that are about 200 to 300 times more than sucrose. Lower glycemic index and fewer calories have. The most well-known diterpene glycosides in stevia leaves are stevioside, ribaodioside (A, B, C, D, E), steviolbioside, dulcoside A [20]. also Stevia plant leaves contain high amounts of phenol, flavonoid, beta-carotene, niacin, dalcoside, rhabdioxide, riboflavin, vitamin C, calcium, chromium, iron, magnesium, potassium, protein, fiber and thiamin. Stevia has received special attention due to its desirable properties such as antibacterial, diuretic, anti-inflammatory, anti-carcinogenic and antioxidant properties, as well as its function as blood sugar and high blood pressure inhibitors [21, 22]. In recent years, many efforts have been made to improve the nutritional value, reduce calories and increase bioactive compounds in all kinds of cereal-based drinks by adding plant extract (safflower seed + *A. aspera* + manyprickle *acanthopanax* + *Kalopanax septemlobus*) in the drink. Sunsik (percentage of barley + brown rice + Audley + black beans + oats) [23], fermented drink based on oats containing inulin extract from sunflower root [24], low-calorie drink based on aloe vera containing stevia sweetener [25], fermented drink gluten-free based on sprouted barley [26], gluten-free fermented drink based on buckwheat and lentils [11], production of gluten-free fermented drink based on quinoa [5] and... so Considering the change in lifestyle and consumers' interest in preparing and consuming functional foods, the aim of this research is to produce a gluten-free, beneficial drink based on judocer containing ginger extract at levels (1.5, 3, 4.5%) and replacing stevia. with sucrose at levels (25, 50, 75%) and checking the physicochemical and sensory characteristics of the production sample during the storage period (21 days).

2- Materials and methods

1-2- Materials

Peeled and debittered oat grains from Mediyaf Company (Tehran, Iran). Stevia powder from Peggaman Shimi Company (Tehran, Iran), sugar (pure 98%) and Ginger plant They were obtained from the local market of Marand city. Also, all the chemicals used were purchased from Merck (Germany) and from the laboratory materials

distribution center of Daya Elixir (Tehran, Iran). *Lactobacillus plantarum* (PTCC 1896) It was purchased from the Scientific and Industrial Research Organization of Iran as an active and live culture and kept in the refrigerator until use.

2-2-Methods

1-2-2-preparation of ginger extract

Ginger plant was bought from the local market of Marand city and in order to prepare ginger extract, its root was cut into pieces, washed and dried in the shade. Then, the dried roots were turned into powder in a kitchen blender (Bosch, Germany) and after sieving with a 60 micron mesh, 100 grams of ginger powder was mixed with one liter of ethanol at 150 rpm for 48 hours at 25 °C was stirred. Then the obtained mixture was smoothed with Whatman number one filter paper. The rotary device was concentrated at a temperature of 35°C and dried in an oven under vacuum at a temperature of 40°C in order to completely remove the organic solvent; Finally, the powder is obtained. Until use, it was kept in the refrigerator in a light-insulated and air-insulated glass container [27].

2-2-2- preparation of judosar milk

In order to prepare judoser seed milk, peeled, debittered and cleaned seeds from Mediyaf Company (Tehran, Iran) They were bought and then washed and soaked for 12 hours at room temperature in water at a ratio of 1 (judocer) to 6 (water). After the mentioned period of time, the material was filtered using a mill homogenizer and the extracted milk through muslin cloth [28].

2-2-3- Microbial culture and determining the amount of starter culture

Lactobacillus plantarum PTCC 1896) It was purchased from the Scientific and Industrial Research Organization of Iran as an active and live culture and kept in the refrigerator until use. In order to determine the amount of starter culture to add bacteria to Joduser's milk, McFarland half standard was used.

3-2-2-preparation of gluten-free beneficial drink

Shahid's beneficial drink formulation was prepared pure and without sweeteners, ginger extract and probiotic bacteria based on studies. Evaluation of the effect of zinnia extract and replacing stevia with sucrose in 9 treatments was done according to Table 1. To prepare the samples, first, the fermented milk prepared from

the previous step was mixed with 1.5% pectin (for the purpose of stability and preventing 2 phases) and pasteurized at 75 degrees for 15 minutes; and after cooling, *Lactobacillus plantarum* probiotic microorganisms (1%) Under sterile conditions, it was added to pasteurized judoser milk and the samples were kept for 16 hours at 37°C for fermentation in a greenhouse; After this time, in order to stop the fermentation process, the product is cooled to the temperature of the refrigerator and then stevia at levels (25, 50, 75% w/w) is replaced with sugar and ginger extract at levels (1.5, 3, 4.5% weight-volume) was added according to table 1. Finally, beneficial fermented beverages at a temperature of 1± They were stored at 4°C and used for analysis at 7-day to 21-day intervals[29].

Table 1. Treatments used to evaluate the effect of stevia and ginger extract

Treatment	Sugars %	Stevia%	Ginger extract%
Control	0	0	0
T1	75	25	1.5
T2	50	50	1.5
T3	25	75	1.5
T4	75	25	3
T5	50	50	3
T6	25	75	3
T7	75	25	4.5
Q8	50	50	4.5
T9	25	75	4.5

4-2-2- Tests

1-4-2-2- Brix measurement

breaks (Solids soluble in water) production samples using a refractometer device (model PAL-1, Made in Japan) was determined at a temperature of 20 degrees Celsius and was expressed based on one gram of sucrose per hundred grams of sample [30].

2-4-2-2- pH measurement

The pH of the produced beverage samples was measured using a digital pH meter (Metrohm, model 827, made in Switzerland) at a temperature of 25 °C after calibrating the pH meter with a buffer solution (pH equal to 7 and 4) [30].

3-4-2-2- Measurement of acidity

In order to check the titratable acidity of the samples, 10 ml of the samples are mixed with 20 ml of distilled water and a few drops of phenolphthalein reagent are added to the resulting solution and using 0.1 normal soda until a pink color appears. (1/8 pH=) titer and the acidity of the samples was calculated using the following relationship [30].

$$\frac{(\text{Liter ml}) \text{Consumable profit volume} \times 0.64}{\text{Sample volume}} = \text{acidity (according to lactic acid)}$$

4-4-2-2- Measurement of total solids (dry extract)

To check the total solids, 10-20 grams of the sample is placed in a vacuum oven at a temperature of 70 degrees and under a pressure of less than 250 mmHg until the sample is dry; Then it was taken out of the oven and weighed after cooling. This process continued until a constant weight was reached, and then the amount of total solids was obtained based on the following relationship [30].

$$\text{Total solid material} = \frac{IN_2 - IN_1}{m} \times 100$$

that W_1 Weight of empty container, W_2 The weight of the container with the dried sample, m is the weight of the original sample.

5-4-2-2- Measurement of antioxidant capacity

Size Determination of antioxidant capacity according to the DPPH method (2,2-Diphenyl-1-picrylhydrazyl) Done. For this purpose, 300 microliters (0.3 ml) of the desired samples were diluted in 96% methanol solvent (1.7 ml) and then diluted with 2 ml of methanolic solution. DPPH (0.1 mM in 96% methanol) mixed and kept in the dark for 30 minutes. Then, the absorbance of the samples was measured by a spectrophotometer at a wavelength of 517 nm [31]. The amount of antioxidant activity of the

samples was calculated according to the following formula:

DPPH radical inhibition percentage

$$= \frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Blank}}} \times 100$$

That A_{Blank} Attracting the witness sample and A_{Sample} Absorption is the main example.

7-4-2-2- Measuring the viability of bacteria

To count bacteria *Lactobacillus plantarum* Porplate method and culture medium (MRS Agar) were used. For this purpose, first 10 ml of the sample was transferred to a sterile filter bag that contained 90 ml of 0.1% peptone water, and homogenized by Stomaker machine for 60 seconds, and with the help of 0.1% peptone water solution, successive dilutions were prepared; Then 1 ml of the desired dilutions prepared from the samples were cultured as a surface culture. The resulting plates were kept in a greenhouse under anaerobic conditions and at a temperature of 37 degrees Celsius for 72 hours, and then the number of living cells was counted with a colony counter device during the storage period (1, 7, 14 and 21 days) and the bacterial growth rate was \log_{10} CFU/ml was reported [32].

8-4-2-2- Sensory evaluation

Sensory characteristics of different samples of gluten-free multipurpose drink containing ginger extract and stevia natural sweetener, including color, aroma, taste, mouthfeel and overall acceptance, were evaluated using a 5-point hedonic test by 20 random evaluators during the storage period. It is worth mentioning that the samples were scored by choosing one of the options 1=unacceptable, 2=relatively satisfactory, 3=average, 4=good, 5=excellent by judges [33].

5-2-2- Statistical analysis

Statistical analysis of data was done using SPSS version 26 software. All tests were performed in three replications in the form of a factorial experiment based on a completely randomized design. Two-Way-ANOVA was used to statistically compare the characteristics of the gluten-free super-beneficial drink based on oats containing extract and stevia during the storage period. Statistical comparisons were made at the 95% confidence level. Also, Duncan's test was used to compare the averages, and all the results are averaged \pm The standard deviation was presented.

3. Results and Discussion

1-3- BRICS analysis

The results of Brix variance analysis of gluten-free ultra-beneficial drinks containing different levels of ginger extract and stevia sweetener during the storage period (21 days) are presented in Table 2. As can be seen, by adding different levels of ginger extract and increasing the percentage of replacing stevia with sucrose, the Brix content of the samples decreased significantly ($P < 0.05$). Also, Brix of gluten-free drink samples decreased during the storage period in all treated samples; As the storage time increased from the moment of production to after fermentation, the Brix level decreased significantly, but on the first day of storage, due to the presence of sucrose in the sample, a significant increase in the Brix of the produced drink was observed compared to the control sample ($P < 0.05$). Given that Gooseberry is a rich source of polysaccharides, sugars, amino acids and other compounds that increase the activity of microorganisms and enzymes; Therefore, during the fermentation process by bacteria, sugars are converted into organic acids and volatile compounds; As a result, Brix decreases as an indicator of water-soluble compounds, and in the first week, due to the presence of stevia and sucrose in the sample and lack of consumption by *Lactobacillus plantarium* microorganisms, it led to an increase in Brix [34]. On the other hand, based on the studies, Brix of the samples increases with the increase of sucrose percentage; Therefore, in this research, by replacing stevia with sucrose (non-regenerative sugar) and reducing the amount of sucrose, the Brix content of the samples decreased [35]; Also, by increasing ginger extract as a prebiotic composition [36-38], the growth of bacteria and, as a result, the consumption of nutrient compounds increased, and as a result, the Brix of the samples decreased; Also, the reduction of Brix by adding Providing suitable conditions for the growth of *Lactobacillus plantarium* bacteria, the pH level decreased and acidity increased, but at higher levels, due to the antimicrobial property of gingerol in ginger, the growth and activity of the bacteria was prevented, and as a result, the pH level increased at the level of 4.5% [17]. On the other hand, the increase in pH and decrease in acidity during the replacement of sugar with stevia can be attributed to the decrease in Maillard browning reactions due to the decrease in the amount of sucrose and the increase in non-regenerative sugars, as well as the inability of stevia sugars (stiosides) to participate in fermentation reactions [40]. Similar results in this regard by Masha et al. (2020), in non-alcoholic fermented beverage mahewu with the addition of aloe

ginger extract can be due to the low amount of total solids in ginger extract [5]. Similar results by Fasreen And colleagues (2017), in millet-based fermented drink [39] • Sheikholeslami et al. (2022), by adding stevia in apple-lemon mixed juice [40] and Ebrahimi Jamet al. (2019), by adding mint extract Gluten-free fermented drink based on quinoa [5] It has been reported.

2-3- pH and acidity analysis

The results of analysis of variance (Tables 3 and 4) of pH and acidity of gluten-free healthy drinks containing different levels of ginger and stevia extracts during the storage period (21 days) show that, Level pH and acidity The samples decreased and increased significantly with the passage of storage time in all the treated samples ($P < 0.05$); Also, by increasing the percentage of replacing stevia with sucrose pH Samples slightly increased and acidity decreased ($P < 0.05$); On the other hand, based on the results presented by increasing the percentage of ginger extract up to 3% Compared to the control sample, pH decreased and acidity increased, but with increasing the percentage of extract to 4.5%, an increase in pH was observed and the amount of acidity decreased slightly. Considering that *Lactobacillus plantarium* probiotic bacteria was used in the prepared treatments; Therefore, in the production samples, the pH and acidity of the treatments decreased and increased, respectively, compared to the pH (5.45) and acidity (0.28) of the control sample. The reason for the decrease in pH and increase in acidity during the storage period can be attributed to the production of lactic acid by the consumed probiotic bacteria and the death of some bacteria [41]. Also, by increasing the percentage of ginger to 3% due to the synergistic effect of ginger on the growth of *Lactobacillus plantarium* bacteria and the inhibitory effect on the growth of unwanted microorganisms and

vera powder [42], Alizadeh et al. (2021), by adding stevia and inulin in mango nectar containing *Lactobacillus plantarium* bacteria [43] and Hajj Ghafarloo et al. (2020), synbiotic buttermilk containing ginger extract [17] is mentioned.

3-3-Analysis of total solids

The results of the analysis of variance of the total solids of the manufactured drinks during the storage period (21 days) are presented in Table 5. As can be seen, during the storage period, the amount of total solids (dry matter) decreased significantly ($P < 0.05$). Also, by increasing the levels of replacing stevia with sucrose and adding different levels of ginger extract, the amount of total solids decreased significantly at all times ($P < 0.05$). According to the results, the reason for the decrease in dry matter during the

storage period and with the addition of ginger extract can be Growth and activity of bacteria and consumption of nutrient compounds attributed by the probiotic bacteria used in the formulation [5]. And considering that by replacing stevia with sucrose, the amount of sucrose consumed in the formulation decreased, and on the other hand, due to the greater sweetness of stevia and as a result, the amount of total solids decreased [44]. Similar results by Ahmad and colleagues (2019), by adding stevia in peach drink [45], Hatami et al. (2021), by adding rice bran extract and honey in probiotic drink [46].

4-3- Antioxidant capacity analysis

The results of variance analysis of antioxidant capacity of gluten-free probiotic drinks produced during the storage period (21 days) are shown in Table 6. According to the presented results, by adding different levels of ginger extract and increasing the percentage of replacing stevia with sucrose, the antioxidant capacity of the samples increased significantly at all times ($P < 0.05$). Also, the antioxidant capacity of gluten-free drink samples decreased during the storage period in all treated samples ($P < 0.05$). The extract obtained from ginger contains high phenolic and antioxidant compounds, which are the most important antioxidant compounds of ginger 6-Gingerol and its derivatives [47] and

on the other hand, the high phenolic antioxidant compounds in the stevia sweetener used in the formulation led to an increase in the antioxidant capacity of the samples [48]; So, in samples containing high levels of both compounds, the antioxidant capacity was higher than other samples. Also, the reason for the decrease in antioxidant capacity during the storage period can be due to the oxidative changes caused by dissolved oxygen in the drink, which accelerates the oxidation of phenolic compounds by polyphenoloxidase enzyme and the reaction of other antioxidant compounds with free radicals, as well as increasing the absorption and interaction of compounds. Phenolic with proteins in the structure of the produced drink leads to a decrease in antioxidant capacity [49]. The results are consistent with the results Chen et al. (2018), in investigating the antioxidant capacity of fermented papaya juice during storage [50], Teneva et al. (2022), adding extracts of medicinal plants (lady's mantle, lavender, rosehip, meadowsweet) in Aronia nectar [51] and Apriliyanti et al. (2021), the drink contained Melinjo bark, mint leaves and stevia leaves [52].

Table 2. The effect of different levels of ginger extract and stevia on brix content of gluten-free functional beverages during the storage period

Treatment/Day	1 th day	7 th day	14 th day	21 th day
Control	001 ^{Aye} 0±11/8	0/001 ^{Aye} ±8/11	0/002 ^{Aye} ±8/10	0/001 ^{Aye} ±8/10
1.5%Ginger extract/ 25%Stevia	^{Aa} 0/003± 25/12	Not ⁰ 0/003± 12/22	0/001 ^{That} ± 12/18	0/001 ^{And} ± 12/01
1.5%Ginger extract/ 50%Stevia	0/001 ^{And} ± 12/20	^{Bc} 0/002± 12/16	^{Cc} 0/002± 12/10	^{Dc} 0/003±11/94
1.5%Ginger extract/ 75%Stevia	^{Ad} 0/002± 12/17	^{Be} 0/001± 12/11	^{Cf} 0/003±12/02	0/002 ^{Df} ± 11/86
3%Ginger extract/ 25%Stevia	^{Ab} 0/001± 12/21	^{Bb} 0/002± 12/19	^{Cb} 0/03± 12/13	0/001 ^{Db} ± 11/96
3%Ginger extract/ 50%Stevia	^{Of} 0/001± 12/15	^{Bd} 0/001± 12/12	^{Cd} 0/002± 12/07	0/001 ^{Of} ± 11/87
3%Ginger extract/ 75%Stevia	^{Ah} 0/003± 12/08	^{Bh} 0/002± 12/00	^{Cg} 0/004± 11/92	0/002 ^{Dg} ± 11/83
4.5%Ginger extract/ 25%Stevia	^{But} 0/003±12/16	^{Bf} 0/003± 12/10	0/002 ^{This} ± 12/04	0/003 ^{Dd} ± 11/89
4.5%Ginger extract/ 50%Stevia	0/002 ^{At} ± 12/09	^{Bg} 0/001± 12/03	^{Ch} 0/001± 11/90	0/004 ^{Dh} ± 11/81
4.5%Ginger extract/ 75%Stevia	^{Ai} 0/001± 11/95	^{With a} 0/004± 11/82	^{There} 0/003±0 11/76	0/001 ^{From} ± 11/74

Different capital letters in each row indicate significant differences between different days.

Different small letters in each column indicate significant differences for different treatments.

Table 3. The effect of different levels of ginger extract and stevia on pH content of gluten-free functional beverages during the storage period

Treatment/Day	1 th day	7 th day	14 th day	21 th day
Control	01 ^{Aa} 0±45/5	0/01 ^{Aa} ±5/44	0/01 ^{Aa} ±5/44	0/02 ^{Aa} ±5/43
1.5%Ginger extract/ 25%Stevia	^{At} 0/03± 65/3	^{Bj} 0/02± 3/43	0/02 ^{Cj} ± 3/35	0/01 ^{Dj} ± 3/24
1.5%Ginger extract/ 50%Stevia	0/02 ^{Of} ± 3/68	^{Bh} 0/01± 3/50	^{Ch} 0/02± 3/39	^{Dh} 0/03±3/30
1.5%Ginger extract/ 75%Stevia	^{But} 0/01± 3/71	^{Bg} 0/01± 3/54	^{Cg} 0/01±3/42	0/02 ^{Df} ± 3/36
3%Ginger extract/ 25%Stevia	^{Aye} 0/02± 3/56	^{With a} 0/03± 3/48	^{There} 0/01± 3/37	0/01 ^{From} ± 3/28
3%Ginger extract/ 50%Stevia	^{Ai} 0/01± 3/60	^{Bf} 0/02± 3/57	^{Cf} 0/02± 3/45	0/03 ^{Dg} ± 3/34
3%Ginger extract/ 75%Stevia	^{Ah} 0/04± 3/63	^{Be} 0/01± 3/69	^{This} 0/01± 3/58	0/02 ^{Of} ± 3/41
4.5%Ginger extract/ 25%Stevia	^{Ad} 0/01±3/78	^{Bd} 0/03± 3/70	0/03 ^{Cd} ± 3/61	0/01 ^{Dd} ± 3/54
4.5%Ginger extract/ 50%Stevia	0/02 ^{And} ± 3/84	^{Bc} 0/01± 3/75	^{Cc} 0/01± 3/67	0/02 ^{Dc} ± 3/59

4.5%Ginger extract/ 75%Stevia	^{Ab} 0/02± 3/89	^{Bb} 0/01± 3/81	^{Cb} 0/02±0 3/74	0/01 ^{Db} ± 3/64
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Different capital letters in each row indicate significant differences between different days.
Different small letters in each column indicate significant differences for different treatments.

Table 4. The effect of different levels of ginger extract and stevia on acidity content of gluten-free functional beverages during the storage period

Treatment/Day	1 th day	7 th day	14 th day	21 th day
Control	01 ^{Aye} /0±29/0	0/01 ^{Aye} ±0/29	0/01 ^{Aye} ±0/29	0/02 ^{Aye} ±0/28
1.5%Ginger extract/ 25%Stevia	^{Ab} 0/02± 51/0	^{Bc} 0/03± 0/57	0/01 ^{Cc} ± 0/62	0/02 ^{Dc} ± 0/68
1.5%Ginger extract/ 50%Stevia	0/01 ^{But} ± 0/44	^{Be} 0/02± 0/50	^{This} 0/02± 0/56	^{Df} 0/03±0/61
1.5%Ginger extract/ 75%Stevia	^{At} 0/01± 0/39	^{Bg} 0/03± 0/43	^{Ch} 0/02±0/49	0/02 ^{From} ± 0/54
3%Ginger extract/ 25%Stevia	^{Aa} 0/02 ± 0/55	^{Not} 0/03± 0/60	^{Cb} 0/01± 0/66	0/01 ^{Db} ± 0/71
3%Ginger extract/ 50%Stevia	^{And} 0/01 ± 0/49	^{Bd} 0/02± 0/53	^{Cd} 0/01± 0/60	0/03 ^{Of} ± 0/64
3%Ginger extract/ 75%Stevia	^{Of} 0/04± 0/42	^{Bi} 0/01± 0/48	^{Cg} 0/01±0/52	0/01 ^{Dh} ± 0/58
4.5%Ginger extract/ 25%Stevia	^{Ad} 0/01±0/47	^{Bb} 0/02± 0/58	0/03 ^{That} ± 0/69	0/03 ^{And} ± 0/72
4.5%Ginger extract/ 50%Stevia	0/03 ^{Ah} ± 0/38	^{Bh} 0/01± 0/41	^{Cf} 0/01± 0/54	0/02 ^{Dd} ± 0/67
4.5%Ginger extract/ 75%Stevia	^{At} 0/02±0/32	^{With a} 0/02± 0/37	^{There} 0/01±0 0/45	0/02 ^{Dg} ± 0/59

Different capital letters in each row indicate significant differences between different days.
Different small letters in each column indicate significant differences for different treatments.

Table 5. The effect of different levels of ginger extract and stevia on total solids content of gluten-free functional beverages during the storage period

Treatment/Day	1 th day	7 th day	14 th day	21 th day
Control	001 ^{Aye} /0±82/7	0/002 ^{Aye} ±7/82	0/002 ^{Aye} ±7/82	0/001 ^{Aye} ±7/81
1.5%Ginger extract/ 25%Stevia	^{Aa} 0/02± 15/11	^{Not} 0/02± 10/66	0/01 ^{That} ± 10/25	0/02 ^{And} ± 10/12
1.5%Ginger extract/ 50%Stevia	0/03 ^{And} ± 10/78	^{Bc} 0/03± 10/34	^{Cb} 0/02± 10/18	^{Dc} 0/01±9/81
1.5%Ginger extract/ 75%Stevia	^{Of} 0/02± 10/51	^{Be} 0/02± 10/22	^{Cd} 0/03±10/09	0/02 ^{Dd} ± 9/62
3%Ginger extract/ 25%Stevia	^{Ab} 0/01 ± 11/01	^{Bb} 0/01± 10/43	^{Cc} 0/02± 10/11	0/03 ^{Db} ± 9/84
3%Ginger extract/ 50%Stevia	^{But} 0/01 ± 10/59	^{Bi} 0/01± 10/21	^{Cf} 0/04± 9/74	0/01 ^{Df} ± 9/57
3%Ginger extract/ 75%Stevia	^{At} 0/04± 10/37	^{Bh} 0/02± 9/91	^{Ch} 0/03± 9/53	0/04 ^{Dh} ± 9/28
4.5%Ginger extract/ 25%Stevia	^{Ad} 0/03±10/73	^{Bd} 0/02± 10/30	0/02 ^{This} ± 9/86	0/02 ^{Of} ± 9/61
4.5%Ginger extract/ 50%Stevia	0/01 ^{Ah} ± 10/34	^{Bg} 0/03± 10/08	^{Cg} 0/01± 9/59	0/01 ^{Dg} ± 9/33
4.5%Ginger extract/ 75%Stevia	^{At} 0/02± 10/19	^{With a} 0/04± 9/66	^{There} 0/01±0 9/41	0/03 ^{From} ± 9/18

Different capital letters in each row indicate significant differences between different days.
Different small letters in each column indicate significant differences for different treatments.

Table 6. The effect of different levels of ginger extract and stevia on DPPH content of gluten-free functional beverages during the storage period

Treatment/ Day	1 th day	7 th day	14 th day	21 th day
Control	01 ^{Aye} /0±13/21	0/02 ^{Bj} ±19/54	0/04 ^{Cj} ±16/35	0/03 ^{Dj} ±12/57
1.5%Ginger extract/ 25%Stevia	^{At} 0/09± 92/41	^{With a} 0/08± 36/54	0/09 ^{There} ± 30/43	^{From} 9/0± 25/43
1.5%Ginger extract/ 50%Stevia	0/08 ^{Ah} ± 46/20	^{Bh} 0/01± 41/01	^{Ch} 0/08± 36/14	^{Dh} 0/07±31/35
1.5%Ginger extract/ 75%Stevia	^{Of} 0/06± 52/49	^{Bi} 0/08± 47/44	^{Cf} 0/09±42/35	0/04 ^{Df} ± 37/20
3%Ginger extract/ 25%Stevia	^{At} 0/17 ± 48/72	^{Bg} 0/01± 45/45	^{Cg} 0/17± 40/30	0/09 ^{Dg} ± 34/43
3%Ginger extract/ 50%Stevia	^{But} 0/09 ± 56/67	^{Be} 0/01± 53/45	^{This} 0/9± 46/30	0/07 ^{Of} ± 39/43
3%Ginger extract/ 75%Stevia	^{Ad} 0/14± 64/20	^{Bd} 0/10± 59/38	^{Cc} 0/05± 54/24	0/06 ^{Dc} ± 47/43
4.5%Ginger extract/ 25%Stevia	^{And} 0/09±66/30	^{Bc} 0/08± 62/54	0/09 ^{Cd} ± 51/43	0/08 ^{Dd} ± 44/43
4.5%Ginger extract/ 50%Stevia	0/06 ^{Ab} ± 79/23	^{Bb} 0/01± 70/51	^{Cb} 0/08± 63/41	0/05 ^{Db} ± 54/63
4.5%Ginger extract/ 75%Stevia	^{Aa} 0/09± 86/10	^{Not} 0/01± 79/44	^{That} 0/2±0 72/35	0/9 ^{And} ± 60/51

Different capital letters in each row indicate significant differences between different days.
Different small letters in each column indicate significant differences for different treatments.

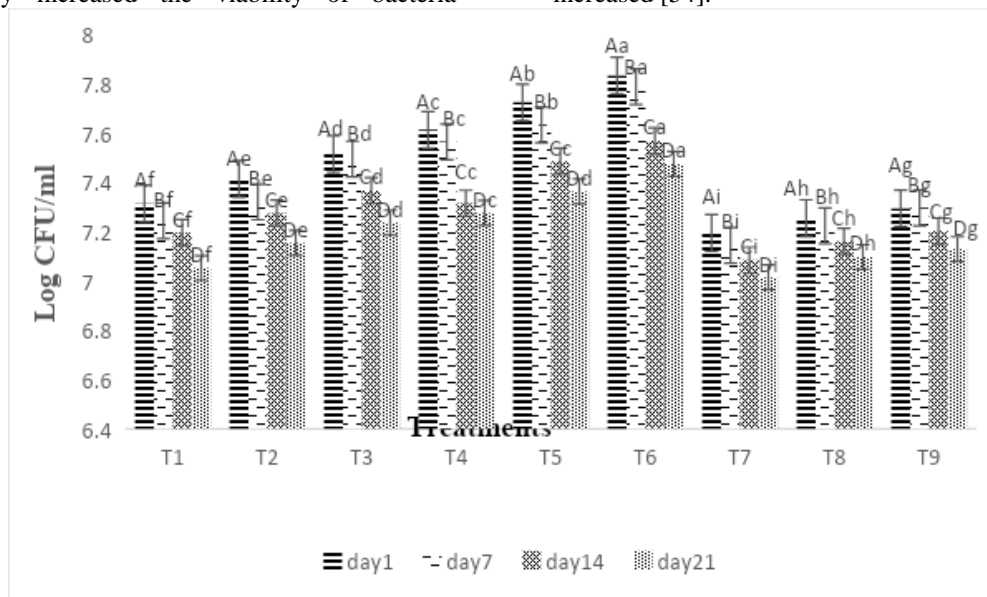
3-5-Analysis of the viability of bacteria

The results of the analysis of variance obtained from the study of the viability of the probiotic *Lactobacillus plantarium* in gluten-free ultra-beneficial drinks containing different levels of ginger and stevia extracts during the storage period (21 days) are shown in Figure 1. As can be seen, during the storage period, the survival rate of bacteria

decreased significantly ($P<0.05$); which can be due to the decrease in pH of the environment and the increase in acid and hydrogen peroxide production due to fermentation and activity of bacteria, and on the other hand, despite the decrease in bacteria, the amount of live bacteria during the storage period is within the standard range (10^8 - 10^7 Logarithmic unit per milliliter) was preserved. Also, by increasing the percentage levels of ginger extract up to 3%, a

positive and synergistic effect on the growth of *Lactobacillus plantarium* bacteria was shown, which is due to the prebiotic properties of ginger extract; But at the level of 4.5% due to its antimicrobial properties, a decreasing effect on the amount of bacteria was observed [17]. On the other hand, the replacement of high levels of stevia in the formulation of gluten-free multipurpose drink significantly increased the viability of bacteria

($P < 0.05$); which is due to the high antioxidant capacity and phenolic compounds in these compounds[43]. Similar results by Ozdemir et al. (2020), by adding stevia and sugar beet bioactive compounds in fermented milk [53]. Also Shariati et al. (2020), stated that by adding coriander extract and watercress gum in buttermilk fermented with *Lactobacillus plantarium*, the survival rate of bacteria increased [54].



Different capital letters indicate significant differences between different days.

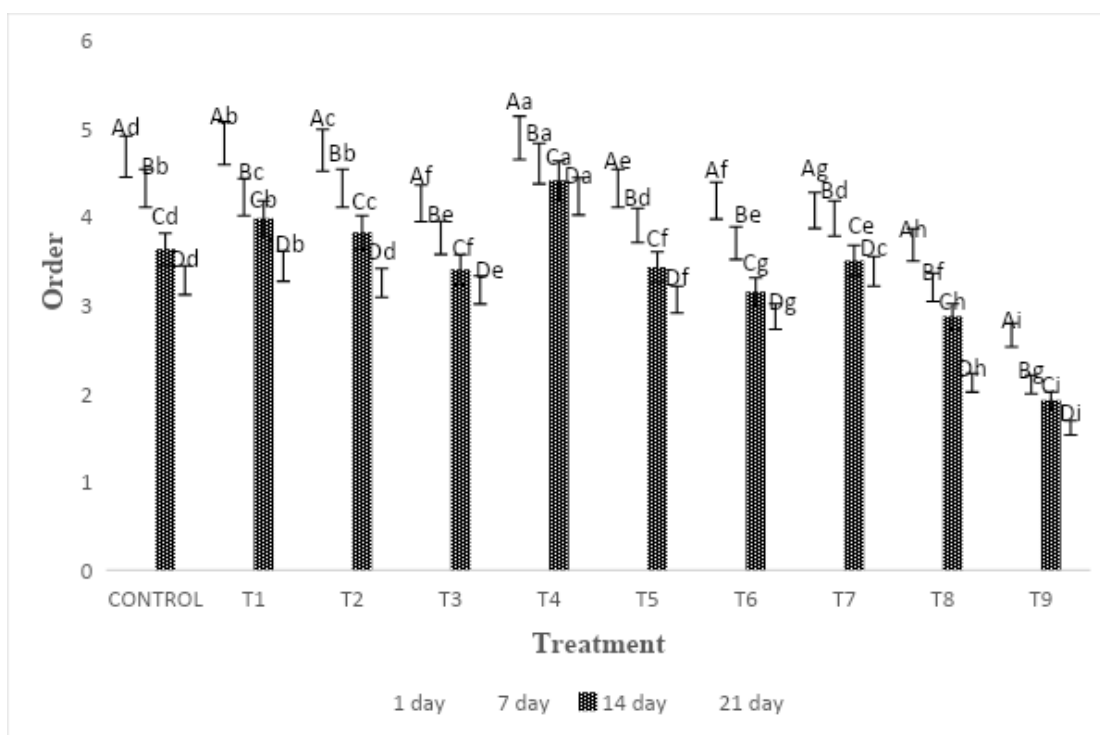
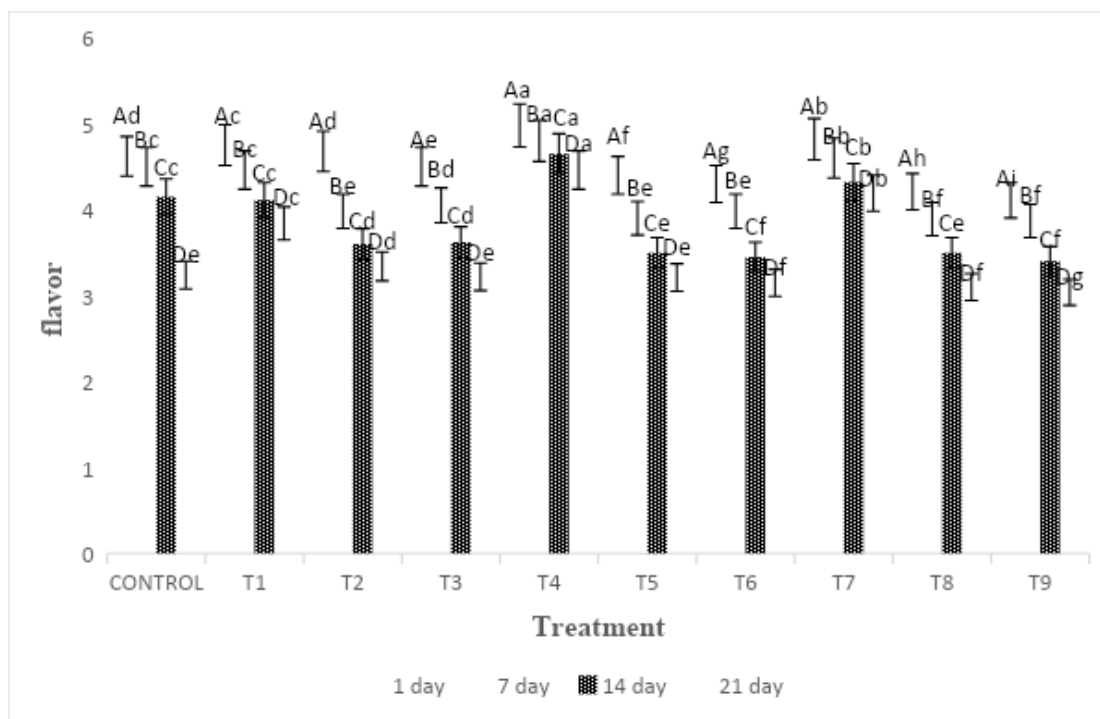
Different small letters indicate significant differences for different treatments.

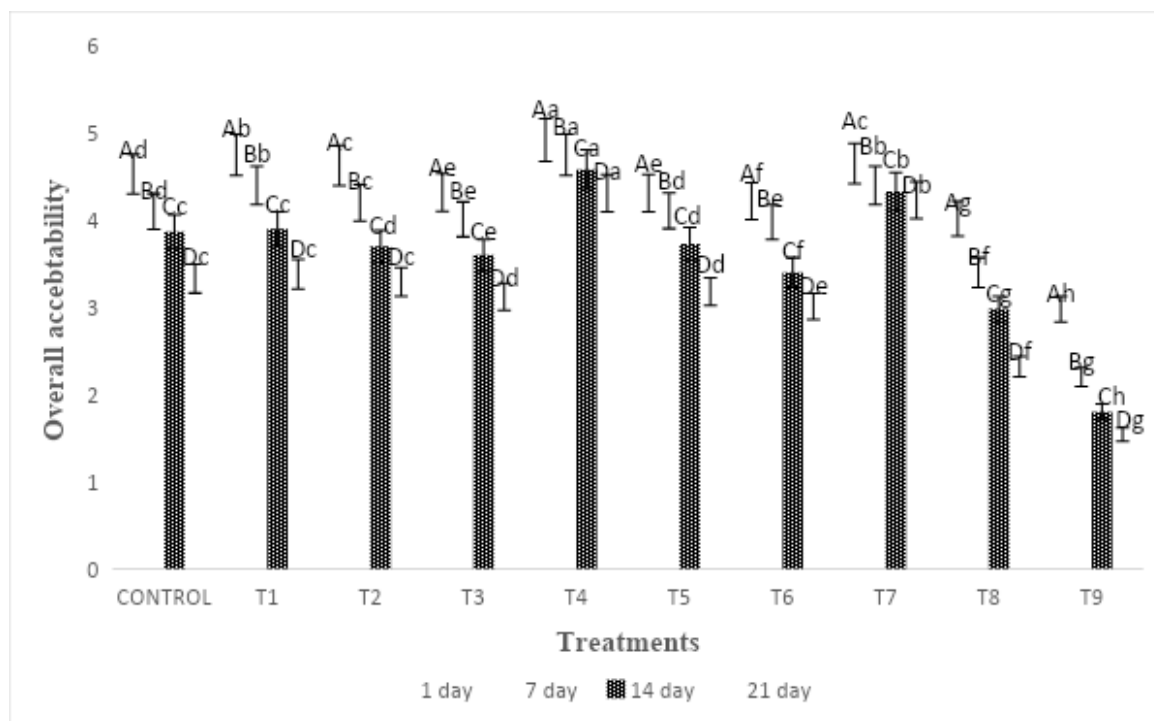
Figure 1. The effect of different levels of ginger extract and stevia on the survival rate of bacteria in gluten-free functional beverages during the storage period

6-3-sensory analysis

The results of changes and analysis of variance of sensory evaluation including taste, smell and overall acceptance of gluten-free super-beneficial drinks produced during the storage period are presented in Figure 2; Based on the results obtained By increasing the percentage of replacing stevia with sucrose, the sensory evaluation of samples decreased significantly in all investigated factors ($P < 0.05$); But by increasing the levels of ginger extract up to 3%, the sensory evaluation of the samples increased significantly and then decreased. So that the lowest score was given to the samples containing high levels of ginger and

stevia extracts due to the bitter taste created. On the other hand, the samples containing 3% ginger extract and replacing 25% stevia with sucrose received the highest score in all the examined sections (taste, smell and general acceptance). Similar results regarding sensory evaluation by Rock et al. (2015), by adding stevia in passion fruit juice [55] and Aiello et al. (2020), by adding ginger and cinnamon extracts in kefir [56]. As the storage time increased, the sensory characteristics of the drinks decreased significantly, but nevertheless, on the 21st day, the sample containing 3% extract Ginger and replacing 25% of stevia with sucrose received the highest score.





Different capital letters indicate significant differences between different days.
 Different small letters indicate significant differences for different treatments.

Figure 2. The effect of different levels of ginger extract and stevia on sensory evaluation of gluten-free functional beverages during the storage period

4- General conclusion

In recent years, due to improper diet, the risk of chronic diseases has increased; to The need to develop safe foods enriched with nutrients and bioactive compounds in order to prevent and control diseases has received much attention from researchers and industrialists.; Therefore, in this research, a beneficial gluten-free drink based on judo juice containing ginger extract and stevia sweetener was prepared and its properties were investigated. The results showed that during the storage period in all the investigated treatments, the amount of Brix, total solid matter, pH and antioxidant capacity decreased significantly and the acidity of the samples increased ($P < 0.05$). The addition of ginger extract led to a decrease in Brix, total solids and pH of the produced drink samples and an increase in the antioxidant capacity and acidity of the samples; Also,

replacing stevia with sucrose led to a decrease in brix, total solid matter, acidity, but the pH and antioxidant capacity increased significantly. The results of investigating the viability of *Lactobacillus plantarium* probiotic bacteria It showed that despite the reduction of bacteria, the amount of live bacteria was maintained within the standard range. Based on the results of sensory evaluation, a sample containing 3% ginger extract and 25% stevia received a higher score from the evaluators. Finally, based on all The obtained results can be concluded that the sample containing 3% ginger extract and 25% stevia sweetener is a favorable sample in terms of physicochemical properties, sensory and bacterial viability and can be produced as a useful multipurpose drink with high nutritional properties.

5- Resources

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مقاله علمی-پژوهشی

تولید نوشیدنی فراسودمند فاقد گلو تن بر پایه جودوسر حاوی عصاره زنجبیل و کاربرد استویا به عنوان جایگزین شکر

انیس طالبی^{۱*}، لیلا رفیعی^۲

۱- دانشجوی دکتری علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه ارومیه، ارومیه، ایران

۲- دانشجوی دکتری علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه ارومیه، ارومیه، ایران

چکیده

اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۲/۳/۱۰

تاریخ پذیرش: ۱۴۰۲/۷/۸

کلمات کلیدی:

نوشیدنی فاقد گلو تن،

پروبیوتیک،

عصاره زنجبیل،

استویا.

DOI: 10.22034/FSCT.20.143. 46

DOR: 20.1001.1.20088787.1402.20.143.4.6

* مسئول مکاتبات:

anis.ta1994@gmail.com

an.talebi@urmia.ac.ir

امروزه با تغییر فرهنگ زندگی و استفاده از رژیم غذایی نامناسب نیاز به توسعه غذاهای ایمن، غیر آلرژی زا و غنی شده با مواد مغذی و ترکیبات زیست فعال به منظور بهینه سازی سیستم ایمنی و پیشگیری و کاهش بیماری ها افزایش یافته است؛ لذا هدف از این پژوهش تهیه نوشیدنی فراسودمند فاقد گلو تن بر پایه جودوسر حاوی عصاره زنجبیل و شیرین کننده استویا بود. بدین منظور اثر عصاره زنجبیل در سطوح ۱/۵، ۳ و ۴/۵٪ و جایگزینی استویا با ساکارز در سطوح ۲۵، ۵۰ و ۷۵٪ در قالب آزمایش فاکتوریل بر پایه طرح کاملاً تصادفی در فرمولاسیون نوشیدنی فراسودمند فاقد گلو تن بر پایه جودوسر حاوی باکتری پروبیوتیک لاکتوباسیلوس پلانترایوم به منظور بررسی ویژگی های فیزیکی و شیمیایی، حسی و میکروبی در طی مدت زمان نگهداری (۲۱ روز) مورد ارزیابی قرار گرفت. نتایج نشان داد که در طی مدت زمان نگهداری در تمامی تیمارهای مورد بررسی میزان بریکس، ماده جامد کل، pH و ظرفیت آنتی اکسیدانی به طور معناداری کاهش و میزان اسیدیته نمونه ها افزایش یافت ($P < 0.05$) و از طرفی با افزودن عصاره زنجبیل میزان بریکس، ماده جامد کل و pH نمونه های نوشیدنی تولیدی کاهش و ظرفیت آنتی اکسیدانی و اسیدیته نمونه ها افزایش یافت ($P < 0.05$)؛ همچنین جایگزینی استویا با ساکارز منجر به کاهش بریکس، ماده جامد کل، اسیدیته گردید ولی میزان pH و ظرفیت آنتی اکسیدانی به طور معناداری افزایش یافتند. نتایج بررسی زنده مانی باکتری های پروبیوتیک لاکتوباسیلوس پلانترایوم نشان داد که در تمامی نمونه ها، رشد باکتری ها با گذشت زمان روند کاهشی داشته ولی با وجود کاهش باکتری ها میزان باکتری های زنده در محدوده استاندارد حفظ گردید و افزودن عصاره زنجبیل و شیرین کننده استویا اثر مثبتی بر زنده مانی باکتری ها داشت. براساس نتایج ارزیابی حسی نیز نمونه ی حاوی ۳٪ عصاره زنجبیل و ۲۵٪ استویا از نظر ارزیابان امتیاز بالاتری کسب کرد. در نهایت براساس تمامی نتایج حاصله می توان بیان کرد که افزودن عصاره زنجبیل و شیرین کننده استویا در نوشیدنی فراسودمند فاقد گلو تن منجر به بهبود خصوصیات نوشیدنی تولیدی گردید و می توان به عنوان یک نوشیدنی فراسودمند جدید با خواص تغذیه ای مطلوب معرفی کرد.