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Production of functional probiotic goat yoghurt supplemented with black grape (*Vitis labrusca* L.) juice concentrate*

Kara üzüm (*Vitis labrusca* L.) suyu konsantrasyonu takviyesi ile üretilmiş fonksiyonel probiyotik keçi yoğurdu üretimi

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ABSTRACT

Objective: To enhance the functional properties of yoghurt, one of the most preferred fermented milk products in Türkiye, black grape (*Vitis labrusca* L.) juice concentrate and the probiotic microorganism (*Lactobacillus acidophilus*) were added to goat milk yoghurt.
Material and Methods: Goat milk yoghurt was prepared with varying concentrations of black grape juice concentrate, 0% v/v (Sample K), 0.5% v/v (Sample A), 1% v/v (Sample B), 1.5% v/v (Sample C) and 2% v/v (Sample D) While some properties (dry matter, oil, ash, protein) were analyzed only on the 1st day of storage, others (pH, titration acidity, antioxidant activity, phenolic compound concentration and microbiological properties) were evaluated on days 1,7,14, and 21 in samples containing black grape juice concentrate and probiotic bacteria.
Results: When the antioxidant capacity values of the samples are compared, Sample B exhibited the highest value. Additionally, Sample D was found to have the highest phenolic content. The total phenolic content of the yoghurts, excluding Sample B, increased when comparing the 1st and 21st days of storage. It was also observed that yoghurts maintained their probiotic qualities throughout the storage period.
Conclusion: The yoghurt samples successfully preserved their probiotic properties throughout the process. Additionally, it was determined that the black grape juice concentrate (*Vitis labrusca* L.) contained phenolic substances and exhibited antioxidant activity. As a result, a functional product with acceptable analysis was obtained.

Keywords: Functional yogurt, goat milk yogurt, grape (*Vitis labrusca* L.), probiotic

ÖZ

Amaç: Ülkemizde en çok tercih edilen fermente süt ürünlerinden biri olan yoğurda çeşitli fonksiyonel özellikler kazandırılmaya çalışılması hedeflenerek keçi sütünden yapılan yoğurtlara kara üzüm (*Vitis labrusca* L.) suyu konsantrasyonu ve probiyotik mikroorganizma (*L. acidophilus*) ilave edilmiştir.
Materyal ve Yöntem: Keçi sütlerine %0 v/v (K), %0.5 v/v (A örneği), %1 v/v (B örneği), %1.5 v/v (C örneği) ve %2 v/v (D örneği) oranlarında kara üzüm suyu konsantrasyonu ve probiyotik bakteri eklenerek üretilen yoğurtların bazı özellikleri (kuru madde, yağ, kül, protein) depolamanın sadece 1. günü belirlenirken, diğer bazı özellikleri de (pH, titrasyon asitliği, antioksidan aktivite, fenolik madde konsantrasyonu ve mikrobiyolojik özellikler) depolamanın 1.,7., 14. ve 21. günlerinde belirlenmiştir.
Araştırma Bulguları: Örneklerin antioksidan kapasite değerleri karşılaştırıldığında B örneğinin en yüksek değere sahip olduğu görülmektedir. Bunun yanı sıra D yoğurdunun en yüksek fenolik madde içeriğine sahip yoğurt olduğu tespit edilmiştir. Yoğurtların B yoğurdu hariç toplam fenolik madde içeriği de depolamanın 1 ve 21. günleri kıyaslandığında artış belirlenmiştir. Depolama süresince yoğurtların probiyotik niteliklerini korudukları saptanmıştır.
Sonuç: Yoğurt örnekleri depolama süresi boyunca probiyotik özelliklerini korumuşlardır. Kara üzüm suyu (*Vitis labrusca* L.) konsantrasyonunun, fenolik madde içerdiği ve antioksidan aktivite gösterdiği belirlenmiş olup, sonuç olarak analizler açısından kabul edilebilir, fonksiyonel bir ürün eldesi sağlanmıştır.

Anahtar sözcükler: Fonksiyonel yoğurt, keçi sütü yoğurdu, kara üzüm (*Vitis labrusca* L.), probiyotik

INTRODUCTION

Although researchers do not have a common definition of functional foods, they are mostly expressed as foods and food components having positive effects on body metabolic activities rather than meeting basic nutritional needs, thus increasing resistance to diseases and contributing to overall well-being (Niva, 2007; Krystallis et al., 2008; Messina et al., 2008).

The properties of a food to be considered functional are as follows: it should act as a probiotic microorganism, contain prebiotic substances or, biologically active ingredients, and be present in food at levels beneficial to the body. Functional foods help reduce the risk of diseases such as cancer, cardiovascular diseases, diabetes, diarrhoea, cholesterol, ulcers, and high blood pressure (Ziemer & Gibson, 1998; Erbaş, 2006; Dayısoylu et al., 2014).

The most important species of probiotic bacteria, defined as live microbiological food components that support intestinal microflora and have proven health benefits, include *Lactobacillus*, *Enterococcus* and *Bifidobacterium* (Klaenhammer, 2000; Krasaekoopt et al., 2006; De Vuyst et al., 2008).

Helping alleviate symptoms of lactose intolerance, constipation, and diarrhoea, supporting the immune system, and exhibiting antitumor and anticarcinogenic effects are among the most important benefits of probiotic microorganisms (Kınık & Gürsoy, 2006; Lye et al., 2009; Çelem, 2023). Due to similar health-supporting effects, the use of probiotics in industrial food production has gained a new dimension (Delikanlı & Özcan, 2014).

When adding probiotics to a product to enhance its functionality; it is essential to use microorganisms with proven probiotic effects and ensure their viability is maintained throughout the entire production, incubation and storage process (Stanton et al., 1998). Since lactic acid bacteria cannot survive in the digestive system, probiotic microorganisms such as bifidobacteria have begun to be added to fermented dairy products such as yoghurt (Kalantzopoulos, 1997). It is recommended to consume 106-109 probiotic bacteria per day (Kınık & Gürsoy, 2006).

In addition to the rise in death rates from diseases such as chronic heart diseases and cancer, as well as risk factors like alcohol consumption, smoking and stress, known to negatively affect health, the effects of foods on health have become an important area of study. To prevent diseases and promote healthier living, research on natural foods and their benefits has increased in both quantity and significance. It is known that plant-based antioxidants help prevent the effects of oxygen and harmful substances that contribute to cellular deformation (Morales et al., 2023; Kaya, 2025).

It has been stated that antioxidant substances influence all cells in the body and help reduce the harmful effects of free radicals, which are known to contribute to diseases such as cardiovascular diseases, cancer, circulatory system, certain eye disorders and aging (Harman, 2009). The foods that naturally contain the highest level of antioxidants are grapes, grapefruit, tomatoes, and apple juice, respectively (Saldamlı, 2014).

Phenolic compounds constitute the most significant group of water-soluble antioxidants known for their positive impact on health. Phenolic compounds such as phytric acid, tocopherol and ascorbic acid are naturally found in fruits and vegetables, tea and cereal grains. It is known that they have a protective effect against harmful internal and external factors, as well as the adverse effects of pathogens on reproduction and growth. As a result of findings related to the antioxidant activity of phenolic compounds, studies in this field have increased (Bravo, 1998; Meral & Doğan, 2006).

Research has shown that phenolic compounds possess antidiabetic, antimicrobial, antiviral, antiallergic, anti-inflammatory, antithrombotic, and antipathogenic properties as well as having barrier effects against diabetes mellitus, cardiovascular diseases, cancer, osteoporosis, and neurodegenerative disorders (Macdougall, 2002; Scalbert et al., 2005).

The labrusca group grape, also known as the strawberry grape, American grape or, Isabella grape, is a grape variety cultivated in the humid climate of the Black Sea Region (Çelik et al., 2009). Its antioxidant effect is higher than that of other grape varieties due to its rich total phenolic content (Rockenbach et al., 2011). Studies have indicated that grape juice surpasses other fruit juices in antioxidant capacity and polyphenol content, is recommended for infant nutrition when breast milk is insufficient, and possesses anti-aging properties (Burak & Çimen, 1999).

Resveratrol, known for its antimutagenic, antioxidant, cancer-preventing, and cholesterol-lowering effects, is found in high amounts in the skin of in fragrant black grape (Çelik, 2003). Grapes and grape-derived products are widely used in studies aimed at enriching phenolic compound and antioxidant substance content for product development. Data from these studies prove that they have a high phenolic component content (Aras, 2006).

The aim of this study was to investigate some chemical and microbiological properties of yoghurts produced from goat milk with black grape (*Vitis labrusca* L.) juice concentration and probiotic microorganism (*L. acidophilus*) added.

MATERIALS and METHODS

Materials

The black grapes (*Vitis labrusca* L.) used in this study were obtained ready-made from the Department of Horticulture, Faculty of Agriculture, Ege University. Any rotten or deformed fruit was removed and stored at +4°C until used within the same week. In the next step, the grapes were first ground through a strainer and then filtered through cheesecloth to separate the pulp and juice. After determining the amount of juice obtained, the separated pulp and juice were mixed again. The mixture was first heat-treated at 80°C for 1 minute and then cooled to 40°C. Sugar was then added to the mixture, amounting to 10% of the fruit juice. The amount of sugar added here was determined through preliminary experiments. The mixture, which contained no additives, was kept at the same temperature for 1 hour and then kept at +4°C for addition to yogurt as soon as possible. Raw goat milk was purchased from Baltalı Gıda Tanıcılık San. And Trade. Ltd. Skimmed milk powder obtained from Pınar Süt AŞ was used in yoghurt production. 80-gram plastic packaging from the Dairy Pilot Enterprise of Ege University Faculty of Agriculture was used (Coveris, TR-34-K-025344). Yoghurt culture (*Lactobacillus bulgaricus* + *Streptococcus thermophillus*) and probiotic culture (*Lactobacillus acidophillus*) used for probiotic yoghurt production were obtained from the Chr Hansen (Christian Hansen, Denmark) company.

Methods

Yoghurt production

For production, the tools and cold storage facilities of Ege University Faculty of Agriculture, Dairy Pilot Plant was used. For the production, 0.831 kg of milk powder (Pınar, TR 35-0069, İzmir, Türkiye) was added to 15 liters of raw goat milk (3.5% fat) at +4ºC, followed by heat treatment at 90ºC for 10 minutes. Commercial yogurt culture and probiotic culture (*L. acidophilus*) were added to milk cooled to 45°C and placed in 5-kilogram plastic yogurt containers. At this stage, black grape juice concentrations of 0% v/v, 0.5% v/v, 1% v/v, 1.5% v/v, and 2% v/v were added to yogurt milk in 5-kilogram plastic yogurt containers at the same temperature (45°C). These ratios were determined based on preliminary tests conducted before production. After mixing, yogurt samples were transferred to 80-gram yogurt containers. The samples were incubated at 45°C until the pH reached 4.6. Following incubation, the yogurt samples were first stored at ambient temperature (20+/-1°C) for 25-30 minutes. After this process, the produced yoghurts were stored at 4°C for 21 days. Production was carried out in two replicates.

Analyses made on raw milk and yoghurt samples

Determinations of dry matter, ash, fat (Gerber method) and protein content in yoghurt samples were conducted. Protein analyses were performed with nitrogen determination, and the result was multiplied by a factor of 6.38 (AOAC, 1990). The pH value was measured using Hanna Instruments, Microprocessor pH-meter (Hanna Instruments USA, Woonsocket, RI 02895). Titratable acidity was determined according to AOAC (1990), while antioxidant activity was assessed 1,1-diphenyl-2-picrylhydrazyl (DPPH) reduction method (Pavithra & Vadivukkarasi, 2015). Measurements were conducted using the Thermo Scientific Skanlt Software for the Varioskan spectrophotometer at 517 nm. The radical quenching capacity of DPPH was calculated using the following formula:

$$\%Inhibition= [(Labs-Labs)] / Labs \times 100$$

Determination of total phenolic content

To determine the total phenolic substance concentration of yoghurt samples, the samples were dissolved in methanol at a 1:1 ratio and centrifuged at 9000 rpm at 4°C for 15 minutes. Then, 20 µL of the filtrate obtained by filtering through filter paper was pipetted onto the plates and allowed to stand. Afterwards, 100 µL of Folin-Ciocalteu reagent was added for colour development. The mixture was incubated at room temperature for 5 minutes. Next, 80 µL of 7.5% Na_2CO_3 was added, and the samples were incubated again in a dark environment for an hour. After incubation, absorbance readings were taken at 760 nm on the Scientific Skanlt Software for Varioskan spectrophotometer (Li et al., 2006; Selçuk & Yılmaz, 2009; Peker, 2012; Ergezer, 2013; Tekyiğit & Uysal, 2023). Total phenolic content was calculated using the equation (y=0.0069218x+0.0257427) obtained from the gallic acid standard curve (R^2:0.959).

Microbiological counts in yoghurt samples

M17 agar was used as the selective medium for *Streptococcus thermophilus* enumeration. Colonies were counted after 48 hours of incubation at 37ºC (Akgün, 2009). Enumeration of *Lactobacillus delbrueckii* ssp. *bulgaricus* was performed according to the International Dairy Federation standard, using the pour plate method on de Man, Rogosa and Sharpe agar (IDF, 1997).

For *Lactobacillus acidophilus* enumeration, MRS-Sorbitol Agar was used. After cultivation, colonies were counted following 72 hours of incubation at 37˚C under anaerobic conditions using the pour plate method, and the results were expressed as colony-forming units per milliliter (cfu/mL) (Dave & Shah, 1996).

Statistical analysis

Analysis of variance (ANOVA) was applied to statistically evaluate and examine the samples, and the data found to be significant as a result of the ANOVA were determined at the $p<0.05$ level according to the Duncan multiple comparison test (IBM SPSS v25).

RESULTS and DISCUSSION

The statistical analysis revealed no significant difference in the average dry matter content of the yoghurt samples($p>0.05$). Total dry matter and total protein content in milk are critical parameters in determining yoghurt quality. Increasing the dry matter content by adding skimmed milk powder is a common technique used to improve texture and prevent defects such as weak gel structure and syneresis (Atamer et al., 2001; Ceylan & Biberoğlu, 2013). Statistical analysis showed that the ash content of samples B and K differed significantly from the other samples ($p<0.05$). (Atamer et al., 2001; Ceylan & Biberoğlu, 2013). Similarly, the fat content of A and B was found to be significantly different from that of the other samples. Furthermore, protein content analysis indicated a significant difference between A and K compared to the other samples ($p<0.05$).

Table 1. Quantities of dry matter, fat, protein and ash on yoghurt samples (Day 1st)

***Çizelge 1**. Yoğurt örneklerine ait kuru madde, kül, yağ ve protein sonuçları (1. Gün)*

Parameter/Sample	A	B	C	D	K
Dry matter (%)	16,89±0,40^{x}	16,71±0,22^{x}	16,79±0,19^{x}	16,75±0,09^{x}	16,95±0,47^{x}
Ash (%)	1,18 ±0,06^{x}	1,06±s0,10^{y}	1,24±0,01^{x}	1,26±0,06^{x}	1,17±0,04^{xy}
Fat (%)	3,40±0,00^{y}	3,13±0,05^{z}	3,71 ±0,05^{x}	3,71±0,05^{x}	3,80 ±0,00^{x}
Protein (%)	5,19±0,05^{y}	5,42±0,01^{x}	5,42±0,08^{x}	5,40±0,01^{x}	5,30±0,14 xy

^{x,y,z}: Values shown with different letters in the same lines indicate significant differences at the p<0.05 level.

The analysis showed that the pH values of the samples ranged from 4.61 to 4.29 over the 21-day storage period. The highest pH value (4.61) was recorded in sample K on Day 1, while the lowest pH value (4.29) was observed in Sample C on Day 21. Statistical analysis indicated the pH changes during storage were significant (p<0.05). Overall, the pH values of all yoghurt samples decreased throughout the storage period.

Previous studies have reported that the pH values of goat yoghurt enriched with milk protein-based products range between 4.4 and 4.6. *L. bulgaricus* and *Streptococcus thermophilus*, which have greater lactic acid production capacity than probiotic strains, cause pH changes in milk and dairy products (Yaygın, 1999; Bonczar et al., 2002; Şahan et al., 2007; İşleten & Karagül-Yüceer, 2008; Gürsel et al., 2016).

Table 2. pH values of yoghurt samples

***Çizelge 2.** Yoğurt örneklerinin pH değerleri*

Sample/Day	1st day	7th day	14th day	21st day
A	4,53±0,015^{zX}	4,50±0,00^{yY}	4,44±0,000^{zZ}	4,38±0,005^{yT}
B	4,57±0,026^{yX}	4,48±0,0057^{zY}	4,41±005^{tZ}	4,38±0,000^{zT}
C	4,59±0,005^{xyX}	4,47±0,000^{tY}	4,32±0,0057^{vZ}	4,29±0,000^{tT}
D	4,57±0,005^{yX}	4,54±0,005^{xY}	4,48±0,000^{xZ}	4,38±0,000^{zT}
K	4,61±0,010^{xX}	4,50±0,005^{yY}	4,45±0,000^{yZ}	4,44±0,000^{xZ}

^{x,y,z,t}: Values shown with different letters in the same columns indicate significant differences at the p<0.05 level.

^{X,Y,Z,T}: Values shown with different letters in the same line indicate significant differences at the p<0.05 level.

Analysis results showed that titratable acidity values in yoghurt samples ranged from 1.11 to 1.29% lactic acid (l.a) over the 21-day storage period. The highest acidity value (1.29% l.a.) was recorded in sample C on Day 21while the lowest acidity (1.11% l.a.) was observed in sample A on Day 1.

Table 3. Lactic acid percentages of yoghurt samples

***Çizelge 3.** Yoğurt örneklerinin % laktik asit miktarları*

Sample/Day	1st day	7th day	14th day	21st day
A	1,11±0,039^{xY}	1,18±0,018^{yX}	1,19±0,000^{zX}	1,21±0,005^{zX}
B	1,13±0,010^{xZ}	1,22±0,005^{xX}	1,20±0,000^{yY}	1,22±0,005^{yX}
C	1,13±0,005^{xT}	1,21±0,000^{xZ}	1,26±0,000^{xY}	1,29±0,005^{xX}
D	1,14±0,010^{xZ}	1,17±0,000^{zY}	1,17±0,005^{vY}	1,20±0,000^{zX}
K	1,14±0,005^{xZ}	1,19±0,005^{yY}	1,18±0,000^{Y}	1,23±0,000^{yX}

^{x,y,z,t}: Values shown with different letters in the same column indicate significant differences at the p<0.05 level.

^{X,Y,Z,T}: Values shown with different letters in the same line indicate significant differences at the p<0.05 level.

Titratable acidity of yoghurt samples generally increased throughout storage, except for sample B, where a decrease was observed between Day 7 and Day 14. However, when comparing the acidity values from Day 1 to Day 21, an overall increase was recorded in sample B by the end of the storage period.

Statistical analysis showed that differences between samples and changes over storage days were significant ($p<0.05$). Overall, the titratable acidity of all yoghurt samples tended to increase during storage.

A study by Sezgin et al (1993) demonstrated that starter cultures increase titratable acidity throughout storage due to their metabolic activities.

Regarding total antioxidant capacity value, the highest value (82.10%) was observed in sample B on Day 1, whereas the lowest value (32.97%) was found in sample K on Day 7. The variation in antioxidant capacity between samples and throughout storage was found to be statistically significant ($p<0.05$).

Table 4. Antioxidant capacity % values of yoghurt samples

Çizelge 4. *Yoğurt örneklerine ait antioksidan kapasite % değerleri*

Sample/Day	1st day	7th day	14th day	21st day
A	$55,81±0,14^{tT}$	$71,51±0,89^{xX}$	$63,51±0,20^{tY}$	$58,27±0,98^{vZ}$
B	$82,10±0,04^{xX}$	$67,88±0,68^{yZ}$	$74,29±0,93^{xY}$	$61,21±0,16^{tT}$
C	$62,72±1,06^{zY}$	$33,11±1,08^{zZ}$	$67,40±1,15^{yX}$	$67,07±0,75^{zX}$
D	$46,02±1,59^{vZ}$	$33,05±0,90^{zT}$	$66,32±0,04^{yzY}$	$80,62±0,28^{xX}$
K	$67,95±0,58^{yY}$	$32,97±0,92^{zT}$	$65,76±0,19^{zZ}$	$74,27±0,80^{yX}$

^{x,y,z,t,v}: Values shown with different letters in the same column indicate significant differences at the $p<0.05$ level.

^{X,Y,Z,T}: Values shown with different letters in the same line indicate significant differences at the $p<0.05$ level.

In a study, probiotic yoghurt was produced by adding pineapple puree to goat milk using *L. reuteri* LR12, and *L. lactis* LL10 strains. It was reported that the total antioxidant capacity of samples containing *L. reuteri* LR12 ranged from 28%—to 89%, while those containing *L. lactis* LL10 varied between 15 and 90% (Al-Dhabi et al., 2020). In another study, the total antioxidant capacity values in goat milk yoghurt enriched with red and black rice bran flour were found to be 45.20% and 38.27%, respectively; the value of the control group goat milk yoghurt was reported as 77.10% (Haskito et al., 2010). Additionally, it has been stated that incorporating natural ingredients such as fruits, vegetables, and grains into milk and dairy products can enhance antioxidant activity, thereby offering protection against diseases linked to oxidative stress (Zubaidah et al., 2010; Samichah & Syauqy, 2014; Abbas et al., 2016).

In a study conducted on fruit yogurts prepared using pomegranate and sour cherry juice concentrates, the antioxidant activity of samples prepared with sour cherry concentrate decreased more during storage than those prepared with pomegranate concentrate (Açıkgözoğlu, 2008).

Kalt et al (1999) reported that the amount of anthocyanins, a flavonoid with strong antioxidant activity, decreased in strawberries, blueberries, and rabbiteye blueberries during storage. Anthocyanins are lost during storage, resulting in a decrease in antioxidant activity. This may be due to various enzymes, pH, temperature and oxygen. In a study on tea, known for its high antioxidant activity, Manzocco et al (1998) observed a decrease in the antioxidant properties of tea extracts over a 30-day storage period.

As a result, the decrease in antioxidant activity of the samples during the storage period or their failure to increase properly may be associated with damage to the compounds that have antioxidant effects due to various reasons.

As a result of the analyses, when the yoghurts were examined in terms of total phenolic content, the highest value (30.49 mg gallic acid/g) was observed in sample D on Day 21 of storage, while the lowest value (17.79 mg gallic acid/g) was recorded in sample A on Day 1. It was observed that the total phenolic content (mg gallic acid/g DM) increased during storage in all yoghurt samples except for B and K. The higher phenolic content in sample D, which contained the highest concentration of grape juice (2% v/v), was expected. Açıkgözoğlu (2008) reported that the phenolic content of fruit yoghurts produced by adding different concentrations like pomegranate and sour cherry concentrates (7.5%v/v, 10%v/v, 12.5%v/v, 15%v/v) ranged between 18.36 mg gallic acid/g and 47% mg gallic acid/g and was directly proportional to the amount of fruit concentrate added.

Table 5. Total phenolic substance values of yoghurt samples (mg GAE / g)

Çizelge 5. *Yoğurt örneklerine ait toplam fenolik madde değerleri (mg GAE / g)*

Sample/Day	1st day	7th day	14th day	21st day
A	17,79±0,13^{zY}	21,78±0,35^{yX}	21,56±0,01^{zX}	21,89±0,58^{yX}
B	20,53±0,37^{yY}	23,24±0,40^{xX}	23,23±0,17^{yX}	17,86±0,74^{tZ}
C	20,80±0,86^{xyX}	21,96±0,07^{yX}	21,11±1,50^{zX}	21,39±0,68^{yX}
D	21,55±0,39^{xT}	24,15±0,69^{xZ}	26,06±0,74^{xY}	30,49±0,06^{xX}
K	18,45±0,41^{zZ}	21,18±0,97^{yY}	25,49±0,89^{xX}	19,74±0,45^{zZ}

^{x,y,z,t,}: Values shown with different letters in the same column indicate a significant difference at the *p*<0.05 level.

^{X,Y,Z,T}: Values shown with different letters in the same line indicate a significant difference at the *p*<0.05 level.

It has been reported that anthocyanins, which constitute a significant portion of flavonoids among phenolic compounds, degrade under the influence of heat (Gil et al., 2000). The lower phenolic content observed suggests that the fruit juice concentrate may have been adversely affected by heat exposure during yoghurt incubation, leading to the degradation of phenolic compounds.

Based on the additions, the total phenolic content of the yogurt samples is directly proportional. It is believed that the yogurt samples containing fruit juice concentrates directly affected the total phenolic content, along with the total phenolic content from milk. Furthermore, it is thought that the phenolic content and fruit juice concentration may have been negatively affected by the increased temperature and acidity during yogurt incubation.

As shown in the table, the highest *Streptococcus thermophilus* count during the 21-day-storage period was observed in Sample K on Day 1 (13.93 log cfu/g), while the lowest value was recorded in sample K on Day 21 (5.82 log cfu/g). The differences in counts between samples and across storage days were found to be statistically significant (*p*<0.05). When examining the changes over time, a decrease was observed between Days 1 and 7, followed by an increase between Days 7 and 14, and a subsequent decrease again on Day 21.

Table 6. *Streptococcus thermophilus* counts in yoghurt samples (log cfu/g)

Çizelge 6. *Yoğurt örneklerinin Streptococcus thermophilus sayıları (log kob/g)*

Sample/Day	1st day	7th day	14th day	21st day
A	13,80±0,14^{xX}	10,84±0,72^{xZ}	11,57±0,12^{xY}	8,33±0,00^{yT}
B	13,54±0,09^{xX}	9,16±0,00^{yZ}	9,40±0,23^{zZ}	10,51±1,08^{xY}
C	11,30±0,35^{yX}	9,38±1,30^{yY}	10,16±0,28^{yXY}	10,23±0,96^{xXY}
D	11,95±0,93^{yX}	8,39±0,67^{yzY}	9,55±0,12^{zY}	9,56±0,44^{xyY}
K	13,93±0,34^{xX}	7,05±0,50^{zZ}	9,82±0,41^{yzY}	5,82±0,34^{zT}

^{x,y,z}: Values shown with different letters in the same column indicate a significant difference at the *p*<0.05 level.

^{X,Y,Z,T}: Values shown with different letters in the same line indicate a significant difference at the *p*<0.05 level.

A study on probiotic goat milk yoghurt enriched with Cupuaçu pulp, a tropical fruit, reported that *Streptococcus thermophilus* counts averaged 11.34 log cfu/g throughout storage and decreased over time (Costa et al, 2015).

The results obtained for *Lactobacillus bulgaricus* in yoghurt samples are presented in Table 7. Statistical analysis indicated that variations in *L. bulgaricus* counts between samples and storage days were significant (*p*<0.05).

Table 7. *L. delbrueckii* subsp. *bulgaricus* counts in yoghurt samples (log cfu/g)

Çizelge 7. *Yoğurt örneklerinin L. delbrueckii* subsp. *bulgaricus sayıları (log kob/g)*

Sample/Day	1st day	7th day	14th day	21st day
A	$8,46±0,09^{zZ}$	$9,30±0,15^{xY}$	$9,65±0,33^{xXY}$	$9,76±0,12^{yX}$
B	$8,99±0,02^{yZ}$	$9,23±0,12^{xY}$	$9,77±0,05^{xX}$	$9,87±0,08^{yX}$
C	$9,41±0,05^{xY}$	$8,09±0,38^{yZ}$	$10,13±0,59^{xX}$	$8,68±0,09^{zZ}$
D	$9,78±0,08^{xX}$	$8,54±0,36^{yY}$	$9,49±0,25^{xX}$	$7,43±0,08^{tZ}$
K	$7,77±0,45^{tZ}$	$9,27±0,01^{xY}$	$9,58±0,19^{xY}$	$10,77±0,10^{xX}$

^{x,y,z,t}: Values shown with different letters in the same column indicate a significant difference at the *p*<0.05 level.

^{X,Y,Z}: Values shown with different letters in the same line indicate a significant difference at the *p*<0.05 level.

During storage, the highest *L. bulgaricus* count was recorded in sample K on Day 21 (10.77 log cfu/g) while the lowest count was observed in sample D on Day 21 (7.43 log cfu/g). An increase in *L. bulgaricus* counts was observed throughout storage in samples A, B and K, whereas fluctuations were noted in C and D.

The results for *L. acidophilus* counts in the samples are presented in Table 8. Statistical analysis indicated that variations in counts between samples and storage days were significant(*p*<0.05). During storage, the highest count (11.08 log cfu/g) was observed in sample K on Day 21, while the lowest count (7.49 log cfu/g) was recorded in sample C on Day 7.

Table 8. *Lactobacillus acidophilus* counts in yoghurt samples (log cfu/g)

Çizelge 8. *Yoğurt örneklerinin Lactobacillus acidophilus sayıları (log kob/g)*

Sample/Day	1st day	7th day	14th day	21st day
A	$7,75±0,24^{zY}$	$7,57±0,19^{yY}$	$9,78±0,07^{yX}$	$9,77±0,06^{zX}$
B	$9,42±0,18^{yY}$	$7,94±0,33^{yZ}$	$10,27±0,11^{xX}$	$10,44±0,19^{yX}$
C	$8,18±0,48^{zY}$	$7,49±0,85^{yY}$	$7,93±0,13^{zY}$	$9,99±0,13^{zX}$
D	$9,27±0,10^{yY}$	$8,25±0,21^{xyZ}$	$9,99±0,16^{xyX}$	$9,17±0,28^{tY}$
K	$10,63±0,23^{xY}$	$8,87±0,02^{xT}$	$9,81±0,29^{yZ}$	$11,08±0,12^{xX}$

^{x,y,z}: Values shown with different letters in the same column indicate a significant difference at the *p*<0.05 level.

^{X,Y,Z,T}: Values shown with different letters in the same line indicate a significant difference at the *p*<0.05 level.

The addition of fructose to milk has been reported to promote the growth of acidophilus (Saxena et al., 1994). In a study on probiotic goat yoghurt production, Costa et al. (2015), reported that acidophilus counts ranged between 11.29 and 9.11 log cfu/g during a 28-day storage period.

One study found that *L. delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, and *L. acidophilus* counts decreased toward the middle of storage and increased later in the day in probiotic yogurts containing no pine honey and those containing varying amounts of pine honey (2%, 4%, and 6%). It has been reported that increasing the amount of honey added positively affected the microbiological values of the yogurts (Dirican, 2017).

As presented in the table, the number of probiotic bacteria in the samples did not fall below 10^7 cfu/g, indicating that the yoghurts retained their probiotic properties throughout storage.

CONCLUSION

When the antioxidant capacity values of yoghurts were analyzed, although a direct correlation between fruit juice concentrate and antioxidant capacity was expected, fluctuations were present throughout the storage period. However, when comparing the 1st and 21st days of storage, an increase in antioxidant capacity was detected in all yoghurt samples except for sample B. A review of similar studies indicates that adding natural ingredients such as fruits or vegetables to milk and dairy products can increase antioxidant activity. Furthermore, the content and ratio of the added ingredient influence antioxidant activity during storage, while various enzymes, pH, temperature, and oxygen can reduce antioxidant activity. Consequently, the lack of a consistent increase or decrease in antioxidant activity during storage may be related to the aforementioned reasons, and further detailed research is recommended.

As for the analyses for total phenolic substance content, the highest value was recorded in sample D on Day 21, while the lowest value was present in sample A on Day 1. In spite of fluctuations observed during storage, sample D consistently exhibited the highest phenolic content among all samples. Similar to the trend in antioxidant capacity, an increase in total phenolic content was observed in all samples, except for sample B, when comparing the 1st and 21st days of storage. A review of the literature indicates that the addition of fruit concentrates rich in phenolic compounds can increase the amount of phenolic compounds in yogurt. The phenolic content of yogurts is directly proportional to the amount of fruit concentrate and the amount of various additives added. Based on this result, it can be concluded that the addition of high-phenolic fruit can be used as a natural preservative in yogurts. Furthermore, considering that increased temperature and acidity during yogurt incubation can negatively affect phenolic content and fruit juice concentration, more detailed examination of all processes used during the production of such yogurts is recommended, and careful, meticulous, and meticulous attention is recommended.

Based on the findings, microbiological analyses revealed differences in the numbers of L. bulgaricus and *S. thermophilus* bacteria among samples. In this study, *S. thermophilus* counts were generally higher than *L. bulgaricus* counts, and fluctuations in *L. bulgaricus* and *S. thermophilus* counts were observed in most samples throughout storage. Increased acidity, decreased pH, and incubation conditions are likely to be responsible for these changes. It is recommended that the effects of the proportions of fruits added to yogurt in the form of concentrates, purees, etc. on yogurt bacterial counts be examined in more detail and across different yogurt bacteria in future studies.

Despite fluctuations throughout the storage period, a general increase in *L. acidophilus* counts was detected when comparing Day 1 and Day 21. These findings confirm that the yoghurts maintained their properties throughout storage. Given that fluctuations during storage are also observed in other yogurt bacteria, it is thought that *L. acidophilus* and other yogurt bacteria interact. It is predictable that irregular increases during storage will occur in studies using different yogurt cultures together.

Based on the results, the produced goat milk yoghurt has the potential to contribute to the food industry as a functional product due to its antioxidant activity, phenolic compounds, and probiotic properties. The addition of grape juice concentrate may improve consumer interest. Considering that the demand for foods with functional properties has increased in recent years, it is thought that the yogurt to be developed using the results we obtained in this study will provide commercial benefits by taking its place in this market. Future research could explore the use of concentrates from other grape species and berries to further enhance product diversity and functionality.

Data Availability

Data will be made available upon reasonable request.

Author Contributions

Conception and design of the study: İTG, HRU, AT; sample collection: İTG; analysis and interpretation of data: İTG, HRU; statistical analysis: İTG, HRU; visualization: İTG, HRU, AT; writing manuscript: İTG, HRU, AT.

Conflict of Interest

There is no conflict of interest between the authors in this study.

Ethical Statement

We declare that there is no need for an ethics committee for this research.

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