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Effect of green tea powder supplementation on probiotic potential, antioxidant, physicochemical, and sensory properties of probiotic ice cream

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ABSTRACT

In recent years, the demand for healthy functional foods has increased and food industry is trying to meet these new needs. This study aimed to assess the effect of different concentrations of green tea powder (GTP) on the physicochemical characteristics, sensory properties, antioxidant activity, and culture viability of probiotic ice cream over a 90-day storage period. Seven ice cream samples containing 0–3% GTP and *Lactobacillus casei* were produced and evaluated at 0, 30, 60, and 90 days of storage. Folin-Ciocalteu and diphenyl picrylhydrazyl (DPPH) methods were applied to determine the total polyphenol content and antioxidant property, respectively. The results showed that the titratable acidity, antioxidant activity, total polyphenols, viscosity, melting resistance, and viable count of *L. casei* increased with increasing GTP concentration ($p<0.05$). Also over time, there was no significant difference in viscosity and total solids, but the total phenol, antioxidant activity, and viability of probiotic bacteria decreased significantly ($p<0.05$). The results of the sensory evaluation revealed that the flavor and color of the samples containing 1.5 and 2% GTP had the highest scores ($p<0.05$). Samples containing 1.5 % GTP that had the highest score of color, flavor, and overall acceptability, high antioxidant activity and viable count of *L. casei* $\geq 10^7$ cfu/g during storage could be introduced as a new product with health-promoting properties.

1. Introduction

Ice cream is a frozen dairy multiphase product prepared from milk, sweeteners, emulsifiers, stabilizers, and either with or without the addition of other substances and ingredients. Ice cream is accepted by all age groups and contains substances such as dairy raw materials, minerals and vitamins, and can act as a food carrier for probiotic microorganisms [1]. Probiotic microorganisms such as *Bifidobacteria* and lactic acid bacteria (LAB) are living bacteria that have beneficial effects on human health by increasing the gut microbiota and inhibiting carcinogenesis [2]. Previous studies have shown the antibacterial activity of probiotic microorganisms against drug-resistant human pathogenic bacteria [3, 4]. Other beneficial effects of probiotics include, lowering blood cholesterol level [5], prevention or treatment of antibiotic-related and infectious diarrhea [6], and immunomodulatory effects [7].

At present, the significance of diet for the prevention of some diseases is well known. Therefore, consumers prefer foods that have simple but obvious health benefits. Functional food refers to food that contains one or more health-enhancing compound(s) beyond traditional foods [1, 8]. Although ice cream is one of the most widely consumed dairy products in the world and has good potential for use as a probiotic vehicle, it is generally poor in terms of natural colors, antioxidants and polyphenols.

Recently, studies on ice cream technology concentrate on developing new ice cream formulas using health benefits such as spices and herbs [9, 10]. Also, numerous functional ice cream has been made, for instance, probiotic ice cream, dietary fiber fortified ice cream, and ice cream containing natural antioxidants [11-13]. Antioxidant compounds are momentous in foods because they can diminish free radical-mediated degradation of cells and tissues in an organism, vegetables, and legumes [10]. One of the most important sources of antioxidants is herbal infusions (especially tea). Many studies have examined the effect of tea, tea extracts, and tea polyphenols in reducing the risk of various types of cancer and cardiovascular diseases. The health claims include reduced risk of neural problems, pulmonary ailments, diabetes, and antibacterial and antiviral effects. Flavenoids namely, catechins and their derivatives are among the most momentous bioactive compounds that are responsible for these health-giving properties [14]. These properties make tea not only very popular as a beverage, but its nutritional merits with the tendency of the consumers to find new flavor perceptions in dairy products have caused the emergence of new tea-flavored yogurts, as well as researchers' increased interest in developing yogurts with the addition of tea extracts [15].

There are two important criteria for determining the effectiveness and successful use of a probiotic products: a) consumer acceptance and (b) viability of probiotic strains during processing and storage period [16]. Previous studies showed that addition of green tea extract could increase the bacterial population of probiotic milk [17]. In another study by Najgebauer-Lejko et al., green tea infusion had no effect on the level of *Bifidobacterium lactis* BB-12 and *Streptococcus thermophilus* in yogurts, and the viability of *Lactobacillus acidophilus* LA-5 depended on the concentration and type of probiotic milk [15]. However, to our knowledge there is no information on the effect of green tea powder on culture viability of probiotic ice cream containing *Lactobacillus casei.* Therefore, this study performed to evaluate the effect of different concentration of GTP on physicochemical, antioxidant, sensory properties, and bacterial survival of probiotic ice creams containing *Lactobacillus casei* during storage at -24 ºC for 90 d.

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2. Materials and methods

2.1 Green tea experiments

Green tea leaves were purchased from Golestan Company, Iran and were ground to an average particle size of 20 mesh by using analytical mill (IKA M20 Universal). Moisture content of green tea powder was assessed using an oven (Fan Azma Gostar, Iran) at 105 °C and dried until constant weight [18]. Fiber and ash content were determined by standard methods [19]. The polyphenol content of green tea powder was assessed using Folin-Ciocalteu method as recommended by the International Organization for Standardization (ISO) [20] and catechins content was analyzed by the ISO 14502-2 method [21].

2.2 Probiotic ice cream manufacture

Ice cream was prepared in Domino Ice cream Company, Tehran, Iran. Sterilized, cow milk (3% fat) (65%) (Domino Dairy, Iran) and sterilized cream (30% milk fat) (14%) (Domino Dairy, Iran) entered the formulation tank and was heated slowly. After the cream was well mixed at 50 °C, the mixture of dry materials, including sugar (16%) (Shahroud, Iran), skim milk powder (4%) (Aryarama, Iran), and stabilizer/emulsifier mixture (guar gum, mono- and diglycerides of fatty acids, carrageenan, and locust bean gum) (0.7%) (Cremodan SE 709 VEG, Danisco, Denmark) was added. Then vanilla (0.07%) or Green tea powder (GTP) as flavoring material was added while the mixer was working. The prepared mixture was then pasteurized at 72 °C for 10 minutes. Afterward, the ice cream mixture was homogenized in a one-step laboratory homogenizer (APV, Denmark) at 80 °C with a pressure of 40 Bar. The mixture was then cooled to 4 °C and kept for 12 hours at this temperature to complete the ice cream production process. The starter culture of *Lactobacillus casei* 431® (Chr. Hansen, Denmark) was added to all mixes of ice cream to assess approximately 10^8 cfu/g, mixed well and kept for 4 h at 5 °C. The mixtures were then frozen (Tetra Pak Hoyer FF 4000, Denmark) at -40 °C for 10 min and packed in plastic cups and stored at -24 °C.

In this study, seven different kinds of probiotic ice cream samples including: control sample with vanilla and without any green tea (code 0), ice cream with 0.5 (code 1), 1 (code 2), 1.5 (code 3), 2 (code 4), 2.5 (code 5), and 3% of GTP (code 6) were produced. The ice creams were manufactured in three independent trials. Physicochemical, microbial, antioxidant, and sensory analysis were carried out at 0, 30th, 60th, and 90th days of storage at -24 ºC.

2.3 Physicochemical tests

The ice cream samples were assessed for total solids, titratable acidity, and pH as described previously [22]. Rheological measurements of the ice cream samples were assessed at 25 °C after aging using a Brookfield DV2T Rheometer as described previously (Brookfield, USA) [23].

To evaluate the melting resistance of ice cream samples, plastic cups containing ice cream were cut and the samples were immediately placed on a wire mesh with 2 mm pores on the glass funnel at 25 °C. The amount of ice cream emptied into the cup was weighed every 10 min for 30 min and the melting resistance was measured as follows:

$$\text{Melting resistance }\% = \frac{A1 - A2}{A1} \times 100$$

Where A1 is the weight of initial sample (30 g) and A2 is the weight of melted sample, [24].

2.4 Antioxidant activity Measurement

To determine the antioxidant activity, 50 g of ice cream samples were added to 25 mL of methanol (Merck, Germany) and were placed for 12 h at 25 °C. Then, the mixtures were filtered through filter paper (Whatman No.4) (Sigma, Aldrich) and 3 mL of each filtrate was added to 1.2 mL methanol and 1.5 mL DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) (0.10 mM in methanol) (sigma Aldrich). After incubation for 90 min at room temperature, the absorbance was determined against a blank (methanol) at 517 nm (UNICO 2150-UV Spectrophotometer, China). Inhibition free radical DPPH in percent (I %) was determined as follows:

$$I\,\% = \frac{A\,control - A\,sample}{A\,control} \times 100$$

Where A_{control} represents the absorbance of the control reaction, which contains all reagents except test component. A_{sample}, indicates the absorbance of the test component. The concentration of sample needed to scavenge 50% of free radicals (IC50) was determined from the regression equation [25].

2.5 Measurement of polyphenol content

For the extraction of phenolics, 0.5-1 g of ice cream sample was added to 50 mL methanol (Merck, Germany), and the mixture was stirred for 4 h on a magnetic hotplate (WiseStir MSH-30D, Germany). At the next step, the mixture was sonicated for 20 min (UP400S, Hielscher, Germany). Then, the methanolic extract was filtered (Whatman No.4) and aliquots were stored in tubes at −20 °C until analysis. Total phenolics constituents of the mentioned extracts was measured using Folin-Ciocalteu reagent (Sigma, Aldrich) as described previously [26].

2.6 Enumeration of probiotic bacteria

To determine the viability of *L. casei* during storage time, sampling was done on production day, 30th, 60th, and 90th day of storage. The samples were then melted at room temperature and serially diluted in physiological saline. The diluted suspension was pour plated onto the De Man, Rogosa and Sharpe (MRS) Agar (Merck, Germany) culture medium and incubated anaerobically at 37 ºC for 72 h. The number of viable cells was stated as log cfu/ g.

2.7 Sensory evaluation test

Sensory analysis of ice cream samples was carried out using nine trained tasters. The tasters examined the samples by tasting and recording their perceptions using a five-point grade hedonic method. Factors of taste, texture, color, and general acceptance was evaluated in five scores, very good (5), good (4), medium (3), bad (2), very bad (1) [27].

2.8 Statistical analysis

All tests were repeated three times and the results were presented as mean± standard deviation (SD). To evaluate the data, SPSS 16 software was used and the significant differences between mean values were evaluated by Duncan's test. The P-value ≤ 0.05 was considered significant. In the case of sensory analysis, the mean values were taken from the grade scores recorded by the tasters.

3. Results and discussion

3.1 Physicochemical results of green tea powder

The ash, moisture, polyphenols, catechin, and fiber content of green tea powder were found to be 4.49±0.01, 4.67±0.01, 11.41±0.02, 7.49±0.01 and 16.21±0.05 g/ 100g, respectively.

3.2 pH and acidity of ice cream

The results of pH and acidity of ice cream are shown in Table 1. Our finding showed that the titratable acidity and pH of samples containing 3% GTP were significantly higher and lower than other treatments, respectively ($p<0.05$). Subsequent ratings of the highest acidity and lowest pH were assigned to samples with 2.5 and 2% GTP, respectively. The control had the lowest acidity and highest pH ($p<0.05$). As shown in Table 1, increasing the GTP level in ice cream increased acidity (between 0.16 and 0.25 % lactic acid) and decreased pH (between 6.32 and 6.46) of all samples. In this study, the pH of GTP was measured to be 5.5, and the pH reduction of ice cream samples with the addition of GTP can be attributed to the low pH of GTP. Our finding are consistent with the results of

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Dönmez et al. (2017) that previously reported changes in the pH values of yogurt with the addition of green tea powder [28]. Various studies have shown that the duration of storage has no significant effect on pH and acidity of probiotic ice cream [29, 30], which was in line with our study. Acidity is one of the indicators showing the microbiological activity of bacteria. In our study, samples with higher levels of GTP showed higher acidity, which may be due to the higher viability of *Lactobacillus* in the presence of higher amounts of GTP.

Table 1. pH and acidity (% lactic acid) (% l.a) (means ± SD) of probiotic ice cream samples containing different concentrations of GTP during storage time (n=3).

Treatment	**Storage days**			
	0	30	60	90
pH				
Code 0	6.46 ±0.01^{aA}	6.43±0.03 ^{aA}	6.40±0.02^{aAB}	6.37±0.06 ^{aB}
Code 1	6.42±0.02^{abA}	6.39±0.05 ^{abA}	6.38±0.01^{abAB}	6.33±0.02 ^{abB}
Code 2	6.40±0.02^{bcdA}	6.38±0.04 ^{bcA}	6.36±0.06 ^{bcAB}	6.30±0.01 ^{bcB}
Code 3	6.38±0.01^{bcdA}	6.36±0.02 ^{bcdA}	6.33±0.01 ^{bcdAB}	6.28±0.05 ^{bcdB}
Code 4	6.36±0.01^{cdeA}	6.34±0.05 ^{cdeA}	6.31±0.02 ^{cdeAB}	6.26±0.03 ^{cdeB}
Code 5	6.34±0.01^{deA}	6.32±0.06 ^{deA}	6.30±0.04^{deAB}	6.25±0.01 ^{deB}
Code 6	6.32±0.01^{eA}	6.30±0.02 ^{eA}	6.28±0.03^{eAB}	6.24±0.01 ^{eB}
Acidity (% l. a)				
Code 0	0.16±0.01 ^{gC}	0.17±0.01 ^{gBC}	0.18±0.01 ^{gAB}	0.19±0.01 ^{gA}
Code 1	0.17±0.01 ^{fC}	0.18±0.01 ^{fBC}	0.19±0.01 ^{fAB}	0.20±0.01 ^{fA}
Code 2	0.18±0.02 ^{eC}	0.19±0.01 ^{eBC}	0.20±0.01 ^{eAB}	0.21±0.01 ^{eA}
Code 3	0.19±0.02 ^{dC}	0.20±0.01 ^{dBC}	0.21±0.01 ^{dAB}	0.22±0.00 ^{dA}
Code 4	0.20±0.01 ^{cC}	0.21±0.01 ^{cBC}	0.22±0.01 ^{cAB}	0.23±0.01 ^{cA}
Code 5	0.23±0.02 ^{bC}	0.24±0.00 ^{bBC}	0.25±0.00 ^{bAB}	0.26±0.00 ^{bA}
Code 6	0.25±0.02 ^{aC}	0.26±0.00 ^{aBC}	0.27±0.00 ^{aAB}	0.28±0.01 ^{aA}

$^{a\text{-}b}$ different characters represent significant differences (P<0.05) per column. $^{A\text{-}B}$ different characters represent significant differences (P<0.05) per row. Samples ingredients: Code 0: probiotic ice cream with no GTP, Code 1 to 6: probiotic ice cream containing 0.5%, 1, 1.5, 2, 2.5 and 3% GTP, respectively.

3.3 Total solids and analysis of apparent viscosity

The results of comparing the mean dry matter of the samples are displayed in Table 2 and indicate that the dry matter of samples containing 3% GTP was higher than other treatments significantly (p<0.05). The control sample had the lowest dry matter content (p<0.05), and by increasing the percentage of green tea, the dry matter of the samples increased significantly (p<0.05). Also, no significant statistical difference in the dry matter of the samples was observed over time (p<0.05). Table 2 also represents the results of the mean viscosity of the ice cream samples. The highest viscosity was obtained in the samples with 3% followed by 2.5 % of green tea powder. However, a significant difference was observed between the above-mentioned treatments (P<0.05). Control samples had the lowest viscosity and then the lowest viscosity was obtained in ice cream samples with 0.5% GTP. No significant difference was found between these treatments (p>0.05). Viscosity is a momentous physical property of ice cream that has a great impact on its sensory quality, especially the melting resistance and texture of ice cream [31]. Our finding showed increasing GTP level increased viscosity index of all samples. Similarly, Karaman and Kayacier (2012) in their studies, proved that adding black tea or some herbal teas (linden, chamomile, and sage) in the formulation of dairy products led to improved viscosity [10]. In another study, Najgebauer-Lejko et al. (2020), found an increase in the viscosity index of yogurt with the addition of green and Pu-erh teas in comparison to the control yogurt [32]. However, Shokery et al. (2017) reported no significant differences in

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rheological properties of freshly made yogurt with and without the addition of green tea extract; but observed a significant increase in viscosity after 15 days of storage [8]. Contrary to their finding, the storage period had no significant effect on viscosity index of our samples.

Table 2. Dry matter (%) and viscosity results (means ± SD) of probiotic ice cream containing different concentrations of GTP during storage time (n = 3).

Treatment	**Storage days**			
	0	30	60	90
Dry matter (%)				
Code 0	38.30±0.03 fA	38.29±0.02 fA	38.26±0.01 fA	38.20±0.03 fA
Code 1	38.55±0.03 eA	38.50±0.05 eA	38.30±0.07 eA	38.25±0.09 eA
Code 2	38.60±0.08 eA	38.57±0.02 eA	38.35±0.01 eA	38.30±0.05 eA
Code 3	39.13±0.13 dA	39.10±0.10 dA	38.95±0.15 dA	38.70±0.18 dA
Code 4	39.80±0.10 cA	39.71±0.20 cA	39.10±0.11 cA	38.95±0.14 cA
Code 5	40.30±0.01 bA	40.25±0.11 bA	40.00±0.20 bA	39.60±0.11 bA
Code 6	41.00±0.04 aA	40.89±0.08 aA	40.68±0.09 aA	40.10±0.07 aA
Viscosity (Pa. s)				
Code 0	144.66±2.08 fA	144.64±2.00 fA	144.62±1.00 fA	143.66±0.70 fA
Code 1	145.24±1.02 fA	145.20±1.20 fA	145.15±1.30 fA	144.50±1.32 fA
Code 2	151.74±0.95 eA	151.70±0.85 eA	151.65±0.49 eA	150.60±0.62 eA
Code 3	163.00±0.80 dA	163.10±0.90 dA	163.00±0.60 dA	162.10±0.70 dA
Code 4	171.00±0.90 cA	171.10±0.80 cA	171.00±0.60 cA	170.20±0.76 cA
Code 5	174.00±0.65 bA	174.20±0.70 bA	174.00±0.90 bA	173.30±0.75 bA
Code 6	178.00±0.80 aA	178.20±0.60 aA	178.00±0.70 aA	177.50±0.80 aA

a-b different characters display significant differences (P<0.05) per column. A-B different characters display significant differences (P<0.05) per row. Samples ingredients: Code 0: probiotic ice cream with no GTP, Code 1 to 6: probiotic ice cream containing 0.5%, 1, 1.5, 2, 2.5 and 3% GTP, respectively.

3.4 Melting resistance analysis

The results of the melting resistance of samples (after 10 min) showed that by increasing the percentage of GTP, the melting resistance of samples was increased significantly (P<0.05) (Table 3). However, no significant difference was found between samples containing 1 and 1.5% GTP (p>0.05). The results of the melting resistance of samples after 20 and 30 min showed that by increasing the percentage of GTP, the melting resistance of samples was increased significantly (P<0.05). The melting time of ice cream is the time required for the melting of ice cream. The melting resistance of favorable ice cream is high when served at room temperature. The ice cream with higher melting rates is not considered favorable by consumers because it loses its texture and appearance at room temperature fast. To achieve a high-quality ice cream, its melting time is better to be between 10 -15 minutes. In this study, increasing the amount of GTP significantly increased the melting resistance (after 10, 20 and 30 minutes) in all treatments, which can be due to the increased viscosity.

Table 3. Melting resistance (%) (means ± SD) of probiotic ice containing different concentrations of green tea powder at intervals of 10, 20 and 30 minutes (n=3).

Treatment	**Melting resistance (%)**		
	After 10 min	After 20 min	After 30 min
Code 0	69.00±0.25 eA	60.00±0.50 gB	46.00±0.50 gC
Code 1	72.00±0.25 dA	62.00±0.15 fB	54.00±0.10 fbC
Code 2	85.00±0.20 cA	65.00±0.90 eB	58.00±0.20 eC
Code 3	86.00±0.25 bcA	72.00±0.15 dB	61.00±0.40 dC
Code 4	87.00±0.20 bA	77.00±0.30 cB	63.00±0.20 cC
Code 5	89.00±0.40 aA	79.00±0.10 bB	66.00±0.10 bC
Code 6	90.00±1.50 aA	81.00±0.50 aB	71.00±0.40 aC

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[a-b] different characters represent significant differences (P<0.05) per column. [A-B] different characters represent significant differences (P<0.05) per row. Samples ingredients: Code 0: probiotic ice cream with no GTP, Code 1 to 6: probiotic ice cream containing 0.5%, 1, 1.5, 2, 2.5 and 3% GTP, respectively.

3.5 Total phenolic compounds and antioxidant activity analysis

The results of total phenolic contents and antioxidant activity (IC50) of ice cream samples are presented in Table 4. The level of total phenolics in ice cream samples varied between 3.00 and 25.40 µg gallic acid equivalent (GAE)/ mL prior to the storage. With increasing green tea, the total phenol content of the samples increased significantly (p<0.05). Also, over time, phenol content of all samples were significantly decreased (p<0.05). The results indicated that the highest IC50 (lowest antioxidant activity) was in the control and lowest IC50 (highest antioxidant activity) was in samples containing 3% GTP (P<0.05). Also, over time, the rate (IC50) of all samples increased significantly (p<0.05). In the present study, by increasing the percentage of GTP, the antioxidant activity of the samples increased. An increase in antioxidant activity can be attributed to the green tea phenolics. Various studies have been reported that adding green tea and moringa extract [8] and black tea and some herbal teas (linden, chamomile, and sage) [10], to dairy products can increase phenolic compounds and antioxidant activity of these products, respectively.

Table 4. Total phenol and IC50 (means ± SD) of probiotic ice cream samples containing different concentrations of GTP during storage time (n=3).

Treatment	**Storage days**			
Total Phenol (µg GAE/mL)	0	30	60	90
Code 0	3.00 ± 0.08^{gA}	$2.12\ 0.05^{fB}$	2.00 ± 0.02^{gC}	1.49 ± 0.04^{gD}
Code 1	7.78 ± 0.12^{fA}	6.54 ± 0.10^{eB}	5.42 ± 0.09^{fC}	4.28 ± 0.12^{fD}
Code 2	15.76 ± 0.15^{eA}	14.26 ± 0.25^{dB}	12.76 ± 0.25^{eC}	11.33 ± 0.10^{eD}
Code 3	21.48 ± 0.10^{dA}	19.22 ± 0.20^{cB}	18.43 ± 0.10^{dC}	17.24 ± 0.20^{dD}
Code 4	23.00 ± 0.10^{cA}	22.10 ± 0.10^{bB}	21.10 ± 0.20^{cC}	19.12 ± 0.20^{cD}
Code 5	24.52 ± 0.15^{bA}	23.22 ± 0.15^{aB}	22.12 ± 0.25^{bC}	20.42 ± 0.25^{bD}
Code 6	25.40 ± 0.20^{aA}	23.40 ± 0.15^{aB}	21.65 ± 0.25^{bD}	20.70 ± 0.10^{aD}
IC50				
Code 0	9.00 ± 0.08^{aD}	10.12 ± 0.04^{aC}	11.00 ± 0.09^{aB}	12.49 ± 0.08^{fA}
Code 1	7.10 ± 0.12^{bD}	8.54 ± 0.10^{bC}	9.42 ± 0.09^{bB}	10.28 ± 0.12^{fA}
Code 2	4.59 ± 0.15^{cD}	5.26 ± 0.25^{cC}	6.76 ± 0.25^{cB}	7.33 ± 0.10^{eA}
Code 3	2.59 ± 0.10^{dD}	3.22 ± 0.20^{dC}	4.43 ± 0.10^{dB}	5.24 ± 0.20^{dA}
Code 4	1.67 ± 0.10^{eD}	2.40 ± 0.10^{eC}	3.65 ± 0.20^{fB}	4.70 ± 0.20^{cA}
Code 5	1.47 ± 0.15^{eD}	2.22 ± 0.15^{eC}	3.12 ± 0.25^{fB}	4.42 ± 0.25^{bA}
Code 6	1.23 ± 0.20^{fD}	2.10 ± 0.10^{fC}	3.10 ± 0.20^{eB}	4.12 ± 0.10^{aA}

[a-b] different characters display significant differences (P<0.05) per column. [A-B] different characters show significant differences (P<0.05) per row. Samples ingredients: Code 0: probiotic ice cream with no GTP, Code 1 to 6: probiotic ice cream containing 0.5%, 1, 1.5, 2, 2.5 and 3% GTP, respectively.

3.6 Viability analysis of probiotic bacteria

The results of comparing the viability of *L. casei* in probiotic ice samples are presented in Table 5. The viable count of *L. casei* was in the range of 6.04 - 8.8 log cfu/ g. Furthermore, ice creams with 2.5 and 3% of GTP provided higher viable counts on day 30, 60 and 90 of storage compare to lower concentration of GTP (p<0.05). One of the most important issues in the production of probiotic products is the minimum changes in the population of probiotics during the storage of the product. To evaluate the effects of probiotics on humans, the number of live microorganisms must be more than log 6 cfu/ g to provide the appropriate amount of daily dose of 10^6-10^9 live bacteria [33]. Some researchers have examined the effect of different concentrations of green tea extract, green tea epigallocatechin gallate, and catechin hydrate on the growth and viability of probiotic bacteria and stated that the growth of *Lactobacillus helveticus* has increased significantly by supplementation of the medium with green tea extracts [34].

In the present study, samples with 2.5 and 3% of GTP provided higher viable counts on day 30, 60 and 90 of storage compare to lower concentration of GTP. It is possible to express that during the storage period, samples that had more antioxidant activity showed higher viability. In general, it can be stated that the antioxidant role of green tea in the viability of probiotic bacteria was obvious. Also, over time, the survival of *L. casei* was significantly reduced in all samples (p<0.05). One of the reasons for the reduction of microorganisms' viability can be attributed to the reduction of their antioxidant effect over time. Our findings are in concordance with the results obtained by Marhamatizadeh et al. (2013) that reported a direct relationship between the increase in the bacterial population of probiotic milk and increasing the concentration of the green tea extract, which was attributed to the phenolic compounds in the extract [17]. In another study, Shah et al. (2010) used green tea extract as a source of antioxidants to enrich fruit juice and report that green tea can increase the viability of probiotic bacteria [35]. However, Cakmakci et al. (2019) found that the addition of green tea powder into milk had no effect on the viability of yogurt bacteria during fermentation [14]. This suggests that various factors including type and composition of tea, probiotic microorganism used, or even the type of product can affect the survival of selected microorganism.

Table 5. Viability of *Lactobacillus casei* (means ± SD) in probiotic ice cream samples containing different concentrations of GTP during storage (in log cfu/g) (n = 3).

Treatment	Storage days			
	0	30	60	90
Code 0	8.59±0.07 ^{bA}	8.07 ±0.06 ^{cB}	7.04±0.08 ^{dC}	6.04±0.04 ^{fD}
Code 1	8.60±0.06 ^{bA}	8.20±0.08 ^{cB}	7.07±0.04 ^{dC}	6.40±0.04 ^{eD}
Code 2	8.63±0.09 ^{bA}	8.60±0.12 ^{bB}	7.10±0.03 ^{cC}	6.90±0.03 ^{dD}
Code 3	8.66±0.06 ^{bA}	8.64±0.11 ^{bB}	7.90±0.03 ^{cC}	7.00±0.03 ^{cdD}
Code 4	8.70±0.05 ^{abA}	8.60±0.06 ^{bB}	8.00±0.02 ^{bC}	7.10±0.02 ^{cD}
Code 5	8.79±0.07 ^{aA}	8.87±0.09 ^{aB}	8.11±0.05 ^{aC}	7.20±0.08 ^{bD}
Code 6	8.80±0.03 ^{aA}	8.82±0.03 ^{aB}	8.13±0.03 ^{aC}	7.30±0.05 ^{aD}

$^{a\text{-}b}$ different characters display significant differences (P<0.05) per column. $^{A\text{-}B}$ different characters show significant differences (P<0.05) per row. Samples ingredients: Code 0: probiotic ice cream with no GTP, Code 1 to 6: probiotic ice cream containing 0.5%, 1, 1.5, 2, 2.5 and 3% GTP, respectively.

3.7 Analysis of flavor, texture, color, and overall acceptance

The results of the comparison of the mean flavor of samples are presented in Figure 1A. The highest flavor score was obtained in ice cream with 1.5% and 2% GTP, respectively, and there was no significant difference with control (p>0.05). The lowest flavor score was observed in ice cream with the highest amount of GTP (3%). Furthermore, in the present study, storage time did not affect the flavor characteristic of samples (P>0.05).

The highest score of texture was observed in control, and ice cream samples containing 0.5-2% GTP; and no significant difference was found between these treatments (p>0.05) (Figure 1B).

The highest score of color belonged to ice cream with 1.5 and 2% GTP, which did not differ significantly from control (Figure 1C and 2). The lowest color score was also observed in samples containing 0.5, 1, and 3% GTP, and no significant difference was found between them (p>0.05).

Total acceptance results showed that the highest score belonged to ice cream with 1.5 and 2% GTP (Figure 1D). The lowest total acceptance score was also observed in samples with 0.5 and 3% GTP, respectively.

Undoubtedly, the characteristics of flavor and texture of ice cream are the most important factor of acceptance of the consumer [36]. In our study, the lowest flavor score was observed in ice cream with the highest amount of GTP (3%), which caused some astringent taste due to higher amounts of polyphenols. Viscosity is an important physical characteristic of ice cream, which has a deliberate effect on its sensory quality and in particular texture evaluation [31]. Contrary to what we expected, the lowest texture score was given to the ice cream samples containing 3 and 2.5% GTP ($p>0.05$). The cause of this occurrence is probably due to the creation of a roughness texture that occurs following the use of excessive amounts of GTP. Some features such as texture, aroma, taste, and color affect the overall acceptance and sense of tasters. In a study by Glibowski et al. (2019), the effect of different tea extracts (black, red, green, and white tea) on sensory parameters of stirred probiotic yogurts was investigated. Sensory evaluation showed that yogurts supplemented with green tea extract were the most accepted. However, the addition of extracts prepared from black, white, and red tea resulted in lower values of all the sensory properties tested [37].

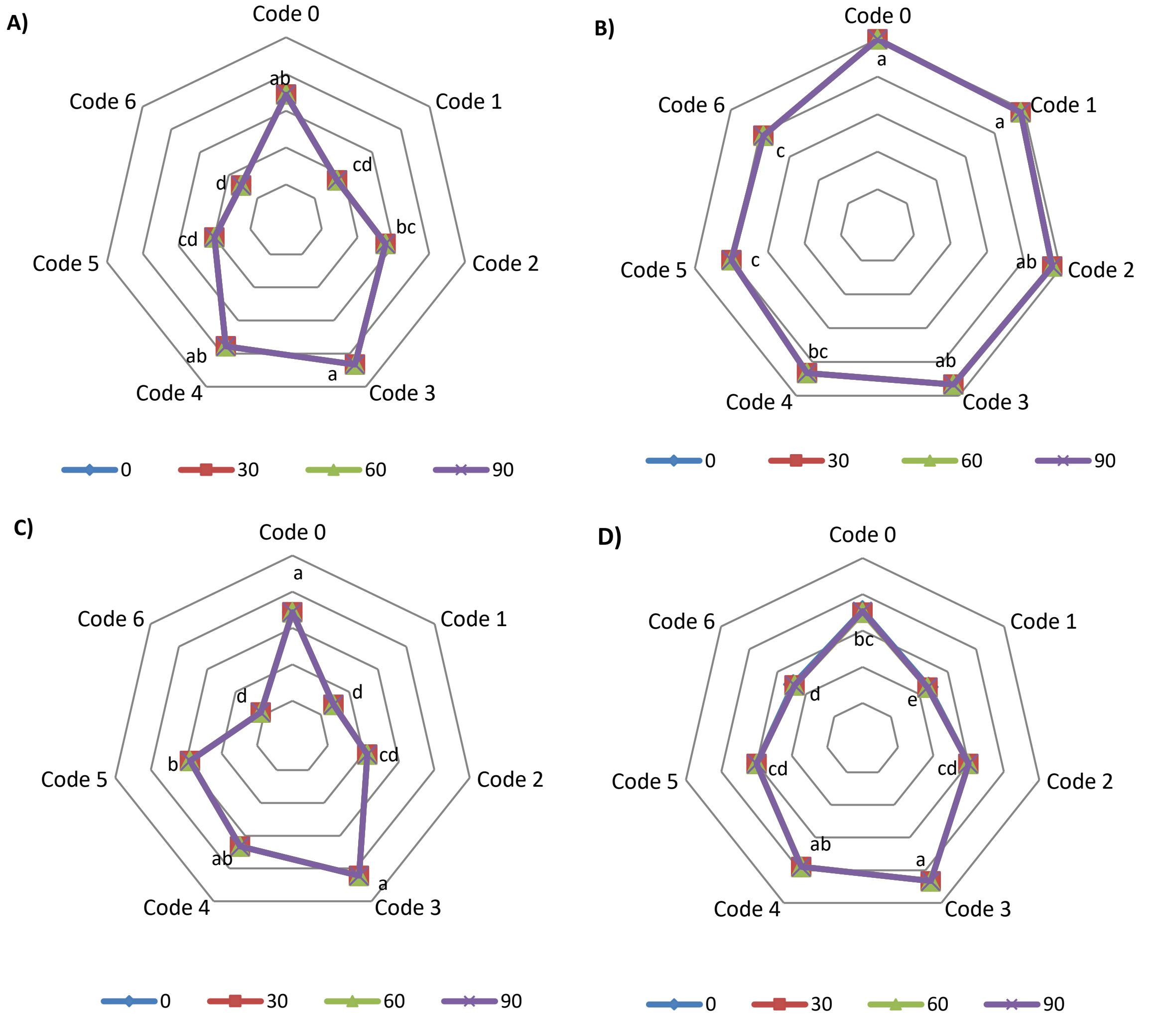

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Figure 1. Flavor (A), texture (B), color-appearance (C), and total acceptance scores (D) of probiotic ice cream samples containing different concentrations of GTP during storage. Different characters (a to e) display significant differences among samples (P<0.05). Samples ingredients: Code 0: probiotic ice cream with no GTP, Code 1 to 6: probiotic ice cream containing 0.5%, 1, 1.5, 2, 2.5 and 3% GTP, respectively

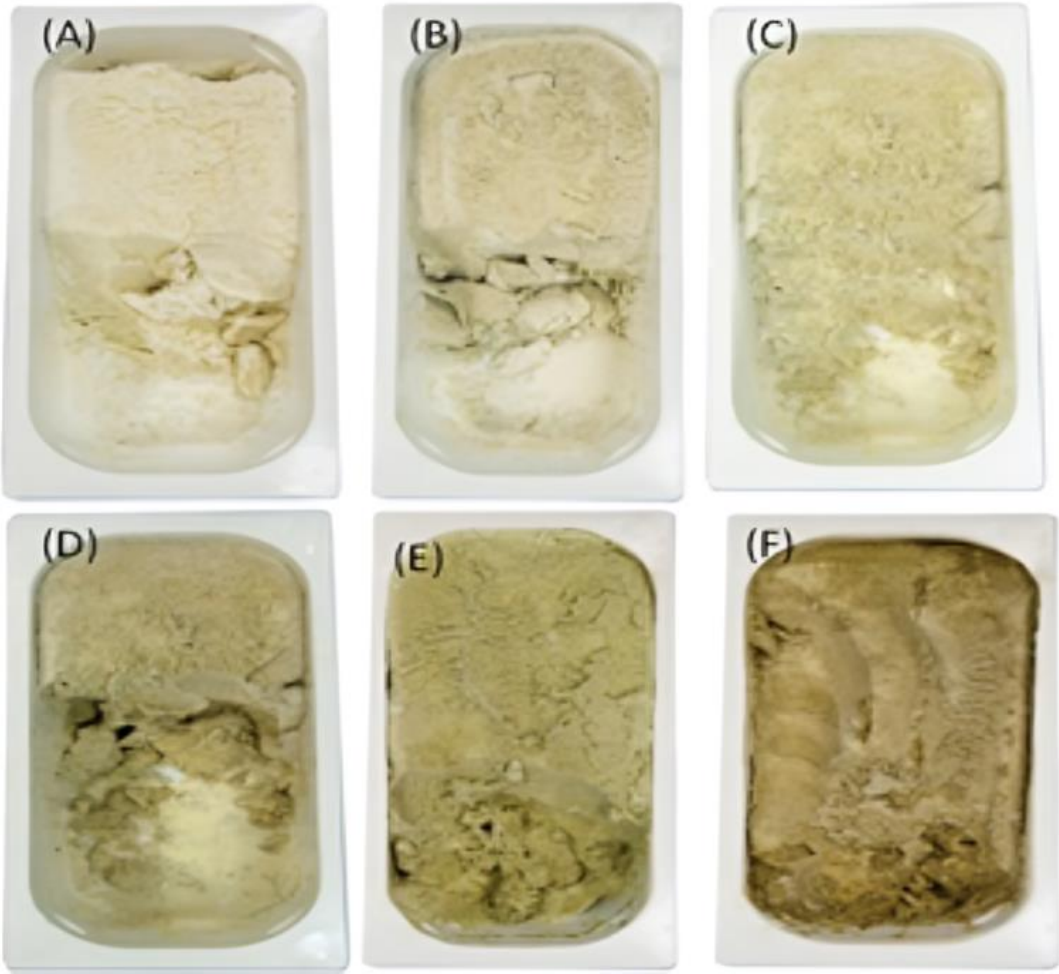

Figure2. Images taken from probiotic ice cream samples containing different amounts of GTP at day 0. A: probiotic ice cream contains 0.5% GTP, B: probiotic ice cream contains 1.0% GTP, C: probiotic ice cream contain 1.5% GTP, D: probiotic ice cream contains 2.0% GTP, E: probiotic ice cream contains 2.5% GTP, F: probiotic ice cream contains 3.0% GTP.

4. Conclusion

The addition of green tea powder in a dose-dependent manner increased the melting resistance, viscosity, and antioxidant property of ice cream compared to control samples. Probiotic ice cream samples with concentrations ≥ 1.5% green tea powder maintained the viability of *Lactobacillus casei* at the level above 7 log cfu/g during a 90-day storage period. However, higher levels than 1.5% contribute to loss of sensory quality. It can be concluded that green tea powder can be employed as a suitable natural supplement for the production of probiotic ice cream due to its physicochemical characteristics and nutritional value, but suitable amounts of tea need to be carefully applied. Based on the results obtained in this study we introduced a new formulation of probiotic ice cream, which could maintained the viable count of *Lactobacillus casei* at the level above 7 log cfu/g during storage. This property alongside the high antioxidant activity and overall acceptability make it as new probiotic ice cream with health promoting properties, which can be produced industrially.

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چکیده

در سال های اخیر، تقاضا برای غذاهای سالم و کاربردی افزایش یافته است و صنایع غذایی در تلاش است تا این نیازهای جدید را برطرف کند. این مطالعه با هدف ارزیابی اثر غلظت‌های مختلف پودر چای سبز بر ویژگی‌های فیزیکوشیمیایی، ویژگی‌های حسی، فعالیت آنتی‌اکسیدانی و زنده مانی باکتری های پروبیوتیک در یک دوره نگهداری ۹۰ روزه انجام شد. هفت نمونه بستنی حاوی ۰ تا ۳ درصد پودر چای سبز و *لاکتوباسیلوس کازئی* تولید و در روزهای ۰، ۳۰، ۶۰ و ۹۰ مورد ارزیابی قرار گرفت. از روش های فولین سیکالتو و دی فنیل پیکریل هیدرازیل برای تعیین محتوای پلی فنل کل و خاصیت آنتی اکسیدانی استفاده شد. نتایج نشان داد که اسیدیته، فعالیت آنتی اکسیدانی، پلی فنل کل، ویسکوزیته، مقاومت به ذوب و تعداد سلول های زنده *لاکتوباسیلوس کازئی* با افزایش غلظت پودر چای سبز افزایش یافت ($p<0.05$). همچنین با گذشت زمان، تفاوت معنی داری در ویسکوزیته و مواد جامد کل مشاهده نشد، اما فنل کل، فعالیت آنتی اکسیدانی و زنده مانی باکتری های پروبیوتیک به طور معنی داری کاهش یافت ($p<0.05$). نتایج ارزیابی حسی نشان داد که طعم و رنگ نمونه‌های حاوی ۱/۵ و ۲ درصد پودر چای سبز بالاترین امتیاز را به خود اختصاص دادند ($p<0.05$). نمونه‌های حاوی ۱/۵ درصد پودر چای سبز که دارای بالاترین امتیاز رنگ، طعم و مقبولیت کلی، فعالیت آنتی‌اکسیدانی بالا و تعداد سلول های زنده *لاکتوباسیلوس کازئی بیش از* 10^7 cfu/g در طول دوره نگهداری بودند، می‌توانند به عنوان یک محصول جدید با خواص ارتقاء دهنده سلامت معرفی شوند.