Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.08122

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Survivability of probiotic strains, *Lactobacillus fermentum* CECT 5716 and *Lactobacillus acidophilus* DSM 20079 in grape juice and physico-chemical properties of the juice during refrigerated storage

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Abstract

In this study, non-fermented probiotic grape juices were produced with the inoculation of two different strains of lactic acid bacteria (*Lactobacillus fermentum* CECT 5716 and *Lactobacillus acidophilus* DSM 20079) separately. The viability of probiotic microorganisms, as well as the grape juices' physicochemical properties were evaluated throughout 21 days of storage at 4 °C. In grape juice, both strains had similar survival rates, and viable cell counts remained above the recommended therapeutic minimum (10^7 CFU mL^{-1}) throughout the monitored storage time. Both of the probiotic samples showed a drop in pH, total phenolic content and viscosity during storage, as well as an increase in acidity. The values of lightness, redness, and yellowness in grape juice containing *L. acidophilus* DSM 20079 were reduced over cold storage period; otherwise, color characteristics for the control and sample with added *L. fermentum* CECT 5716 were maintained. This study showed that grape juice is an effective vehicle for delivering the *Lactobacillus* strains studied, with their probiotic activity remaining for 21 days at 4 °C, making it a probiotic beverage alternative for non-dairy product users. Furthermore, *L. fermentum* CECT 5716 can be more suitable due to its maintenance of color properties in grape juice over the storage.

Keywords: probiotic; grape juice; viability; *Lactobacillus*.

Practical Application: Non-dairy probiotic beverage production with grape juice medium.

1 Introduction

Today, with the changing living conditions, the development of nutritional awareness and the increase in the level of education have led consumers to attach importance to nutrition and food production. Consumers expect food to provide health benefits as well as nutrition and quality. Probiotics are a type of food that are selective live, microbial, dietary supplements that are given in sufficient amounts to provide health advantages beyond those provided by natural nutrition (Champagne et al., 2018; Perricone et al., 2015). In 2001, the WHO and FAO considered probiotics as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'. Finally, in 2014, the International Scientific Association for Probiotics and Prebiotics (ISAPP) modified the latter definition slightly and defined probiotics as 'live microorganisms, that when administered in adequate amounts, confer a health benefit on the host' (Zendeboodi et al., 2020; Hossain et al., 2021).

Health and medical professionals are increasingly promoting the benefits of probiotics to human health (Perricone et al., 2015). Probiotic bacteria-fortified foods are becoming increasingly popular among consumers and there has been an increase in demand for probiotic-based beverages in recent years (Shori, 2016).

Traditionally, probiotics have been used extensively in dairy beverages. Previous studies identified appropriate probiotic strains for incorporation into dairy products such as yoghurt, dairy drinks, cheese an ice cream (Lucatto et al., 2020; Yerlikaya et al., 2020; Prezzi et al., 2020; Mituniewicz-Małek et al., 2019; Acu et al., 2021). Non-dairy beverages such as fruits, vegetables, and cereal juices, on the other hand, may constitute an appropriate vehicle to give probiotics to consumers who are sensitive to milk proteins or have severe lactose intolerance (Kun et al., 2008). Since fruit juice-based functional beverages containing probiotics have appealing flavor profiles for people of all ages and are seen as healthful and refreshing foods, there is a genuine interest in their development (Tuorila & Cardello, 2002; Yoon et al., 2004; Sheehan et al., 2007). Furthermore, they do not contain starter cultures, which compete with probiotic microorganisms for nutrients (Costa et al., 2013; Ding & Shah, 2008; Sheehan et al., 2007). In particular, tea and fruit juices are matrices rich in bioactive components such as vitamins, minerals, and polyphenols, making them promising candidates for probiotic addition (Amorim et al., 2018). However, the use of probiotic cultures in a variety of food matrices and beverages could be a difficult task. Different probiotic species behave differently to substrate acidity, dissolved oxygen, post-acidification in fermented beverages, metabolic products, temperatures, and dry and gastrointestinal tract environments (Guérin et al., 2003; Vinderola & Reinheimer, 2003). Probiotic cultures' survival in fruit juices is influenced by the juice conditions as well as

Received 12 Jan., 2022
Accepted 20 Feb., 2022
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the probiotic strain selected. Furthermore, probiotic cultures have the potential to affect the sensory properties of products, particularly the aroma and flavor features (Antunes et al., 2013; Luckow & Delahunty, 2004; Mostafa et al., 2021).

For many years, it was thought that the presence of phenolic compounds, among other plant matrix ingredients, would inhibit probiotics; nevertheless, studies have revealed that phenolic acids, flavonoids, and betacyanins have a prebiotic impact, promoting the growth of probiotic bacteria (Luciano et al., 2018; Morais et al., 2019). Overall, microbial metabolism in fruit-derived matrices can improve the bioaccessibility and usefulness of phenolic substances (Morais et al., 2019).

Grapes are a widely consumed fruit in Mediterranean regions. In Turkey, the harvest of grape fruit, for the season 2020, was 4,1 million tons, representing an increase of 2.5% as regard the previous season (Food and Agriculture Organization, 2020). Grapes (*Vitis vinifera* L.) enrich nutrients and have shown beneficial effects on human health (Xu et al., 2019), due to the presence of minerals, fiber, vitamins (provitamin A, vitamin C and vitamin E), and particularly phenolics, including phenolic acids, stilbenes, flavonols, flavan-3-ols, and anthocyanins derivatives (Rodriguez-Casado, 2016). The biological effects of grape phenolic compounds as antioxidants, antimicrobials, and enzyme modulators are well documented (Silva et al., 2018). From a nutritional point of view, whole grape juice is comparable to fresh fruit, as it largely maintains constituents such as acids, sugars, minerals, vitamins, and phenolic compounds (Rizzon & Meneguzzo, 2007).

Hence, the objective of this study was to evaluate the viability of *Lactobacillus fermentum (CECT 5716)* and *Lactobacillus acidophilus (DSM 20079)* probiotic cultures and the physicochemical characteristics of non-fermented grape juice during cold storage (4 °C for 21 days).

2 Materials and methods

2.1 Material

In this work, Cabernet Sauvignon and Hamburg Misketi varieties (*Vitis vinifera* L.) of black grapes from Viticulture Research Institute, Tekirdag, Turkey were used. Probiotic cultures including *Lactobacillus fermentum* CECT 5716 and *Lactobacillus acidophilus* DSM 20079 were obtained from culture collection of Selcuk University, Department of Food Engineering (Konya, Turkey).

2.2 Preparation of probiotics

A glycerol stock culture tubes of *L. acidophilus* DSM 20079 and *L. fermentum* CECT 5716 were inoculated individually in 10 mL MRS (de Man, Rogosa, Sharpe) broth (Merck, Darmstadt, Germany) and incubated at 37 °C for 24 h under aerobic conditions. The cultures were then transferred to 95 mL MRS and incubated under the same conditions until the spectrophotometrically measured cell density reached 0.600, which corresponds to 9.00 log CFU mL^{-1} on the MacDFarland scale. The cell density was spectrophotometrically determined at 590 nm. This culture was added to the grape juice as an inoculum (Mariano, 2000).

2.3 Preparation of grape juices

In the production of grape juice, 30% Cabernet sauvignon and 70% Hamburg Misketi, one of the grape varieties grown in the Marmara region, were used. The grapes harvested from the vineyard were first washed in the washing machine and then the stems were separated with the help of a stalk separator. After the harvested grapes were washed with distilled water twice, the stems were separated by a stalk separator. The grape fruits were then crushed using a fruit processor. Heat treatment was applied to the obtained mash at 50 °C for 1 h in order to transfer the crust color and phenolic substance content to the must. It was then pressed in a balloon press and kept in an upright tank overnight to precipitate the must residue. The top residue-free must was kept at -2 °C and crystallization of tartaric acid in the form of tartrate salts was achieved by detartarization. Nearly 90% of the formed crystal structure was removed with the help of thin-plate filters. Grape juices were placed in glass flasks, pasteurized at 80 °C for 20 min in a water bath and cooled in an ice bath until reaching 37 °C. Formulations with probiotic cultures were supplemented with 2% activated probiotic cultures. The grape juices were then stored at 4 ± 2 °C for 21 days prior to use. No additive was added to the juice.

2.4 Formulations

Three formulations of black grape juice were prepared; GJ (no probiotic addition), GJLA (juice added probiotic culture of *L. acidophilus* DSM 20079) and GJLF (juice added probiotic culture of *L. fermentum* CECT 5716). For the probiotic grape juices, 4 mL of activated probiotic bacteria (1,5 x 10^8 CFU mL^{-1}) was inoculated into 200 mL of fruit juices. All prepared juices were stored in glass bottles at 4 ± 2 °C for 21 days. On days 1, 7, 14, and 21 of the refrigerated storage experiment, the pH, titratable acidity, total soluble solids (TSS), total phenolic contents, and survival probiotic counts of grape juices were determined. On the first and 21st days of storage, the color characteristics and viscosity were assessed. The entire experiment was repeated twice, and the analyses were conducted three times.

2.5 Viable probiotic counts determination

The viable counts of *L. acidophilus* DSM 20079 and *L. fermentum* CECT 5716 were obtained by serial dilution with sterile peptone water until 10^{-6} dilution. Aliquots of 0.1 mL of dilution were plated, in triplicate in plates containing MRS agar (Merck®) (spread plate method). The plates were incubated for 72 h at 37 °C under anaerobic conditions and the results were expressed as CFU mL^{-1} juice (Pimentel et al., 2015).

2.6 Physical and chemical characteristics of grape juices

The pH was evaluated by directly immersing the glass electrode in grape juice samples using a previously calibrated digital pH meter (Mettler TOLEDO, ABD). The titratable acidity was calculated by titrating the samples with 0.1 N NaOH and

represented as a percentage of tartaric acid (Cemeroğlu, 2018). The contents of total soluble solids were determined using a portable refractometer (Worldbest 2WA) at 20 ± 1 °C and expressed in °Brix.

The total phenolic content of the samples was determined using Folin–Ciocalteu method spectrophotometric assay as described by Singleton et al. (1999). The calibration curve is made with gallic acid at various concentrations ranging from 0.500 mg mL^{-1} to 0.100 mg mL^{-1}. The results were measured in milligrams of gallic acid equivalents (GAE) per milliliter of grape juice.

The color of the grape juice samples was estimated using a Konica Minolta colorimeter Chroma meter CR-5 (Osaka, Japan), and the results were recorded as L*, a*, and b*. The L* values represent the level of lightness (0-100), the a* red to green (+ = red and – = green) and the b* yellow to blue (+ = yellow and – = blue) (Falcão et al., 2013). Analyses were performed in triplicate.

The apparent viscosity was measured using a rheometer (TA Discovery HR-20). The experimental measurements were obtained after the establishment of the sample equilibrium temperature (10 ± 1 °C) using a thermostatic bath coupled to the viscometer. The measurements were carried out at a constant rate of 10^6 s^{-1}, and the values were expressed in mPa°s.

2.7 Experimental design and statistical analysis

The study's experimental design included two different *Lactobacillus* strains and four different measured storage-time intervals (except for color and viscosity analysis). The analyses were carried out in triplicate in two different experiments, and the results are given as averages standard deviations. The IBM SPSS Statistics 21.0 software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) was used to perform one-way analysis of variance (ANOVA) and Duncan multiple comparison tests on the experimental data at a significance level of $p < 0.05$.

3 Results and discussion

3.1 Survival of probiotics during cold storage

The survival of probiotic cultures in grape juice formulations during refrigerated storage is shown in Table 1. The counts of *L. acidophilus* DSM 20079 and *L. fermentum* CECT 5716 in grape juice samples kept at 4 °C were over 7 log CFU mL^{-1} during the storage period, which is the recommended threshold value for a product to exert its probiotic benefits on the host (Maciel et al., 2014).

On the first day of storage, the number of viable cells in grape juice samples was 7.61 and 7.00 log CFU mL^{-1} for *L. acidophilus* DSM 20079 and *L. fermentum* CECT 5716, respectively. During storage, both probiotic counts increased on day 14, indicating rapid adoptation to the grape juice matrix, and then slightly decreased until the end of storage, with similar counts on days 1 and 28 ($p < 0.05$). The storage time had significant influence ($p < 0.05$) on both probiotic cultures survival. Viable counts of *L. acidophilus* DSM 20079 and *L. fermentum* CECT 5716 declined similarly by 7.7% and 7.6% from the 14th to the last day of storage, respectively. The decreases observed in bacterial concentration are associated with an increase in bacterial susceptibility over time due to cold storage. In addition, it is thought that the incrase in acidity and/or the presence of oxygen in the medium may negatively affect the bacterial count. On the last day of storage, the formulations supplemented with probiotic culture presented similar ($p > 0.05$) counts, and both grape juice formulations could be considered probiotic products (> 10^7 CFU mL^{-1}) during the cold storage period. The results indicate that the probiotics used (*L. acidophilus* DSM 20079 and *L. fermentum* CECT 5716) are resistant strains and that the grape juice was a suitable carrier for probiotic supplementation.

Our findings are consistent with those previously published for fruit beverages. Chen et al. (2019) tested the survivability of *L. rhamnosus*, *L. acidophilus*, *L. casei*, and *L. plantarum* in apple juice after 28 days of refrigerated storage. Although the cultures in apple juice gradually lost viability during cold storage, the viable cell counts remained over 6 log CFU mL^{-1} after 28 days of cold storage at 4 °C, and *L. acidophilus* was more resistant to cold storage than other bacteria in apple juice.

Similarly, the viability of *L. plantarum*, *L. delbrueckii*, and *L. rhamnosus* in grape juice was investigated during 4 weeks of cold storage, and the results revealed that while counts of all bacteria decreased significantly after the 21st day of storage, the level of all bacteria was still above 10^6 CFU mL^{-1} at the end of the cold storage. Zhu et al. (2020) reported that *L. saanfranciscensis* grew well in apple, orange and tomato juice reaching above 6-7 log CFU mL^{-1} at the end of storage at 4 °C. The viable cell counts were higher than 7.00 log CFU mL^{-1} during the storage period, according to the current study's storage assay (21 days).

3.2 Physicochemical characteristics

The physicochemical characteristics of grape juice formulations are shown in Table 2. The grape juices showed TSS (17.45-19.40 °Brix); pH (3.42-3.64) and titratable acidity (0.44-0.74% tartaric acid) values typical for grape juice and similar to those reported by other authors (Burin et al., 2010; Garcia et al., 2018).

Table 1. Viability (log CFU mL^{-1}) of the *L. acidophilus* DSM 20079 and *L. fermentum* CECT 5716 in grape juices during storage at 4 °C for 21 days.

Product	Microorganism	Log CFU mL^{-1}			
		Day 1	Day 7	Day 14	Day 21
Grape Juice	*L. acidophilus* DSM 20079	$7.61 \pm 0{,}06^{bc,A}$	$7.68 \pm 0.12^{ab,A}$	$7.93 \pm 0.23^{a,A}$	$7.32 \pm 0.04^{c,A}$
	L. fermentum CECT 5716	$7.00 \pm 0.3^{b,B}$	$7.79 \pm 0.2^{a,A}$	$7.82 \pm 0.11^{a,A}$	$7.22 \pm 0.23^{ab,A}$

Values are expressed as mean ± standard deviation (n = 3). Different superscript uppercase letters in the same column indicate significant differences ($p < 0.05$) between the samples for the same period of storage. Different superscript lowercase letters in the same row indicate significant differences ($p < 0.05$) between storage days for the same studied sample.

Table 2. Physicochemical characteristics of the grape juice formulations.

Parameter	Storage time (days)	Formulation		
		Control	GJLA	GJLF
Total soluble solids (°Brix)	1	17.70 ± 0.00^{b,BC}	17.45 ± 0.07^{b,C}	19.40 ± 0.00^{a,A}
	7	18.00 ± 0.00^{a,BC}	18.40 ± 0.14^{a,B}	19.35 ± 0.07^{a,A}
	14	18.00 ± 0.00^{a,C}	18.25 ± 0.07^{a,B}	18.65 ± 0.07^{b,A}
	21	18.20 ± 0.00^{c,AB}	18.30 ± 0.00^{a,A}	18.05 ± 0.07^{c,B}
pH	1	3.47 ± 0.00^{a,A}	3.49 ± 0.01^{a,A}	3.64 ± 0.00^{b,B}
	7	3.45 ± 0.01^{ab,A}	3.47 ± 0.01^{ab,A}	3.44 ± 0.01^{a,A}
	14	3.43 ± 0.01^{b,A}	3.46 ± 0.00^{ab,A}	3.43 ± 0.01^{a,A}
	21	3.42 ± 0.00^{bc,A}	3.44 ± 0.01^{b,A}	3.43 ± 0.00^{a,A}
Titratable acidity (%)	1	0.74 ± 0.11^{a,A}	0.52 ± 0.01^{b,AB}	0.44 ± 0.01^{b,B}
	7	0.73 ± 0.07^{a,A}	0.47 ± 0.01^{b,B}	0.45 ± 0.00^{b,B}
	14	0.71 ± 0,01^{a,A}	0.68 ± 0,02^{a,A}	0.71 ± 0,02^{a,A}
	21	0.74 ± 00,1^{a,A}	0.71 ± 0,01^{a,A}	0.74 ± 0,01^{a,A}
Total phenolic content (GAE in mg.mL^{-1})	1	845.00 ± 65.57^{a,B}	995.00 ± 36.05^{a,A}	742.50 ± 25.00^{b,B}
	7	864.50 ± 45.53^{a,A}	782.50 ± 36.05^{c,A}	860.83 ± 14.43^{a,A}
	14	912.50 ± 35.00^{a,A}	877.50 ± 31.22^{b,A}	900.83 ± 24.66^{a,A}
	21	945.83 ± 63.31^{a,A}	855.83 ± 28.86^{bc,A}	865.83 ± 44.81^{a,A}
Color				
L*	1	21.21 ± 0.00^{a,A}	15.74 ± 0.01^{b,B}	11.22 ± 0.02^{c,C}
	21	18.24 ± 0.03^{a,A}	10.75 ± 0.04^{c,BC}	11.06 ± 0.02^{c,C}
a*	1	45.85 ± 0.02^{a,AB}	40.55 ± 0.01^{b,BC}	36.92 ± 0.04^{c,AC}
	21	44.08 ± 0.03^{a,AB}	35.05 ± 0.01^{c,C}	35.76 ± 0.02^{c,C}
b*	1	34.35 ± 0.05^{a,AB}	26.16 ± 0.04^{a,BC}	19.04 ± 0.05^{a,AC}
	21	30.19 ± 0.06^{b,AB}	18.06 ± 0.02^{b,C}	18.66 ± 0.04^{a,C}
ΔE		5.81	10.99	1.23
Viscosity (mPa°s)	1	6.5 ± 0.00^{a,A}	7.0 ± 0.00^{a,A}	7.0 ± 0.00^{a,A}
	21	3.6 ± 0.00^{b,B}	5.0 ± 0.00^{b,A}	3.5 ± 0.00^{b,B}

Values are expressed as mean ± standard deviation (n = 3). Different superscript uppercase letters in the same row indicate significant differences ($p < 0.05$) between the samples for the same period of storage. Different superscript lowercase letters in the same column indicate significant differences ($p < 0.05$) between storage days for the same studied sample. Formulation: Control; grape juice without added probiotic, GJLA; grape juice with *L. acidophilus* DSM 20079, GJ LF; grape juice with *L. fermentum* CECT 5716. L ranging from 0 (black) to 100 (white), a* ranging from red (+a*) to green (−a*), b* ranging from yellow (+b*) to blue (−b*).

On the 1st day of storage, juice supplemented with *L. fermentum* (GJLF) presented a higher TSS ($p < 0.05$) value than juice supplemented with *L. acidophilus* (GJLA) and the control sample. During the storage period, the control and GJLA formulations had an increase in TSS values of 2.8% and 4.9%, respectively, while the TSS value of the GJLF formulation decreased by 7.0% ($p < 0.05$). These findings are most likely connected to the *Lactobacillus* strains' ability to utilise the sugars in the juice as substrates for metabolism, resulting in the creation of organic acids.

GJLA and control samples presented similar pH ($p > 0.05$) values, while GJLF had a higher pH value ($p < 0.05$) on the 1st day of storage. Throughout the storage period, pH values in grape juice declined ($p < 0.05$) slightly in grape beverages containing probiotic bacteria, as well as in the control sample. However, pH of the grape juices with or without added probiotic culture was similar ($p > 0.05$) after the day 7 of refrigerated storage (Table 2). Similar pH values of the samples at the end of storage may be linked to the high buffering capacity of the juices (Nualkaekul & Charalampopoulos, 2011).

The titratable acidity of the probiotic-added samples was lower compared to the control sample on the first day of storage ($p < 0.05$). During the storage period, the acidity value of the control sample was maintained ($p > 0.05$), whereas the acidity of the GJLA and GJLF probiotic samples was significantly increased ($p < 0.05$) from 0.52 to 0.71 and 0.44 to 0.74%, respectively. Regarding acidity, the results obtained can be correlated with the viability of probiotic cultures, where the higher the viability of bacteria, the higher the degree of acidity. Probiotic bacteria can metabolize the simple sugars in grape juice, and dead probiotic cells can release enzymes that hydrolyze the sugars in the media, increasing the acidity of the final product (Rodrigues et al., 2012; Ding & Shah, 2008). Simple sugars contained in juices can be converted into organic acids by *Lactobacillus* bacteria.

Previous researches on non-fermented probiotic fruit juices indicated that pH and acidity alterations varied depending on the probiotic strain and kind of fruit juice used. A study that proves these statements is presented by Garcia et al. (2018), who assessed the viability of five fruit-derived and freeze-dried potentially probiotic *Lactobacillus* strains in apple, orange, and grape juices. These authors reported that the values of the monitored °Brix, pH, or titratable acidity during storage (4 °C, 21 days) varied depending on the added strain and the type of fruit juice. Miranda et al. (2019) reported the acidity

increment of the orange juice after the addition of *L. casei*, and the addition of probiotics had no effect on pH and TSS values of the orange juice.

The total phenolic contents of probiotic-containing samples varied from 742 to 995 GAE in mg.mL^{-1}, and non-probiotic-containing sample was from 845 to 945 GAE in mg.mL^{-1} (Table 2). The antioxidant activity of phenolic compounds, as well as their contribution to the color and sensory features of food products, are recognized to provide significant health benefits for humans (Porto et al., 2018). The addition of the *L. acidophilus* (DSM 20079) probiotic culture had significant impact on the total phenolic content of grape juice ($p < 0.05$), contrary of the *L. fermentum* (CECT 5716) addition (Table 2). The total phenolic content did not vary ($p > 0.05$) in control sample over the monitored storage time. During the storage period, a fluctuating change was observed in sample containing *L. acidophilus* in terms of total phenolic content ($p < 0.05$), while it was increased in sample containing *L. fermentum* ($p < 0.05$) on the 7th day and stayed constant ($p > 0.05$) until the end of storage. However, the total phenolic contents of the samples with or without probiotics were similar ($p > 0.05$) at the end of storage.

Nematollahi et al. (2016) reported that the total phenolic content of cherry juice supplemented with *L. rhamnosus* ATCC 7469 declined somewhat from 216.20 to 191.75 GAE in mg.100 mL^{-1} after 28 days of cold storage, which is similar to our findings for sample containing *L. acidophilus* (GJLA). The addition of *L. casei*, on the other hand, had no effect on the total phenolic content of orange juice, according to Miranda et al. (2019). The authors also reported phenolic compound preservation in cold storage (comparing days 1 and 28). The presence of oxygen, high temperatures, light exposure, and low pH are all factors that alter the phenolic content of juices. The increase in the phenolic content of the GJLF sample may be due to the ability of enzymes such as β-galactosidase and α-amylase produced by this bacterium to convert phenolic glucosides in sugar-bound form to free phenolic acids. The low storage temperature (4 °C) and the use of glass flasks may have contributed to the low loss or preservation of total phenolic content in GJLA and the control sample, respectively.

The color component (L*, a*, and b*) in grape juice samples was measured on the first and last days of cold storage, as shown in Table 2. Considering the color parameters, the addition of probiotic culture resulted in darker (lower L* values), less red and yellow (lower a* and b* values) products ($p < 0.05$), comparing the probiotic products (GJLA and GJLF) to the control. These decreases were more pronounced in the *L. fermentum* supplemented sample when compared to the GJLA sample (comparing the products on the 1st day of storage). The probiotic cultures were added as an active probiotic culture, which resulted in a reduction in the color intensity and a darker color. Control and GJLF formulations behaved in a similar manner during the storage period (comparing the products at days 1 and 28 of storage), with maintenance of the color parameters (L*, a* and b*) ($p > 0.05$). However, during storage at 4 °C, the lightness of GJLA was reduced ($p < 0.05$). The greater turbidity generated by the *L. acidophilus* biomass increase is responsible for these results. A decrease ($p < 0.05$) in the color component a* (redness) and b* (yellowness) was also observed for GJLA sample during the storage (Table 2). These changes are linked to the degradation of the carotenoid pigments in grape fruit, which is increased by heat, humidity, and oxygen exposure (Damasceno et al., 2008; Dhuique-Mayer et al., 2007). Miranda et al. (2019) found that the addition of probiotic culture (*L. casei*) had no effect on the color parameters of orange juice and the color values were maintenanced during the cold storage. Garcia et al. (2018) searched at the color values of apple, grape, and orange juices supplemented with freeze-dried *L. plantarum* 49, *L. brevis* 59, *L. paracasei* 108, *L. fermentum* 111, and *L. pentosus* 129 strains for 21 days at 4 °C and found that the L* value decreased in apple and grape juices while increasing in orange juice. Over the measured storage time, the a* value increased in apple and grape juices but did not change in orange juice. Over time, the b* value of apple juice remained constant, declined in grape juice, and climbed in orange juice.

The ΔE value varied in juices that did or did not contain *Lactobacillus* cells. On day 21 of storage, both the control and GJLA juices had ΔE values of > 2, indicating visible color changes (Lee & Coates, 2003). Enzymatic darkening actions of fruit polyphenolic substances, as well as pigments such as anthocyanin and carotenoids, which are plentiful in grape juice, are principally responsible for changes in sample color (Zepka et al., 2014). On the other hand, lower ΔE values were noted for sample GJLF, whose ΔE value was equal to 1.23. This result reflects differences that are barely visible to the human eye, indicating that the inclusion of *L. fermentum* strengthened the pigment stability in this formulation. Garcia et al. (2018) obtained > 2 ΔE values in orange and grape juices with or without added probiotics for 21 days of refrigerated storage.

As indicated in Table 2, the viscosity of grape juice samples was determined on the first and last days of cold storage. The addition of the probiotic culture did not result in alteration in the viscosity of the grape juice ($p > 0.05$), indicating that the probiotic juices retained the same rheological characteristics as the pure product. The viscosity values decreased ($p < 0.05$) on day 21 of storage in grape juice containing *L. acidophilus* DSM 20079 or *L. fermentum* CECT 5716, as well as in the control samples. The higher viscosity ($p < 0.05$) of sample containing *L. acidophilus* DSM 20079 at the end of the storage period compared to the other samples may be related to the larger total soluble solid content of this sample

4 Conclusion

The grape juice was found to be a good medium for the integration of *L. acidophilus* DSM 20079 or *L. fermentum* CECT 5716, since the products maintained appropriate counts (> 10^7 CFU/mL) during the 21-day refrigerated storage period.

From the monitoring physicochemical characteristics of the grape juice samples during the storage period, it was noted that the probiotic grape juice formulations behaved similarly, with a decrease in the pH and viscosity values and an increase in acidity (comparing days 1 and 28). However, no differences ($p > 0.05$) in total phenolic content, pH, or acidity values were observed on day 21 in grape juice containing probiotic cells

or in the control sample. The L*, a*, and b* values decreased ($p < 0.05$) in GJLA and were maintained ($p > 0.05$) in GJLF during the 21 days of storage. The changes over time in total soluble solids, total phenolic contents, and color values varied depending on the added strain. Color is a key aspect in food acceptance, and the ability to maintain the original color without the use of preservatives is a technological benefit. As a result, grape juice with *L. fermentum* CECT 5716 is more suitable and a decent alternative to probiotic-containing functional products.

Acknowledgements

We would like to thank the Viticulture Research Institute, Tekirdag, for the support provided for this work and also the Selcuk University, Department of Food Engineering, for providing the probiotic bacteria.

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