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Effect of grape (*Vitis vinifera* L. cv. Dimrit) seed extract powder on total phenolic content and antioxidant properties of ayran drink

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Abstract. In this study, the lyophilized extracts of grape (*Vitis vinifera* L. cv. Dimrit) seeds [0 (control), 4, 5 and 6 mg/100 mL)] were incorporated into ayran drinks, and the total solids, fat, pH, acidity (% lactic acid) and CIELAB color values, total phenolic content, antioxidant activity values, some microbiological and sensory properties of ayran drinks were determined during storage at 4±1 °C for 10 days. The addition of grape seed extract powder (GSEP) had an insignificant effect on the pH, acidity and L* values of ayran drinks ($p > 0.05$), but the a* and b* color values of drinks increased as its concentration increased. The incorporation of GSEP into ayran drinks increased the total phenolic content and antioxidant activity values of samples significantly in comparison to control samples ($p < 0.05$). Addition of GSEP to ayran drinks did not change the counts of lactobacilli and lactococci in ayran drinks during storage. Panelists in sensory evaluation liked the flavor and color properties of control and ayran drinks with 4 mg/100 mL GSEP more than other samples ($p < 0.05$). Results indicated that GSEP might have a great potential to increase the antioxidant activity of ayran drinks, but its concentration is primarily limited by its bitter taste in drinks.

***Keywords*:** antioxidant activity; ayran; grape seed; phenolic content.

1. Introduction

Acidified milk drinks are popular worldwide and have many health benefits and high nutritional value. Ayran (or yogurt drink) is an acidified milk drink produced by diluting and homogenizing yogurt with water or the fermentation of standardized milk with starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) [1, 2]. Plain yogurt drinks such as doogh, lassi, chaas and ayran are popular in a wide geographical region from Central Asia to the Balkans, including Anatolia and the Middle East while in Europe and the US, yogurt drinks with fruits and sweeteners are preferred mostly [2]. It is reported that the consumption of ayran per capita in Turkey in 2019 is 18.1 kg [3].

Grapes, one of the most common cultivated plants in the world [4], are cultivated all over the world with the total area under vines reaching, in 2019, 7.4 million hectares with Spain (13.1%), China (11.5%), France (10.7%), Italy (9.6%) and Turkey (5.9%) representing more than 50% of the world's vineyard [5]. Grapes are easy to cultivate because their climatic and soil requirements are not very selective, and their fruits can be consumed in a variety of ways [6]. Grape berries generally consist of skin, pulp and seed parts and 80-90% of berry weight is juice. The number of seeds in the grain varies between 0-4 [7]. Production of fruit juice, wine and molasses produces grape seeds as a by-product, which are rich in phenolic compounds with antioxidant properties. Gallic acid, catechin, epicatechin, and dimeric, trimeric, oligomeric or polymeric procyanidins are major phenolic constituents of grape seeds [8]. Therefore, grape seeds are valuable for human health and have been used as a food supplement and a source of natural antioxidants [9]. It has been stated that the proanthocyadins contained in grape seed extract, which is a natural antioxidant source, are 50 times stronger than vitamin C and 20 times stronger than vitamin E [10]. Grape seed production is estimated as approximately 30 thousand tons per year in Turkey [11]. Grape seed oil consumption may increase HDL cholesterol level while reducing LDL level [12]. The oil yield, bioactive components and aroma profiles of grape seeds also differ according to grape genotype [13]. Grape seed extract is known as a powerful antioxidant source that may protect human body against certain diseases like Alzheimer and cancer [14].

In a number of studies, grape seed powder extracts were added into biscuits, yogurt, ice cream, vegetable oils, red and white meat products as a natural antioxidant source [15-23]. In addition, there are some studies [24-27] on the use of grape seed extract to increase the antioxidant activity of yogurt while there is a lack of study on increasing the antioxidant activity of ayran drink by its incorporation. In this study, our aim was to increase the total phenolic content (PC) and antioxidant activity value of ayran drink by adding different ratios of GSEP into drinks. The effect of GSEP addition on some physico-chemical properties (dry matter, pH, acidity and color values) of ayran drinks were

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determined during storage at 4±1 °C for 10 days in addition to their microbiological and sensory properties.

2. Experimental

2.1. Materials

Fresh grapes of *Vitis vinifera* L. cultivar Dimrit were obtained from a local producer in Burdur (Turkey). Trolox® [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid] and gallic acid [$(HO)_3C_6H_2CO_2H$] obtained from Fluka (St. Louis, MO, USA) and potassium persulfate ($K_2S_2O_8$), Folin-Ciocalteu reagent, chromatographic grade ethanol (CH_3CH_2OH), buffered peptone water, MRS and M17 agars obtained from Merck (Darmstadt, Germany) were used in analyses. Chromatographic grade methanol (CH_3OH) and ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonate)] were purchased from Sigma-Aldrich (St. Louis, MO, USA) and sodium carbonate (Na_2CO_3) from Riedel-de Haen (Charlotte, NC, USA).

2.2. Methods

2.2.1. Preparation of grape seed extract powder

Grape seed ethanolic extract was prepared with slight modifications as described by Yilmaz and Toledo [8]. After seeds were carefully separated from fresh berries, drying at 40 °C for 24 h was performed in a convective oven (Wisecube, Daihan, Korea). Seeds were ground by a grinder, and a sieve was used to standardize the particle size of ground seed powder. After grape seed powder and aqueous ethanol (70% by volume) (1:10, w/v) were mixed and sonicated for 10 min in a water bath, this mixture was shaken for 15 min on an orbital shaker at 200 rpm. Following the centrifugation of samples in Falcon® tubes at 8000 rpm for 10 min at 10°C, clear supernatants were separated, and this procedure was applied again for solid residues. Supernatants in amber bottles were stored at -24 °C. By using a rotary evaporator, ethanolic phases were evaporated at 40 °C under vacuum, and remaining (aqueous) phase was dried in a freeze-drier to obtain freeze-dried extract powder.

2.2.2. Ayran production and addition of grape seed extract powder into ayran

Plain yoghurt with different production dates from a local dairy plant (BurSüt, Organized Industrial Zone, Burdur, Turkey) was used for the production of ayran drinks. For this purpose, plain yogurt was diluted by drinking water (3:1, w/v) and mixed with table salt at a ratio of 0.70%. This mixture was mixed with a stand mixer (Arzum AR1069 Cust Mix Stand Mixer, Istanbul, Turkey) using its whisker unit at a mixing level of 4 for 3 min to produce ayran samples. This bulk ayran drink was divided into four equal volumes including control. Preliminary experiments showed that a bitter taste was highly noticeable in ayran drinks when more than 6 mg of GSEP was added into 100 mL of ayran. Therefore, GSEP concentrations added to 100 mL of ayran drinks were adjusted to be 4, 5 and 6 mg. The stand mixer was used to produce a homogenous ayran drinks with GSEP. Control and GSEP added samples were stored in Schott bottles (0.5 L) with caps screwed at 4 °C for 10 days.

2.2.3. Physicochemical analysis

The total solid content of ayran drinks was determined by the method of ISO/IDF [28], fat content by Gerber method [29] and total nitrogen content [30] by Dumas method using the Dumatherm® analyzer. A conversion factor of 6.38 was used to calculate the protein content of drinks. The pH values of ayran drinks were determined by a pH meter. Titratable acidity values were determined on the 1st, 5th and 10th days of storage according to Oysun [29].

The CIE (Commission International de L'Eclairage) L*, a* and b* color values of ayran drinks were determined on the 1st, 5th and 10th days of storage using a colorimeter (Model CR-400, Konica Minolta, Japan). Color readings were performed using the D65 illuminator, 10° observer angle and 8 mm diameter diaphragm. Measurements were conducted at room temperature, using a 5 mL sample in an optical glass cell provided by the manufacturer of colorimeter, and taking the averages of 4 measurements at 3 second intervals. For L* (luminance value) color values, 0 and 100 indicate black and white color, respectively. While positive a* and b* color values indicate red and yellow, negative a* and b* color values indicate green and blue, respectively.

2.2.4. Antioxidant activity assay

ABTS assay according to Thaipong et al. [31] was used to determine the antioxidant activity of extracts and ayran drinks on the 1st and 10th days of storage. ABTS dissolved in distilled water was mixed with 2.6 mM $K_2S_2O_8$ (1:1, v/v). This mixture was kept in dark for 12-16 hours at room temperature for the formation of ABTS radicals. ABTS working solution was prepared by diluting this solution with CH_3OH so that the final absorbance was 1.20±0.02 in cuvettes. A fresh calibration curve of Trolox® was prepared for quantification on the day of each experiment. The regression equation was y = - 0.0311 (±0.0054) x + 1.0119 (±0.0397), where y is absorbance value and x is the concentration of Trolox® solution) with $R^2 \geq 0.986$. Sample or standard (150 µL) and ABTS working solution (2850 µL) were mixed in a test tube and allowed to react for 30 min in dark. Then, the absorbance of colored solutions was obtained at 734 nm by using a spectrophotometer (Optizen POP, Mecasys, Daejeon, Korea), and antioxidant activity values were calculated as µmol Trolox® equivalent (TE) per 100 mL ayran drinks and expressed as mean ± standard deviation.

2.2.5. Total phenolic content

The total phenolic contents of ayran drinks and extracts were determined by the micro-adapted Folin-Ciocalteu (FC) method [32]. The FC reagent was diluted with distilled water at a rate of 1:10 (v/v), and the concentration of Na_2CO_3 solution was 20% (w/v). The fresh stock solution of gallic acid (500 mg/L) was used to obtain a calibration curve on the day of each experiment, where a linear region was between 5 and 100 mg/L. The regression equation was y = -0.0094 (±0.0006) x + 0.0233 (±0.0027), where y is absorbance value and x is the concentration of gallic acid solution) with $R^2 \geq 0.999$. Sample or standard (2 mL) and dilute FC reagent (10 mL) were first mixed, and then Na_2CO_3

solution (8 mL, 20% w/v) was added in 1-8 minutes, and the reaction was allowed to continue for 2 h in dark. Finally, the absorbance values of colored solutions were obtained by a spectrophotometer (Optizen POP, Mecasys, Daejeon, Korea) at 760 nm, and total phenolic contents were calculated as mg gallic acid equivalent (GAE) per 100 mL ayran drinks and expressed as mean ± standard deviation. The total phenolic contents of ayran samples were determined on the 1st and 10th days of storage at 4°C.

2.2.6. Microbiological analysis

Ayran drinks were aseptically sampled, and bacterial counts were carried out on the 5th and 10th days of storage. Buffered peptone water was used to dilute samples (10 mL). The enumeration was carried out using the pour plate technique described by Da Silva et al. [26]. The enumeration of *Lactobacillus* spp. was obtained on MRS agar, following 48 h incubation at 37±1 °C under anaerobic conditions while *Lactococcus* spp. was enumerated on M17 agar, following 48 h incubation at 37±1 °C under aerobic conditions.

2.2.7. Sensory analysis

Ranking test was used in sensorial flavor and color evaluations of ayran drinks according to Bodyfelt et al. [33]. Panelists included staff and students of the Department of Food Engineering in Burdur Mehmet Akif Ersoy University (Burdur, Turkey). A different 3-digit numeric code was randomly assigned to each ayran drink. Samples in white plastic cups were served to panelists in varying orders by using individual booths equipped with daylight. Panelists ranked ayran samples from 'the most liked' to 'the least liked' by the flavor and color of samples. Panelists were allowed to drink water to cleanse their palate between samples.

2.2.8. Statistical analysis

All experiments were duplicated while all analyses were carried out in two parallels. The analysis of variance (ANOVA) was conducted to determine the effect of GSEP concentration and storage time on the physicochemical properties, antioxidant activity, phenolic content, microbiological and sensory properties of ayran drinks by using Statistical Analysis System (SAS) version 9.0 (SAS Institute Cary, NC, USA). Differences between means were tested by the Duncan multiple comparison test at a $p < 0.05$ level.

3. Results and discussion

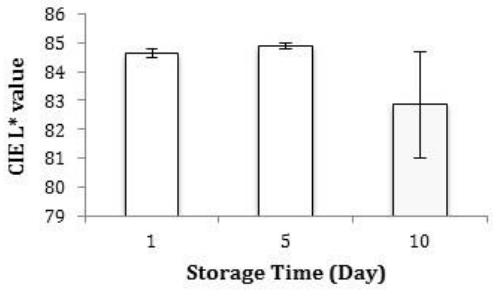
3.1. Physico-chemical properties

The total solids, fat and protein content of the control drinks on the 1st day of storage were determined as 9.79±0.04, 2.55±0.06 and 2.65±0.34%, respectively. The pH and acidity values of ayran drinks during storage are given in Table 1. The addition of GSEP to ayran drinks and storage time had a statistically insignificant effect on the pH values of drinks ($p > 0.05$). Da Silva et al. [26] reported that the addition of grape extract (1.5 and 3.0 g/L) into yoghurt did not influence the pH, titratable acidity (%), protein, ash, fat and moisture contents of yoghurt samples. However, Brahmi et al. [23] and Demirbüker Kavak and Akdeniz [27] reported that the addition of grape seed extract to yogurt slightly decreased the pH values of the yogurt samples. In our study, pH values of drinks were compatible with those reported in plain ayran drinks by Tonguc [34] and Gursoy et al. [35]. Acidity values (%) of samples varied between 0.48±0.02 and 0.54±0.04 during storage, which were in good agreement with previous studies [35, 36]. Acidity values of all samples on the 1st, 5th and 10th days of storage (0.48, 0.51 and 0.53%, respectively) increased during storage ($p < 0.05$) (Table 1), and interaction between storage time and GSEP concentration was statistically insignificant ($p > 0.05$).

Table 1. pH and acidity values (%) of ayran drinks with grape seed extract powder (GSEP) at different concentrations during 10 days of storage ($n = 2$) (mean ± standard deviation).

GSEP concentration in ayran drinks (mg/100 mL)	pH			Acidity (Lactic acid, %)		
	Storage days			Storage days		
	1	5	10	1	5	10
0	4.16±0.04	4.14±0.02	4.20±0.30	0.48±0.02	0.51±0.01	0.53±0.06
4	4.17±0.02	4.11±0.02	4.25±0.24	0.49±0.02	0.51±0.01	0.53±0.04
5	4.19±0.02	4.12±0.03	4.27±0.23	0.48±0.02	0.50±0.01	0.53±0.03
6	4.19±0.02	4.16±0.07	4.28±0.21	0.48±0.02	0.52±0.02	0.54±0.04

Color is an important factor of yogurt-like fermented products, influencing consumer`s acceptability of a product [37]. A high L* color value is an important quality parameter for ayran drink [35]. Storage time influenced the L* and b* color values of ayran drinks significantly ($p < 0.05$) (Figure 1).



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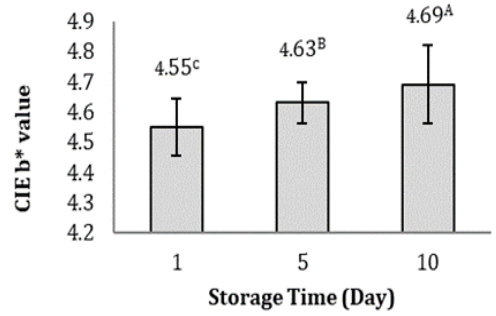
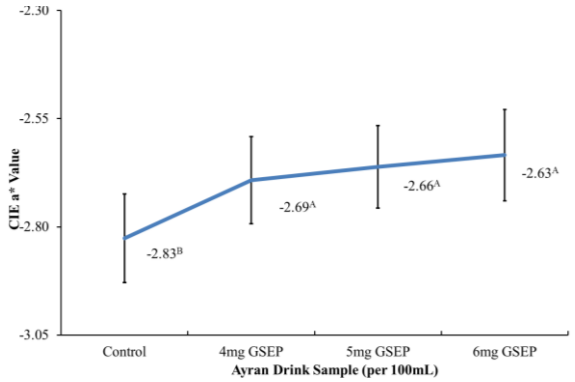


b.

Figure 1. Changes in CIE L* (a) and b* (b) color values of ayran drinks during 10 days of storage (Different superscripts in each figure show statistical significances ($p < 0.05$).

Significant differences in a* and b* values were found among ayran drinks containing different amounts of GSEP ($p > 0.05$) (Figure 2). Demirkol and Tarakçı [38] reported that adding freeze- and oven-dried grape pomace samples to yogurt at different rates (1, 3 and 5%) decreased the L* and b* color values of samples

while increased a* color values. Similarly, the addition of 3% organic Bordeaux grape (*Vitis labrusca* L.) seed flour into yogurt was reported to decrease the L* values of yogurt samples while increasing their a* and b* values by da Rocha Zanetti et al. [39]. In our study, an increase in the concentration of GSEP added to ayran drinks decreased L* color values and increased a* color values, which was similar to results reported by Demirkol and Tarakçı [38] and da Rocha Zanetti et al. [39]. The differences in b* values of samples reported in the literature could be due to variations in GSEP concentrations and grape variety used as a source of seeds.



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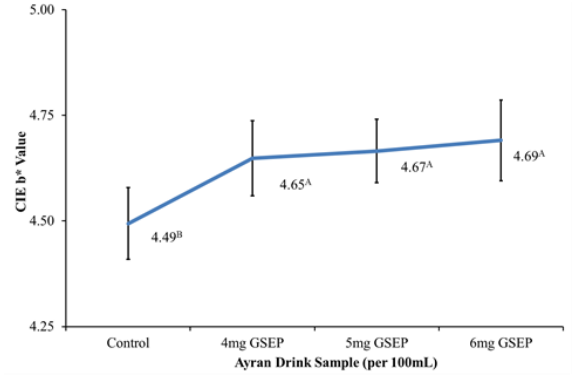


b.

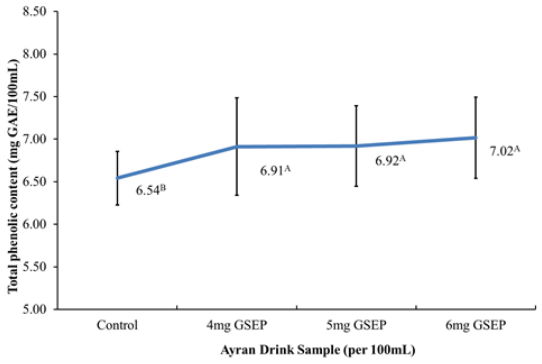
Figure 2. Changes in CIE a* (a) and b* (b) color values depending on the concentration of grape seed extract powder added to ayran drinks. *: Control (without extract), 4, 5 and 6 mg/100 mL grape seed extract powder added ayran drink (Different superscripts in each figure show statistical significances ($p < 0.05$).

3.2. Total phenolic content and antioxidant activity

Phenolic compounds are significant constituents of plant foods, and they influence the sensory properties of foods such as flavor, color and astringency directly. In addition, the presence of phenolic compounds in human diet have some beneficial effects to health because of their chemo-preventive activities against carcinogenesis and mutagenesis, which are mainly associated with their antioxidant activities [40]. The antioxidant activity (by DPPH assay) and total phenolic contents of GSEP were determined as 6,224 µmol TE/g dry matter and 66,909 mg GAE/g dry matter, respectively. Total PC and antioxidant values of ayran drinks during 10 days of storage are presented in Table 2. The addition of GSEP to ayran drinks significantly influenced the total PC and antioxidant activity values of samples ($p < 0.05$). The total phenolic contents and antioxidant activity values of ayran drinks with GSEP were higher than those of control samples (Figure 3). Increased antioxidant activity via the fortification of samples with GSEP was in good agreement with previous reports [25, 27]. Similarly, Altın [41] reported that the addition of cocoa shell (1:10 v/v) encapsulated by different methods to ayran drinks increased the total PC and antioxidant activity of samples. In a study by Ünal et al. [42], it was reported that the addition of green tea and black tea extracts at two different rates (2 and 4%) in the production of ayran drinks increased the total PC and antioxidant activity of ayran samples. Individual effect of storage time on total PC of samples was statistically significant ($p < 0.05$). Total phenolic contents of drinks increased during storage (Figure 4). GSEP concentration and storage time interaction had an insignificant influence on the total PC and antioxidant values of ayran drinks ($p > 0.05$).

Table 2. Changes in total phenolic content and antioxidant activities of ayran drinks with grape seed extract powder (GSEP) at different concentrations on the 1st and 10th days of storage ($n = 2$) (mean ± standard deviation).

GSEP concentration in ayran drinks (mg/100 mL)	Phenolic content (mg GAE/100 mL)		Antioxidant value (µmol TE/ 100 mL)	
	Storage time (Day)		Storage time (Day)	
	1	10	1	10
0	6.31±0.30	6.75±0.14	1.40±0.12	1.34±0.07
4	6.46±0.34	7.36±0.34	1.48±0.15	1.39±0.04
5	6.50±0.22	7.34±0.15	1.49±0.14	1.44±0.05
6	6.69±0.28	7.34±0.42	1.50±0.13	1.47±0.05



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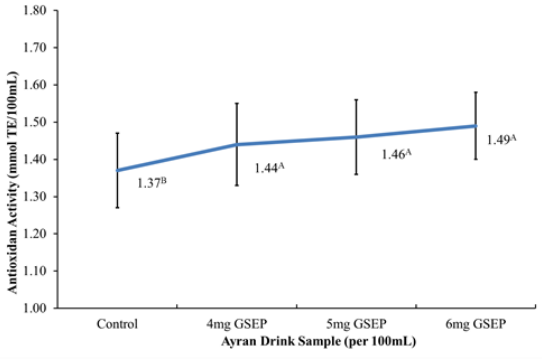

b.

Figure 3. Effect of grape seed extract powder on total phenolic contents (A) and antioxidant activities (B) of ayran drinks (Different superscripts show statistical significances at $p < 0.05$).

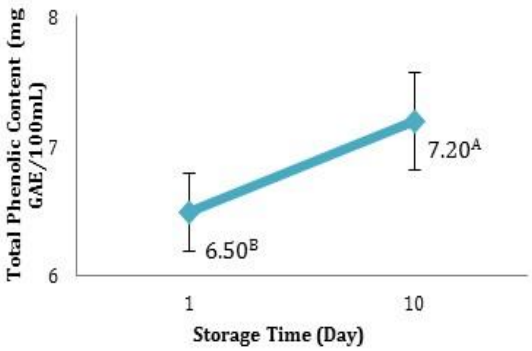

Figure 4. Changes in total phenolic contents of ayran drinks after 10 days of storage at 4 °C (Different superscripts show statistical significances at $p < 0.05$).

3.3. Microbiological properties

Some of lactic acid bacteria (LAB) species may grow in plant-derived food substrates, which can be rich in phenolic compounds, and most of these compounds may inhibit LAB growth [40]. However, antimicrobial effect is related to different factors such as type and concentration of phenolic compound present in the medium, type and strain of bacteria [40]. *Lactobacillus* spp. count of the control ayran drinks on the 1st day of storage was 6.52±0.01 log cfu/mL while the *Lactococcus* spp. count was 7.75±0.73 log cfu/mL. It was determined that the concentration of GSEP in ayran drinks did not influence the lactobacilli and lactococci counts on the 5th and 10th days of storage (Figure 5). In the literature, it was reported that grape pomace addition had no effect on the lactobacilli and lactococci counts of yogurt during 21 days of storage [38]. Similarly, Cossu et al. [43] reported that the chemical and microbiological characteristics of yogurts might not be influenced by the incorporation of selected edible polyphenolic extracts.

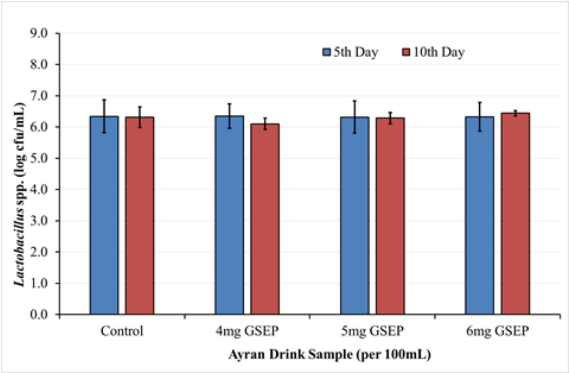

Figure 5. Changes in *Lactobacillus* spp. (A) and *Lactococcus* spp. (B) counts of ayran drinks containing grape seed powder extract at different concentrations on the 5th and 10th days of storage.

3.4. Sensory properties

The addition of GSEP into ayran drinks significantly influenced their sensory flavor and color scores ($p < 0.05$) (Figure 6). The flavor and color scores of the control and ayran drinks with 4 mg/100 mL GSEP were found statistically similar ($p > 0.05$). Similarly, differences in the flavor and color scores of ayran samples with 5 and 6 mg/100 mL GSEP were insignificant ($p > 0.05$). Panelists liked control and ayran samples with 4 mg/100 mL GSEP more than others ($p < 0.05$). Previously, it was reported that the addition of grape seed extract (121.80-224.53 mg GAE/g) to strained yogurt might adversely influence the sensory flavor scores of yogurt [24]. Demirbüker Kavak and Akdeniz [27] reported that high amounts of grape seed extract addition (> 0.15 g/100 g) to yogurt samples were not appreciated by panelists from sensorial point of view. Our results were in good agreement with those findings.

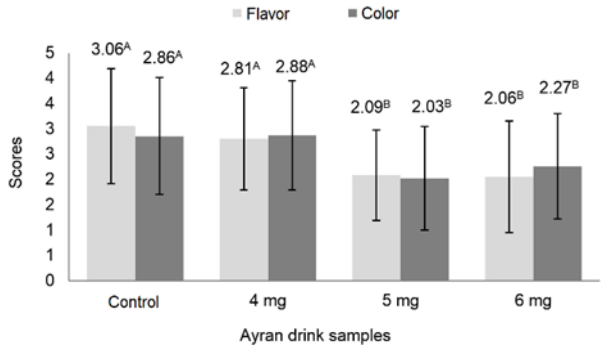

Figure 6. Ranking scores of sensory flavor and color for ayran drinks containing grape seed powder extract at different concentrations (Different letters show statistical significances at $p < 0.05$).

4. Conclusions

The addition of GSEP did not influence some physicochemical properties (pH, acidity and L* color values) and microbiological properties of ayran drinks ($p > 0.05$); however, a* and b* color values of samples increased as its concentration in ayran drinks increased. In terms of flavor and color, control and ayran samples with 4 mg/100 mL GSEP were liked more by the panelists than other samples ($p < 0.05$). It was demonstrated that GSEP could be successfully used to increase the antioxidant activity of ayran drinks. On the other hand, high concentrations of GSEP may influence the sensory flavor and color properties of ayran drinks (> 4 mg/100 mL) adversely. Therefore, ayran drink could be used as a carrier for phenolic compounds of GSEP, and concentrations lower than 5 mg/100 mL sample are highly recommended to minimize any adverse effect on the sensorial properties of drinks.

Conflict of interest

Authors declare no conflict of interest.

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