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Influence of forest fruit purees (strawberry or blackberry) on the biochemical, microbiological, sensory properties, and consumer preferences of standard and probiotic cultured yogurts

Einfluss von Waldfruchtpüree (Erdbeere oder Brombeere) auf die biochemischen, mikrobiologischen und sensorischen Eigenschaften sowie die Verbraucherpräferenzen von Standard- und probiotischen Kulturjoghurts

Binnur Kaptan[1]), Serap Kayisoglu[2])

Summary

In this study, antioxidant activity, total phenolic content, microbiological content, sensory evaluation and consumer preferences of both standard and probiotic cultured yogurts prepared by adding 15% strawberry or blackberry puree as forest fruit were examined.

In standard yogurt production, *Streptococcus thermophilus (S thermophilus)* and *Lactobacillus delbrueckii* subsp. *bulgaricus (L. bulgaricus)* cultures, *Lactobacillus acidophilus* LA-5 *(L. acidophilus)* and *Bifidobacterium bifidum* BB-12 *(B. Bifidum)* cultures were used in the production of probiotic yogurt.

As a result of the analyzes performed on all yogurt samples on the 1st, 7th, 14th and 21st days; It was determined that antioxidant activity and total phenolic content were higher in probiotic yogurt with strawberry puree. Both forest berry purees added to the probiotic yogurt effectively supported the growth of probiotic bacteria. Notably, the probiotic bacteria number in the strawberry-added PSY yogurt sample reached maximum values of 8.11 log cfu/g for *L. acidophilus* and 7.58 log cfu/g for *B. bifidum* on the 7th day. Blackberry puree added probiotic yogurt with received the highest score in the sensory evaluation made by trained panelists. In terms of consumer preferences, both standard and probiotic yogurts with blackberry puree added were preferred more than control and strawberry puree yogurt samples.

In addition, in this study, it was determined that in addition to improving the functional properties in fruit yogurt production, the selection of fruit types to be added is important in terms of sensory evaluation and consumer preferences.

Keywords: probiotic yogurt, forest berries, antioxidant activity, phenolic content, *Lactobacillus acidophilus, Bifidobacterium bifidum*

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Introduction

Yogurt is a fermented dairy product composed of a casein network formed through isoelectric precipitation during the fermentation process by lactic acid bacteria (LAB). Standard yogurt is a functional dairy product that contains *Lactobacillus bulgaricus* and *Streptecoccus thermophilus* bacteria (FAO, 1997), whereas probiotic yogurt, in addition to the standard starter cultures, incorporates probiotic cultures such as Lactobacillus and Bifidobacterium. In previous studies, yogurt was made to be a better environment for LAB growth and also a source of bioactive compounds by the addition of various fruits containing valuable molecules (Khoulod Blassy et al., 2020). Due to their significance as healthy sources of bioactive compounds with antioxidant, anti-inflammatory, and antimicrobial activities, fruits have increasingly been utilized in yogurt production in recent years. Fruits particularly, berries, have pharmacological and biochemical properties mainly due to the antioxidant activity of fiber and various other compounds.

Blackberries (Rubus fructicosus) have gained attention as a nutritionally significant fruit, owing to their abundant anthocyanins, ellagitannins, and other phenolic compounds, which together contribute to their considerable antioxidant capacity (Demir, 2023). Similarly, strawberries (Fragaria ananassa) are among the most commonly consumed berries, renowned for their high nutritional value. This value is correlated not only to their rich content of antioxidant micronutrients, such as vitamin C and folate, but also to a diverse and abundant array of polyphenolic antioxidants, including flavonoids, hydrolysable tannins, condensed tannins, and phenolic acids (Seeram et al., 2006; da Silva Pinto et al., 2010). These fruits are rich in basic sugar compounds (fructose, glucose) and organic acids (citric and ascorbic acid) (Odriozola-Serrano et al., 2008). Additionally, strawberries and blackberries are widely accepted as an excellent source of bioactive phenolic compounds, including flavonoids, phenolic acids, and tannins, both individually and synergistically (Jiang et al., 2022).

Therefore, in the present study, the antioxidant activity, phenolic content, microbiological properties, sensory evaluations, and consumer preferences of standard and probiotic yogurts prepared with blackberry or strawberry puree were investigated considering culture and fruit types used. The study was conducted under 21-days storage in refrigerator conditions (±4°C).

The some properties of blackberry and strawberry fruits were presented in Table S1, in comparison with the literature data (1–6) and the Standard Reference values taken from the National Nutrient Database (USDA – United States Department of Agriculture, 2013).

Materials and methods

Materials

Raw cow's milk used to prepare standard and probiotic yogurts with added strawberry and blackberry puree was sourced from Tekirdag Namik Kemal University Research and Application Farm (Tekirdag, Turkey). Skimmed milk powder, used in yogurt preparation, was obtained from Aynes Dairy Co. (Denizli, Turkey), while the forest fruits (blackberries, strawberries) and sugar were commercially provided. The freeze-dried (FD-DVS) standard yogurt (SY) starter culture (YC-X11), consisting of *S. thermophilus* and *L. bulgaricus*, and probiotic yogurt (PY) starter culture (ABY-2), consisting of *L. acidophilus*, *B. bifidum*, *S. thermophilus* and *L. bulgaricus* were obtained from Chr.Hansen-Peyma (Istanbul, Turkey).

The cultures were maintained according to the manufacturer's instructions at –18°C.

Preparation of fruit purees

Undamaged, ripe blackberry and strawberry fruits were randomly selected from the market shelves in Tekirdag province, Turkey. The remaining stem and leaf parts of the fruits were manually cleaned and washed under running water. Subsequently, 200 g of each washed fruit was softened by soaking in water preheated to 65±2°C for 2-3 minutes and turned into puree by mixing. Fifteen percent (w/w) sugar was added to the prepared purees and mixed well. The resulting blackberry and strawberry purees were heat-treated at 90°C for 10 min at 90 ± 1°C for 5 minutes. The hot purees were then transferred to brown glass jars, sealed airtight, and stored in the refrigerator at +4°C until used in yogurt production. Some properties of the fruit purees used in production are given in Supplementary Table S1

Production of standard and probiotic yogurt samples with added blackberry or strawberry puree

The raw cow milk (3% fat, pH 6.6, lactic acid 0.17%) used in the production of standard and probiotic yogurt (SY and PY), was standardized to 14% total solids content using skim milk powder and heat-treated at 90°C for 10 min for 10 minutes at 95°C. The milk was divided into two equal parts. The first portion milk, for the production of SY, was cooled to 43 ± 2°C and inoculated with 2.5% (v/w) SY culture YC-X11. It was then incubated for approximately 2.5 hours until the pH reached 4.7 from 6.6. The second portion of milk, for the production of PY, was cooled to 37 ± 2°C. The PY culture ABY-2 (2.5% v/w) was inoculated and incubated for approximately 6 hours until the pH reached 4.7. After the incubation process, both SY and PY were initially kept at room temperature (20±1°C), and then refrigerated (4±1°C) overnight. Each of SY and PY, whose clots were broken with a mechanical mixer, was divided into three equal parts. The first parts of yogurt were coded as standard control yogurt (SCY) and probiotic control yogurt (PCY). Blackberry or strawberry puree (15% w/w) was added to the remaining 2nd and 3rd parts. Yogurts containing strawberry puree were coded as SSY for standard yogurt, PSY for probiotic yogurt, and yogurts containing blackberry puree were coded as SBY for standard yogurt and PBY for probiotic yogurt (Table 1). The prepared yogurt samples were divided into 100 mL plastic containers and stored at 4±1°C for 21 days. Samples were analyzed on days 1, 7, 14 and 21 of storage. The production was repeated three times.

pH and Titratable Acidity

The pH value of the homogenized samples, prepared by adding water in a 1:1 ratio, was measured using a digital pH meter (pH 211, Hanna Instruments, Portugal). Titratable acidity (TA), also expressed as the percentage of lactic acid, was determined according to AOAC (2012). Titrated with 0.1 N NaOH with continuous stirring until a consistent pink color was formed in the presence of 0.2% phenolphthalein added to the yogurt mixture. It was calculated using the following formula (1).

$$\% TA = \frac{Ax\ B\ x\ C}{Weight\ of\ sample\ in\ grams} x100 \qquad (1)$$

Where, A (mL) is 0.1 N NaOH; B is N NaOH factor; C (milliequivalent) is weight of lactic acid (The milliequivalent weight of lactic acid is 90/1000 or 0.09).

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TABLE 1: *Culture and blackberry or strawberry puree combinations used for trials of yogurt.*

Sample code	Yogurt trial	Sample definition
SCY	Standard Contol Yogurt	*S. thermophilus* + *L. bulgaricus*
SBY	Standard Yogurt with Blackberry puree	*S. thermophilus* + *L. bulgaricus* + Blackberry puree
SSY	Standard Yogurt with Strawberry puree	*S. thermophilus* + *L. bulgaricus* Strawberry puree
PCY	Probiotic Control Yogurt	*S. thermophilus* + *L. bulgaricus* + *L. acidophilus* + *B. bifidum*
PBY	Probiotic Yogurt with Blackberry puree	*S. thermophilus* + *L. bulgaricus* + *L. acidophilus* + *B. bifidum* + Blackberry puree
PSY	Probiotic Yogurt with Strawberry puree	*S. thermophilus* + *L. bulgaricus* + *L. acidophilus* + *B. bifidum* + Strawberry puree

Total phenolic content and antioxidant activity

Extractions of yogurt samples prepared with fruit puree for the determination of total phenolic content and antioxidant were carried out following the method described by Shori and Baba (2013). A total of 20 mL of extraction solution (ethanol/water, 70:30, v/v) was added to yogurt samples (2 g) and mixed with a magnetic stirrer for 3 min at room temperature. The mixture was then kept at room temperature for 20 min and centrifuged at 5000 rpm at 4°C for 10 min (Universal 32R Hettich Centrifuge, Germany). Collected sample extracts were filtered through a 0.45 µm cellulose acetate filter (Milex, Millipore Millex, Bedford, MA, USA) and stored at –20°C in amber bottles until used for total phenolic content and antioxidant activity analyses.

To determine the total phenolic content of yogurt extracts, 40 µL of the extract was diluted with 3.16 mL of distilled water and mixed with 200 µL of Folin-Ciocalteau solution (Sigma-Aldrich, Germany). After 3 minutes, 600 µL of 20% Na_2CO_3 solution was added, and the mixture was left at room temperature in the dark for 2 hours. Absorbance measurements were made at 760 nm using a spectrophotometer (Hitachi U 2000 UV/Vis Model 121-002). Total phenolic content was calculated as mg gallic acid equivalents (GAE) per 100 g sample using a gallic acid standard curve (calibration curve linearity range: $R^2 = 0.99$) (Singleton et al., 1999).

The antioxidant activity of yogurt samples was determined according to the DPPH (2-2-diphenyl-2picrylhydrazyl) radical scavenging activity method (Apostolidis et al., 2007). A 200 mL volume of the sample extract was dissolved in ethanol in a test tube, mixed with 2.4 mL of DPPH solution (Sigma-Aldrich, Germany), and kept in the dark for 30 min. The absorbance was then measured at 517 nm against the blank sample (containing 200 µL of distilled water). The results were calculated as a percent inhibition of free radicals using equation (2) below.

$$\text{DPPH } (\%) = \frac{\left[Abs_{517}^{control} - Abs_{517}^{sample\ exract}\right]}{\left[Abs_{517}^{control}\right]} x100 \quad (2)$$

Microbiological Analysis

After being homogenized with a stomacher, yogurt samples (25 g) were diluted, and decimal dilutions were performed in sterile physiological solution (225 g). Plating and incubation of microbial suspensions were carried out as follows: *S. thermophilus* on M17-agar, incubated at 37°C for 72 h; *L. bulgaricus* on Man Rogosa and Sharpe (MRS) agar, incubated at 37°C for 48 h; *L. acidophilus* on MRS agar containing 0.15% (w/v) bile, incubated at 37ºC for 72 h (Vinderola et al., 2000) (irregular, small star-shaped colonies were evaluated as *L. acidophilus*); *B. bifidum* on MRS-NNLP agar (consisting of lithium chloride 3 mg/mL, nalidixic acid 15 µg/mL, neomycin sulfate 100 µg/mL, and paromomycin sulfate 200 µg/mL), incubated at 37°C for 5 d (white colonies with a diameter of 0.5–3.0 mm were evaluated) (Dave and Shah 1996). All media were purchased from Merck (Darmstadt, Germany). Probiotic cultures were anaerobically incubated in 2.5 L anaerobic jars using the Gas Pak system (Merck, Darmstadt, Germany). Results are expressed as the logarithm of colony formation units per gram of product (log cfu/g). Microbiological counts of the yogurts were performed on days 1, 7, 14, and 21 of storage.

Sensory evaluation by trained panelists

Sensory evaluation of yogurt samples was carried out by academic staff (5 women, 7 men) aged 33-55, trained in 12 sensory tests, at Tekirdağ Namık Kemal University, Department of Food Engineering. Participants were given 6 yogurt samples and asked to rate their taste, aroma, appearance, texture, and general acceptability properties based on a 5-point hedonic scale (where 5 is excellent, 1 is unacceptable) (Brennan et al., 2008). On the days the sensory evaluation was performed (days 1, 7, 14, 21), yogurt samples coded randomly with three-digit numbers, were presented to the panelists in 100 g plastic containers. Panelists were served water and unsalted crackers during the evaluation to clear their mouth before every sample test.

Evaluation of consumer preferences

The research on consumer preference for yogurt samples was conducted at Tekirdag Namık Kemal University Campus, involving 120 consumers (72 women and 48 men; aged 23–58) who were randomly selected from among personnel such as technicians, civil servants, and students. Six yogurt samples, each coded with three digits, were stored in heatproof-lid portable coolers at 10±2°C until served. The consumers who were served the yogurts were asked to list their favorite as top preference and least favorite as last preference after testing the samples a few times. Water and unsalted crackers were provided for mouth cleansing between tests. Care was taken during the consumer selection process to ensure that they were not vegetarian and did not have dairy allergies.

Statistical analysis

Data were analyzed by ANOVA and Duncan's multiple range test ($p < 0.05$) using the SPSS 20.0 software for Windows and are expressed as the mean ± standard deviation (SD). All analyses were performed in triplicate.

Results and discussion

Changes in pH and titratable acidity of yogurts

In control yogurt samples (SCY and PCY), both with and without fruit puree, pH decreased, and TA increased ($p < 0.05$) during 21 days of storage at +4°C (Table 2). It has been reported that *L. bulgaricus* and *S. thermophilus* are responsible for the acidity that develops during storage in control yogurts (SCY and PCY) without fruit puree (Vinderola et al., 2000).

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TABLE 2: *pH and TA values during storage period of yogurt samples.*

Parameter	Storage days	SY samples SCY	SY samples SSY	SY samples SBY	PY samples PCY	PY samples PSY	PY samples PBY
pH	1	4.42±0.09^{ab}	4.34±0.04^{a}	4.35±0.11^{a}	4.54±0.02^{b}	4.48±0.11^{b}	4.51±0.06^{b}
	7	4.22±0.04^{a}	4.15±0.03^{a}	4.18±0.05^{a}	4.45±0.02^{b}	4.37±0.13^{b}	4.38±0.14^{b}
	14	4.17±0.05^{ab}	4.07±0.03^{a}	4.11±0.04^{a}	4.40±0.03^{c}	4.29±0.16^{bc}	4.33±0.09^{c}
	21	4.13±0.05^{ab}	4.06±0.04^{a}	4.08±0.06^{a}	4.38±0.03^{c}	4.26±0.12^{bc}	4.25±0.14^{bc}
	Total	4.24±0.13^{A}	4.16±0.12^{A}	4.18±0.12^{A}	4.44±0.07^{B}	4.35±0.14^{B}	4.37±0.14^{B}
TA (LA %)	1	0.89±0.06^{b}	0.92±0.11^{b}	0.96±0.05^{b}	0,71±0.03^{a}	0.72±0.03^{a}	0.76±0.05^{a}
	7	0.94±0.03^{b}	0.98±0.07^{b}	1.00±0.04^{b}	0.74±0.03^{a}	0.76±0.04^{a}	0.78±0.07^{a}
	14	0.99±0.08^{b}	1.03±0.03^{b}	1.05±0.03^{b}	0.81±0.05^{a}	0.80±0.03^{a}	0.83±0.04^{a}
	21	1.07±0.03^{b}	1.12±0.03^{b}	1.14±0.05^{b}	0.86±0.03^{a}	0.88±0.06^{a}	0.91±0.03^{a}
	Total	0.97±0.08^{B}	1.01±0.09^{B}	1.04±0.08^{B}	0.78±0.07^{A}	0.79±0.07^{A}	0.82±0.07^{A}

Lowercase letters are used to indicate the differences between the averages of the samples on the days of measurement, and uppercase letters are used to indicate the differences between the overall averages of the samples (P<0.05).

While pH values of all yogurt samples varied between 4.16 and 4.44, TA values were determined to vary between 0.78% and 1.04%. At the end of storage, the average pH in SY samples decreased by approximately 6.4%, while the pH value in PY samples decreased by approximately 4.7%. Compared to yogurts containing standard cultures, lower levels of pH decrease and TA increase were observed in yogurts containing probiotic cultures. The decrease in pH and increase in TA during storage were greater in standard and probiotic yogurts containing strawberry puree (SSY, PSY) and blackberry puree (SBY, PBY) than in control yogurts (SCY and PCY), and the change was statistically significant ($p < 0.05$). The effect on pH and TA changes in yogurts with the same culture (SBY and SSY; PBY and PSY) containing blackberry or strawberry puree (15%) was found to be insignificant ($p > 0.05$). However, the pH and acidity changes between yogurts containing different cultures (SBY and PBY; SSY and PSY) were found to be statistically significant ($p<0.05$). It has also been reported in previous studies that TA increases and pH decreases in yogurts containing fruit concentrate, pulp or fruit puree. Varedesara et al., (2021) reported that pH decreased and TA values increased in various fruit-added yogurts due to high activity during storage. In the study, it was observed that the changes in pH and TA results determined in control and fruit puree added yogurt samples were compatible with the results stated in previous studies.

Changes in total phenolic content of yogurts

Total phenolic content (TPC) increased during 21 days of storage at 4°C in SY and PY samples with added strawberry or blackberry puree, as well as control yogurts without puree (Figure 1). During storage, TPC was found to be higher in PCY than SCY and the difference between control samples was insignificant ($p > 0.05$) but significant ($p < 0.05$) between control yogurts and yogurts containing fruit puree. It has been shown that the phenolic substance content in pure yogurts (SCY, PCY) is due to the presence of phenolic compounds such as estrogen in milk at the beginning of storage and polyphenols, which are mostly released from the feed (Pape-Zambito et al. 2010). Increased phenolic content during storage has been associated with the hydrolysis of milk proteins by LAB (Damin et al., 2009) and the release of amino acids containing phenolic side chains such as tyrosine (Korhonen 2009). Additionally, the conversion of polyphenolic compounds into smaller, more removable or stable units by and the complex structures formed due to polyphenol-protein interaction (El-Said et al., 2014) contributed to this increase. Depending on the change in culture content, strawberry or blackberry puree was more effective in increasing the TPC of PYs containing *L. acidophilus* and *B. bifidum* as compared with SYs during storage ($p < 0.05$). At the end of the storage period, the highest TPC value was observed in the PSY sample (159.9 mg GAE/g). The TPC values of SBY and SSY were determined 116.3 mg GAE/g and 102.4 mg GAE/g, respectively. Moreover, the addition of strawberry puree was more effective in increasing the TPC of the yogurt ($p < 0.05$) as compared with blackberry.

Strawberries are rich in bioactive phenolic compounds, including hydroxycinnamic acids, ellagic acids, ellagitannins, xavan-3-ols, xavonols, and anthocyanins, as well as flavonoids and phenolic acids (Chen et al., 2015). In some studies, certain fruit varieties and processing methods have been shown to increase TPC in yogurt-type fermented dairy products (Delgado-Fernández et al., 2019), with fruit-free control yogurts having lower TPC (Bueno et al., 2014). In this study as well, probiotic yogurt with added strawberry puree (PSY) stood out due to its high TPC content.In this study also, probiotic yogurt with added strawberry puree (PSY) stood out due to its high TPC content.

Changes in antioxidant activity of yogurts

The antioxidant potential of any fruit is determined by phyto-chemicals that scavenge free radicals. Fruits that have high levels of phytochemicals have high antioxidant activity. The antioxidant activities of the yogurt samples were compared during the 21-day storage using the percentage of DPPH radical scavenging ability (Figure 2). Throughout the storage period, no significant difference in antioxidant activities was observed between fruit puree-free SCY and PCY control samples. However, there was a statistically significant difference in antioxidant activities among the other yogurt samples ($p<0.05$). In the control yogurts (SCY, PCY), the continuation of proteolysis of organic acid derivatives and milk proteins (Lourens-Hattingh and Viljoen 2001) during storage was suggested as a possible source of DPPH inhibitors. Moreover, low-mole-

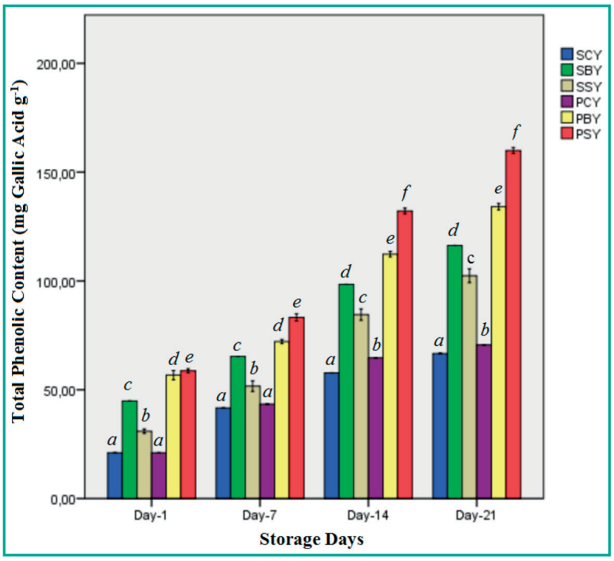

FIGURE 1: *Total phenolic content of yogurt samples during the refrigerated storage period.*

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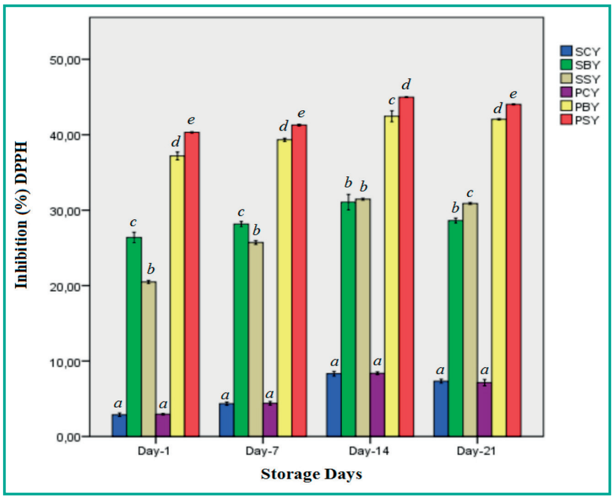

FIGURE 2: *Antioxidant activity (% DPPH inhibition) of yogurt samples during refrigerated storage period.*

cular-weight thiols (Niero et al., 2017), ascorbate (Nielsen et al., 2001), tocopherol, retinol, and carotenoids (Noziere et al., 2006), originating from the milk structure are also noted to contribute to antioxidant activity. Consistently, the antioxidant activity was higher in PY yogurt samples with added fruit compared to SY samples. The antioxidant activity of all yogurt samples increased up to day 14 of storage and then decreased. On the 14th day, the antioxidant activities of the PSY, PBY, SSY, SBY, PCY, and SCY yogurt samples were observed to be 44.98%, 42.45%, 31.45%, 31.06%, 8.37%, and 7.97%, respectively, with the highest values. The decrease observed after 14 days of storage was related to the binding effect between polyphenol and milk proteins, which decreased their antioxidant activity by reducing the free hydroxyl number (Dubeau et al., 2010). Previous studies have reported the antioxidant potential of fruits used in the development of nutraceutical probiotic products (Bobinaitė et al., 2012) The radical scavenging activity of various fruit jams used to flavor yogurt was determined to be the highest in strawberry jam (46.60%), followed by blackcurrant (45.35%) and pumpkin (42.73%) jam (El Samh et al., 2013). The findings obtained in the present study are in accordance with previous reports that used various fruit purees, jams, extracts, or pow-ders (El Samh et al., 2013; El-Said et al., 2014). Taha et al. (2007) demonstrated that the use of strawberries in milk-based beverages or flavored yogurt yielded the highest antioxidant activity among other fruits and vegetables.

El-Said et al. (2014) demonstrated that in traditional standard stirred yogurt, the DPPH radical scavenging activity was 17.91% in the control yogurt and 43.81 in the yogurt with added pomegranate peel extract (35%). Furthermore, increasing the amount of pomegranate peel extract enhanced the antioxidant activity of the stirred yogurts by up to 25%.

Microbiological analysis

In probiotic yogurt samples, the count of *S. thermophilus* began decreasing after the 7th day, while standard yogurt samples showed a decrease from the first day. *L. bulgaricus* count started decreasing after the 7th day in all yogurt samples (Figure 3). Both *S. thermophilus* and *L. bulgaricus* counts were higher in probiotic yogurt samples (PSY and PBY) until the 14th day. However, on the 21st day, there was no statistically significant difference in all yogurt samples for *S. thermophilus* , except for SCY. By the end of the storage period, *L. bulgaricus* did not show a significant difference in all yogurt samples ($p > 0.05$). The highest count of *S. thermophilus* (9.85 log cfu/g) was observed in the PSY yogurt sample on the 7th day, while the lowest (8.57 log cfu /g) was observed in the SCY yogurt sample on the 21st day. The highest count of *L. bulgaricus* (8.75 log cfu/g) was observed in the PSY yogurt sample on the 1st day, and the lowest count (7.32 log cfu/g) was observed in the SCY yogurt sample on the 21st day. Depending on the acidity development, *L. delbrueckii* ssp. *bulgaricus* lost its viability in all yogurts by the end of the storage period, consistent with previous studies demonstrating better improvement in starter cultures in the presence of certain sugars (such as glucose and maltose) and a significant relationship between growth and fruit concentrates. (Hassanein et al., 2014).

Changes in *L. acidophilus* and *B. bifidum* content (log cfu/g) of probiotic yogurts (PCY, PBY and PSY) are given

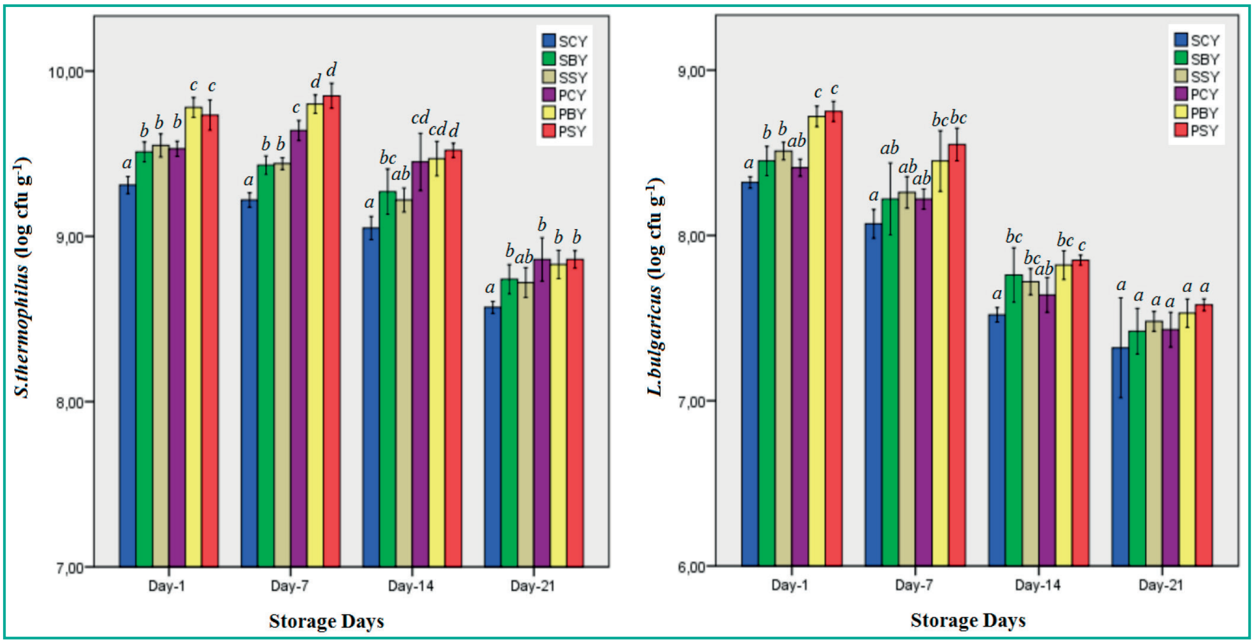

FIGURE 3: *S. thermophilus and L. bulgaricus (log cfu /g) in probiotic yogurt samples during refrigerated storage period.*

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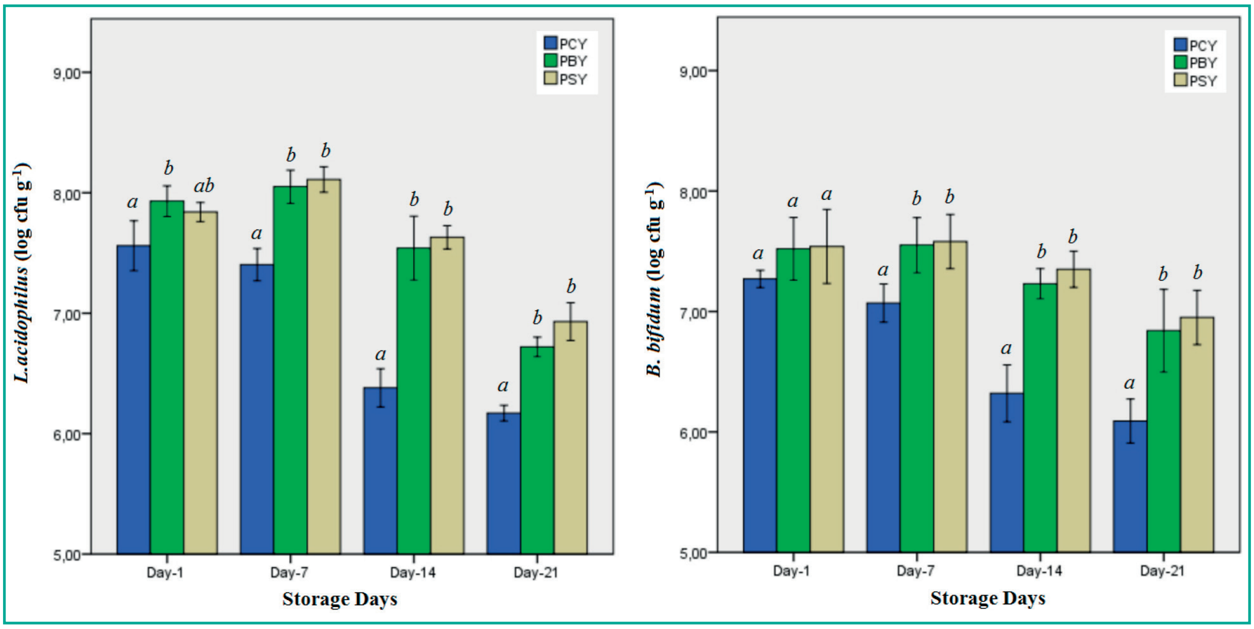

FIGURE 4: *L. acidophilus and B. bifidum in probiotic yogurt samples during refrigerated storage period.*

graphically in Figure 4. Yogurts enriched with fruit puree exhibited higher numbers of *L. acidophilus* and *B. bifidum* than the probiotic control (PCY). Among fruit purees on all storage days, the number of both *L. acidophilus* and *B. bifidum* (in log cfu/g) was found to be slightly higher in PSY (strawberry puree added) yogurt compared to PBY (blackberry puree added) yogurt (Figure 4). In statistical analysis, there was no significant difference between PSY and PBY ($p > 0.05$), but PCY was significantly different from both ($p<0.05$). Starting from the 14th day, decreases in the numbers of *L. acidophilus* and *B. bifidum* were observed in all yogurt samples. The maximum values of *L. acidophilus* and *B. bifidum* were determined in the PSY yogurt sample on the 7th day with 8.11 log cfu/g and 7.58 log cfu/g, respectively. The numbers of live *L. acidophilus* and *B. bifidum* in yogurts with fruit puree decreased after the 14th day of storage, but remained at the recommended level of 10^6 log cfu/g at the end of storage (21 days at +4°C) (Kurmann and Rasic, 1991).

The prebiotic effect of the complex polysaccharide pectin and pectic-oligosaccharide compounds in fruits such as strawberries and blackberries may have increased the growth rate of probiotic bacteria. On the other hand, the absence of growth-promoting substances in the control sample, which did not contain strawberry or blackberry puree, may have caused the number of probiotic bacteria to decrease. Phenolic compounds found in fruits, including anthocyanin pigments (such as pelargonidin-3-monoglucoside, cyanidin-3-monoglucoside, and delphinidin-3-monoglucoside) have been reported to play an important role in stimulating the growth rate of probiotic bacteria (Werlein et al., 2005) . The increased viability of *L. acidophilus* and *B. bifidum* is attributed to phenolic compounds, which are the main components of the fruits (Mullen et al., 2002). Molan et al., (2009) showed that phenolic components in fruits increased the growth of Lactobacillus and Bifidobacterium. A similar increase in the growth rate of *L. acidophilus* and *B. bifidum* has also been reported in other studies (Feng et al., 2019).

Sensory evaluation by trained panelists

The sensory scores for control (SCY and PCY) and fruit puree-added yogurts, rated on a scale from 1 to 5 by trained panelists, were illustrated in Figure 5 and Supplementary Table S2. Throughout storage, sensory ratings for all yogurts decreased. The addition of strawberry or blackberry puree in standard and probiotic yogurts positively influenced taste, aroma, texture, appearance, and general acceptability scores ($p < 0.05$) compared to control yogurts. Probiotic fruit yogurts (PSY and PBY) with *L. acidophilus* and *B. bifidum* received higher ratings ($p < 0.05$) during storage than standard yogurts (*S. thermophilus* and *L. bulgaricus*) with fruit puree (SSY and SBY). Significant differences ($p < 0.05$) were noted in scores among yogurts with the same fruit puree (SSY-PSY and SBY-PBY) and different fruit

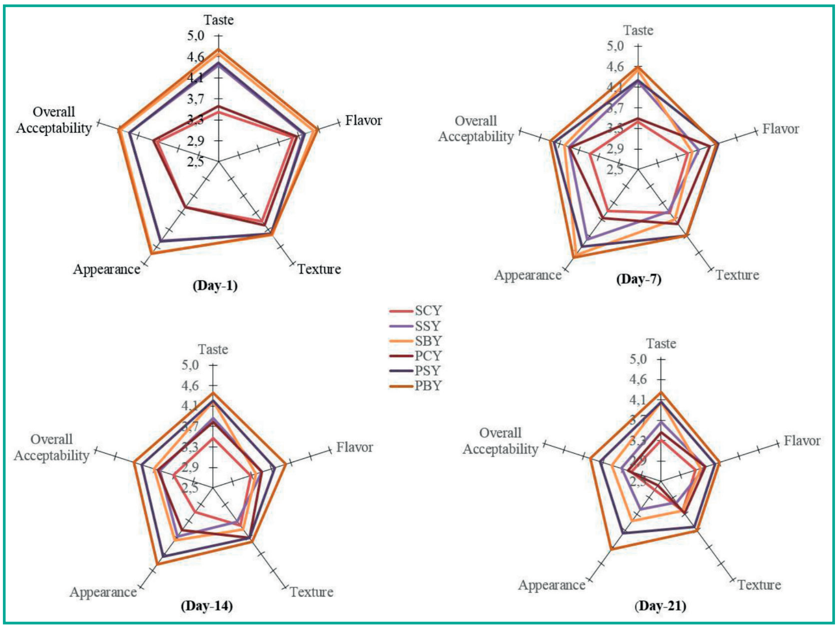

FIGURE 5: *Change of sensory properties for yogurt samples on storage days.*

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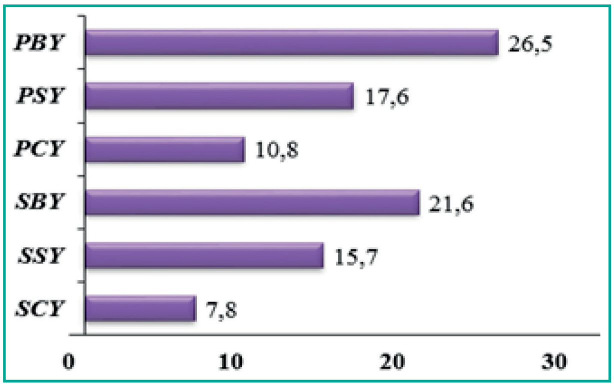

FIGURE 6: *Consumer preferability rates (%) for yogurt samples during storage on the day 1.*

puree (SSY-SBY and PSY-PBY). At storage onset, PBY had the highest taste score (4.7), while SSY scored the lowest (3.7) at the end. Here, the same amount of sucrose was used in the production of both strawberry and blackberry purees. Therefore, the type of fruit was effective in scoring. Werlein et al., (2005) also stated that fruit yogurts with natural sugar content are preferred over control yogurts.

Feng et al. (2019) also highlighted that fruit yogurts receive higher general acceptability scores in sensory evaluations.

Consumer preference evaluation for the yogurts

The graph showing the evaluation made with 120 non-trained consumers to determine their preferences for yogurt samples with and without strawberry and blackberry puree is shown in Figure 6. As a result of the evaluation made according to the preferences of non-trained consumers, probiotic yogurt containing blackberry puree was the most preferred yogurt with a preference rate of 26.5% at the beginning of storage. Consumers' preference for control yogurts (SCY and PCY) was relatively low. Consumers preferred yogurts containing fruit puree, especially yogurts containing blackberry puree (SBY PBY). In this study, it is thought that the taste and aroma specific to fruits is an important factor in why yogurts containing fruit puree are preferred by consumers more than control yogurts.

Consumers' general opinions regarding yogurt preferences were also evaluated. The more intense acidic taste, semi-liquid structure, and typical yogurt taste perception felt in the fruit -free control yogurts (SCY and PCY) were among the important consumer opinions that caused the increase in the preference of yogurts with fruit puree.

Conclusions

In this study, different fruit purees prepared under the same conditions and with the same sucrose ratio were added to yogurt; Different fruit purees exhibited varying levels of enhancing effects on the antioxidant activity and total phenolic content of both standard and probiotic yogurts. The number of probiotic bacteria in probiotic yogurts prepared with fruit puree was determined to be higher during storage compared to probiotic control yogurt. Yogurts with strawberry fruit puree had a slightly enhancing effect on the growth of probiotic bacteria compared to yogurts with blackberry fruit puree, but it was not statistically significant. The probiotic yogurt sample containing blackberry puree received the highest score from experienced panelists in terms of sensory properties. In the evaluations regarding consumer preferences, it was determined

SUPPLEMENTARY TABLE S1: *The some properties of blackberry and strawberry fruits (g/100 g fresh weight), the range of values found in the literature and the USDA database values.*

Compositions	Fruit	
	Blackberry[a]	Strawberry[b]
pH	2.84 ± 0.02	3.18 ± 0.03
Literature	2.51–4.12	3.27–3.43
TSS (Brix)	11.08 ± 0.31	10.42 ± 0.25
Literature	2.75–22.1	4.50–6.52
Antioxidant capacity – DPPH (EC_{50} – g f.w./g DPPH)	1872.16 ± 115.34	3256.42 ± 241.43
Total phenolics (mg GAEs/100 g f.w.)	985 ± 3.74	446.24 ± 23.24
Literature	176–1020	200–300

Mean value ± standard deviation of fruit weight; n = 3.
Abbreviations: TSS: total soluble solids; DPPH: 2-diphenyl-1-picryhydrazyl radical scavenging activity; GAE: gallic acid equivalent.

[a]: Literature data for blackberry: Acosta-Montoya et al., 2010, de Souza et al., 2014; Segantini et al 2015; Mikulic-Petkovseket al., 2021. [b]: Literature data for strawberry: Pantelidis et al., 2007, de Souza et al., 2014; Segantini et al 2015; Miller et al., 2019.

Supplementary Table S2: *Average scores for the sensory properties of control and standard and probiotic culture yogurts containing forest fruit puree (strawberry and blackberry) during their storage period.*

Parameter	Storage (day)	Standard yogurt samples SCY	SSY	SBY	Probiotic yogurt samples PCY	PSY	PBY
Taste	1	3.47±0.10^{a}	4.38±0.03^{b}	4.61±0.04^{c}	3.59±0.05^{a}	4.43±0.12^{b}	4.7±0.15^{c}
	7	3.45±0.10^{a}	4.25±0.10^{b}	4.48±0.13^{bc}	3.52±0.22^{a}	4.28±0.08^{b}	4.54±0.06^{c}
	14	3.50±0.22^{a}	3.9±0.22^{ab}	4.24±0.13^{b}	3.52±0.11^{a}	4.15±0.05^{bc}	4.4±0.20^{c}
	21	3.33±0.08^{a}	3.70±0.10^{b}	4.1±0.23^{c}	3.48±0.18^{a}	4.10±0.22^{c}	4.3±0.13^{c}
	Total	3.44±0.13^{A}	4.06±0.30^{B}	4.36±0.24^{C}	3.53±0.14^{A}	4.24±0.18^{C}	4.49±0.20^{D}
Flavor	1	4.03±0.20^{a}	4.24±0.06^{b}	4.45±0.11^{c}	4.11±0.03^{a}	4.26±0.03^{b}	4.53±0.02^{c}
	7	3.53±0.23^{a}	3.76±0.15^{ab}	4.0±0.26^{ab}	3.63±0.25^{b}	4.16±0.23^{c}	4.13±0.11^{c}
	14	3.30±0.10^{a}	3.40±0.15^{ab}	3.48±0.76^{ab}	3.36±0.07^{ab}	3.8±0.36^{bc}	4.03±0.20^{c}
	21	3.23±0.57^{a}	3.38±0.10^{ab}	3.43±0.76^{ab}	3.30±0.05^{ab}	3.63±0.11^{bc}	3.73±0.15^{c}
	Total	3.52±0.35^{A}	3.69±0.38^{B}	3.84±0.45^{C}	3.60±0.35^{A}	3.96±0.33^{C}	4.10±0.32^{D}
Texture	1	3.94±0.10^{a}	4.25±0.04^{b}	4.27±0.11^{b}	4.03±0.20^{a}	4.23±0.11^{b}	4.26±0.15^{b}
	7	3.53±0.15^{a}	3.73±0.20^{a}	4.10±0.10^{a}	3.60±0.10^{a}	4.13±0.12^{b}	4.33±0.14^{b}
	14	3.30±0.05^{a}	3.48±0.07^{a}	3.53±0.15^{a}	3.38±0.07^{ab}	3.73±0.15^{b}	3.83±0.25^{b}
	21	3.23±0.30^{a}	3.03±0.28^{a}	3.23±0.20^{a}	3.30±0.10^{ab}	3.63±0.25^{b}	3.73±0.40^{b}
	Total	3.50±0.32^{A}	3.62±0.48^{AB}	3.78±0.45^{BC}	3.57±0.31^{A}	3.93±0.30^{CD}	4.04±0.35^{D}
Appearance	1	3.60±0.22^{a}	4.41±0.16^{b}	4.71±0.04^{b}	3.60±0.26^{a}	4.42±0.03^{b}	4.72±0.11^{b}
	7	3.53±0.04^{a}	4.22±0.11^{b}	4.60±0.12^{c}	3.70±0.16^{a}	4.40±0.08^{b}	4.67±0.12^{c}
	14	3.10±0.15^{a}	3.70±0.13^{b}	3.80±0.18^{b}	3.36±0.07^{b}	4.20±0.05^{c}	4.40±0.18^{c}
	21	2.72±0.20^{a}	3.20±0.32^{b}	3.50±0.16^{b}	2.61±0.15^{a}	3.80±0.27^{c}	4.20±0.31^{d}
	Total	3.24±0.39^{A}	3.88±0.52^{B}	4.15±0.55^{C}	3.31±0.47^{A}	4.21±0.29^{C}	4.49±0.27^{D}
Overall Acceptability	1	3.76±0.12^{a}	4.32±0.05^{b}	4.51±0.02^{c}	3.83±0.07^{a}	4.33±0.05^{b}	4.55±0.10^{c}
	7	3.51±0.08^{a}	3.99±0.23^{b}	4.29±0.11^{b}	3.61±0.13^{b}	4.24±0.08^{c}	4.42±0.03^{c}
	14	3.30±0.12^{a}	3.62±0.05^{b}	3.76±0.10^{b}	3.41±0.03^{b}	3.97±0.12^{c}	4.16±0.06^{c}
	21	3.13±0.07^{a}	3.341±0.12^{b}	3.54±0.04^{c}	3.20±0.13^{a}	3.79±0.15^{d}	3.99±0.05^{e}
	Total	3.42±0.26^{A}	3.81±0.39^{B}	4.02±0.41^{C}	3.51±0.25^{A}	4.08±0.24^{C}	4.28±0.23^{D}

Lowercase letters are used to indicate the differences between the averages of the samples on the days of measurement, and uppercase letters are used to indicate the differences between the overall averages of the samples ($P<0.05$).

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that the consumer preference rate of standard and probiotic culture yogurts containing blackberry puree was higher than other yogurt samples. As a result, it has been observed that sensory and consumer preferences play an important role in the selection of fruit types to increase functional properties in fruit yogurt production.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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