

Probiotic properties, sensory qualities, and storage stability of probiotic banana yogurts

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Abstract: The quality properties of probiotic yogurt samples made with banana marmalade (BM), which can be a probiotic product, were examined. Yogurt samples were produced from cow milk inoculated with yogurt cultures and probiotic cultures (*Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and an equal mixture of the 2 strains), and then 15% BM was added to each yogurt sample. Acidity, pH, bacteria counts, and sensory analysis of the yogurt samples were investigated on days 1, 3, 5, 7, 10, and 14 during storage at 4 °C. *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, *L. acidophilus*, and *B. bifidum* counts generally decreased during the storage period. The highest *L. acidophilus* count (8.145 log cfu/g) was found in *L. acidophilus* + *B. bifidum*-mixed yogurt on the third day. The highest *B. bifidum* count (6.38 log cfu/g) was recorded in *B. bifidum*-fermented yogurt on the first day. Yeast and mold counts of all yogurts increased during the storage period. Coliform bacteria and *Staphylococcus aureus* were not found in the yogurt samples. The sensory quality and probiotic properties of all of the yogurts decreased after 7 days. The highest sensory score was observed in the control and the yogurts produced by adding *B. bifidum*.

Key words: Probiotic yogurt, banana yogurt, banana, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*

Probiyotikli muzlu yoğurtların probiyotik özellikleri, duyuşal kalitesi ve depolama stabilitesi

Özet: Bu araştırmada, probiyotik bir ürün olabilmesi mümkün, muz marmelatı ile üretilen probiyotik yoğurt örneklerinin bazı kalite özellikleri incelenmiştir. İnek sütünden yoğurt kültürleri (*Lactobacillus delbrueckii* subsp. *bulgaricus* ve *Streptococcus salivarius* subsp. *thermophilus*) ve farklı probiyotik kültürler (*Lactobacillus acidophilus*, *Bifidobacterium bifidum* ve ikisinin eşit karışımı) ile yoğurtlar üretilmiştir. Daha sonra yoğurtlara % 15 oranında muz marmelatı (MM) ilave edilmiştir. Bütün yoğurt örnekleri 4 °C'de 14 gün depolanmış ve depolamanın 1, 3, 5, 7, 10 ve 14. günlerinde; asitlik, pH, mikroorganizma sayıları ve duyuşal analizler yapılmıştır. *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus salivarius* subsp. *thermophilus*, *L. acidophilus* and *B. bifidum* sayıları genel olarak azalmıştır. En yüksek *L. acidophilus* (8,145 log kob/g) sayısı *L. acidophilus* + *B. bifidum* karışımı ile üretilen yoğurtlarda depolamanın 3. gününde ve en yüksek *B. bifidum* sayısı 6,38 log kob/g olarak *B. bifidum* ile üretilen yoğurtta ilk günde tespit edilmiştir. Probiyotik kültürlü muzlu yoğurtlar, 7. günden sonra probiyotik özelliklerini kaybetmiştir. Maya ve küf sayıları ise bütün yoğurt örneklerinde depolama süresince artmıştır. Hiç bir yoğurt örneğinde koliform ve *Staphylococcus aureus* tespit edilememiştir. Tüm yoğurt örneklerinin duyuşal kalitesi 7. günden sonra azalmıştır. En yüksek duyuşal değerleri kontrol ve *B. bifidum* ilaveli yoğurtlar almıştır.

Anahtar sözcükler: Probiyotik yoğurt, muzlu yoğurt, muz, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*

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Introduction

Most strains of classic yogurt cultures cannot survive in the intestinal tract, and thus their prophylactic use is limited. Microbiological investigations of yogurt report poor microbial survival due to the low pH of the yogurt media, and this has led to the investigation of probiotic formulations in different yogurt preparations (1). Probiotic yogurt is a classic example of a functional food (2). Special types of yogurts are often manufactured for dietetic and/or therapeutic purposes and are known as bioyogurts. Yogurt contains live probiotic microorganisms, which reportedly have beneficial health effects (3-5). The incorporation of *L. acidophilus* and *Bifidobacterium* species into yogurt starter culture may contravene some existing handicaps of yogurt and lead to greater therapeutic value in the resultant milk product (6). The presence of viable lactic acid bacteria in a high concentration in fermented milks, such as yogurt, has been correlated with several consumer health benefits (7,8).

There are several studies on yogurt spoilage (9,10) and the beneficial effects of lactic acid bacteria (11). Due to the presence of both low pH levels and lactic acid bacteria, yogurt is a selective environment for the growth of certain contaminating microorganisms; the introduction of fruit and sugar into yogurt amplifies the risk of spoilage by providing additional fermentable substrates (10). This makes yogurt a less selective growth environment, and yogurt products are likely to support the growth of a number of yeast species (12).

Fruit mixes improve the nutritional value and the taste of yogurt, and fruit enhancement plays a considerable role in yogurt consumption and sales. Varieties of fruit yogurts have been formulated as probiotic fruit yogurts, and the survival of probiotic bacteria in yogurts has been investigated during cold storage (13). Banana (*Musa* sp.) fruit contains considerable amounts of amylose, starch, dietary fiber, protein, vitamins, and minerals (14,15). The production of fruit juices, concentrates, and purees may be a good use for excess and rejected stocks. Common processed banana products include banana puree, banana powder or flour, banana chips, canned banana slices, banana jam, banana vinegar, and banana wine (16,17). Among these, banana puree is

the most important product and is used in the bakery, ice cream, and baby food industries (14). In addition, banana puree is used in fruit-flavored yogurt production. The functionality of yogurt increases with the addition of probiotic microorganisms. The most commonly used probiotic microorganisms in dairy food are *L. acidophilus* and *B. bifidum*, which are chosen for their nutritive, therapeutic, and symbiotic characteristics (18,19).

The aim of this study was to investigate the possibility of using probiotic bacteria (*L. acidophilus*, *B. bifidum*, or an equal mixture of these 2 strains) in the production of banana marmalade (BM)-mixed yogurt products and to contribute to the manufacturing of new functional foods. This study will also help increase the fruit-flavored probiotic yogurt consumption of children and improve consumer health.

Materials and methods

Bacterial cultures, raw cow milk, and banana

L. acidophilus DSMZ 20079 and *B. bifidum* DSMZ 20456 strains were imported from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Yogurt cultures (*L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*) were provided by the Atatürk University Department of Food Engineering (Erzurum, Turkey). Each culture was maintained in 12% sterile reconstituted skim milk supplemented with 2% glucose and 1% yeast extract. All cultures were stored at 4 °C, and working cultures were propagated successively 3 times prior to use (20).

The cow milk used in this study was supplied by the Atatürk University Agricultural Faculty's Food Engineering Department Pilot Dairy Plant (Erzurum, Turkey). Banana was obtained from a supermarket in Erzurum.

Manufacture of yogurts

Yogurt samples were manufactured from cow milk. The dry matter of homogenized milk was increased to 17.32% by evaporation under vacuum pressure (450 mmHg) at 60 °C. Evaporated milk was heated to 90 °C for 5 min and cooled to 37 ± 1 °C for incubation. The yogurt culture was added to the milk, and the

milk was divided into 4 equal parts of 10 kg each. The first part was left unaltered for the control group. *L. acidophilus* was added to the second part (3%, w/w), *B. bifidum* was added to the third part (3%, w/w), and *L. acidophilus* + *B. bifidum* culture was added to fourth part (1:1, 3%, w/w). *L. acidophilus* and *B. bifidum* were added to the milk after the preliminary incubation at 37 ± 1 °C for 18 h. All of the cultured milk groups were then left for a final fermentation at 43 ± 1 °C. The fermentations were terminated when the acidity level reached a pH value of 4.7. Following incubation, yogurt samples were cooled to 5 °C and held at this temperature for 1 day.

A mixture of banana puree and an equal amount of sucrose was heated at 85 °C for 20 min and then cooled to 5 °C. The resulting BM mixture was added to the yogurts at a ratio of 15 g BM to 100 g yogurt (dry matter was 23.29% after the addition of BM). The final product was mixed and then placed in 150-mL sterile glass jars and stored at 4 °C. All yogurt samples were subjected to microbiological, sensory, acidity, and pH analyses on days 1, 3, 5, 7, 10, and 14.

Microbiological analysis

The samples studied (25 g) were weighed aseptically into sterile Stomacher bags diluted with 225 mL of buffered peptone water and homogenized using a Stomacher (HG 400, Mayo International, Milan, Italy). Thus, the first dilution of 10^{-1} was obtained; the other dilutions were prepared from this first 10^{-1} dilution to dilutions of 10^{-7} . Plate count agar, violet red bile agar, potato dextrose agar, and Baird-Parker medium were used for the enumeration of total aerobic mesophilic bacteria (TAMB), coliform bacteria, yeast and mold, and *S. aureus*, as described by Harrigan (21). De Man, Rogosa, and Sharpe (MRS agar, Merck, Darmstadt, Germany), MRS LP, MRS bile, and M17 agars were used for the enumeration of lactic acid bacteria, *B. bifidum*, *L. acidophilus*, and *S. thermophilus*, respectively, as described by Vinderola and Reinheimer (22). The agar plates were incubated for 2 days at 30-32 °C (TAMB), 1 day at 35-37 °C (coliform bacteria), 5-7 days at room temperature (yeast and mold), 1 day at 35-37 °C (*S. aureus*), 3 days at 35-37 °C in an anaerobic jar (lactic acid bacteria and bifidobacteria), or 2 days at 35-37 °C (*S. thermophilus*). All media were provided by Merck except for the Baird-Parker medium (Fluka, Steinheim, Germany).

Physical and chemical analysis

The pH value of the yogurt samples was measured at 17-20 °C with a digital pH meter (pH 211, Hanna Instruments, Amorim, Portugal). The titratable acidity (lactic acid, %) was determined after mixing yogurt samples with 10 mL of distilled water and titrating with 0.1 N NaOH using 0.5% phenolphthalein indicator. Yogurt and milk samples were then analyzed for total dry matter by the gravimetric method (23).

Confirmation of identity of *L. acidophilus* and *B. bifidum*

Identification of *L. acidophilus* and *B. bifidum* colonies was performed by microscopic analysis and with an API identification test kit. The API 50 CH system (ref.: 50300; bioMerieux, Marcy l'Etoile, France) was used to identify *L. acidophilus*, and API 20 A was used for *B. bifidum*. The API identification systems were used according to the manufacturer's directions. Test identification rates were 94.6% for *L. acidophilus* and 97.2% for *B. bifidum*.

Sensory analysis

Stored yogurt samples were assessed by 6 panelists using a sensory rating scale of 1 (poor) to 9 (excellent) for some sensory parameters (odor, texture, syneresis, flavor, acidity, sweetness, and overall acceptability), as described by Bodyfelt et al. (24). All yogurt samples were presented to the panelists in the glass jars (150 mL) and at 4-6 °C. The panel of assessors was an external group of nonsmokers who were very familiar with fermented dairy products and who were evaluated for sensory acuity and consistency. Water and bread were provided to panel members to cleanse their palates between samples.

Statistical analysis

A factorial arrangement was set up to study the influence of probiotic treatments (4) and storage time (6) using 2 replicates. A total of 48 samples were investigated for microbiological, sensory, acidity, and pH analyses on days 1, 3, 5, 7, 10, and 14. All analyses were conducted twice. Data obtained from analysis of the samples were evaluated by variance analysis, and the differences among means were detected by Duncan's multiple range tests (SPSS 1999).

Results

The initial titratable acidity values of the control samples and yogurts with *L. acidophilus*, *B. bifidum*, and *L. acidophilus* + *B. bifidum* were 0.85%, 0.83%, 0.74%, and 0.85%, respectively; these values had increased to 1.04%, 1.00%, 1.10%, and 0.94% at the end of 14 days.

Changes in counts of TAMB, yeast and mold, *S. thermophilus*, and *L. delbrueckii* subsp. *bulgaricus* in yogurt samples are shown in Figures 1-4. Coliform bacteria and *S. aureus* were below the detection limit for all yogurt samples (<10 log cfu/g). Probiotic bacteria counts in BM yogurt samples with probiotic culture decreased significantly after 7 days (Figures 2-4). The *B. bifidum* count was 6.21 on the first day and decreased to 3.40 log cfu/g at the end of 14 days; the *L. acidophilus* count was 8.04 on the first day and decreased to 5.37 log cfu/g at the end of 14 days in yogurt with *L. acidophilus* and *B. bifidum* (Figure 4). The *B. bifidum* count was 6.38 on the first day and decreased to 4.03 log cfu/g at the end of 14 days in yogurt with *B. bifidum* (Figure 3). The *L. acidophilus* count was 7.20 on the first day and decreased to 4.90 log cfu/g at the end of 14 days in yogurt with *L. acidophilus* (Figure 2).

The lowest counts of *L. acidophilus* (4.90 log cfu/g) were found in a single strain of *L. acidophilus*-mixed

yogurt at day 10. The highest counts of *L. acidophilus* (8.15 log cfu/g) were found in *L. acidophilus* + *B. bifidum*-mixed yogurt after 3 days of storage. The effect of storage time on *L. acidophilus* and *B. bifidum* counts was statistically significant ($P < 0.01$).

The average count of *L. acidophilus* in monoculture-mixed yogurt samples was 6.42 log cfu/g. The average count of *L. acidophilus* in yogurts mixed with *L. acidophilus* + *B. bifidum* increased slightly to 6.75 log cfu/g. The differences between these 2 values were not statistically significant. The effect of storage time on *L. acidophilus* counts was statistically significant ($P < 0.01$). Although counts of *L. acidophilus* decreased gradually during storage time, yogurt samples held probiotic values of 10^6 log cfu/g until day 7. The effect of storage time on *B. bifidum* counts was statistically significant ($P < 0.05$). The average count of *B. bifidum* in monoculture-mixed yogurt samples was 5.74 log cfu/g. The average count of *B. bifidum* in yogurts mixed with *L. acidophilus* + *B. bifidum* decreased slightly, to 5.45 log cfu/g. The difference between these 2 values was not statistically significant. The effect of storage time on *B. bifidum* counts was statistically significant ($P < 0.01$). Although counts of *B. bifidum* decreased gradually during storage time, the yogurt samples retained probiotic values of 10^6 log cfu/g until day 7.

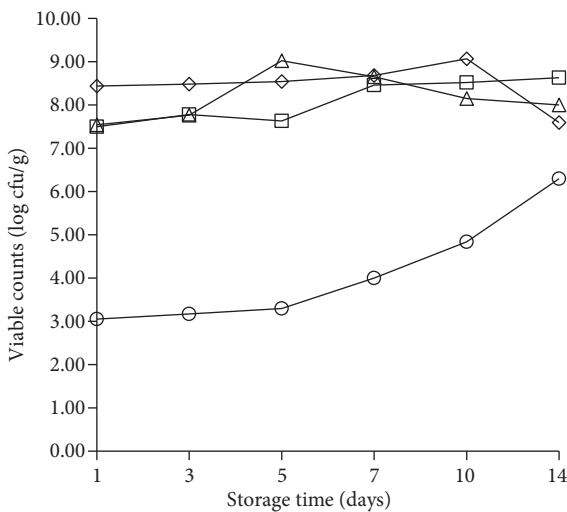


Figure 1. Viable counts of control yogurts during storage at 4 °C for 14 days: Δ *L. delbrueckii* subsp. *bulgaricus*, □ TAMB, ◇ *S. thermophilus*, and ○ yeast and mold.

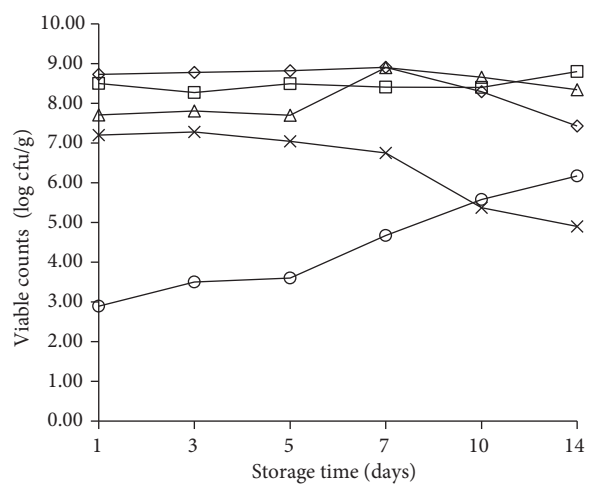


Figure 2. Viable counts of BM yogurts inoculated with *L. acidophilus* during storage at 4 °C for 14 days: Δ *L. delbrueckii* subsp. *bulgaricus*, □ TAMB, ◇ *S. thermophilus*, ○ yeast and mold, and × *L. acidophilus*.

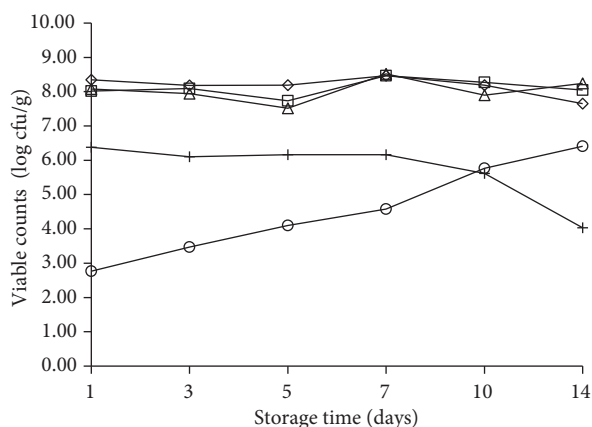


Figure 3. Viable counts of BM yogurts inoculated with *B. bifidum* during storage at 4 °C for 14 days: Δ *L. delbrueckii* subsp. *bulgaricus*, □ TAMB, ◇ *S. thermophilus*, ○ yeast and mold, and + *B. bifidum*.

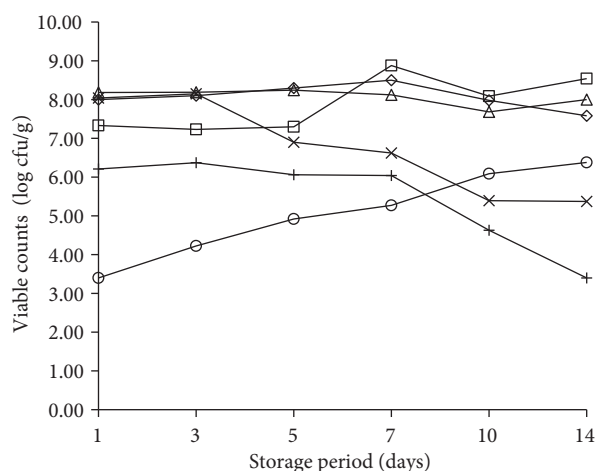


Figure 4. Viable counts of BM yogurts inoculated with *L. acidophilus* and *B. bifidum* during storage at 4 °C for 14 days: Δ *L. delbrueckii* subsp. *bulgaricus*, □ TAMB, ◇ *S. thermophilus*, ○ yeast and mold, x *L. acidophilus*, and + *B. bifidum*.

High sweetness scores, acidity, and general acceptance were obtained by the control yogurts; BM yogurt with *L. acidophilus* received a low score of general acceptance. Statistically significant differences ($P < 0.01$) were found among the control and the other yogurts for the score of sweetness and acidity; however, statistically significant differences ($P < 0.05$) were not found in BM yogurts with *L. acidophilus*, *B. bifidum*, or the *L. acidophilus* and *B. bifidum* combination. A high score of general acceptance was found in the control yogurts and yogurts with *B. bifidum*. High sweetness scores, acidity, and general acceptance were obtained on the first day of storage and decreased up until day 14. No significant differences ($P < 0.05$) were found among the control and other yogurts between days 1 and 7. However, statistically significant differences ($P < 0.01$) were found in the sensory parameter scores for all yogurts after day 7, with the exception of odor and syneresis scores. Similarly, most of the sensory parameter scores decreased after 7 days. However, all scores were above 7 until day 14 of storage.

During the 10-day storage period, general acceptability scores did not fall below 7.5 for BM yogurt samples. General acceptability values of the control samples and yogurts with *L. acidophilus*, *B. bifidum*, and *L. acidophilus* + *B. bifidum* were 7.95, 7.35, 7.68, and 7.60, respectively.

Discussion

Changes in acidity and pH values of the yogurt samples

During the storage period, significant differences were found between the control and other yogurt samples for titratable acidity values. Titratable acidity values of the control and other yogurts tended to increase. *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are responsible for the postacidification of yogurt during cold storage (25). The initial pH values of the control yogurts and yogurts with *L. acidophilus*, *B. bifidum*, and *L. acidophilus* + *B. bifidum* were 4.49%, 4.60%, 4.59%, and 4.52%, respectively. At the end of 14 days, these values were 4.16%, 4.11%, 4.07%, and 4.16%. The pH of the control and other yogurts continued to decrease up until day 14 of storage. Significant differences were found between the pH of the control and the other yogurts. Öztürk and Öner (26) also reported that the titratable acidities of concentrated grape juice-flavored yogurts and control yogurts increased after 7 days of storage at 4 °C and paralleled the change in titratable acidity, while the pH of control and juice-flavored yogurts decreased during 14 days of storage. Similar results were also reported by others (27-29). These results may be due to acid production in the yogurt during storage as a result of lactose fermentation (9).

Changes in some microorganism counts in the yogurt samples

The viable counts of all samples did not change statistically up to day 7; after 7 days the counts decreased, with the exception of yeast and mold ($P < 0.05$). Çon et al. (30) reported similar results for sour cherry, orange, strawberry, and banana yogurts. TAMB, yeast, and mold counts of BM yogurts with *L. acidophilus* increased during the storage period. The TAMB count of BM yogurts with *B. bifidum* had not changed by the end of 14 days. Yeast and mold counts increased significantly during the storage period. This increase was paralleled in BM yogurts with *L. acidophilus*; *S. thermophilus* and *B. bifidum* counts had decreased by day 14, but *L. delbrueckii* subsp. *bulgaricus* counts increased. The TAMB, yeast, and mold counts of BM yogurts with added *L. acidophilus* and *B. bifidum* increased during the 14 days. This increase also occurred in the *L. acidophilus* and *B. bifidum* yogurts. *S. thermophilus* and *B. bifidum* counts decreased. This decrease was similar to the decrease observed in BM yogurts with *B. bifidum*. Similarly, *L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus* counts decreased over 14 days. Coliform bacteria and *S. aureus* were not found in any yogurt samples. A similar result was reported by Birollo et al. (7).

Overall, results were quite similar among the control yogurts and the yogurts containing different probiotic cultures (Figures 1-4). Canganella et al. (27) found that the *S. thermophilus* count remained at 8-9 log cfu/mL throughout the entire experiment, whereas the number of lactobacilli (5-6 log cfu/mL) was stable for 2-3 weeks but then diminished rapidly. In our study, the trend for *Lactobacillus* and lactic *Streptococcus* counts was similar to that reported by Canganella et al. (27).

Changes in *L. acidophilus* and *B. bifidum* counts in yogurt

The number of probiotic organisms in a probiotic product should meet the suggested minimum value of >6 log cfu/g to achieve optimal potential therapeutic

effects (9). Vinderola et al. (28) also stated that probiotic microflora counts decrease during storage. The rate of this loss in cell viability depended on the yogurt type and the use of lactic starter. Con et al. (30) stated that different fruit-flavored yogurts should not be stored longer than 7 days. In this research, counts of *L. acidophilus* and *B. bifidum* decreased gradually with storage time; the yogurt samples had probiotic values of 10^6 log cfu/g up until day 7, and then sensory qualities decreased.

Sensory analysis results

A positive correlation was found between general acceptability and *S. thermophilus* count, and a negative correlation was observed between general acceptability and yeast and mold count ($P < 0.01$). The sensory quality of all yogurts decreased after 7 days. The sensory quality of control yogurts was higher than that of the yogurts with probiotic cultures. Yogurts with *B. bifidum* were preferred to probiotic yogurt samples by the panelists. At the beginning of storage, all yogurts were superior, mainly because of their more intense flavor and better consistency. However, after 7 days, the acidity of the yogurts increased, and the sensory scores of all samples began to decrease. The overall acceptability scores of samples increased during storage for up to 7 days (scores greater than 7) and then decreased (highest score: 9). This could be attributed to the development of acidity. The highest sensory scores occurred in the control yogurt and the yogurts produced by adding *B. bifidum*.

The results suggest that the probiotic cultures tested in this study have the potential to contribute to fruit-flavored yogurt production technology and product taste. Yogurt samples retained probiotic values of 10^6 log cfu/g up until day 7 of storage at 4 °C; after 7 days, sensory qualities also began to decrease. The highest sensory scores were recorded in the control and in yogurts produced with *B. bifidum*.

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