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The physicochemical and sensory properties, and the impact on probiotic viability during storage of probiotic ice cream containing cocoa, walnut, persimmon, and cinnamon

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ABSTRACT

In this study, the effect of probiotics in flavored ice creams was investigated and ice creams containing different concentrations of cocoa, walnut, date and cinnamon were prepared. Two different probiotics (*Lactobacillus acidophilus* and *Bifidobacterium animalis* subps. *lactis* BB-12) were added to each sample and stored at -18°C for 60 days. The effects of storage on pH, titratable acidity, dry matter, fat and ash, sensory properties and probiotic viability were investigated. Increasing ingredient concentrations and storage time did not affect the dry matter, fat and ash values of the ice creams ($p>0.05$). Especially in ice creams containing 20% walnut and 0.4% cinnamon, pH increased significantly ($p<0.05$), and titratable acidity decreased ($p<0.05$). Probiotic counts were performed on days 1, 15, 30, 45 and 60. Although probiotic bacteria decreased during storage, by day 45 of storage all ice cream samples were above the minimum probiotic concentration required to produce a probiotic effect.

Keywords: probiotic, *Lactobacillus*, *Bifidobacterium*, ice cream, prebiotic

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INTRODUCTION

Ice cream is a dairy product widely consumed in all age groups all over the world due to its taste, cooling effect and high nutritional value[1,2]. The addition of various ingredients to ice cream, which has a higher carbohydrate, fat and protein content compared to milk, further increases its nutritional value[3]. In recent years, with the increasing awareness of consumers about health and nutrition, interest in the consumption of foods containing more bioactive substances, probiotics and prebiotics has increased[4]. Defined by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) as "living microorganisms that provide health benefits to the host when administered in adequate amounts", probiotics have been reported to have health benefits such as protection of intestinal flora, anticarcinogenic effect, lowering serum cholesterol levels and blood pressure[5,6]. Probiotic foods are expected to contain more than 106 colony forming units (CFU) of live probiotic microorganisms per ml or gram. The most important points to be considered in the selection of probiotic microorganisms are that they remain viable during the processing and storage of foods, during intestinal transit, and that they confer potential health benefits on consumers[7]. *Lactobacillus* and *Bifidobacterium* species are the most widely used microorganisms in the probiotic food industry[2,8]. However, it has been reported that *Lactobacillus* are more resistant than *Bifidobacterium* because they are more resistant to low pH values and can adapt more easily to environments such as milk[9].

The addition of probiotic cultures to ice cream gives the product functional properties. In addition, the use of prebiotic sources creates a symbiotic effect by increasing the viability of probiotic microorganisms[10]. Ice cream is a good food for probiotic microorganisms because its nutrients such as milk protein, fat and lactose provide a suitable environment for probiotics[2,11]. Plants containing bioactive compounds such as phenolic compounds, chlorophyll and carotenoids increase the survival of probiotic microorganisms by reducing their oxidative stress[12]. Cinnamon is one of the most widely used spices with prebiotic properties and therapeutic applications and has strong antioxidant and anti-inflammatory activities[10]. Cocoa contains flavonoids and phenolic acids, which have high antioxidant activity. Furthermore, cocoa has prebiotic activity and can support the growth of beneficial bacteria[13]. Walnuts contain many nutrients such as unsaturated fatty acids, fiber, minerals, vitamins, phytosterols and polyphenols[14]. Persimmon is a rich source of vitamins, minerals and dietary fiber and may enhance the health benefits of probiotic ice cream[15,16].

The aim of the study was to produce ice creams containing *Lactobacillus acidophilus* and *Bifidobacterium animalis* subps. *lactis* BB12 (*Bifidobacterium* BB-12) with different concentrations of ingredients such as cocoa, walnuts, persimmon and cinnamon and to investigate their organoleptic properties and the effect of added ingredients on the viability of probiotics and physicochemical properties of ice cream.

METHODOLOGY

Materials

Ice cream was produced with UHT milk obtained from grocery stores (Pınar, Izmir, Turkey). *Lactobacillus acidophilus* (ATCC 4356, Kwik-Stik) and *Bifidobacterium* Bb-12 (Chr. Hansen, Hoersholm Denmark) were used as probiotics. White sugar (Bor Şeker, Niğde, Turkey), salep (Salep Evi, Samsun, Turkey), cream (İçim, Sakarya, Turkey), skim milk powder (Bağdat, Ankara, Turkey), emulsifier (Danisco, Turkey), cocoa (Dr. Oetker, Izmir, Turkey), walnut (Tadım, Kocaeli, Turkey), cinnamon (Baghdad, Ankara, Turkey) and persimmon from the market were used in the production of ice cream.

Preparation of probiotic ice cream

A total of 17 different ice creams were prepared, including one control (no ingredient). These ice creams were prepared in 4 different flavors; cocoa, walnut, cinnamon and persimmon (2 different proportions of each flavor). In addition, 2 different probiotic microorganisms, *L. acidophilus* and *Bifidobacterium* BB-12, were used.

Ice creams were produced in Istanbul Esenyurt University laboratory using WMF brand D-89343 model ice cream machine (China). The ingredients used in ice cream production and their usage percentages are given in Table 1.

Table 1. Ingredients used in functional ice cream production and their usage percentages

Components	K*	A1*	A5*	C10*	C20*	H10*	H30*	T4*	T8*
Milk	74.8%	74.1%	71.1%	67.3%	59.8%	67.3%	52.4%	74.5%	74.2%
Skim milk powder	2.2%	2.2%	2.1%	2%	1.8%	2%	1.6%	2.2%	2.2%
Emulsifier	0.5%	0.5%	0.5%	%0.4	0.4%	0.4%	0.3%	0.5%	0.5%
Cream	5.2%	5.2%	4.9%	4.7%	4.1%	4.7%	3.7%	5.2%	5.2%
Salep	0.8%	0.7%	0.7%	0.7%	0.6%	0.7%	0.5%	0.8%	0.7%
Sugar	16.5%	16.3%	15.6%	14.8%	13.2%	14.8%	11.5%	16.4%	16.3%
Cocoa		1%	5%						
Walnut				10%	20%				
Cinnamon								0.4%	0.8%
Persimmon						10%	30%		

* K: Plain ice cream, control; A1: ice cream with 1% cocoa; A5: ice cream with 5% cocoa; C10: ice cream with 10% walnuts; C20: ice cream with 20% walnuts; H10: ice cream with 10% persimmon; H30: ice cream with 30% persimmon; T4: ice cream with 0.4% cinnamon; T8: ice cream with 0.8% cinnamon

Probiotic strains were incubated in MRS Broth at 37°C for 24 hours to obtain the desired probiotic density[17]. The density was determined using McFarland standards.

Probiotic cultures (*L. acidophilus* and *Bifidobacterium* BB-12) were inoculated at 107 level into the ice cream mixture kept in the refrigerator overnight. Then, probiotic ice cream samples were produced by mixing in an ice cream machine for 20 minutes.

Microbiological analysis

Ice creams were inoculated with *L. acidophilus* and *Bifidobacterium* BB-12 and stored at -18°C. Live probiotic bacteria counts were determined on days 0, 15, 30, 45 and 60 during storage. Maximum Recovery Diluent (Neogen VCM0085A) was used for serial dilutions of ice creams. The viability of *L. acidophilus* and *Bifidobacterium* BB-12 during storage was determined by incubation for 72 hours at 37°C under anaerobic conditions on MRS Agar supplemented with 0.05% Clindamycin (Merck 1.10660) and MRS Agar supplemented with 0.05% L-cysteine, respectively. Colony numbers were calculated by converting to log CFU g^{-1} [18,19].

Physicochemical analysis

Ice cream samples were thawed at room temperature and pH values were determined by pH meter (HANNA instruments, HI 221). Titratable acidity, total solid, fat (Gerber method) and ash values were determined according to AOAC 947.05, TS ISO 3728, AOAC 952.06, AOAC 930.30, respectively. All measurements were performed on day 0 and 60 days of storage with 3 repetitions[20-23].

Sensory analysis

All ice cream samples were sensory evaluated for taste, texture, color and overall acceptability by 20 panelists (17 female, 3 male) trained in sensory evaluation. A hedonic scale ranging from 1 to 5 was used in the evaluation process (1 being very bad and 5 being very good). All samples were coded with 3-digit numbers. In addition, during the evaluation, panelists were asked to clean their mouths with water after each tasting.

Statistical analysis

IBM SPSS 25.0 program was used for all statistical analyses. Significant differences between samples were determined using One-Way ANOVA test and Independent t-test. A $p<0.05$ level was used to define significant differences. Graphic plots were made in GrapPad Prism 9.1.1 program (mean ± standard deviation).

RESULTS and DISCUSSION

Survivability of probiotics during storage time

To study the effect of different concentrations of certain ingredients on the viability of two different probiotics in ice cream, the samples were stored at -18°C for 60 days. Table 2 shows the changes in the number of probiotic bacteria during storage of probiotic ice creams containing different concentrations of ingredients. During 60 days of storage, the number of probiotic bacteria decreased in ice creams except C10L and C20L ($p< 0.01$), while the change in the number of probiotics in C10L and C20L samples was insignificant ($p>0.01$). On the 45th day of storage, the decrease in probiotic microorganisms in A1L, C10L, C20L, H10L, A1B, A5B, C10B, C20B samples was statistically insignificant. The decrease in probiotic bacteria populations during storage at -18°C may be thought to be due to the limited ability of microorganisms to adapt to low temperatures[24].

Table 2. Survival of *Lactobacillus acidophilus* and *Bifidobacterium animalis* subps. lactis BB-12 (log CFU g-1) in ice cream samples over a 60-day storage period

Days	KL	A1L	A5L	C10L	C20L	H10L	H30L	T4L	T8L
0	7.38 ± 0.13^{a}	6.84 ± 0.20^{a}	6.98 ± 0.08^{a}	6.91 ± 0.49^{a}	6.08 ± 0.34^{a}	6.92 ± 0.28^{a}	6.78 ± 0.51^{a}	6.95 ± 0.62^{a}	7.10 ± 0.61^{a}
15	6.41 ± 0.09^{b}	6.71 ± 0.24^{a}	5.97 ±0 .89^{b}	7.02 ± 0.23^{a}	6.32 ± 0.43^{a}	6.58 ± 0.23^{a}	6.51 ± 0.37^{ab}	6.35 ± 0.30^{ab}	6.79 ± 0.26^{ab}
30	6.26 ± 0.10^{b}	6.46 ± 0.39^{a}	6.35 ± 0.18^{ab}	6.52 ± 0.47^{a}	6.17 ± 0.72^{a}	6.61 ± 0.41^{a}	6.63 ± 0.44^{a}	5.8 4± 0.84^{b}	6.72 ± 0.35^{ab}
45	6.13 ± 0.14^{b}	6.40 ± 0.16^{a}	6.34 ± 0.37^{ab}	6.58 ± 0.48^{a}	6.20 ± 0.22^{a}	6.72 ± 0.09^{a}	6.53 ± 0.47^{ab}	6.37 ± 0.34^{ab}	6.09 ± 0.27^{bc}
60	5.76 ± 0.39^{c}	5.30 ± 0.27^{b}	5.57 ± 0.36^{b}	6.50 ± 0.25^{a}	6.28 ± 0.19^{a}	5.50 ± 0.54^{b}	5.61 ± 0.61^{b}	6.27 ± 0.13^{ab}	5.86 ± 0.55^{c}
Days	**KB**	**A1B**	**A5B**	**C10B**	**C20B**	**H10B**	**H30B**	**T4B**	**T8B**
0	7.65 ± 0.22^{a}	7.17 ± 0.21^{a}	6.93 ± 0.20^{a}	7.13 ± 0.18^{a}	6.94 ± 0.12^{c}	7.43 ± 0.08^{ab}	7.67 ± 0.16^{a}	7.05 ± 0.08^{a}	7.13 ± 0.05^{a}
15	6.9 5± 0.11^{b}	6.95 ± 0.19^{a}	6.89 ± 0.08^{a}	7.17 ± 0.11^{a}	7.28 ± 0.06^{a}	7.27 ± 0.17^{ab}	6.99 ± 0.08^{c}	6.60 ± 0.49^{ab}	6.91 ± 0.07^{bc}
30	6.97 ± 0.29^{b}	6.96 ± 0.11^{a}	6.65 ± 0.31^{a}	6.98 ± 0.09^{a}	7.11 ± 0.03^{b}	7.17 ± 0.29^{b}	7.42 ± 0.25^{ab}	6.10 ± 0.07^{c}	6.94 ± 0.06^{b}
45	6.94 ± 0.23^{b}	6.90 ± 0.14^{a}	6.77 ± 0.10^{a}	7.04 ± 0.08^{a}	6.95 ± 0.04^{c}	7.49 ± 0.05^{a}	7.13 ± 0.14^{bc}	6.33 ± 0.25^{bc}	6.81 ± 0.06^{c}
60	5.22 ± 0.15^{c}	5.80 ± 0.07^{b}	5.96 ± 0.39^{b}	5.81 ± 0.20^{b}	5.52 ± 0.13^{d}	6.13 ± 0.08^{c}	5.77 ± 0.16^{d}	5.26 ± 0.10^{d}	5.77 ± 0.06^{d}

* Mean ± standard deviation. $^{a-d}$ -- Mean values shown with different letters in the columns show statistically significant difference ($p<0.01$). KL: control sample containing *L. acidophilus*, A1L: sample containing 1% cocoa and *L. acidophilus*, A5L: sample containing 5% cocoa and *L. acidophilus*, C10L: sample containing 10% walnuts and *L. acidophilus*, C20L: sample containing 20% walnuts and *L. acidophilus*, H10L: sample containing 10% persimmon and *L. acidophilus*, H30L: sample containing 30% persimmon and *L. acidophilus*, T4L: sample containing 0.4% cinnamon and *L. acidophilus*, T8L: sample containing 0.8% cinnamon and *L. acidophilus*. KB: Control sample containing *Bifidobacterium* BB-12, A1B: sample containing 1% cocoa and *Bifidobacterium* BB-12, A5B: sample containing 5% cocoa and *Bifidobacterium* BB-12, C10B: sample containing 10% walnuts and *Bifidobacterium* BB-12, C20B: 20% walnuts and *Bifidobacterium* BB-12, H10B: 10% persimmon and *Bifidobacterium* BB-12, H30B: 30% persimmon and *Bifidobacterium* BB-12, T4B: 0.4% cinnamon and *Bifidobacterium* BB-12, T8B: 0.8% cinnamon and *Bifidobacterium* BB-12.

Probiotic foods should contain probiotic microorganisms in numbers ranging from 106 to 109 CFU g^{-1} (6 to 9 log CFU g^{-1}) during shelf life[25]. At the end of 60th day, it was observed that C10L, C20L, T4L and H10B samples had probiotic food characteristics. In other words, 10% and 20% walnuts and 0.4% cinnamon in ice creams containing *L. acidophilus* and 10% persimmon in ice creams containing *Bifidobacterium* BB-12 are thought to be effective on probiotic viability. However, it was observed that the number of probiotic bacteria was above 6 log CFU g^{-1} in all samples during 30 and 45 days of storage.

L. acidophilus was inoculated at an average level of 6.88 and *Bifidobacterium* BB-12 at an average level of 7.23 in the ice cream samples produced. When the logarithmic decreases in the number of probiotic microorganisms were compared; the number of probiotic bacteria survived at a higher level compared to the control samples except for the samples containing 0.4% cinnamon (T4L and T4B). This finding suggests that the applied concentrations of all ingredients except 0.4% cinnamon may be due to the prebiotic effect up to 30 and 45 days.

When the logarithmic decrease of the control samples without any ingredient (CL and CB) at the end of 60 days was analyzed, it was observed that the logarithmic decrease of the ice cream containing *L. acidophilus* was lower. This finding supports the hypothesis that *Lactobacillus* species are more resistant than *Bifidobacterium* species[9]. Although there was a significant decrease in the number of probiotics on the 15th and 45th days of the control samples ($p<0.01$), the number of viable probiotic bacteria was above 6 log CFU g^{-1} at the end of the 45th day. Differently, in a study by Turgut and Çakmakçı (2009) investigating the possibility of using some probiotic bacteria species in ice cream production, no significant difference was observed in the numbers of *L. acidophilus* and *B. bifidum* in ice cream samples after 15 and 45 days of storage at -20°C[26].

All ingredients were applied at two different concentrations. When the effect of increasing concentrations on probiotic viability was evaluated, it was observed that only increasing the walnut concentration increased the number of probiotics ($p<0.01$).

When probiotic viability was evaluated in ice cream samples with 1% and 5% cocoa at the end of 60 days of storage, a significant decrease in the number of probiotics was observed ($p<0.01$). In a study by Laličić-Petronijević et al. on probiotic viability in milk and dark chocolate, it was reported that the survival rate of *L. acidophilus* in dark chocolate containing 75% cocoa part was very good at 4 and 25°C for 180 days; *B. lactis* decreased faster[27]. The difference in the viability of *L. acidophilus* with increasing cocoa portion is thought to be due to different storage temperatures.

The change in *L. acidophilus* count with increasing walnut concentration was insignificant ($p>0.01$), while *Bifidobacterium* BB-12 count increased until day 30 ($p<0.01$). At the end of 60 days, the number of *L. acidophilus* was at the desired level for probiotic food (10^6 to 10^8 CFU g^{-1}), while *Bifidobacterium* BB-12 remained below this level. In the study of Salik and Aslaner on probiotic ice cream containing saruc, the decrease in the number of probiotic bacteria on days 15 and 30 as the saruc concentration increased was statistically insignificant. However, similarly, the probiotic microorganism maintained its viability throughout 60 days of storage and remained at the level of 106 to 108 CFU g^{-1}[28].

Although the antibacterial effect of cinnamon against food pathogens has been reported in various studies[29,30], when the effect of increasing cinnamon concentration on the viability of two different probiotic bacteria was investigated in this study, it was found that increasing cinnamon concentration provided a higher survival rate of both probiotic bacteria on day 30. Similarly, Gunes-Bayir et al. found that cinnamon increased the number of probiotic bacteria in yogurts produced by adding the same amount of propolis and increasing concentrations (0.3%, 1%, 2.5%) of cinnamon[31].

During the 45-day storage period, the logarithmic decrease in the ice cream samples with persimmon was very low compared to the control samples, so it is seen that persimmon also shows prebiotic effect. There is no study on the effect of persimmon on probiotic viability.

Since most probiotic bacteria thrive in anaerobic environment, the fact that ice cream is not stored in anaerobic environment may explain the decrease in the number of probiotic microorganisms. Furthermore, the decrease in the probiotic population during the freezing process may be due to damage to bacterial cells due to thermal shock caused by the freezing process[32,33].

Physicochemical properties of probiotic ice cream products

The evaluation of the physicochemical properties of probiotic ice cream samples is given in Table 3. On the 60th day of storage, the ash values of probiotic ice creams ranged between 1.08% and 1.28%, fat values ranged between 5.91% and 6.31%, and dry matter values ranged between 32.70% and 44.76%. The changes in dry matter, ash, acidity and fat values of all ice cream samples during the 60-day storage period were insignificant ($p>0.05$). Also, increasing ingredient concentrations did not affect ash, fat and dry matter values.

Table 3. Physico-chemical properties of ice cream products produced with *L. acidophilus* and *Bifidobacterium* BB-12 at 0 and 60 days of storage

L. acidophilus (log CFU g^{-1})										
	Days	**KL**	**A1L**	**A5L**	**C10L**	**C20L**	**H10L**	**H30L**	**T4L**	**T8L**
pH	0	6.1 9± 0.06^{b}	6.44 ± 0.13^{b}	6.38 ± 0.07^{b}	6.33 ± 0.13^{b}	6.79 ± 0.03^{a}	6.40 ± 0.13^{b}	6.41 ± 0.17^{b}	6.25 ± 0.14^{a}	6.25 ± 0.18^{b}
	60	6.89 ± 0.10^{a}	6.93 ± 0.03^{a}	6.97 ± 0.05^{a}	6.54 ± 0.04^{a}	6.73 ± 0.02^{b}	6.92 ± 0.03^{a}	6.91 ± 0.02^{a}	6.24 ± 0.02^{b}	6.87 ± 0.03^{a}
Titration	0	0.38 ± 0.02^{a}	0.29 ± 0.02^{a}	0.22 ± 0.02^{a}	0.33 ± 0.03^{a}	0.18 ± 0.02^{a}	0.25 ± 0.03^{a}	0.26 ± 0.03^{a}	0.26 ± 0.02^{b}	0.27 ± 0.01^{a}
	60	0.14 ± 0.02^{b}	0.12 ± 0.01^{b}	0.10 ± 0.05^{b}	0.22 ± 0.01^{b}	0.18 ± 0.01^{a}	0.11 ± 0.01^{b}	0.12 ± 0.01^{b}	0.33 ± 0.02^{a}	0.13 ± 0.01^{b}
Dry matter	0	36.92 ± 0.07^{a}	37.04 ± 0.08^{a}	38.10 ± 0.94^{a}	37.90 ± 0.13^{a}	39.12 ± 0.53^{a}	38.73 ± 0.18^{a}	40.50 ± 0.86^{a}	36.96 ± 0.25^{a}	37.01 ± 0.56^{a}
	60	37.63 ± 0.98^{a}	37.15 ± 0.18^{a}	39.37 ± 0.46^{a}	37.87 ± 0.54^{a}	39.29 ± 1.06^{a}	37.99 ± 1.99^{a}	41.25 ± 0.37^{a}	36.99 ± 0.62^{a}	38.01 ± 0.53^{a}
Ash	0	1.19 ± 0.04^{a}	1.24 ± 0.06^{a}	1.22 ± 0.04^{a}	1.18 ± 0.04^{a}	1.25 ± 0.04^{a}	1.13 ± 0.13^{a}	1.26 ± 0.04^{a}	1.22 ± 0.07^{a}	1.27 ± 0.03^{a}
	60	1.16 ± 0.06^{a}	1.21 ± 0.08^{a}	1.25 ± 0.03^{a}	1.23 ± 0.06^{a}	1.24 ± 0.04^{a}	1.13 ± 0.08^{a}	1.21 ± 0.04^{a}	1.19 ± 0.06^{a}	1.28 ± 0.03^{a}
Fat	0	6.10 ± 0.16^{a}	6.25 ± 0.17^{a}	6.16 ± 0.22^{a}	6.33 ± 0.07^{a}	6.32 ± 0.19^{a}	6.17 ± 0.34^{a}	6.19 ± 0.45^{a}	6.21 ± 0.35^{a}	5.99 ± 0.58^{a}
	60	6.11 ± 0.43^{a}	6.14 ± 0.15^{a}	6.19 ± 0.23^{a}	6.23 ± 0.26^{a}	6.31 ± 0.29^{a}	6.26 ± 0.19^{a}	6.24 ± 0.23^{a}	6.17 ± 0.17^{a}	6.11 ± 0.16^{a}
Bifidobacterium animalis subps. lactis BB-12 (log CFU g^{-1})										
	Days	**KB**	**A1B**	**A5B**	**C10B**	**C20B**	**H10B**	**H30B**	**T4B**	**T8B**
pH	0	6.12 ± 0.06^{b}	6.27 ± 0.07^{b}	6.40 ± 0.13^{b}	6.29 ± 0.08^{b}	6.41 ± 0.11^{b}	6.19 ± 0.01^{b}	5.97 ± 0.05^{b}	6.32 ± 0.06^{b}	6.27 ± 0.02^{b}
	60	6.95 ± 0.03^{a}	6.87 ± 0.02^{a}	6.81 ± 0.02^{a}	6.85 ± 0.05^{a}	6.92 ± 0.02^{a}	6.79 ± 0.01^{a}	6.87 ± 0.03^{a}	7.97 ± 1.74^{a}	6.89 ± 0.02^{a}
Titration	0	0.38 ± 0.02^{a}	0.28 ± 0.01^{a}	0.25 ± 0.01^{a}	0.28 ± 0.01^{a}	0.16 ± 0.02^{a}	0.35 ± 0.03^{a}	0.40 ± 0.01^{a}	0.32 ± 0.04^{a}	0.35 ± 0.02^{a}
	60	0.13 ± 0.02^{b}	0.13 ± 0.02^{b}	0.18 ± 0.01^{b}	0.15 ± 0.01^{b}	0.10 ± 0.01^{b}	0.17 ± 0.02^{b}	0.13 ± 0.01^{b}	0.06 ± 0.02^{b}	0.14 ± 0.02^{b}
Dry matter	0	32.05 ± 0.10^{a}	32.31 ± 0.37^{a}	34.22 ± 0.76^{a}	38.51 ± 0.59^{a}	42.98 ± 0.83^{a}	34.93 ± 0.18^{a}	39.66 ± 0.57^{a}	34.28 ± 1.11^{a}	34.53 ± 0.93^{a}
	60	32.70 ± 0.25^{a}	33.24 ± 0.43^{a}	34.73 ± 0.57^{a}	39.13 ± 1.41^{a}	44.76 ± 2.57^{a}	34.47 ± 1.62^{a}	38.65 ± 0.85^{a}	36.66 ± 3.55^{a}	36.71 ± 5.09^{a}
Ash	0	1.24 ± 0.09^{a}	1.22 ± 0.05^{a}	1.25 ± 0.08^{a}	1.24 ± 0.12^{a}	1.26 ± 0.09^{a}	1.08 ± 0.08^{a}	1.19 ± 0.06^{a}	1.21 ± 0.11^{a}	1.22 ± 0.07^{a}
	60	1.21 ± 0.08^{a}	1.19 ± 0.16^{a}	1.22 ± 0.03^{a}	1.19 ± 0.09^{a}	1.23 ± 0.13^{a}	1.13 ± 0.09^{a}	1.21 ± 0.04^{a}	1.19 ± 0.06^{a}	1.22 ± 0.06^{a}
Fat	0	5.85 ± 0.43^{a}	6.11 ± 0.76^{a}	5.96 ± 0.55^{a}	6.28 ± 0.27^{a}	6.23 ± 0.47^{a}	6.20 ± 0.20^{a}	6.24 ± 0.08^{a}	6.22 ± 0.19^{a}	6.00 ± 0.19^{a}
	60	6.07 ± 0.22^{a}	5.91 ± 0.35^{a}	6.11 ± 0.27^{a}	6.30 ± 0.17^{a}	6.20 ± 0.18^{a}	6.10 ± 0.36^{a}	6.27 ± 0.11^{a}	6.21 ± 0.26^{a}	6.25 ± 0.11^{a}

* Mean ± standard deviation. $^{a-b}$ -- Mean values shown with different letters in the columns show statistically significant difference ($p<0.05$). KL: control sample containing *L. acidophilus*, A1L: sample containing 1% cocoa and *L. acidophilus*, A5L: sample containing 5% cocoa and *L. acidophilus*, C10L: sample containing 10% walnuts and *L. acidophilus*, C20L: sample containing 20% walnuts and *L. acidophilus*, H10L: sample containing 10% persimmon and *L. acidophilus*, H30L: sample containing 30% persimmon and *L. acidophilus*, T4L: sample containing 0.4% cinnamon and *L. acidophilus*, T8L: sample containing 0.8% cinnamon and *L. acidophilus*. KB: Control sample containing *Bifidobacterium* BB-12, A1B: sample containing 1% cocoa and *Bifidobacterium* BB-12, A5B: sample containing 5% cocoa and *Bifidobacterium* BB-12, C10B: sample containing 10% walnuts and *Bifidobacterium* BB-12, C20B: 20% walnuts and *Bifidobacterium* BB-12, H10B: 10% persimmon and *Bifidobacterium* BB-12, H30B: 30% persimmon and *Bifidobacterium* BB-12, T4B: 0.4% cinnamon and *Bifidobacterium* BB-12, T8B: 0.8% cinnamon and *Bifidobacterium* BB-12.

The reason for the lack of a significant difference in ash values may be the low ingredient concentrations. The studies of Şentürk et al. (2023) and Haghani et al. (2021) support these results[2,34]. Differently, Kotan (2018) stated that the ash content in ice cream decreased with increasing blueberry concentration[35].

The highest fat content was found in walnut ice cream (6.20% to 6.33%). Regarding the change in fat values, different results were obtained in some studies. Karaman et al. (2014) and Haghani et al. (2021) reported that fat content decreased as the concentration increased in ice creams with CCP[2,36].

Dry matter results were similar to the study of Haghani et al., and it was reported that dry matter content increased with increasing CCP levels[2]. Akalın et al. (2017) reported that the addition of 5 different dietary fibers (apple, orange, oat, bamboo and wheat) to ice cream increased the total solids content[37]. The increase in the dry matter content of ice cream samples can be attributed to moisture loss during storage[38]. However, it has been reported that ice creams with lower dry matter have more water content and therefore form more ice crystals during freezing, which affects the ice cream texture[39]. In the study by Şentürk et al. differently, the dry matter content decreased with the increase in the amount of blueberry and jujube fruit. This decrease was explained by the high moisture content of the added fruits[34].

The addition of cocoa, walnuts, persimmon and cinnamon to ice cream generally increased pH, but this increase was statistically significant ($p<0.05$) in ice cream samples containing 20% walnuts and 0.4% cinnamon. Titratable acidity decreased, this decrease was statistically significant in ice cream samples containing 0.4% cinnamon and *L. acidophilus* ($p<0.05$). In the study by Şentürk et al., there were no regular changes in pH values with the addition of blueberry and jujube fruit purees to ice cream; titratable acidity decreased insignificantly compared to the control sample[34]. In the study by Öztürk et al., the pH value decreased insignificantly, while the titratable acidity increased insignificantly in probiotic ice creams enriched with white and blue myrtle fruits[40].

Changes in pH and acidity were irregular with increasing ingredient concentration. The pH increased and titratable acidity decreased with increasing walnut and cinnamon concentrations. This change was statistically significant in ice creams containing *L. acidophilus* ($p<0.05$), but insignificant in ice creams containing *Bifidobacterium* BB-12 ($p>0.05$). The change in pH and acidity due to the increase in concentration of other ingredients was not significant ($p>0.05$). It is thought that fatty acid and acidic phenolic compounds contained in walnut and cinnamon with high antioxidant content affect pH and acidity[41,42].

Sensory analyses of probiotic ice cream products

The results of the sensory evaluation of the ice cream samples in terms of taste, structure/texture, color and general palatability are given in Figure 1 and Figure 2. As a result of the metabolic activities of probiotic microorganisms, components that may adversely affect the taste and aroma of the product may be formed. For example, acetic acid is produced during fermentation and storage of *Bifidobacterium* spp.[32].

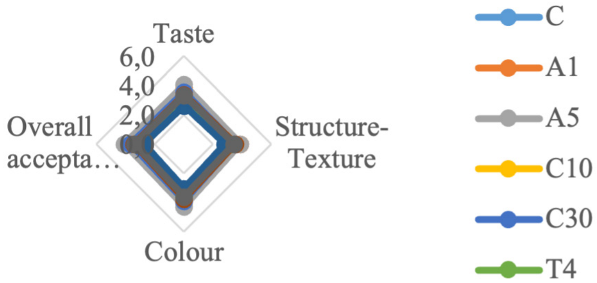

Figure 1. *L. acidophilus* sensory evaluation results

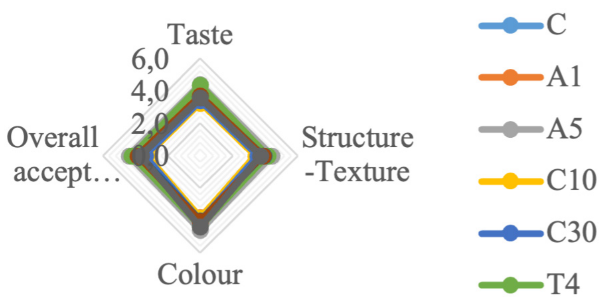

Figure 2. *Bifidobacterium* BB-12 sensory evaluation results

The lowest taste, structure/texture, color and overall liking scores belonged to sample T8L with scores of 2.6, 2.8, 3.0, 2.8, respectively. The highest taste, structure/texture, color and overall liking scores belonged to sample A5B with scores of 4.4, 4.4, 4.4, 4.6, 4.4, respectively.

Adding various concentrations of ingredients to ice cream affected the overall acceptability either positively or negatively. As the concentration of dates increased, the overall acceptability score decreased. The most preferred ice cream was the one containing 5% cocoa.

In conclusion; it is very important to use the appropriate probiotic microorganism at the appropriate dose in probiotic food production[43,44]. *Bifidobacterium* and *Lactobacillus* are commonly used probiotic microorganisms in foods and

Lactobacilli have been reported to be more resistant[2,9]. In this study, *Lactobacillus acidophilus* and *Bifidobacterium* BB-12 were used separately and the effect of the added ingredients was examined. At the end of 60 days, it was observed that there was a lower logarithmic decrease in CL compared to the control samples and the decrease in the walnut ice cream containing *L. acidophilus* was insignificant.

The viability of probiotic microorganisms in food during production and storage is very important in terms of the number of microorganisms that can show the probiotic effect during consumption[7]. The data obtained from the study showed that different concentrations of cocoa, walnuts, persimmon and cinnamon added to ice cream had a positive effect on probiotic viability until the end of the 45th day of storage. In other words, according to this study, the required level of probiotic viability in ice cream continued for 45 days.

Walnut showed the strongest prebiotic effect on day 45. Because both the number of probiotic microorganisms increased with the increase in walnut concentration and the highest number of probiotics was seen in ice cream with 20% walnut on the 45th day.

Sensory wise, the most liked ice cream was the ice cream with 5% cocoa and the least liked ice cream was the ice cream with 0.8% cinnamon.

STATEMENT OF ETHICS

This study does not require any ethical permission.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

Planing and Design: C.H., B.Ç.S., M.A.; Materials and Methods: B.Ç.S., M.A.; Laboratory Studies and Literature Review: B.Ç.S., M.A., B.E., A.G.Ç.; Article Writing, Review and Editing: B.Ç.S., M.A., B.E., A.G.Ç. and C.H.

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