JFBE 6(2): 81-91, 2023

Journal of Food and Bioprocess Engineering

Journal homepage: https://jfabe.ut.ac.ir

Original research

Production of Hibiscus Tea (*Hibiscus sabdariffa*) Probiotic Dietary Drink based on Stevia and Inulin

Mohammadreza Abdolmaleki [a], Lida Shahsavani [a,*], Toktam Mostaghim [a]

[a] *Department of Food Industry, Faculty of Agriculture, Islamic Azad University, Shahr-e-Qods Branch, Tehran, Iran*

ABSTRACT

In recent years, the consumption of healthy drinks has increased due to the increase in consumer awareness. In this research, the production of hibiscus tea probiotic dietary drinks based on stevia and inulin sweeteners was investigated. In order to prepare probiotic drinks, *Lactiplantibacillus plantarum* bacteria, hibiscus tea at levels of 2.5, 5 and 7%, inulin at levels of 2, 4, 6% and stevia at levels (0.1, 0.2 and 0.3 percent) were used and pH, acidity, brix, formalin index, turbidity, total phenol, antioxidant activity (DPPH, FRAP, ABTS), sensory characteristics of the samples were investigated on day 1 and total microbial count and survival of probiotics on days 1, 3 and 15. According to the obtained results, at the highest levels of hibiscus tea and the lowest levels of inulin, the pH of the samples decreased significantly and the acidity increased (p<0.05). At the highest levels of hibiscus tea and inulin, Brix, Turbidity and formalin index of samples increased significantly (p<0.05). In the highest levels of hibiscus tea, inulin and stevia, total phenol and antioxidant activity and population of probiotics in the samples increased significantly (p<0.05). Sample H5, I4, S0.2 and sample H5, I6, S0.2 obtained the highest sensory score compared to other samples (p<0.05). Due to the higher sensory scores, antioxidant activity and proper survival of probiotics, sample H5, I4, S0.2 was selected as the best sample.

Keywords: Hibiscus tea; Inulin, Stevia; Probiotic

Received 25 Nov 2023; Revised 28 Dec 2023; Accepted 30 Dec 2023

1. Introduction

The World Health Organization's emphasis on the health benefits of functional foods and beverages has helped to increase their popularity globally. In addition, consumers have become more aware of the importance of their food composition and nutrition. In recent years, the growth of the market of functional foods has been significant (Gupta *et al*., 2023).

Probiotic food products are becoming a part of considerations generally due to their prospective health benefits and quickly developing worldwide markets. The reported health benefits of expending probiotics include possible roles for the administration and prevention of diarrhoea, inflammatory bowel disease, lactose intolerance, allergies, cancer, respiratory tract infections, constipation, urinary tract infections, *helicobacter pylori* infection and high blood cholesterol (Nagpal *et al*., 2012). Lactic acid bacteria (LAB) constitute a differing gather of Gram-positive, catalase-negative bacteria creating lactic acid as the most end-product of carbohydrate fermentation (Mokoena *et al*., 2021). With more than 220 substantial species, Lactobacillus genus is certainly the most and different LAB group (Bernardeau *et al*., 2008). *Lactobacillus plantarum* is a Gram-positive, nonmotile, non-sporeforming bacterium. Although *Lactobacillus* spp. are usually recognized as catalase-negative, true catalase and manganese-containing pseudocatalase activities have been found, under special conditions, in a few strains of *Lb. plantarum.* Some strains also exhibit nitrate- and haematin-dependent nitrite reductases (Corsetti and Gobbetti, 2002). Teas are widely consumed, and hibiscus species have been noted for their health-giving properties and chemical composition. There are more than 300 varieties of hibiscus. Among them, *Hibiscus sabdariffa*.L is widely consumed worldwide, especially in subtropical and tropical countries such as India, Sudan, and Egypt (Maciel *et al.,* 2018). Different parts of the plant are used

*Corresponding author.
E-mail address: shahsavani.l@gmail.com (L. Shahsavani).
https://doi.org/10.22059/JFABE.2023.368666.1159

to make jams, jellies, soft drinks, while their calyxes are mainly used to make traditional tea by steeping in cold or hot water for 5 minutes and then filtering (Monteiro *et al.,* 2017). The extract of *H. sabdariffa* calyces is rich in organic acids (such as hibiscus acid, gallic acid, malic acid, and citric acid), anthocyanins (such as cyanidin-3-sambubioside, delphinidin-3-sambubioside, and cyanidin-3-glucoside) (Preciado- Saldaña *et al.,* 2019; Izquierdo-Vega *et al*., 2020). There are various compounds and alkaloids in the petals of this plant. For this reason, it is used in traditional medicine for the prevention and treatment of kidney and bladder stones, as well as antibacterial, antifungal, hypocholesterolemic, antispasmodic, blood pressure reducing agents. The use of hibiscus tea has a special place in the traditional medicine of countries. The infusion of its leaves or flowers is considered as a diuretic, cholesterol reducer, disinfectant, blood pressure reducer, blood viscosity reducer (Islam, 2019).

On the other hand, in recent decades, sugar substitutes have become popular in order to reduce energy intake, control body weight, diseases such as diabetes and blood sugar reduction. Many synthetic sweeteners have side effects for their consumers, including phenol sensitivity, carcinogenicity of some of them. For this reason, attention has been paid to natural sweeteners, including stevia which have many desirable qualities (Aghabeyk *et al.,* 2017). Research has shown that excessive consumption of sugar-sweetened beverages may lead to adverse effects on human health (Malik *et al*., 2022). Therefore, replacing sugar with artificial or natural sweeteners in the formulation of food products is a challenge for the food industry (Ozuna, & Franco-Robles, 2022). Stevia (*Stevia rebaudiana*) leaves are a good source of diterpenoid glycosides, which are the main responsible for the sweet taste of stevia, and their sweetening power is about 300 times that of sucrose, and at the same time, it has few calories. Stevia leaf extract, besides its sweetening properties, has many therapeutic properties, such as reducing blood sugar and blood pressure, anti-inflammatory, anti-tumor, and regulating the immune system.

Various toxicological studies have been conducted on stevia sweeteners, and most of these studies have confirmed that its extract is not mutagenic or carcinogenic. The sweetening compounds of the extract of this plant are stable at pH = 3-9 and temperature of 100 °C, they do not ferment and their color does not change due to cooking (Safari *et al*., 2013). Several researches have been conducted in the field of using stevia in various beverages. Saraswati and Taniwiryono (2020) used 2% (weight-volume) hibiscus tea and 0.1, 0.3 and 0.5% (w/v) stevia while preparing a hibiscus tea drink containing stevia for diabetic patients. The results showed that the sample produced with 2% hibiscus tea and 0.3% stevia leaves had a bright red color, no bitter taste, no aftertaste, and the total number of microbes was within the standard range. The carbohydrate content of hibiscus tea of the mentioned sample and hibiscus tea produced with sugar was 0.15% and 1.33%. Inulin is a prebiotic substance that improves the technological properties and increases the nutritional value of food, and also has synergistic properties with other sweeteners and has a sweetness equivalent to 30% of sucrose (Jafari, 2012). Inulin is colorless and has a mild and neutral taste and aroma, without any bad taste and is easily mixed with other substances without changing their taste (Kalyani Nair *et al*., 2010). Reducing sugar from beverages leads to a reduction in viscosity and thus a reduction in mouthfeel and texture. Inulin can interact with other soluble or dispersed molecular species in a hydrated state and provide various technological advantages such as texturizing and thickening, emulsification and stabilization. Therefore, their use can improve the mouthfeel of low-calorie drinks and mask bad flavors in them (Kasapoğlu *et al.,* 2019; Furlán *et al.*, 2017). Fakhri *et al.* (2023) during the optimization of a beneficial low-calorie drink based on lime juice from sucralose as a low-calorie sweetener and inulin/polydextrose mixture, stated that the sample containing a mixture of prebiotics, the ratio of 2:3 was selected as the best formulation. Ghavidel *et al.* (2014) in investigating the effect of adding FOS (Fructooligosaccharides) in apple and orange juice admitted that there was no significant difference with the control sample during the sensory evaluation. The purpose of this research was to produce hibiscus tea probiotic dietary drinks based on stevia and inulin.

2. Material and Methods

2.1. Materials

Hibiscus Tea was prepared from the local market and *Lactiplantibacillus plantarum* (Christian Hansen), stevia (Sweete, Germany) and inulin with degree of polymerization 9-12 (Food chem, china) were purchased. DPPH, ABTS, TPTZ, Folin–Ciocalteu were prepared from Sigma-Aldrich (USA) and other chemicals used for chemical analysis were prepared from Merck (Germany).

2.2. Methods

2.2.1. Preparation of hibiscus tea drink

2.2.2. Revival of tested microorganism and production of hibiscus tea probiotic drink containing different levels of Inulin and Stevia

First, the tested microorganism (*Lactiplantibacillus plantarum*) was revived on De Man, Rogosa and Sharpe agar (MRS) liquid medium and then the linear culture of the activated microorganisms was done on MRS Agar medium. Different levels of hibiscus tea, inulin, stevia were used to prepare probiotic drink samples. In this way, the aqueous extract of hibiscus tea was prepared by placing it in hot water with a temperature of 80 °C for 10 minutes. Then different percentages of inulin and stevia were added according to Table 1. After that, *Lactiplantibacillus plantarum* 10^7-10^8 CFU/ml was inoculated. The samples were kept in the refrigerator (4 °C) for 21 days and the physico-chemical properties, survivals of probiotics were investigated at 1, 15 and 30 days.

2.3. Final product tests (production of hibiscus tea probiotic diet drink based on stevia and inulin)

2.3.1. pH measurement

pH values of the samples were measured by pH meter (Metrohm, Swiss) at 20 ˚C (Markov *et al.,* 2012).

2.3.2. Titratable acidity

The total acidity was measured through titration, involving the addition 0.1 normal sodium hydroxide to samples until a pink color appeared (Rastegari *et al.,* 2013).

$$TA\ (\%) = \frac{Meqw \times N \times Y}{V} \quad (1)$$

Meqwt = the equivalent weight of citric acid, N = Normality of sodium hydroxide, V: NaOH volume consumed, Y: ml volume of sample extract.

Table 1. Introduction of treatments tested in the research (probiotic drink samples produced with different levels of inulin, stevia, hibiscus tea.

Sample	Hibiscus Tea (%)	Inulin (%)	Stevia (%)
H2.5, I2, S0.1	2.5	2	0.1
H2.5, I2, S0.2	2.5	2	0.2
H2.5, I2, S0.3	2.5	2	0.3
H2.5, I4, S0.1	2.5	4	0.1
H2.5, I4, S0.2	2.5	4	0.2
H2.5, I4, S0.3	2.5	4	0.3
H2.5, I6, S0.1	2.5	6	0.1
H2.5, I6, S0.2	2.5	6	0.2
H2.5, I6, S0.3	2.5	6	0.3
H5, I2, S0.1	5	2	0.1
H5, I2, S0.2	5	2	0.2
H5, I2, S0.3	5	2	0.3
H5, I4, S0.1	5	4	0.1
H5, I4, S0.2	5	4	0.2
H5, I4, S0.3	5	4	0.3
H5, I6, S0.1	5	6	0.1
H5, I6, S0.2	5	6	0.2
H5, I6, S0.3	5	6	0.3
H7, I2, S0.1	7	2	0.1
H7, I2, S0.2	7	2	0.2
H7, I2, S0.3	7	2	0.3
H7, I4, S0.1	7	4	0.1
H7, I4, S0.2	7	4	0.2
H7, I4, S0.3	7	4	0.3
H7, I6, S0.1	7	6	0.1
H7, I6, S0.2	7	6	0.2
H7, I6, S0.3	7	6	0.3

2.3.3. Formalin index

In order to measure the formalin index, the samples were titrated with NaOH until pH = 1.8. Then neutralized formaldehyde solution was added. After the solution was potentiometrically titrated to pH=1.8 with NaOH.

$$F = \{(V \times N \times 10)/V0\} \times 100 \quad (2)$$

Where V is the volume of NaOH used, N is the normality of NaOH, and V0 is the volume of the sample (Nemati *et al.*, 2020).

2.3.4 Total phenol

Gallic acid was used to draw the standard curve using extraction solvent (100% methanol) and was diluted to concentrations of 8.82, 5.88, 4.70, 3.53 and 1.18 µm. The solutions were prepared fresh and under low light and the reaction was carried out in the dark. 20 µl of the diluted extract, or gallic acid standard, was mixed with 100 µl of 0.2 normal Folin–Ciocalteu solution and mixed completely. After 6 minutes, 80 µL of 7.5% w/w sodium carbonate was added and mixed completely. The mixture was placed at room temperature for 2 hours and finally the absorbance at 760 nm wavelength was read by spectrophotometer (Hitachi,U-3900/3900H). The results were expressed as µg/g of gallic acid per dry matter (Rupasinghe *et al.*, 2008).

2.3.5. Assessment of antioxidant activity with different methods

2.3.5.1. Inhibition of DPPH free radicals

100 µl of samples were placed with 3 ml of 2-diphenyl-1-picrylhydrazyl (DPPH) solution (0.1 mM) in methanol in the dark and at room temperature for 2 hours and absorption at 515 nm wavelength was read using a spectrophotometer in comparison with the control. DPPH free radical inhibition was calculated based on the percentage according to the following equation (Najgebauer-Lejko *et al*., 2011).

$$\text{Radical inhibition percentage} = 1 - (A_{sample} - A_{blank}) \times 100 \quad (3)$$

A_{blank} is the absorption of the control solution (water) and A sample is the absorption of the samples. The concentration of the sample that showed 50% inhibition (IC50) was obtained from the curve drawn based on the percentage of inhibition (Adams, 2002). All evaluations were done in three repetitions and data analysis was done in the form of completely randomized design.

2.3.5.2. Ferric Reducing Antioxidant Power (FRAP) Method

This experiment was performed based on the ability to regenerate trivalent iron ion and convert it into divalent iron ion. The resulting divalent iron in acidic pH and in the presence of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) reagent forms Fe-TPTZ, which has a blue color and the intensity of the resulting color can be measured with a spectrophotometer at a wavelength of 593 nm. For this purpose, the concentration of 250 µg/ml was taken from the sample and added to the final volume of 2 ml of TPTZ solution, which contains 10 ml of FRAP solution in (40 mM HCl), 20 mM iron chloride and 300 mM buffer acetate with pH = 3.6 and placed at 37 ˚C for 10 minutes and the resulting color intensity was read at 593 nm wavelength against blank. Iron sulfate with concentrations of 1000, 500, 250, and 125 µM were used to draw the standard curve, and the antioxidant power was expressed based on the Fe^{+2} µM scale. The antioxidant compound Quercetin was used as a positive control (Hatamnia *et al.*, 2014).

FRAP value = $[(A_1 - A_0)/(A_c - A_0)] \times 2$, Ac is the absorbance of the positive control, A_1 is the absorbance of the sample, and A_0 is the absorbance of the blank.

2.3.5.3. ABTS radical scavenging assay

For the preparation of the radical, 2' 2-Azino-bis (3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS), first an aqueous solution with a concentration of 7 mM was prepared. To this ABTS solution, potassium persulfate was added until its final concentration reached 2.45 mmol in the solution. The resulting solution was kept at room temperature and darkness for 16 hours. During this period, ABTS radical cation was produced from ABTS molecule. 20 µl of the samples mixed with 2 ml of $ABTS^{\bullet+}$ solution, then its absorbance was read at 734 nm at 2, 4, and 6 minutes after mixing. The results were reported as TEAC number (ABTS radical scavenging power) of the samples based on the trolox standard (Hatamnia *et al.*, 2014).

$$AABTS^{\cdot}\text{radical scavenging activity (\%)} = (\text{Acontrol - Asample})/\text{Acontrol}) \times 100 \quad (4)$$

Where Abs control is the absorbance of ABTS radical in methanol; Abs sample is the absorbance of ABTS radical solution mixed with sample extract/standard.

2.3.6. Brix

In this test, a digital refractometer made by Atago, Japan was used. A few drops of the treatment were placed on the prism, then the start button was pressed and the Brix of the sample was shown on the screen (Al-nemr *et al.,* 2013).

2.3.7. Turbidity

Micro turbidity meter (model HI 93703, HANNA Company, made in America) was used to measure turbidity. So that 15 ml of the sample diluted with deionized distilled water at a ratio of (20:1) was added to the tube of the device after stirring and the turbidity of the samples was measured. Before measuring the turbidity of the samples, the calibration of the turbidity meter was done with NTU standard buffers (0.1, 800 and 20,100) and the turbidity was reported in NTU units (Reisi *et al.,* 2013).

2.3.8. Colorimetric test

The colorimetric test of the samples was performed by a colorimeter according to AACC standard method No. 10-90 (AACC, 2003). In this way, the colorimeter device (Hunterlab, Colorflex, USA) was placed within one centimeter of the sample and L* (lightness), a* (greenness to redness), and b* (blueness to yellowness) were obtained by the device.

2.3.9. Viscosity measurement

The viscosity of the production samples was measured using a Brookfield viscometer (RV-DVII (made in USA). In this experiment, after the initial tests, spindle number 6 was selected as the suitable spindle for viscosity measurement. All tests were performed at a temperature of 5°C and under the same conditions; So that the viscosity of the samples was read and reported at a speed of 70 rpm and after 15 seconds of spindle rotation (Akin *et al.,* 2007).

2.3.10. Investigation of probiotic viability

In order to count the probiotics, serial dilutions of the samples were first prepared. And then it was transferred to the MRS agar culture medium, and the cultured samples were placed in an incubator at 37˚C for 48 hours (Fathi Achacheloi *et al*., 2017).

2.3.11. Evaluation of sensory characteristics

Sensory evaluation was carried out by a group of 10 trained sensory evaluators using the 5-point hedonic method, so that scoring was done by sensory evaluators from score 1 (very bad) to 5 (very good) according to the designed forms (De-Heer *et al.*, 2011).

2.4. Statistical analysis method

The experimental data were compared with one-way ANOVA. Statistically significant differences between mean values (in cases where the overall effect of treatments is significant) were determined using Duncan's multi-range follow-up test. Statistical tests of the obtained results were performed using SPSS version 26 software. A significance level of $p \leq 0.05$ was considered for all data comparisons.

3. Results and Discussion

3.1 Evaluation of the results of the tests performed on probiotic drink samples produced with different levels of hibiscus tea, inulin and stevia

3.1.1. pH and acidity

The results of this research showed that samples H7, I6, S0.1, sample H7, I6, S0.2 and the samples H7, I6, S0.3, which contained the highest levels of hibiscus tea and the lowest levels of inulin, showed the lowest pH and the highest acidity ($p<0.05$). The highest pH level and the lowest acidity level belonged to sample H2.5, I6, S0.3, followed by sample H2.5, I6, S0.2 and sample H2.5, I6, S0.1 ($p<0.05$). The mentioned samples contained the lowest levels of hibiscus tea and the highest levels of inulin. At the highest levels of hibiscus tea and the lowest levels of inulin, the pH of the samples decreased significantly ($p<0.05$). pH is determined by the inverse logarithm of the hydrogen ion concentration by a pH meter device, and titratable acidity includes the total acidity, which is measured based on the dominant organic acid (Ahmadi *et al.,* 2007). The pH of a sample affects the sensory properties of the sample. Low pH produces sour and astringent products. De-Heer *et al.* (2011) during the formulation and sensory evaluation of an herbal drink consisting of hibiscus tea, Moringa and lemon grass stated that the sample of hibiscus tea had the lowest pH value (2.73) and was very acidic. This finding confirms the reports that hibiscus tea calyx is rich in organic acids (Kerharo, 1971, Wong *et al.* 2002). Acids play a significant role in influencing the taste of food products by imparting a sour or sharp taste to food (Bender, 2003). The use of hibiscus tea in herbal tea production showed potentially more acidity than other plants such as moringa and lemon grass. Chumsri *et al*., also reported the pH value of 2.30-2.33 in the investigation of extraction time and temperature on the characteristics of hibiscus tea drink. Hashemi *et al.,* in the study of the effect of replacing the sugar of stevia plant (*Stevia rebaudiana*) with sucrose in saffron diet syrup, stated that the pH value decreased significantly with increasing the percentage of stevia as a sweetener. The lowest pH value was related to the sample containing 100% stevia and the highest pH value was related to the samples containing 100% sucrose. In the current research, the addition of different levels of stevia did not cause a statistically significant difference in the pH of the samples, and the reason for this can be attributed to the low dose used. Ajali *et al.* (2014) acknowledged that no significant effect on the pH of the orange drink was observed by replacing the natural sweetener stevia instead of sugar in the orange diet drink. Khakbaz Hashmati *et al.* (2017) during the production of combined cherry and red grape juice enriched with dietary fiber inulin admitted that the effect of inulin on the pH and acidity of the produced product in combination with cherry and red grape juice is not significant. They attributed this to the neutral nature of inulin. Researchers observed that by adding inulin to various products such as kefir (Tratnik *et al*., 2006), yogurt (Vivek *et al.,* 2013; Guven *et al.,* 2005), there is no significant change in the pH and acidity of the product. Khushgadam *et al.* (2014), in investigating the effect of inulin on the pH and acidity of mixed juice of cherry and red grape, admitted that no significant difference was seen in the samples. Sohrab Vandi *et al.* (2014) investigating the effect of adding inulin and D-tagatose on the physicochemical and sensory properties of Functional grape juice admitted that with the increase in the percentage of inulin, no statistically significant difference was observed in the pH and acidity

of the samples. In the current research, increasing the levels of inulin used led to an increase in the pH of the samples, and the difference in the results can be attributed to the higher dose of inulin used in the current research.

3.1.3. Formalin index

The results of this research showed that sample H7, I6, S0.2 and 26 sample H7, I4, S0.1, which contained the highest levels of hibiscus tea and inulin, showed the highest level of formalin index (p<0.05). The lowest level of formalin index belonged to sample H2.5, I2, S0.1 and sample H2.5, I2, S0.2 (p<0.05). The mentioned samples contained the lowest levels of hibiscus tea, inulin and stevia. At the highest levels of hibiscus tea and inulin, the formalin index of the samples increased significantly (p<0.05). Formalin number indicates the amounts of amino acids and proteins in drinks. This test is important because if the number of amino acids is high, they combine with sugars and enter the Maillard reaction, as a result the color of the drink will be dark. (Heshmati Khakbaz and Khoshghadam, 2017). Hainida *et al*. (2008) in the investigation of hibiscus tea compounds reported its protein content to be 30 to 35% and in their research, 17 essential and non-essential amino acids were identified and they admitted that the seeds were rich in lysine (14-15 g/100 g), arginine (30-35 g/100 g), leucine (15.4-18.6 g/100 g), phenylalanine (11-12 g/100 g) and glutamic acid (21-24 g/100 g). In the current research, it seems that due to the higher amounts of amino acids in hibiscus tea compared to inulin and stevia, its addition has created a greater effect. Heshmati Khakbaz and Khoshghadam (2017) also stated that the enrichment of cherry and red grape mixed drink with inulin dietary fiber as a prebiotic product did not have a significant effect on the formalin number of the produced drinks.

3.1.4. Brix

Based on statistical results, samples H7, I6, S0.3, H7, I6, S0.2 and H7, I6, S0.1, which contained the highest levels of hibiscus tea and inulin, showed the highest amount of Brix (p<0.05). The lowest amount of Brix belonged to samples H2.5, I2, S0.1 and H2.5, I2, S0.2 (p<0.05). The mentioned samples contained the lowest levels of hibiscus tea, inulin and stevia. At the highest levels of hibiscus tea and inulin, the Brix of the samples increased significantly (p<0.05), which can be attributed to the increase of soluble solids. Brix degree indicates the weight percentage of solid materials in a solution to the total weight of the solution, or in other words, the weight percentage of solid materials in the solution. Sohrab Vandi et al. (2014) investigated the effect of adding inulin and D-tagatose on the physicochemical and sensory properties of Functional grape juice. They acknowledged that by increasing the percentage of inulin, the Brix level of the samples increased, so that the treatment with 6% inulin had the highest amount and the control treatment had the lowest amount of Brix. Rihanzadeh et al. (2017) in the production of a low-calorie and long-lasting drink based on hibiscus tea and tree leaves, admitted that the increase of stevia had a direct effect on the amount of Brix, and with the increase of each, the amount of Brix also increased. Kargozari et al. (2014) admitted that the samples containing higher stevia, had higher brix during the production of Dietary and functional lime drink.

3.1.5. Turbidity

The results of this research showed that samples H7, I6, S0.3, H7, I6, S0.2 and H7, I6, S0.1, which contained the highest levels of hibiscus tea and inulin, showed the highest level of turbidity (p<0.05). The lowest amount of turbidity belonged to samples H2.5, I2, S0.1 and H2.5, I2, S0.2 (p<0.05). The mentioned samples contained the lowest levels of hibiscus tea, inulin and Stevia. At the highest levels of hibiscus tea and inulin, the turbidity of the samples increased significantly (p<0.05). The reason for this may be attributed to the higher brix of the mentioned samples. In the study of the effect of the concentration process on the physicochemical and rheological properties of grapefruit juice, they stated that there is a direct relationship between the turbidity of the initial grapefruit juice and concentrates with higher Brix (Motamedzadegan *et al.,* 2018). Cassani *et al.* (2016) reported that the interaction effect of red grape juice and inulin on the final color and turbidity of mixed fruit juice formulations was not significant. But in any case, it is expected that the addition of inulin is effective on the amount of color absorption and turbidity of fruit juice samples. This result is similar to the observations of Yousaf *et al*. (2010) in studying the stability of banana juice enriched by inulin. Hosseini *et al.* (2014) in optimizing the production of low-calorie orange nectar using stevioside and evaluating its physicochemical properties during storage, prepared samples containing a combination of stevia powder and orange concentrate and acknowledged that the amount of turbidity decreased during storage.

3.1.6. Total phenolic content

The results of this research showed that sample H7, I6, S0.3 and then sample H7, I6, S0.2 showed the highest amount of total phenol. (p<0.05). The lowest amount of total phenol belonged to sample H2.5, I2, S0.1, followed by sample H2.5, I2, S0.2 (p<0.05). The mentioned samples contained the lowest levels of hibiscus tea, inulin and stevia. In the highest levels of hibiscus tea, inulin and stevia, the total phenol of the samples increased significantly (p<0.05). The polyphenol content of compounds in herbal tea formulations is important because it is a direct indication of the health-enhancing properties of the plant (De-Heer *et al*., 2011). De-Heer *et al.* (2011) during the formulation and sensory evaluation of an herbal drink consisting of hibiscus tea, Moringa and lemon grass stated that the total phenol content of hibiscus tea was 27.81 mg/g. Phenolic compounds in fruits and vegetables have received special attention due to their relationship in increasing antioxidant activity *in vitro* and *in vivo*, which is related to their ability to destroy free radicals (Kalinowska *et al*., 2014). Among these phenolic compounds, *H. sabariffa* mainly contains organic acids (hydroxycitric acid, hibiscus acid), anthocyanins (Delphinidin-3-sambobioside, cyanidin-3-sambobioside), flavonoids and phenolic acids (gallic acid, quercetin, caffeic acid, chlorogenic acids) (Da-Costa-Rocha *et al.,* 2014). Zhen *et al.* (2016) identified 10 polyphenols such as neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, quercetin, kaempferol and their glycosides along with 5-(hydroxymethyl) furfural in *Hibiscus sabdariffa*. Total phenolic content was determined in several different types of *Hibiscus sabdariffa* from 18.98 to 29.9 ± 0.5 mg GAE/g. According to the findings of the researchers, the amount of total phenol in stevia leaves was reported to be 91 mg/g, which can indicate the good antioxidant properties of stevia. The main compounds identified in stevia leaves are: dicaffeoylquinic acids (diCQA), Chlorogenic acid, Quercetin 3-O-xyloside, Apigenin-7-O-glucoside, 3, 4 dimethoxycinnamic acid, luteolin 7-O-rutinoside, caffeic acid, etc (Shivanna *et al*., 2013).

Table 2. Comparison of pH, acidity, formalin index, Brix, Turbidity and Total phenol of probiotic drink samples produced with different levels of inulin and stevia sour tea. Different small letters in the column indicate significant differences (p<0.05).

Samples	pH	Acidity (%)	Formalin index(mg/100cc)	Brix (%)	Turbidity (NTU)	Total phenol (µg GAE/mL)
H2.5, I2, S0.1	3.17±0.00 ^{d}	0.48±0.01 ^{j}	8.40±0.40 ^{p}	5.55±0.00 ^{b}	0.25±0.00 ^{jk}	833.46±2.82 ^{z}
H2.5, I2, S0.2	3.17±0.00 ^{cd}	0.47±0.00 ^{j}	8.80±0.40 ^{op}	5.55±0.01 ^{b}	0.25±0.00 ^{k}	845.37±1.85 ^{y}
H2.5, I2, S0.3	3.18±0.01 ^{cd}	0.47±0.01 ^{j}	9.06±0.23 ^{mno}	5.55±0.01 ^{a}	0.25±0.00 ^{k}	852.77±1.85 ^{x}
H2.5, I4, S0.1	3.18±0.00 ^{c}	0.46±0.00 ^{jk}	8.93±0.23 ^{no}	5.61±0.00 ^{e}	0.26±0.00 ^{j}	858.33±1.85 ^{w}
H2.5, I4, S0.2	3.18±0.00 ^{c}	0.44±0.01 ^{kl}	9.33±0.23 ^{lmn}	5.61±0.01 ^{d}	0.26±0.00 ^{j}	862.03±1.85 ^{w}
H2.5, I4, S0.3	3.18±0.00 ^{cd}	0.44±0.01 ^{kl}	9.73±0.23 ^{jkl}	5.60±0.01 ^{c}	0.26±0.00 ^{ij}	875.00±1.85 ^{v}
H2.5, I6, S0.1	3.21±0.01 ^{b}	0.43±0.01 ^{l}	9.46±0.23 ^{klm}	5.72±0.00 ^{h}	0.29±0.00 ^{h}	884.87±1.06 ^{u}
H2.5, I6, S0.2	3.22±0.01 ^{b}	0.42±0.02 ^{l}	9.86±0.23 ^{jk}	5.73±0.00 ^{g}	0.29±0.00 ^{h}	894.75±2.82 ^{t}
H2.5, I6, S0.3	3.25±0.01 ^{a}	0.42±0.01 ^{l}	10.13±0.23 ^{j}	5.74±0.01 ^{f}	0.29±0.00 ^{h}	907.09±2.82 ^{s}
H5, I2, S0.1	2.98±0.00 ^{g}	0.72±0.00 ^{f}	16.66±0.23 ^{i}	7.57±0.01 ^{b}	0.97±0.00 ^{g}	1179.32±2.82 ^{r}
H5, I2, S0.2	2.98±0.00 ^{g}	0.72±0.00 ^{f}	17.06±0.23 ^{hi}	7.57±0.01 ^{a}	0.97±0.00 ^{g}	1191.04±2.82 ^{q}
H5, I2, S0.3	2.98±0.00 ^{g}	0.70±0.00 ^{fg}	17.73±0.23 ^{fg}	7.57±0.01 ^{e}	0.96±0.00 ^{g}	1202.16±2.82 ^{p}
H5, I4, S0.1	3.00±0.00 ^{f}	0.69±0.01 ^{gh}	17.46±0.46 ^{gh}	7.64±0.01 ^{d}	0.98±0.00 ^{f}	1225.00±1.85 ^{o}
H5, I4, S0.2	3.00±0.00 ^{f}	0.68±0.00 ^{ghi}	17.73±0.23 ^{fg}	7.64±0.00 ^{c}	0.98±0.00 ^{f}	1234.87±1.06 ^{n}
H5, I4, S0.3	3.01±0.00 ^{f}	0.67±0.01^{hi}	18.13±0.23 ^{ef}	7.64±0.01 ^{h}	0.98±0.00 ^{f}	1247.22±1.85 ^{m}
H5, I6, S0.1	3.02±0.00 ^{e}	0.67±0.00 ^{hi}	17.73±0.23 ^{fg}	7.71±0.01 ^{g}	1.03±0.00 ^{e}	1262.03±1.85 ^{l}
H5, I6, S0.2	3.02±0.00 ^{e}	0.66±0.00 ^{i}	18.13±0.23 ^{ef}	7.72±0.00 ^{f}	1.03±0.00 ^{e}	1282.40±1.85 ^{k}
H5, I6, S0.3	3.03±0.01 ^{e}	0.66±0.01 ^{i}	18.40±0.40 ^{e}	7.71±0.00 ^{b}	1.03±0.00 ^{e}	1300.30±1.06 ^{j}
H7, I2, S0.1	2.81±0.00 ^{j}	1.25±0.00 ^{a}	25.33±0.23 ^{d}	8.42±0.01 ^{a}	1.68±0.00 ^{d}	1642.28±4.66 ^{i}
H7, I2, S0.2	2.82±0.00 ^{j}	1.24±0.01 ^{ab}	25.86±0.23 ^{c}	8.43±0.02 ^{e}	1.68±0.00 ^{d}	1656.48±1.85 ^{h}
H7, I2, S0.3	2.82±0.00 ^{j}	1.22±0.00 ^{b}	26.00±0.40 ^{bc}	8.44±0.02 ^{d}	1.68±0.00 ^{d}	1668.20±2.82 ^{g}
H7, I4, S0.1	2.83±0.00 ^{i}	3.17±0.01 ^{c}	25.73±0.23 ^{cd}	8.50±0.01 ^{c}	1.71±0.00 ^{c}	1689.19±4.27 ^{f}
H7, I4, S0.2	2.84±0.00 ^{i}	1.16±0.01 ^{cd}	26.13±0.23 ^{abc}	8.51±0.01 ^{h}	1.71±0.00 ^{bc}	1702.77±1.85 ^{e}
H7, I4, S0.3	2.84±0.00 ^{i}	1.16±0.01 ^{cde}	26.26±0.23 ^{abc}	8.51±0.01 ^{g}	1.71±0.00 ^{b}	1714.50±3.85 ^{d}
H7, I6, S0.1	2.86±0.00 ^{h}	1.16±0.01 ^{cde}	26.13±0.61 ^{abc}	8.62±0.01 ^{f}	1.73±0.00 ^{a}	1730.55±1.85 ^{c}
H7, I6, S0.2	2.86±0.00 ^{h}	1.14±0.01 ^{de}	26.53±0.23 ^{ab}	8.62±0.02 ^{f}	1.73±0.00 ^{a}	1747.83±2.82 ^{b}
H7, I6, S0.3	2.87±0.00 ^{h}	1.14±0.00 ^{e}	26.66±0.23 ^{a}	8.62±0.01 ^{f}	1.73±0.00 ^{a}	1762.03±1.85 ^{a}

3.1.7. Comparison of antioxidant activity with DPPH free radical inhibition methods, iron reduction rate (FRAP method) and ABTS radical absorption

The results of this research showed that samples H7, I6, S0.3 and H7, I6, S0.2 had the highest antioxidant activity (p<0.05). The lowest amount of antioxidant activity belonged to sample H2.5, I2, S0.1, followed by sample H2.5, I2, S0.2 (p<0.05). The mentioned samples contained the lowest levels of hibiscus tea, inulin and stevia. At the highest levels of hibiscus tea, inulin and stevia, the antioxidant activity of the samples increased significantly (p<0.05). According to what was mentioned above 10 polyphenols such as neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, quercetin, kaempferol and their glycosides along with 5-(hydroxymethyl) furfural in *Hibiscus sabdariffa*. Total phenolic content of *Hibiscus sabdariffa* from 18.98 to 29.9 ± 0.5 mg GAE/g and antioxidant activities from 17.5 to 18.8 ± 152.5 µM trolox/g by ABTS radical cation decolorization method (Zhen *et al*., 2016).

3.1.8. Viscosity

The results of this research showed that sample H7, I6, S0.3 and then sample H7, I6, S0.2 showed the highest viscosity (p<0.05). The lowest viscosity belonged to sample H2.5, I2, S0.1, followed by sample H2.5, I2, S0.2 (p<0.05). The mentioned samples contained the lowest levels of hibiscus tea, inulin and stevia. At the highest levels of hibiscus tea and inulin, the viscosity of the samples increased significantly (p<0.05). Based on the findings obtained in various surveys, the viscosity of beverages and juices changes with the change in its solid content (Raesi *et al.,* 2014). Therefore, according to the changes in the amount of solids in this study, changes in viscosity were also observed. Khakbaz Hashmati *et al.* (2017) in investigating the rheological and physicochemical properties of a new formulation of fruit juice obtained from a combination of cherry and red grapes enriched with dietary fiber inulin as a prebiotic product, acknowledged that the integration of the two types of fruit juice studied in combination with inulin did not have much effect on the quality of the product from a rheological point of view, considering that inulin has been introduced as a texturizing agent in many studies (Akin *et al*., 2007; Al-nemr *et al*., 2013; Juan *et al*., 2013). Of course, this feature of inulin is shown at a concentration above 15% (Al-nemr *et al.,* 2013). Therefore, since the amount of inulin used in their research was more than 15% (50% and 100%), texture creation properties and changes in the viscosity of the products were observed.

3.1.9. Viability of probiotic bacteria during storage

Sample H7, I6, S0.3 followed by samples H7, I6, S0.2 and sample H7, I6, S0.1 showed the highest population of probiotics (p<0.05). The lowest population of probiotics belonged to sample H2.5, I2, S0.1, followed by sample H2.5, I2, S0.2 (p<0.05). The mentioned samples contained the lowest levels of hibiscus tea, inulin and stevia. Over time, the population of probiotics decreased significantly (p<0.05). The reason can be attributed to the antioxidant properties and the appropriate amount of prebiotic inulin in the mentioned samples. The survival of probiotic cells is very important to have health effects on the host (Mortazavian et al., 2007). One of the simple ways to improve the stability of probiotic bacteria in food products is enrichment with prebiotic compounds and antioxidant compounds (Rakin et al., 2013).

Table 3. Antioxidant activity and viscosity of probiotic drink samples produced with different levels of inulin and stevia sour tea. Different small letters in the column indicate significant differences (p<0.05).

Samples	Inhibition of DPPH free radicals (IC50) (mg/ml)	Inhibition of free radicals FRAP (mg/g)	ABTS radical (absorption method) (mg/ml)	Viscosity Centipoise (cP)
H2.5, I2, S0.1	25.84±0.41 ^{a}	588.66±1.41 ^{w}	87.26±1.96 ^{a}	148.67±7.76 ^{ghi}
H2.5, I2, S0.2	24.73±0.75 ^{b}	562.44±0.81 ^{vw}	75.36±1.46 ^{b}	140.67±8.08 ^{i}
H2.5, I2, S0.3	23.89±0.35 ^{c}	564.80±0.81 ^{v}	70.54±1.28 ^{c}	140.67±8.08 ^{i}
H2.5, I4, S0.1	16.72±0.17 ^{d}	571.88±0.16 ^{u}	46.69±0.56 ^{d}	159.00±5.00 ^{bcd}
H2.5, I4, S0.2	15.97±0.15 ^{e}	574.71±2.16 ^{tu}	44.80±0.52 ^{e}	161.67±10.01 ^{abc}
H2.5, I4, S0.3	14.88±0.26 ^{f}	579.90±1.41 ^{t}	41.18±0.44 ^{f}	165.33±8.08 ^{abc}
H2.5, I6, S0.1	11.34±0.57 ^{g}	587.92±2.16 ^{s}	29.47±0.39 ^{g}	171.00±9.64 ^{abc}
H2.5, I6, S0.2	10.31±0.50 ^{h}	594.52±2.16 ^{r}	26.41±0.18 ^{h}	169.00±11.05 ^{abc}
H2.5, I6, S0.3	9.84±0.25 ^{i}	602.07±2.16 ^{q}	25.40±0.29 ^{i}	170.67±3.05 ^{abc}
H5, I2, S0.1	9.15±0.17 ^{j}	731.35±6.48 ^{p}	11.01±0.03 ^{j}	165.33±6.02 ^{abc}
H5, I2, S0.2	8.13±0.03 ^{k}	742.67±2.45 ^{o}	9.89±0.05 ^{k}	148.33±7.57 ^{hi}
H5, I2, S0.3	7.90±0.05 ^{k}	749.75±4.24 ^{n}	9.58±0.02 ^{kl}	154.33±8.02 ^{efg}
H5, I4, S0.1	7.34±0.02 ^{l}	762.96±4.97 ^{m}	8.86±0.02 ^{lm}	157.33±8.50 ^{def}
H5, I4, S0.2	7.08±0.01 ^{lm}	773.34±2.16 ^{l}	8.57±0.03 ^{mn}	159.33±2.51 ^{bcd}
H5, I4, S0.3	6.84±0.03 ^{m}	779.47±3.74 ^{k}	8.26±0.01 ^{mn}	163.33±2.08 ^{abc}
H5, I6, S0.1	6.72±0.01 ^{m}	787.49±0.81 ^{j}	8.13±0.01 ^{mn}	169.00±11.05 ^{abc}
H5, I6, S0.2	6.29±0.02 ^{n}	795.51±2.16 ^{i}	7.57±0.01 ^{no}	165.33±6.65 ^{abc}
H5, I6, S0.3	5.90±0.01 ^{no}	800.23±3.56 ^{i}	7.09±0.01 ^{o}	169.67±7.23 ^{abc}
H7, I2, S0.1	5.68±0.04 ^{op}	893.65±5.89 ^{h}	6.08±0.00 ^{p}	151.00±5.56 ^{fgh}
H7, I2, S0.2	5.38±0.04 ^{pq}	907.80±2.16 ^{g}	5.76±0.00 ^{pq}	165.33±6.65 ^{abc}
H7, I2, S0.3	5.26±0.00 ^{pqr}	913.94±2.83 ^{f}	5.66±0.02 ^{pq}	154.00±1.73 ^{efg}
H7, I4, S0.1	5.11±0.03 ^{qrs}	937.53±3.56 ^{e}	5.47±0.00 ^{pq}	157.67±5.68 ^{cde}
H7, I4, S0.2	5.03±0.03 ^{qrst}	946.49±4.24 ^{d}	5.35±0.01 ^{pq}	168.67±7.63 ^{abc}
H7, I4, S0.3	4.88±0.01 ^{rst}	949.32±5.66 ^{d}	5.23±0.01 ^{pq}	166.00±10.44 ^{abc}
H7, I6, S0.1	4.76±0.01 ^{stu}	959.23±4.24 ^{c}	5.10±0.01 ^{pq}	174.67±2.08 ^{a}
H7, I6, S0.2	4.65±0.03 ^{tu}	968.67±3.56 ^{b}	4.98±0.01 ^{q}	174.00±3.60 ^{a}
H7, I6, S0.3	4.43±0.00 ^{u}	978.57±5.89 ^{a}	4.75±0.01 ^{q}	171.67±8.38 ^{ab}

3.1.10. Colorimetry

The chromatic parameters L* (darkness/brightness), a* (greenness/redness), and b* (blueness/yellowness) values of probiotic dietary drink are presented in Table 4. The results showed that samples H2.5, I2, S0.1, H2.5, I2, S0.2 and H2.5, I2, S0.3 had the highest L* (p<0.05). The lowest L* belonged to samples H7, I6, S0.1, H7, I6, S0.2, and H7, I6, S0.3 (p<0.05). The mentioned samples contained the lowest levels of Hibiscus tea, inulin and stevia. At the highest levels of hibiscus tea, inulin and stevia, the L* of the samples increased significantly (p<0.05). Color index L* measures the degree of darkness and lightness (light-dark, 0-100) (Pino & Gonzalez, 2002). Sample H7, I2, S0.1, sample H7, I2, S0.2, sample H7, I2, S0.3, sample H7, I4, S0.1, sample H7, I4, S0.2, sample H7, I4, S0.3, sample H7, I6, S0.1, sample H7, I6, S0.2 and sample H7, I6, S0.3 showed the highest a* (p<0.05). The lowest a* belonged to sample H2.5, I2, S0.1, sample H2.5, I2, S0.2, sample H2.5, I2, S0.3, sample H2.5, I4, S0.1, sample H2.5, I4, S0.2 and sample H2.5, I4, S0.3 (p<0.05). The mentioned samples contained the lowest levels of hibiscus tea, inulin and stevia. De-Heer *et al.* (2011) during the formulation and sensory evaluation of a* herbal drink consisting of hibiscus tea, Moringa and lemongrass stated that hibiscus tea is also known as red sorrel due to the unique red color of its calyx (Mounigan and Badrie, 2006). Researchers have attributed the reddish color of hibiscus tea calyx to the presence of anthocyanins (highly water-soluble red pigments) (Du and Francis 1973; Mazza and Miniati 2000). Fresh roselle calyx contains natural compounds of organic acids such as malic acid, citric acid and 3-indolyl acetic acid (AL-Kahtani and Hassan, 1990), which play an important role in giving the bright red color of the juice sample. It has been found that in acidic environments, four anthocyanin structures, including flavylium cation, quinonoidol base, carbinol pseudobase, and chalcone exist in equilibrium. And at pHs below 2, the anthocyanin is mainly present as the red flavylium cation. As the pH increased (>4.5), rapid proton loss occurred to form aqueous quinonoid forms (Mazza and Miniati, 1993). The results of the present research showed that samples H7, I6, S0.3 and H7, I6, S0.2 showed the highest b* (p<0.05). The lowest b* belonged to sample H2.5, I2, S0.1 (p<0.05). The mentioned samples contained the lowest levels of hibiscus tea, inulin and stevia.

3.1.11. Sensory evaluation

3.1.11.1. Taste Score

The results of the present research showed that samples H5, I4, S0.2 and H5, I6, S0.2 showed the highest taste score (p<0.05). De-Heer *et al.* (2011) stated that samples with low proportions of Moringa and high proportions of hibiscus tea and lemongrass had a more attractive taste. This observation is consistent with the trend in odor scores and the taste was more influenced by hibiscus tea. Sohrab Vandi *et al.* (2014) investigated the effect of adding inulin and D-tagatose on the physicochemical and sensory properties of functional grape juice. They acknowledged that with the increase in the percentage of inulin, the transparency of the samples decreased significantly, so that the highest transparency was observed in the control treatment. The lowest transparency was assigned to the treatment with 6% inulin.

Table 4. Comparison of viability of probiotic bacteria during storage samples produced with different levels of inulin and stevia sour tea. Different small letters in the column indicate significant differences (p<0.05).

Samples	Total plate count for probiotic Log (CFU/ml)		
	1 day	15 days	30 days
H2.5, I2, S0.1	8.91±0.04 ^{aA}	4.23±0.00 ^{klB}	5.91±0.04 ^{gC}
H2.5, I2, S0.2	8.97±0.04 ^{aA}	4.35±0.00 ^{iB}	5.97±0.04 ^{fC}
H2.5, I2, S0.3	8.03±0.04 ^{aA}	4.41±0.01 ^{hiB}	6.03±0.04 ^{deC}
H2.5, I4, S0.1	8.02±0.03 ^{aA}	4.54±0.07 ^{gB}	6.02±0.03 ^{eC}
H2.5, I4, S0.2	8.16±0.03 ^{aA}	4.76±0.06 ^{eB}	6.16±0.03 ^{cC}
H2.5, I4, S0.3	8.24±0.02 ^{aA}	4.87±0.06 ^{dB}	6.24±0.02 ^{bC}
H2.5, I6, S0.1	8.06±0.01 ^{aA}	4.98±0.02 ^{cB}	6.06±0.01 ^{dC}
H2.5, I6, S0.2	8.14±0.01 ^{aA}	5.08±0.01 ^{bB}	6.14±0.01 ^{cC}
H2.5, I6, S0.3	8.29±0.01 ^{aA}	5.16±0.01 ^{aB}	6.29±0.01 ^{aC}
H5, I2, S0.1	8.21±0.01 ^{aA}	3.93±0.02 ^{nB}	5.21±0.01 ^{nC}
H5, I2, S0.2	8.27±0.00 ^{aA}	3.98±0.01 ^{nB}	5.27±0.00 ^{lmC}
H5, I2, S0.3	8.41±0.00 ^{aA}	4.19±0.01 ^{lB}	5.41±0.00 ^{jC}
H5, I4, S0.1	8.25±0.01 ^{aA}	4.22±0.01 ^{lB}	5.25±0.01 ^{mnC}
H5, I4, S0.2	8.31±0.01 ^{aA}	4.28±0.01 ^{jkB}	5.31±0.01 ^{klC}
H5, I4, S0.3	8.47±0.00 ^{aA}	4.30±0.01 ^{jB}	5.47±0.00 ^{iC}
H5, I6, S0.1	8.70±0.04 ^{aA}	4.56±0.05 ^{fgB}	5.70±0.04 ^{hC}
H5, I6, S0.2	8.88±0.02 ^{aA}	4.59±0.04 ^{fB}	5.88±0.02 ^{gC}
H5, I6, S0.3	8.91±0.02 ^{aA}	4.61±0.04 ^{fB}	5.91±0.02 ^{gC}
H7, I2, S0.1	8.82±0.01 ^{aA}	4.09±0.03 ^{mB}	4.82±0.01 ^{rC}
H7, I2, S0.2	8.02±0.02 ^{aA}	4.20±0.02 ^{iB}	5.02±0.02 ^{qC}
H7, I2, S0.3	8.13±0.02 ^{aA}	4.35±0.01 ^{iB}	5.13±0.02 ^{oC}
H7, I4, S0.1	8.86±0.03 ^{aA}	4.23±0.00 ^{klB}	4.86±0.03 ^{rC}
H7, I4, S0.2	8.07±0.02 ^{aA}	4.36±0.00 ^{iB}	5.07±0.02 ^{pC}
H7, I4, S0.3	8.22±0.02 ^{aA}	4.39±0.01 ^{hiB}	5.22±0.02 ^{nC}
H7, I6, S0.1	8.25±0.01 ^{aA}	4.27±0.01 ^{jkB}	5.25±0.01 ^{mnC}
H7, I6, S0.2	8.33±0.00 ^{aA}	4.40±0.00 ^{hiB}	5.33±0.00 ^{kC}
H7, I6, S0.3	8.41±0.00 ^{aA}	4.44±0.00 ^{hB}	5.41±0.00 ^{jC}

Cardoso *et al*., (2007) in the study of the use of stevia, sucralose and aspartame in peach nectar as sweeteners stated that Peach nectar containing 0.1% sucrose, 0.01% sucralose and 0.05% aspartame has been very similar to the control sample in terms of sensory characteristics, and the consumption of a small amount of stevia has created a suitable sweetness in the juice. And on the other hand, using a high dose of stevia caused a bitter taste in the products. The results of this research were in line with the results of the aforementioned researchers. Sheikhul-Islami *et al*. (2023) investigated the effect of date liquid sugar and stevia extract as a sugar substitute on the qualitative characteristics of apple-lemon mixed juice. In most cases, fruit juice samples containing stevia extract, especially samples containing higher amounts of this compound, obtained a higher sensory score.

3.1.11.2. Odor Score

The results of this research showed that sample H5, I2, S0.2, sample H5, I4, S0.2 and sample H5, I6, S0.2 showed the highest odor score (p<0.05).

3.1.11.3. Mouthfeel Score

The results of this research showed that sample H5, I2, S0.3, sample H5, I4, S0.2, sample H5, I6, S0.1, sample H5, I6, S0.2 and sample H5, I6, S0.3 showed the highest score of mouthfeels (p<0.05). By increasing the percentage of hibiscus tea and decreasing the percentage of inulin, the transparency of the samples increased significantly (p<0.05). Adeline and Julie (2004) used 10% inulin and oligofructose as sugar substitutes in ice cream formulations separately, so the results of their research showed that ice creams containing prebiotic ingredients were better than ice cream in terms of textural characteristics. The ones containing sugar were superior and as a result, the quality of prebiotic ice creams improved by increasing the texture strength. Hashemi et al. (2015) as the amount of stevia is increased, its taste tends to be slightly bitter and it is not very favorable for the consumer, therefore, this effect is less observed in samples with sucrose content of more than 50%. Covering the bitter taste of stevia with other sugars has been reported by another researcher.

3.1.11.4. Color Score

The results of this research showed that sample H5, I2, S0.2, sample H5, I4, S0.2, sample H5, I6, S0.1, sample H5, I6, S0.2, sample H7, I2, S0.3, sample H7, I4, S0.2, sample H7, I4, S0.3 and sample H7, I6, S0.1 showed the highest color scores (p<0.05). The lowest color score belonged to sample H2.5, I2, S0.3 (p<0.05). By increasing the percentage of hibiscus tea and decreasing the percentage of inulin, the transparency of the samples increased significantly (p<0.05). Alizadeh et al., (2014) in investigating the effect of stevia as a sugar substitute in fruit milk, stated that there is no statistically significant difference between the color of the prepared drinks and the control drink.

Table 5. Comparison of color components of probiotic drink samples produced with different levels of inulin and stevia sour tea. Different small letters in the column indicate significant differences (p<0.05).

Samples	L*	a*	b*
H2.5, I2, S0.1	54.36±0.22 ^{a}	43.65±0.16 ^{c}	15.91±0.64 ^{l}
H2.5, I2, S0.2	53.93±0.40 ^{ab}	43.82±0.03 ^{c}	16.39±0.85 ^{k}
H2.5, I2, S0.3	54.24±0.30 ^{a}	43.65±0.28 ^{c}	16.40±0.26 ^{k}
H2.5, I4, S0.1	53.83±0.42 ^{ab}	44.03±0.34 ^{c}	18.35±0.26 ^{j}
H2.5, I4, S0.2	53.45±0.17 ^{bc}	43.74±0.16 ^{c}	18.89±0.54 ^{j}
H2.5, I4, S0.3	53.41±0.25 ^{bc}	43.92±0.15 ^{c}	18.91±0.35 ^{j}
H2.5, I6, S0.1	53.00±0.27 ^{cd}	43.87±0.29 ^{c}	20.35±0.08 ^{i}
H2.5, I6, S0.2	52.92±0.66 ^{cd}	43.80±0.28 ^{c}	20.96±0.29 ^{i}
H2.5, I6, S0.3	52.48±0.43 ^{d}	43.83±0.34 ^{c}	23.68±0.44 ^{h}
H5, I2, S0.1	49.92±0.23 ^{e}	51.00±0.31 ^{b}	24.02±0.63 ^{gh}
H5, I2, S0.2	49.86±0.14 ^{e}	51.14±0.13 ^{b}	24.44±1.03 ^{gh}
H5, I2, S0.3	49.62±0.24 ^{ef}	51.30±0.01 ^{b}	24.75±0.20 ^{g}
H5, I4, S0.1	49.39±0.17 ^{efg}	50.97±0.26 ^{b}	25.91±0.15 ^{f}
H5, I4, S0.2	49.20±0.18 ^{fgh}	50.95±0.32 ^{b}	26.10±0.47 ^{f}
H5, I4, S0.3	49.12±0.14 ^{fgh}	50.85±0.06 ^{b}	26.24±0.37 ^{f}
H5, I6, S0.1	48.87±0.21 ^{ghi}	50.88±0.43 ^{b}	27.92±0.03 ^{e}
H5, I6, S0.2	48.76±0.24 ^{hi}	50.84±0.29 ^{b}	27.90±0.31 ^{e}
H5, I6, S0.3	48.40±0.49 ^{i}	50.93±0.35 ^{b}	28.22±0.74 ^{e}
H7, I2, S0.1	45.78±0.28 ^{j}	58.02±0.40 ^{a}	29.57±0.15 ^{d}
H7, I2, S0.2	45.37±0.21 ^{j}	58.00±0.24 ^{a}	29.97±0.76 ^{d}
H7, I2, S0.3	44.77±0.14 ^{k}	57.93±0.28 ^{a}	30.02±0.44 ^{d}
H7, I4, S0.1	44.53±0.14 ^{k}	58.12±0.30 ^{a}	31.29±0.37 ^{c}
H7, I4, S0.2	44.33±0.16 ^{k}	57.89±0.34 ^{a}	31.55±0.30 ^{c}
H7, I4, S0.3	43.69±0.38 ^{l}	58.14±0.28 ^{a}	31.94±0.53 ^{c}
H7, I6, S0.1	42.98±0.28 ^{m}	58.15±0.43 ^{a}	32.84±0.58 ^{b}
H7, I6, S0.2	42.69±0.56 ^{mn}	57.71±0.20 ^{a}	33.64±0.29 ^{a}
H7, I6, S0.3	42.38±0.39 ^{n}	58.15±0.29 ^{a}	33.84±0.32 ^{a}

Table 6. Sensory evaluation of probiotic drink samples produced with different levels of inulin and stevia sour tea. Different small letters in the column indicate significant differences (p<0.05).

Samples	Taste	Odor	Mouthfeel	Color
H2.5, I2, S0.1	2.33±0.57 ^{e}	3.66±0.57 ^{bcd}	3.33±0.57 ^{c}	3.66±0.57 ^{bcd}
H2.5, I2, S0.2	2.66±0.57 ^{de}	4.00±0.00 ^{abcd}	3.66±0.57 ^{bc}	3.33±0.57 ^{cd}
H2.5, I2, S0.3	2.66±0.57 ^{de}	4.33±0.57 ^{abc}	4.00±0.00 ^{abc}	3.00±0.00 ^{d}
H2.5, I4, S0.1	3.00±1.00 ^{cde}	4.00±0.00 ^{abcd}	3.66±0.57 ^{bc}	3.66±0.57 ^{bcd}
H2.5, I4, S0.2	4.66±0.57 ^{ab}	4.33±0.57 ^{abc}	4.00±0.00 ^{abc}	4.00±1.00 ^{abcd}
H2.5, I4, S0.3	2.66±0.57 ^{de}	4.33±0.57 ^{abc}	4.00±0.00 ^{abc}	4.00±1.00 ^{abcd}
H2.5, I6, S0.1	3.33±0.57 ^{cde}	4.33±0.57 ^{abc}	4.33±0.57 ^{abc}	3.33±0.57 ^{cd}
H2.5, I6, S0.2	4.00±1.00 ^{abc}	4.00±0.00 ^{abcd}	4.33±0.57 ^{abc}	3.33±0.57 ^{cd}
H2.5, I6, S0.3	3.00±1.00 ^{cde}	3.66±0.57 ^{bcd}	4.33±0.57 ^{abc}	3.33±0.57 ^{cd}
H5, I2, S0.1	3.66±0.57 ^{bcd}	4.33±0.57 ^{abc}	4.33±0.57 ^{abc}	4.66±0.57 ^{ab}
H5, I2, S0.2	4.66±0.57 ^{ab}	5.00±0.00 ^{a}	4.66±0.57 ^{ab}	4.33±0.57 ^{abc}
H5, I2, S0.3	3.66±0.57 ^{bcd}	5.00±0.00 ^{a}	5.00±0.00 ^{a}	4.00±0.00 ^{abcd}
H5, I4, S0.1	3.66±0.57 ^{bcd}	4.66±0.57 ^{ab}	4.66±0.57 ^{ab}	4.66±0.57 ^{ab}
H5, I4, S0.2	5.00±0.00 ^{a}	5.00±0.00 ^{a}	5.00±0.00 ^{a}	4.66±0.57 ^{ab}
H5, I4, S0.3	3.33±0.57 ^{cde}	4.00±1.00 ^{abcd}	4.33±1.15 ^{abc}	4.66±0.57 ^{ab}
H5, I6, S0.1	4.66±0.57 ^{ab}	4.66±0.57 ^{ab}	5.00±0.00 ^{a}	5.00±0.00 ^{a}
H5, I6, S0.2	5.00±0.00 ^{a}	5.00±0.00 ^{a}	5.00±0.00 ^{a}	5.00±0.00 ^{a}
H5, I6, S0.3	4.00±1.00 ^{abc}	4.66±0.57 ^{ab}	5.00±0.00 ^{a}	4.33±0.57 ^{abc}
H7, I2, S0.1	3.00±0.00 ^{cde}	3.33±0.57 ^{cd}	3.33±0.57 ^{c}	4.66±0.57 ^{ab}
H7, I2, S0.2	3.66±0.57 ^{bcd}	4.00±0.00 ^{abcd}	3.66±0.57 ^{bc}	3.66±0.57 ^{ab}
H7, I2, S0.3	3.00±0.00 ^{cde}	4.00±0.00 ^{abcd}	4.00±0.00 ^{abc}	5.00±0.00 ^{a}
H7, I4, S0.1	3.00±0.00 ^{cde}	3.66±0.57 ^{bcd}	3.66±0.57 ^{bc}	4.66±0.57 ^{ab}
H7, I4, S0.2	3.66±0.57 ^{bcd}	3.66±0.57 ^{bcd}	4.00±0.00 ^{abc}	4.66±0.57 ^{ab}
H7, I4, S0.3	2.33±0.57 ^{e}	3.00±1.00 ^{d}	3.33±1.15 ^{c}	5.00±0.00 ^{a}
H7, I6, S0.1	3.66±0.57 ^{bcd}	3.66±0.57 ^{bcd}	4.00±0.00 ^{abc}	5.00±0.00 ^{a}
H7, I6, S0.2	4.00±0.00 ^{abc}	4.00±0.00 ^{abcd}	4.33±0.57 ^{abc}	4.33±0.57 ^{ab}
H7, I6, S0.3	2.66±0.57 ^{e}	3.33±0.57 ^{cd}	4.00±0.00 ^{abc}	5.00±0.00 ^{a}

3.1.11.5. Overall acceptability

The results of this research showed that sample H5, I2, S0.2, sample H5, I4, S0.2, sample H5, I6, S0.1 and sample H5, I6, S0.2 showed the highest overall acceptance score ($p<0.05$). The lowest overall acceptance score belonged to sample H2.5, I2, S0.1, sample H7, I2, S0.1 and sample H7, I6, S0.1 ($p<0.05$). By increasing the percentage of hibiscus tea and decreasing the percentage of inulin, the transparency of the samples increased significantly ($p<0.05$). Sohrab Vandi et al. (2014) investigating the effect of adding inulin and D-tagatose on the physicochemical and sensory properties of Functional grape juice admitted that the addition of prebiotic sugar caused a decrease in sensory acceptability according to sensory evaluators. Sohrab Vandi et al. (2014) in investigating the effect of some prebiotics on the physicochemical and sensory properties of diet orange juice admitted that the addition of prebiotics, either alone or in combination with sweeteners, increased the sensory acceptability of the treatments stored at refrigerator. Hashemi et al. (2015) reported that with the increase of stevia sugar, saffron diet syrup scored lower in terms of sensory and the sample containing 25% stevia and 75% sucrose had higher sensory acceptance than other samples.

4. Conclusion

According to the obtained results, at the highest levels of hibiscus tea and the lowest levels of inulin, the pH of the samples decreased significantly and the acidity increased ($p<0.05$). At the highest levels of hibiscus tea and inulin, Brix, Turbidity index of formalin samples increased significantly ($p<0.05$). In the highest levels of hibiscus tea, inulin and stevia, total phenol and antioxidant activity and population of probiotics in the samples increased significantly ($p<0.05$). Sample H5, I4, S0.2 and Sample H5, I6, S0.2 obtained the highest sensory score compared to other samples ($p<0.05$). Due to higher antioxidant activity and higher survival of probiotics in the mentioned sample, sample H5, I4, S0.2 was selected as the best sample.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Adeline K and Julie A, 2004. Sensory Evaluation of Ice Cream made with Prebiotic. Journal of rurals: Review of Undergraduate Research in Agricultural and Life Science 3: 1-9.

Al-Nemr, T. M., Abd El-Razek, A. M., Hasan, H.M. and Massoud, M.I. (2013). Improving of Karish cheese by using enhanced technological texturizing inulin. Alex. J. Agric. Res., 58: 173.

Aghabeyk, S., Ahmadi Golsefidi, M., & Soleimani, M. H. (2017). Quantitative and qualitative extraction of stevioside from Stevia rebaudiana Bertoni. using solid phase molecularly imprinted technique. Eco-phytochemical Journal of Medicinal Plants, 5(2), 86-99.

Alizadeh, M., Azizi-lalabadi, M., Hojat-ansari, H., & Kheirouri, S. (2014). Effect of Stevia as a substitute for sugar on physicochemical and sensory properties of fruit based milk shake. Journal of scientific research and reports, 3(11), 1421-1429.

Bernardeau, M., Vernoux, J. P., Henri-Dubernet, S., & Guéguen, M. (2008). Safety assessment of dairy microorganisms: the Lactobacillus genus. International journal of food microbiology, 126(3), 278-285.

Chumsri, P., Sirichote, A., & Itharat, A. (2008). Studies on the optimum conditions for the extraction and concentration of roselle (Hibiscus sabdariffa Linn.) extract. Songklanakarin Journal of Science & Technology, 30.

Chung, H. J. (2012). Comparison of physicochemical properties and physiological activities of commercial fruit juices. Korean Journal of Food Preservation, 19(5), 712-719.

Corsetti, A., & Gobbetti, M. (2002). Lactobacillus plantarum. In Encyclopedia of dairy sciences (Vol. 3, pp. 1501-1507). Academic Press Ltd..

Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, and Heinrich M. Hibiscus sabdariffa L.—A phytochemical and pharmacological review.2014; Food Chem., vol. 165, pp. 424–443, 2014, pmid:25038696.

Furlán, L.T.R.; Baracco, Y.; Lecot, J.; Zaritzky, N.; Campderrós, M.E. Influence of hydrogenated oil as cocoa butter replacers in the development of sugar-free compound chocolates: Use of inulin as stabilizing agent. Food Chem. 2017, 217, 637–647.

Fakhri, L. A., Ghanbarzadeh, B., & Falcone, P. M. (2023). Development of a Novel Low-Calorie Lime Juice-Based Prebiotic Beverage Using a Combined Design Optimization Methodology. Foods, 12(3), 680.

Fathi Achacheloi Bahram, Mahmoudi Maghas Houshang. The effect of different levels of inulin and chitosan on physical and chemical characteristics, sensory and viability of probiotic bacteria in symbiotic buttermilk. Iranian journal of food science and industry. 1397; 15 (75): 245-235.

Gupta, A., Sanwal, N., Bareen, M. A., Barua, S., Sharma, N., Olatunji, O. J., & Sahu, J. K. (2023). Trends in functional beverages: functional ingredients, processing technologies, stability, health benefits, and consumer perspective. Food Research International, 113046.

Ghavidel, R. A., Karimi, M., Davoodi, M., Jahanbani, R., & Asl, A. F. A. (2014). Effect of fructooligosaccharide fortification on quality characteristic of some fruit juice beverages (apple & orange juice). International Journal of Farming and Allied Sciences, 3(2), 141-146.

Guven, M., Yasar, K., Karaca, O. B., & Hayaloglu, A. A. (2005). The effect of inulin as a fat replacer on the quality of set-type low-fat yogurt manufacture. International journal of dairy Technology, 58(3), 180-184.

Guggisberg, D., Piccinali, P., & Schreier, K. (2011). Effects of sugar substitution with Stevia, Actilight™ and Stevia combinations or Palatinose™ on rheological and sensory characteristics of low-fat and whole milk set yoghurt. International Dairy Journal, 21(9), 636-644.

Hosseini S, Goli S A H, Keramat J. Production Optimization of Low-Calorie Orange Nectar Using Stevioside Sweetener and Evaluation of Its Physicochemical Properties during Storage. Journal of Crop Production and Processing 2015; 5 (16) :39-52.

Hatamnia, A. A., Abbaspour, N., & Darvishzadeh, R. (2014). Antioxidant activity and phenolic profile of different parts of Bene (Pistacia atlantica subsp. kurdica) fruits. Food chemistry, 145, 306-311.

Hainida, K. E., Amin, I., Normah, H., & Esa, N. M. (2008). Nutritional and amino acid contents of differently treated Roselle (Hibiscus sabdariffa L.) seeds. Food chemistry, 111(4), 906-911.

Hashemi, N., Rabiee, H., Tavakolipour, H., & Gazerani, S. (2016). Effect of Stevia (Stevia rebaudiana) as a Substitute for Sugar on Physicochemical, Rheological and Sensory Properties of Dietary Saffron Syrup. Saffron Agronomy & Technology, 4.

Islam, M. M. (2019). Food and medicinal values of Roselle (Hibiscus sabdariffa L. Linne Malvaceae) plant parts: A review. Open J Nutr Food Sci, 1(1), 1003.

Kalinowska M, Bielawska A, Lewandowska-siwkiewicz H, and Priebe W. Plant Physiology and Biochemistry Apples: Content of phenolic compounds vs. variety, part of apple and cultivation model, extraction of phenolic compounds, biological properties. Vol. 84, 2014, pmid:25282014

Kasapoğlu, K.N.; Daşkaya-Dikmen, C.; Yavuz-Düzgün, M.; Karaça, A.C.; Özçelik, B. Enrichment of Beverages with Health Beneficial Ingredients. In Value-Added Ingredients and Enrichments of Beverages; Academic Press: Cambridge, MA, USA, 2019; pp. 63–99.

Kalyani Nair, K., Kharb, S., & Thompkinson, D. K. (2010). Inulin dietary fiber with functional and health attributes—A review. Food Reviews International, 26(2), 189-203.

Khmerian, Rezaamir, Jovindeh, Hossein, Hissari, Javad, and Barzegar, Hassan. (2016). Optimizing and investigating the physicochemical, microbial and sensory properties of the orange probiotic drink produced on the basis of Traveh. Iran Food Science and Industry, 14(65), 185-197.

Kargozari, Mi., Bagheri, L & Mohammadi, A (2016). Dietary and beneficial lime drink, stabilization and investigation of physicochemical and sensory properties. Iran Journal of Food Science and Industry Research, 13(2), 214-226

Maciel, L. G., do Carmo, M. A. V., Azevedo, L., Daguer, H., Molognoni, L., de Almeida, M. M., ... & Rosso, N. D. (2018). Hibiscus sabdariffa anthocyanins-rich extract: Chemical stability, in vitro antioxidant and antiproliferative activities. Food and Chemical Toxicology, 113, 187-197.

Monteiro, M. J. P., Costa, A. I. A., Fliedel, G., Cissé, M., Bechoff, A., Pallet, D., ... & Pintado, M. M. E. (2017). Chemical-sensory properties and consumer preference of hibiscus beverages produced by improved industrial processes. Food Chemistry, 225, 202-212.

Mozaffari-Khosravi, H., Jalali-Khanabadi, B. A., Afkhami-Ardekani, M., & Fatehi, F. (2009). Effects of sour tea (Hibiscus sabdariffa) on lipid profile and lipoproteins in patients with type II diabetes. The Journal of Alternative and Complementary Medicine, 15(8), 899-903.

Markov, S. L., Cvetković, D. D., & Velićanski, A. S. (2012). The availability of a lactose medium for tea fungus culture and kombucha fermentation. Archives of Biological Sciences, 64(4), 1439-1447.

Motamedzadegan, A, Porshaigan, M., Golkar, A., Maqshoodi, S, and Bagheri, Haditha. (2018). Effect of concentration process on physicochemical and rheological properties of grapefruit juice (Citrus paradise). Food processing and preservation, 11(1), 131-142.

Malik, V. S., & Hu, F. B. (2022). The role of sugar-sweetened beverages in the global epidemics of obesity and chronic diseases. Nature Reviews Endocrinology, 18(4), 205-218.

Mokoena, M. P., Omatola, C. A., & Olaniran, A. O. (2021). Applications of lactic acid bacteria and their bacteriocins against food spoilage microorganisms and foodborne pathogens. Molecules, 26(22), 55-70.

Mortazavian, A. M., Ehsani, M. R., Mousavi, S. M., Rezaei, K., Sohrabvandi, S., & Reinheimer, J. A. (2007). Effect of refrigerated storage temperature on the viability of probiotic micro-organisms in yogurt. International Journal of Dairy Technology, 60(2), 123-127.

Nemati, A., Alizadeh-Khaledabad, M., & Ghasempour, Z. (2020). Optimization of orange juice formulation through using lactose-hydrolyzed permeate by RSM methodology. Food Science & Nutrition, 8(8), 4584-4590.

Najgebauer-Lejko, D., Sady, M., Grega, T., & Walczycka, M. (2011). The impact of tea supplementation on microflora, pH and antioxidant capacity of yoghurt. International Dairy Journal, 21(8), 568-574.

Ozuna, C., & Franco-Robles, E. (2022). Agave syrup: An alternative to conventional sweeteners? A review of its current technological applications and health effects. Lwt, 162, 113434.

Preciado-Saldaña, A. M., Abraham Domínguez-Avila, J., Fernando Ayala-Zavala, J., Villegas-Ochoa, M. A., Sáyago-Ayerdi, S. G., Wall-Medrano, A., ... & González-Aguilar, G. A. (2019). Formulation and characterization of an optimized functional beverage from hibiscus (Hibiscus sabdariffa L.) and green tea (Camellia sinensis L.). Food Science and Technology International, 25(7), 547-561.

Rupasinghe, H. V., Yu, L. J., Bhullar, K. S., & Bors, B. (2012). Haskap (Lonicera caerulea): A new berry crop with high antioxidant capacity. Canadian Journal of Plant Science, 92(7), 1311-1317.

Rakin, M., Vukasinovic, M., Siler-Marinkovic, S., & Maksimovic, M. (2007). Contribution of lactic acid fermentation to improved nutritive quality vegetable juices enriched with brewer's yeast autolysate. Food chemistry, 100(2), 599-602.

Rastegari, H, Tehranifar, A., Nemati, S. H & vazifehshenas, M. (2013). The application of salicylic acid before fruit harvesting on the properties of pomegranate after harvesting and storage in cold storage. Horticultural science, 28(3), 360-368.

Shivanna, N., Naika, M., Khanum, F., & Kaul, V. K. (2013). Antioxidant, anti-diabetic and renal protective properties of Stevia rebaudiana. Journal of Diabetes and its Complications, 27(2), 103-113.

Saraswati, A. R., & Taniwiryono, D. (2020). Formulation of ready to drink (rtd) made from roselle (Hibiscus sabdariffa. L) tea and stevia (stevia rebaudiana) leaf safe for diabetics. Indonesian Journal of Applied Research (IJAR), 1(1), 1-9.

Sowalsky, R. A., & Noble, A. C. (1998). Comparison of the effects of concentration, pH and anion species on astringency and sourness of organic acids. Chemical senses, 23(3), 343-349.

Sheikholeslami,M., pedram nia, A., Jalali M. The effect of date liquid sugar and stevia extract as a sugar substitute on the quality characteristics of apple-lemon juice. FSCT 2022; 19 (126) :175-191.

Sohrab Vandi, S., Sarmadi, B., Nemat Elahi, A., Kamili Fanood, R &Mohammad Mortazavian Farsani, S.A. (2014). Investigating the effect of adding inulin and D-tagatose on the physicochemical and sensory properties of Functional grape juice. Journal of Nutritional Sciences and Food Industries of Iran. 10, 4, 115-124.

Safari, O., Ajali, A., Arian Far, A., (2013). the use of natural sweetener stevia as a substitute for sucrose in orange drink and the investigation of its physicochemical and sensory properties, the third national conference of food science and industry, Qochan

Tratnik, L., BOŽANIĆ, R., Herceg, Z., & Drgalić, I. D. A. (2006). The quality of plain and supplemented kefir from goat's and cow's milk. International Journal of Dairy Technology, 59(1), 40-46.

Vivek, K. (2013). Use of encapsulated probiotics in dairy based foods. Int J Food, Agricultural and Veterinary Sciences, 3(1), 188-99.

Wong, P.K., Yusof, S., Ghazali, H.M. and Che-Man, Y.B. (2002) Physico-chemical characteristics of roselle (Hibiscus sabdariffa L.). Nutrition and Food Science 32: 68 – 73.

Zhen, J., Villani, T. S., Guo, Y., Qi, Y., Chin, K., Pan, M. H & Wu, Q. (2016). Phytochemistry, antioxidant capacity, total phenolic content and anti-inflammatory activity of Hibiscus sabdariffa leaves. Food chemistry, 190, 673-680.