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The effect of pine honey on the viability of probiotics and some properties of probiotic yogurt

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ABSTRACT: In this study, it was aimed to investigate the effect of pine honey on the viability of probiotic bacteria (*Lactobacillus acidophilus* LA-5® and *Bifidobacterium animalis* subsp. *lactis* BB-12®) and some properties of probiotic yogurts. The non-fat dry matter of milk used in yogurt production is standardized to 11%. Milk is divided into 3 groups (A, B, and C). A, B, and C milk groups were inoculated with yogurt culture, yogurt culture + *L. acidophilus* LA-5 and yogurt culture + *B. animalis* subsp. *lactis* BB-12 respectively. Each 3 groups of milk are divided into 3 parts. While pine honey was not added to the first part (control group), 5% pine honey was added to the second part and 7% pine honey to the third part of the milk. Produced yogurt samples were stored at +4 °C for 21 days and physico-chemical, structural, microbiological, and sensory analyzes were performed on the 1st, 7th, 14th and 21st days of storage. According to the results obtained in the study, with the addition of pine honey, hardness and viscosity values of yogurt samples decreased, while water holding capacity values increased. Also, pine honey addition did not have a significant effect on *L. acidophilus* LA-5 viability, however, the addition of 7% pine honey statistically significantly increased the viability of *B. animalis* subsp. *lactis* BB-12. Also, taste scores of yogurts containing probiotic cultures are lower than those of the control group yogurt in sensory analysis. The odor, texture, and color scores of the yogurt samples were found to be close values to each other.

Keywords: Pine Honey; yogurt; prebiotic; probiotic; viscosity

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INTRODUCTION

Yogurt, which has been accepted as a reliable product for centuries, is one of the most consumed dairy products (Lourens-Hattingh and Viljoen, 2001). Yogurt is also included in functional foods that not only meet the body's need for essential nutrients but have benefits on human health, thereby creating resistance to diseases and leading a healthier life (Sarkar, 2019; Hadjimbei et al., 2022). Not every food can be considered a functional food. In order for a food to be considered functional, it must have components that are beneficial to human physiology, such as bioactive compounds, probiotic bacteria, and prebiotic substances. Yogurt is considered a functional food due to its functional components such as organic acids, bioactive peptides, and oligosaccharides. However, it is possible to increase the functionality of yogurt with the addition of prebiotics and probiotics (Laudadio et al., 2015; Rashwan et al., 2023).

Probiotics are live microorganisms that regulate the intestinal microflora of the host and are generally taken with food. Probiotics can be added to many foods such as yogurt, sour cream, baby food, fruit juice, milk powder, ice cream, butter, mayonnaise, meat, and oats. It is claimed that probiotics provide benefits such as regulating intestinal flora, preventing diarrhea, strengthening the immune system, regulating cholesterol levels, reducing the risk of cancer, and increasing mineral absorption (Soccol et al., 2010; Tripathi and Giri, 2014). Foods in which probiotics and prebiotics are used together are called symbiotics. The effect of symbiotics is greater than the effect of probiotics and prebiotics when used alone. For this reason, it is recommended to use prebiotics in probiotic food production (Şener et al., 2008; Markowiak and Sliżewska, 2017; Ballan et al., 2020) and probiotics should be present in the food in amounts between 10^7 and 10^8 CFU/mL or CFU/g (Afzaal et al., 2019).

Food components that provide beneficial effects on the health of the host, enable selective proliferation of beneficial microorganisms in the gastrointestinal microflora, and pass into the large intestine without being digested at all or to a large extent are defined as prebiotics. (Huebner et al., 2008; Okur et al., 2008). There are many prebiotics that differ in the types and sequence of monosaccharides in their structure. Oligosaccharides and polysaccharides are the most common prebiotics known. Apart from this, some sugar alcohols (sorbitol, mannitol, xylitol, and lactitol) and refined carbohydrates (resistant starch) are also reported to have prebiotic properties (Andersson et al., 2001; Sezen, 2013).

There are two bacteria in the traditionally produced yogurt starter. However, since these bacteria cannot pass through the gastrointestinal tract alive because they do not have acid and bile resistance. Therefore, they do not have any role in the human intestinal system (Meybodi et al., 2020). Probiotic yogurt, which contains a sufficient number of live probiotic microorganisms until the expiration date, contains bacteria that can pass live through the gastrointestinal tract, unlike traditional yogurts. The continuation of the viability of probiotics, both in the product and in the intestinal tract, depends on the various factors such as available energy sources, pH, and temperature. Prebiotic is a non- or very poorly digestible food ingredient that produces beneficial effects for human health by selectively increasing the growth or activity of bacteria in the colon that create beneficial effects for the host (Davani-Davari et al., 2019). There are many studies using various prebiotics to increase the viability of probiotic bacteria in yogurts (Akalın et al., 2004; Yeo and Liong, 2009; Riazi and Ziar, 2012; Karaca et al., 2019). Honey is also one of the possible sources to be used as a prebiotic. Honey contains 25 different types of oligosaccharides such as panose, hybritose, and raffinose. It is known that these oligosaccharides have similar effects with fructooligosaccharides and glucooligosaccharides, and they increase the growth of bifidobacteria in the intestine and have a prebiotic effect (Shamala et al., 2000; Ilyasov et al., 2012; Karadal et al., 2012). Studies on this subject have shown that there are not many studies on the use of honey as a prebiotic in probiotic yogurt.

In this study, the effect of pine honey used at 5% and 7% levels on the chemical, structural and textural properties of yogurt was investigated. In addition, it was aimed to determine whether pine honey was effective on the viability of the probiotic bacteria used in the study.

MATERIAL AND METHODS

Material

Lyophilized culture (Y411, Maysa Food) which contained *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (1%) were used as yogurt starter cultures and pure freeze-dried *Lactobacillus acidophilus* LA-5® and *Bifidobacterium animalis* subsp. *lactis* BB-12® (BB-12®) (CHR. Hansen, Hørsholm, Denmark) were used as probiot-

ic cultures. Commercial pine honey (batch number: BPM19.0037, Balparmak®, Turkey) was used in this study.

Methods

Yogurt and probiotic yogurt production

For the production of yogurt samples, a heat treatment of approximately 10 minutes at 95 °C was applied to the milk (3% fat) and the milk was then cooled to 43-45 °C. After pasteurization, the milk was divided into 3 groups (A, B, and C). Since honey reduces the viscosity of yogurt, the nonfat dry matter of the milk used in yogurt production was adjusted to 11% with skim milk powder. Pine honey was used as a prebiotic source. According to the sensory evaluation results made with 10 panelists during preliminary trials, it was determined that 5% and 7% honey ratios were more appropriate.

The yogurt and probiotic production diagram are given in Figure 1. The first group (A) was inoculated with yogurt culture (1%), 2nd group (B) milk was inoculated with yogurt cultures and *L. acidophilus* LA-5 (10^8 cfu/mL), and 3rd group (C) milk was inoculated with yogurt culture and *B. animalis* subsp. *lactis* BB-12® (10^8 cfu/mL). Each 3 groups of milk (A, B, and C) were divided into 3 subgroups. Pine honey was not added to the 1st samples of groups (sample 1, 4 and 7), but 5% (samples 2, 5 and 8) and %7 (samples 3, 6, and 9) pine honey was added to some subgroups. The inoculated milk was distributed into 100 mL sterile sample dishes. Sample dishes were incubated at 43-45 °C. When the pH values of the samples reached approximately 4.6, the fermentation of the yogurts was stopped and the samples were stored at +4 °C for 21 days. The study was carried out in 2 replications and the analyzes were performed in 3 parallels.

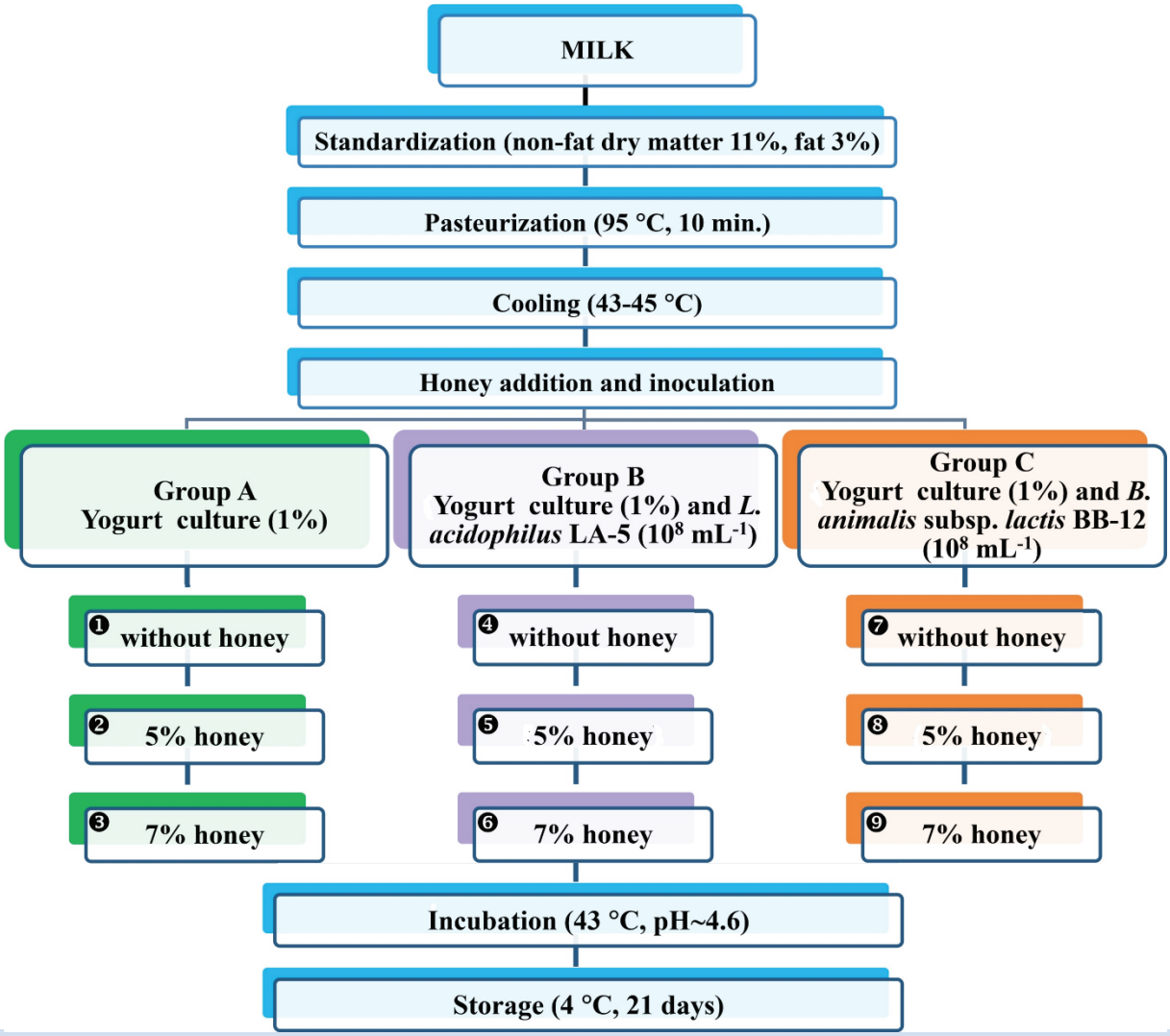

Figure 1. The yogurt and probiotic yogurt production diagram.

Chemical Composition and Physicochemical Analyses

The compositional analyses of pine honey were conducted by the manufacturing company. The total solid of milk was determined by drying in an oven at 105 °C until constant weight (AOAC, 1990). The fat content of milk was determined by the Gerber method (Case et al., 1985). Milk and yogurt samples were analyzed for titratable acidity as a lactic acid percentage according to the method described by AOAC (1995). The pH of the milk and yogurt samples was measured with a pH meter (Thermo Scientific, USA) equipped with a glass electrode.

Water holding capacity

For water holding capacity, 2 g of sample was weighed into centrifuge tubes, then the samples were centrifuged at 13500 x *g* at 10 ºC for 30 minutes in a refrigerated centrifuge (Hettich Universal 32R, Germany) and at the end of the time, the watery part on the top was removed. The water holding capacity of the samples was calculated by proportioning the solid part remaining in the tubes to the weight of the first yogurt sample (Parnell-Clunies et al., 1988).

Syneresis

A 40 g sample was weighed into the tube and then centrifuged at 222 x *g* at 10 ºC for 10 minutes in a refrigerated centrifuge (Hettich Universal 32R, Germany). The syneresis value was calculated by proportioning the clear aqueous phase remaining at the top of the tubes to the initial weighing value of the yogurt sample (Keogh and O'Kennedy, 1998).

Viscosity measurement

Samples were kept at +4 °C for viscosity determination. The samples to be analyzed were taken out of the refrigerator, and mixed with a spatula to make them homogeneous, and viscosity measurements were made. Viscosity analysis in yogurt samples was performed using a rheometer (Brookfield DV-III Ultra, MA, USA) equipped with a helipath stand and a T-bar spindle rotating at a speed of 20 rpm (Gauche et al., 2009).

The texture profile analysis (TPA)

The texture profile analysis (**TPA**) test was carried out using TA-XT Plus texture analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK) equipped 5 kg load cell and a cylindrical probe of 25.4 mm in diameter and results were analyzed in the Texture Expert Exceed Version 2V3 program. Two cycles were applied to a depth of 10 mm at the rate of 1 mm s^{-1}. Hardness, adhesiveness, cohesiveness, gumminess, and springiness factors were determined in the samples (Mudgil et al., 2017).

Microbiological analysis

The selective media were preferred to enumerate for probiotic bacteria. MRS-sorbitol agar was used for *L. acidophilus* LA-5 and MRS-NNLP agar was used for *B. animalis* subsp. *lactis* BB-12. These media were prepared according to the method described by Dave and Shah (1996, 1997). For MRS-sorbitol agar, firstly MRS base medium was prepared without glucose and autoclaved. D-sorbitol solution was sterilized with 0.45 µm MILLIPORE MILLEX ™ GP syringe filter (Millipore, Cork, Ireland). Ten mL of sterile D-sorbitol solution was added per 90 mL of base medium (1% final concentration) at 50 °C. For MRS-NNLP agar, selective agents which were nalidixic acid (50 mg L^{-1}), neomycin sulfate (100 mg L^{-1}), lithium chloride (3000 mg L^{-1}), and paromomycin sulfate (200 mg L^{-1}) (Sigma-Aldrich, USA) were prepared and sterilized with 0.45 µm syringe filter. Twenty mL of NNLP solution was added per 80 mL of MRS agar at 50 °C. In addition, the inhibitor-amino acid L-cysteine was used for enhancing the growth of anaerobic bifidobacteria and lower the oxidation-reduction potential of the medium (Tharmaraj and Shah, 2003).

Ten grams of yogurt sample was transferred to the erlenmayer which was containing 90 mL sterile 0.1% sterile peptone water in order to make an initial (10^{-1}) dilution. Serial dilutions were prepared, and tubes were mixed uniformly with a vortex (VELP Scientifica, Italy). A 0.1 mL of the aliquots from appropriate dilutions were spread plated onto MRS-sorbitol agar, MRS-NNLP agar and M17 agar. M17 plates were incubated aerobically for 72 hours at 37 °C and MRS-Sorbitol plates were incubated at CO2 incubator (5% CO2 - microaerobically) and MRS-NNLP plates were incubated anaerobically in anaerobic jars (using a Gas Pack) for 72 hours at 42 °C, respectively. Plates containing 30 - 300 colonies were enumerated and the results were recorded as colony-forming units. For this purpose, syringe filter-sterilized (0.45 µm) L-cysteine HCl (Merck, Germany) was added to MRS agar (0.05% final concentration) in flask at the same time NNLP solution. Also, M17 agar was used for enumeration of *Str. thermophilus*.per gram (CFU g^{-1}) of yogurt.

Sensory analysis

Sensory analysis was done 1, 7, 14, and 21 days of storage. Yogurt samples were stored at 4 °C and served after the samples out of the refrigerator. The sensory analysis was carried out by 10 panelist who had previous experience in sensory studies of yogurt. A hedonic type scale was used for the evaluation and scoring of yogurt samples. The color, odor, taste, appearance, consistency, and acceptability parameters were assessed by panelists.

Statistical analysis

SPSS (Statistical Package for Social Science) PASW 21 Statistical Package Program was used to evaluate the data obtained in the study. The effects of storage time and honey levels (independent variables) on chemical composition and physicochemical analyses, water holding capacity, syneresis, viscosity measurement, texture profile analysis, microbiological analysis and sensory analysis (dependent variables) were analyzed by Two-Way Analysis of Variance (ANOVA). The Duncan's multiple range test was used to determine the degree of difference. The lowest level of significance was taken as $p < 0.05$ in all statistical tests.

RESULT AND DISCUSSION

The composition of pine honey and milk used in yogurt production has been determined and is presented in Table 1. The components of pine honey were analyzed in the laboratory belonging to the Balparmak® company. The dry matter content of the milk used in yogurt production is 14%, the non-fat dry matter content is 11%, and the fat content is 3%. The diagram related to yogurt and probiotic yogurt production is given in Figure 1.

pH is a measure of the concentration of H^+ ions and is an important parameter in determining the incubation time of yogurt. The pH values of all samples (nine subgroups) have statistically decreased during the storage period. The pH values of samples 8 and 9, however, are consistently higher than the pH values of the other samples in all analysis period (Figure 2). This situation may have occurred due to the buffering properties of protein breakdown products resulting from the proteolytic activities of bifidobacteria. Bifidobacteria have higher proteolytic activity compared to lactic acid bacteria. Additionally, honey may have stimulated the proteolytic activity of bifidobacteria (Abu-Taraboush et al., 1998; Bergamini et al., 2009).

The titratable acidity is used to measure the degree of acidity. The acidity values increased in all samples (nine subgroups) during the storage period. The highest acidity values were obtained from probiotic yogurts without pine honey. The lowest acidity values were found in yogurts with the highest pH values, which included *B. animalis* subsp. *lactis* BB-12 and pine honey (Figure 2). *Str. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* are homofermentative lactic acid bacteria. Homofermentative lactic acid bacteria metabolize hexoses via the Embden-Meyerhof (EMP), with lactic acid as the major end product. However, bifidobacteria are heterofermentative bacteria that metabolize hexoses via the bifid-shunt, producing acetate and lactate in a 3:2 ratio (González-Rodríguez et al., 2013).

Furthermore, some studies have shown that bifidobacteria can utilize a diverse range of oligo- and polysaccharides such as xylo-oligosaccharides, (trans)-galactooligosaccharides, soy bean oligosaccharides,

Table 1. Composition of pine honey and milk used yogurt production

Pine Honey		Milk	
Glucose (%)	26.80	Total solid (%)	14
Fructose (%)	32.30	Non-fat dry matter (%)	11
Turanose (%)	1.80	Fat (%)	3
Isomaltose (%)	1.60	pH	6.65
Total Disaccharide (%)	11.70	Acidity (%)	0.130
Oligosaccharides (%)	7.90	Antibiotic	ND
Color (mm)	70		
Moisture (%)	16.50		
pH	4.60		
Free acidity (meq kg^{-1})	25.300		
Diastase number	15.6		

***ND:** Not detected

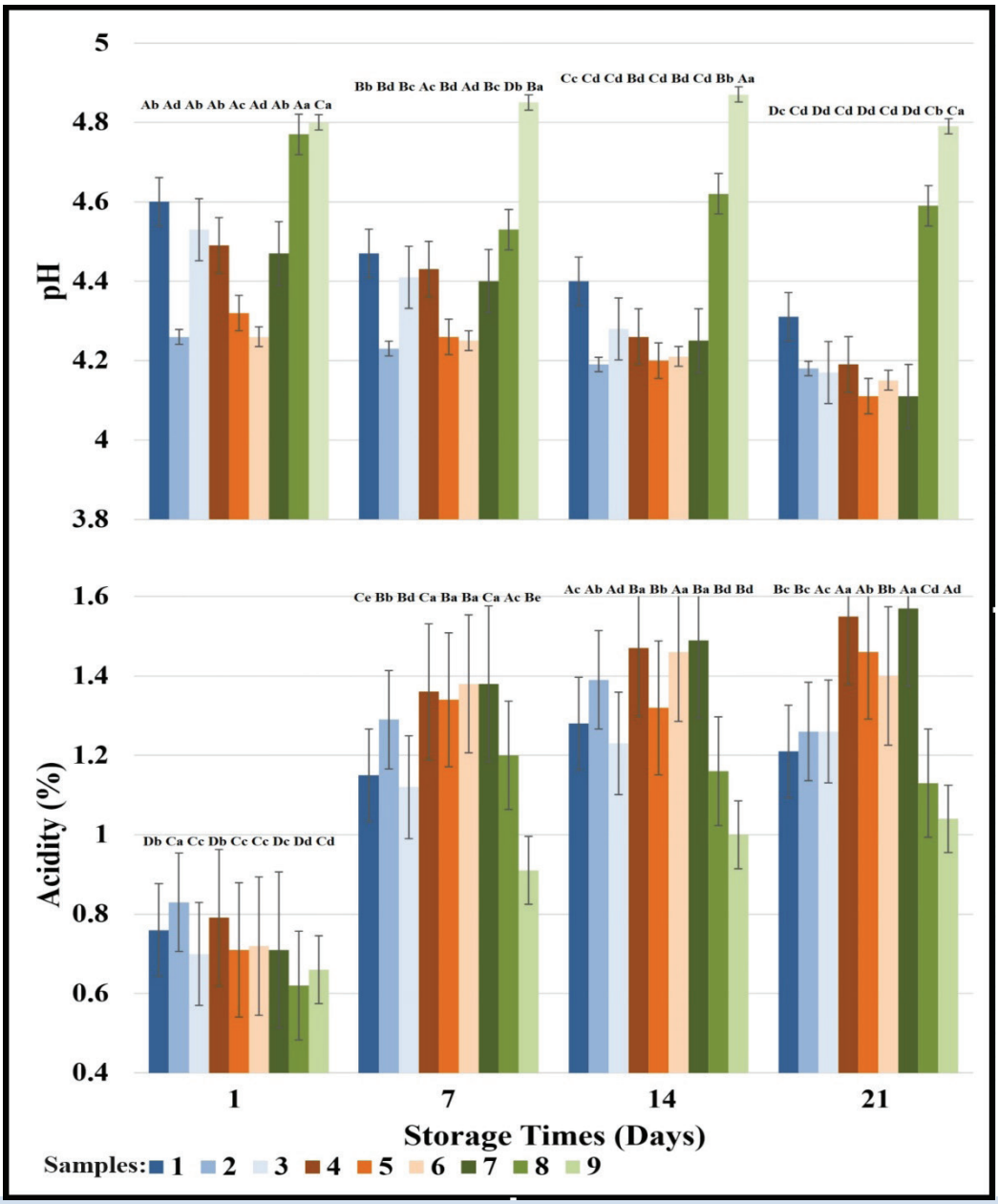

Figure 2. pH and acidity values of yogurt samples.

Different uppercase letters indicate a significant difference between the same samples at different storage times ($p < 0.05$).
Different lowercase letters indicate a significant difference between different samples at the same storage time ($p < 0.05$).

1: Yogurt without pine honey and probiotics; 2: Yogurt with 5% pine honey without probiotics; 3: Yogurt with 7% pine honey without probiotics; 4: Yogurt without pine honey with *L. acidophilus* LA-5; 5: Yogurt with 5% pine honey and *L. acidophilus* LA-5; 6: Yogurt with 7% pine honey and *L. acidophilus* LA-5; 7: Yogurt without pine honey with *B. animalis* subsp. *lactis* BB-12; 8: Yogurt with 5% pine honey and *B. animalis* subsp. *lactis* BB-12; 9: Yogurt with 7% pine honey and *B. animalis* subsp. *lactis* BB-12.

malto-oligosaccharides, and fructo-oligosaccharides (Amaretti et al., 2006; Vrese and Schrezenmeir, 2008; Pokusaeva and Fitzgerald, 2011). This means that oligosaccharides found in honey and lactose found in milk can increase the activity of bifidobacteria. In addition to the organic acids found in yogurt, honey contains various organic acids (0.17% to 1.17%) such as acetic, butyric, citric, formic, gluconic, lactic, malic, pyroglutamic, and succinic acids. Acetic acid has a stronger antagonistic effect on bacteria than lactic acid (Ammar et al., 2015). The acidity values of yogurt samples containing *B. animalis* subsp. *lactis* BB-12 and pine honey were lower than those of the other samples. Because bifidobacteria produce acetic acid

to lactic acid in a 2:1 ratio. Pine honey also contains some acetic acid. Therefore, both the acetic acid produced by *B. animalis* subsp. *lactis* BB-12 and the acetic acid present in pine honey may have inhibited the activities of yogurt bacteria (Figure 4) (Lourens-Hattingh and Viljoen, 2001). pH and acidity values obtained in the study are in line with the pH and acidity values reported in studies related to probiotic yogurt (Çakmakçı et al., 2012; Bahrami et al., 2013; Pour et al., 2022).

Water-holding capacity is a term used to describe the ability of macromolecules such as pectin, starch, and proteins at low concentrations to physically trap large amounts of water, preventing it from leaking out of the structure. In yogurt, water is held in the gel structure formed by milk proteins. Water protein interactions occur through dipole-ion, dipole-dipole, hydrophobic hydration and hydrophobic interactions (Fennema, 1996; Delikanli and Özcan, 2017). The water-holding capacity of yogurt samples showed fluctuating changes during the storage period and ranged between 43% and 60%. The highest water-holding capacity values during storage were observed in 2nd group (B) samples. At the end of the storage period, all three groups (nine samples) showed a slight increase in water-holding capacity for the samples with added pine honey compared to those without pine honey (Table 2). In yogurts produced by Mercan and Akın (2017) using pine honey, an increase in honey concentration has been observed to correspond with an increase in the water-holding capacity of the yogurts. This increase could be attributed to the ability of carbohydrates in honey to hydrate water. Similar results have been reported for yogurt samples with added molasses, apple fiber, and dandelion extract (Sert et al., 2010; Akın et al., 2016; Yao et al., 2017).

The syneresis values of the samples centrifuged at 4 °C varied between 1% and 12%. The syneresis values of all samples increased up to 7 days of storage and then began to decrease. On the 1st, 7th, and 21st days of storage, the highest syneresis values were observed in the 3rd group (C - samples 7, 8 and 9) samples containing *B. animalis* subsp. *lactis* BB-12 (Table 2). Syneresis is influenced by various factors such as the dry matter content, acidity, heat treatment, cooling, and mechanical processes (Arab et al, 2023).

The fact that the 3rd (C) group samples (7, 8 and

Table 2. Water holding capacity and syneresis values of yogurt samples

		Storage Time (Days)			
	Sample no	**1**	**7**	**14**	**21**
Water Holding Capacity (%)	**1**	43.0 ± 0.02 ^{Be}	54.5 ± 0.01 ^{Ab}	46.0 ± 0.02 ^{Bd}	46.0 ± 0.01 ^{Bd}
	2	50.0 ± 0.08 ^{Bd}	52.0 ± 0.00 ^{Ac}	48.0 ± 0.01 ^{Bc}	52.0 ± 0.03 ^{Ab}
	3	57.0 ± 0.03 ^{Ab}	53.0 ± 0.01 ^{Bc}	46.0 ± 0.00 ^{Cd}	50.0 ± 0.02 ^{Bc}
	4	53.0 ± 0.01 ^{Bc}	56.0 ± 0.00 ^{Aa}	52.0 ± 0.02 ^{Ba}	53.0 ± 0.03 ^{Bb}
	5	55.0 ± 0.01 ^{Ac}	57.0 ± 0.03 ^{Aa}	52.0 ± 0.07 ^{Ba}	54.0 ± 0.01 ^{Aa}
	6	60.0 ± 0.11 ^{Aa}	56.0 ± 0.00 ^{Ba}	53.0 ± 0.03 ^{Ca}	53.0 ± 0.03 ^{Ca}
	7	52.0 ± 0.02 ^{Ba}	54.0 ± 0.07 ^{Ab}	46.0 ± 0.02 ^{Cd}	50.0 ± 0.02 ^{Bc}
	8	59.0 ± 0.03 ^{Ab}	54.0 ± 0.02 ^{Bb}	50.0 ± 0.00 ^{Cb}	51.0 ± 0.04 ^{Cc}
	9	59.0 ± 0.05 ^{Ab}	55.5 ± 0.01 ^{Ba}	48.0 ± 0.01 ^{Cc}	52.0 ± 0.05 ^{Bb}
Syneresis (%)	**1**	1 ± 0.02 ^{Cd}	9 ± 0.02 ^{Ab}	3 ± 0.02 ^{Bb}	3 ± 0.04 ^{Bb}
	2	7 ± 0.00 ^{Aa}	5 ± 0.18 ^{Bc}	3 ± 0.01 ^{Cb}	1 ± 0.01 ^{Dc}
	3	2 ± 0.02 ^{Cd}	7 ± 0.01 ^{Ab}	5 ± 0.03 ^{Ba}	1 ± 0.00 ^{Dc}
	4	3 ± 0.00 ^{Bc}	7 ± 0.01 ^{Ab}	2 ± 0.00 ^{Cc}	1 ± 0.00 ^{Cc}
	5	2 ± 0.01 ^{Bd}	8 ± 0.02 ^{Ab}	1 ± 0.00 ^{Cc}	1 ± 0.00 ^{Cc}
	6	3 ± 0.01 ^{Bc}	8 ± 0.04 ^{Ab}	2 ± 0.01 ^{Cc}	1 ± 0.01 ^{Dc}
	7	4 ± 0.00 ^{Bb}	12 ± 0.01 ^{Aa}	1 ± 0.00 ^{Cc}	5 ± 0.03 ^{Ba}
	8	5 ± 0.00 ^{Bb}	10 ± 0.01 ^{Aa}	1 ± 0.00 ^{Cc}	5 ± 0.01 ^{Ba}
	9	5 ± 0.02 ^{Bb}	10 ± 0.02 ^{Aa}	2 ± 0.00 ^{Cc}	6 ± 0.02 ^{Ba}

^{a,b,c,d,e}: Means within a column and each category followed by the different letters are significantly differ ($p < 0.05$).
^{A,B,C,D}: Means within a row and each category followed by the different letters are significantly differ ($p < 0.05$).

1: Yogurt without pine honey and probiotics; 2: Yogurt with 5% pine honey without probiotics; 3: Yogurt with 7% pine honey without probiotics; 4: Yogurt without pine honey with *L. acidophilus* LA-5; 5: Yogurt with 5% pine honey and *L. acidophilus* LA-5; 6: Yogurt with 7% pine honey and *L. acidophilus* LA-5; 7: Yogurt without pine honey with *B. animalis* subsp. *lactis* BB-12; 8: Yogurt with 5% pine honey and *B. animalis* subsp. *lactis* BB-12; 9: Yogurt with 7% pine honey and *B. animalis* subsp. *lactis* BB-12.

9 samples) had the highest syneresis values could be associated with these samples containing *B. animalis* subsp. *lactis* BB-12 and having lower acidity values than the other samples. This is because an increase in acidity exposes charged groups, and these charged groups have a greater capacity to bind water (Zhao et al., 2016; Arab et al., 2023). In addition, the presence of charged groups can contribute to the formation of a tighter gel structure in protein-based systems, such as yogurt (Dönmez et al., 2017; Arab et al., 2023). Also, bifidobacteria have high proteolytic activity and cultures with high proteolytic activity cause increased syneresis in yogurt (Abu-Taraboush et al., 1998; Bergamini et al., 2009; Dönmez et al., 2017). Keogh and O'Kennedy (1998) reported that syneresis values of yogurt samples with added milk fat, protein, and hydrocolloids ranged from 0.0% to 39.10%. In a study conducted by Machado et al. (2017), the water-holding capacity (WHC) values of control and honey-added goat milk yogurts decreased with storage, while syneresis values increased.

The viscosity values of yogurt samples increased over time (Figure 3). Similar results were reported by Machado et al. (2017) for probiotic yogurts with 10% and 15% honey additions. Throughout the storage period, the viscosity values of yogurt samples with added pine honey in all three groups (samples 2, 3, 5, 6, 8, and 9) were lower than those of the control group yogurts (samples 1, 4, and 7).

The gel structure in yogurt is formed as a result of protein-protein interactions that occur between casein and serum proteins due to denatured serum proteins and high acidity. However, in protein-carbohydrate interactions, carbohydrates act like water molecules bound to proteins, partially weakening protein-protein interactions. As a result, a weaker gel structure is formed, leading to a decrease in viscosity (Singh et al., 2009). The carbohydrates in the composition of the pine honey used in our study caused a decrease in viscosity by weakening protein-protein interactions. Sohrabpour et al. (2021) reported similar results for set-type yogurts containing honey and aqueous cinnamon extract (ACE). In this study, it was reported that the addition of up to 3% honey and 1% ACE did not significantly change the viscosity in yogurts, but above these values, honey and ACE significantly reduced the viscosity and caused deformation in the yogurt structure.

Like viscosity values, the hardness values of all samples increased significantly compared to the initial values at the end of the storage period and the highest hardness values in all three groups were obtained from yogurt samples without pine honey (control samples) (Figure 3). This may be due to carbohydrates weakening protein-protein interactions (Singh et al., 2009). Costa et al. (2015) reported in their study that the firmness values of goat milk yogurts with added Cupuassu (*Theobroma grandiflorum*) pulp or inulin decreased during storage.

The adhesiveness in TPA refers to the work required to overcome the attraction force between the food and the probe. The adhesiveness values of yogurt samples generally did not change until the 14th day. However, at the end of storage, in all three groups, it increased slightly in yogurt with 7% pine honey (samples 3, 6, and 9) compared to the control samples and yogurt with 5% pine honey (Table 3). Mudgil et al. (2017) and Domagala et al. (2005) reported that the addition of partially hydrolyzed guar gum and oat maltodextrin, respectively, increased the adhesiveness of yogurt. At the end of storage, the highest adhesiveness values belong to the samples of the 3rd group (C), which also had the highest syneresis.

The cohesiveness values of the samples increased slightly up to the 7th day of storage and then decreased. Similar to adhesiveness, the highest cohesiveness values were observed in the samples of the 3rd group (C) (Table 3). Mohan et al. (2020) found similar results for yogurt samples with manuka honey and reported that there was a linear correlation between syneresis and cohesiveness. In another study, it was found that the addition of cupuassu pulp, inulin, and probiotics did not have a significant effect on the cohesiveness values of goat milk yogurts (Costa et al., 2015).

The gumminess refers to the energy required to break down a semi-solid food and make it ready for swallowing. It is obtained by multiplying the hardness and cohesiveness values in texture profile analysis. The gumminess values of yogurt samples increased during the storage period. Depending on the hardness values, the gumminess values at the end of storage are statistically significantly higher than the initial values. Similar to the hardness values, the highest values in all three groups at the end of storage were obtained from yogurt samples without pine honey (control samples) (Table 3). It has been reported that the gumminess value of yogurt increases with the increase in the level of partially hydrolyzed guar gum (Mudgil et al., 2017). Similar to the study of Mudgil et al. (2017) in our study, probiotic culture affected the gumminess

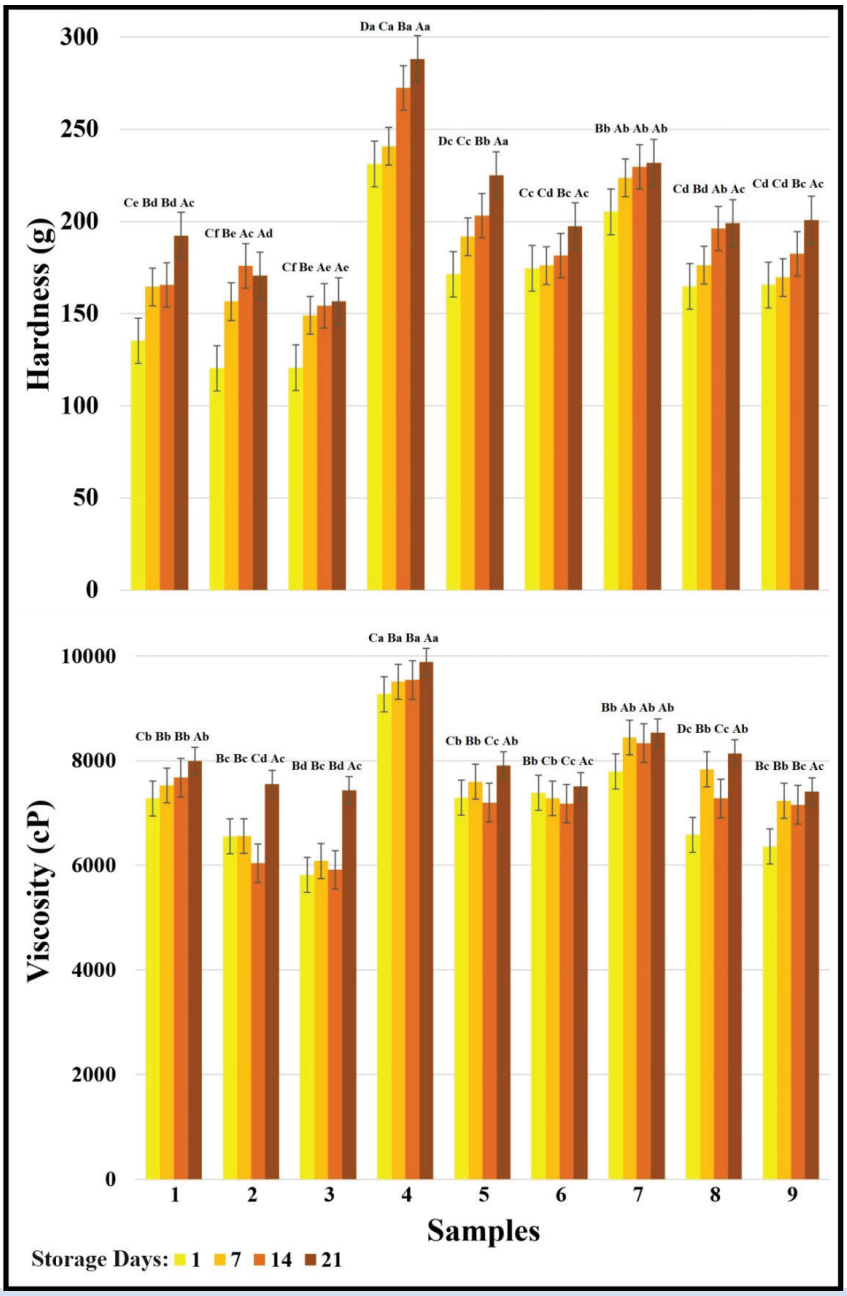

Figure 3. Hardness and viscosity values with and without probiotic yogurt samples.

Different uppercase letters indicate a significant difference between the same samples at different storage times ($p < 0.05$).
Different lowercase letters indicate a significant difference between different samples at the same storage time ($p < 0.05$).
1: Yogurt without pine honey and probiotics; 2: Yogurt with 5% pine honey without probiotics; 3: Yogurt with 7% pine honey without probiotics; 4: Yogurt without pine honey with *L. acidophilus* LA-5; 5: Yogurt with 5% pine honey and *L. acidophilus* LA-5; 6: Yogurt with 7% pine honey and *L. acidophilus* LA-5; 7: Yogurt without pine honey with *B. animalis* subsp. *lactis* BB-12; 8: Yogurt with 5% pine honey and *B. animalis* subsp. *lactis* BB-12; 9: Yogurt with 7% pine honey and *B. animalis* subsp. *lactis* BB-12.

values of yogurts at a statistically significant level and caused an increase.

The springiness is the measure of how well a product physically springs back after the force applied to it is removed. If springiness is high, it requires more chewing energy in the mouth. The springiness of yogurt samples was not significantly affected by the varied culture and pine honey levels. The springiness of the yogurt slightly increased on the 7th day of storage and then decreased (Table 3). Culture differences did not have a statistically significant effect on springiness in hydrolyzed guar gum added yogurts. In contrast, springiness was significantly affected by hydrolyzed guar gum levels (Mudgil et al., 2017).

Table 3. Adhesiveness, cohesiveness, gumminess and springiness values of yogurt samples

		Storage Time (Days)			
	Sample no	**1**	**7**	**14**	**21**
Adhesiveness (g.sn)	**1**	0.02 ± 0.00 ^{Ac}	0.02 ± 0.00 ^{Ad}	0.02 ± 0.00 ^{Ac}	0.02 ± 0.00 ^{Ad}
	2	0.02 ± 0.00 ^{Ac}	0.02 ± 0.00 ^{Ae}	0.02 ± 0.00 ^{Ac}	0.02 ± 0.00 ^{Ad}
	3	0.02 ± 0.00 ^{Bc}	0.02 ± 0.00 ^{Ce}	0.02 ± 0.00 ^{Ac}	0.03 ± 0.00 ^{Ad}
	4	0.03 ± 0.00 ^{Cb}	0.03 ± 0.00 ^{Bc}	0.03 ± 0.00 ^{Bb}	0.04 ± 0.00 ^{Ab}
	5	0.03 ± 0.01 ^{Ca}	0.04 ± 0.00 ^{Aa}	0.04 ± 0.00 ^{Ab}	0.03 ± 0.00 ^{Bc}
	6	0.03 ± 0.00 ^{Ca}	0.04 ± 0.00 ^{Bb}	0.04 ± 0.00 ^{Aa}	0.05 ± 0.00 ^{Aa}
	7	0.03 ± 0.00 ^{Cb}	0.03 ± 0.00 ^{Ab}	0.03 ± 0.00 ^{Bb}	0.03 ± 0.00 ^{Bc}
	8	0.03 ± 0.00 ^{Db}	0.03 ± 0.00 ^{Cc}	0.03 ± 0.00 ^{Bb}	0.04 ± 0.00 ^{Ab}
	9	0.03 ± 0.00 ^{Ba}	0.03 ± 0.00 ^{Bb}	0.03 ± 0.00 ^{Bb}	0.05 ± 0.00 ^{Aa}
Cohesiveness	**1**	0.49 ± 0.00 ^{Bd}	0.53 ± 0.02 ^{Ac}	0.55 ± 0.01 ^{Ac}	0.54 ± 0.01 ^{Ad}
	2	0.57 ± 0.00 ^{Ba}	0.59 ± 0.03 ^{Aa}	0.57 ± 0.02 ^{Bb}	0.57 ±0.01 ^{Bc}
	3	0.55 ± 0.00 ^{Db}	0.58 ± 0.01 ^{Ab}	0.56 ± 0.01 ^{Cb}	0.57 ± 0.02 ^{Bc}
	4	0.53 ± 0.02 ^{Cc}	0.53 ± 0.02 ^{Cc}	0.54 ± 0.01 ^{Bd}	0.56 ± 0.03 ^{Ac}
	5	0.58 ± 0.04 ^{Ba}	0.59 ± 0.02 ^{Aa}	0.57 ± 0.01 ^{Bb}	0.58 ± 0.00 ^{Bb}
	6	0.55 ± 0.01 ^{Cb}	0.58 ± 0.01 ^{Ab}	0.56 ± 0.01 ^{Bb}	0.56 ± 0.03 ^{Bc}
	7	0.55 ± 0.03 ^{Cb}	0.60 ± 0.00 ^{Aa}	0.60 ± 0.00 ^{Aa}	0.59 ± 0.02 ^{Bb}
	8	0.57 ± 0.01 ^{Ba}	0.62 ± 0.09 ^{Aa}	0.61 ± 0.02 ^{Aa}	0.61 ± 0.02 ^{Aa}
	9	0.55 ± 0.02 ^{Cb}	0.61 ± 0.11 ^{Aa}	0.60 ± 0.00 ^{Aa}	0.59 ± 0.02 ^{Bb}
Gumminess (g)	**1**	66.10 ± 3.32 ^{Cc}	87.69 ± 1.85 ^{Bc}	91.90 ± 0.79 ^{Bc}	104.70 ± 10.78^{Ac}
	2	68.75 ± 0.46 ^{Cc}	92.88 ± 8.42 ^{Bc}	100.39 ± 1.08 ^{Abc}	97.36 ± 3.06 ^{Ad}
	3	66.54 ± 0.78 ^{Bc}	87.01 ± 4.32 ^{Ac}	86.43 ± 5.86 ^{Ac}	89.90 ± 5.59 ^{Ad}
	4	121.95 ± 0.57 ^{Ca}	127.41 ± 7.14 ^{Ca}	147.09 ± 5.80 ^{Ba}	161.16 ± 1.02 ^{Aa}
	5	99.66 ± 2.47 ^{Cb}	113.24 ± 1.95 ^{Bab}	115.73 ± 0.03 ^{Bb}	130.47 ± 3.49 ^{Ab}
	6	95.70 ± 9.10 ^{Ca}	101.95 ± 7.85 ^{Bb}	102.51 ± 0.99 ^{Bbc}	111.32 ± 0.29 ^{Ac}
	7	113.01 ± 15.6^{Bab}	134.02 ± 9.02 ^{Aa}	137.76 ± 0.93 ^{Aab}	135.68 ± 6.24 ^{Ab}
	8	94.42 ± 0.44 ^{Ca}	109.42 ± 23.43^{Bb}	120.67 ± 1.91 ^{Ab}	122.44 ± 13.79^{Ab}
	9	91.25 ± 10.63 ^{Ca}	103.04 ± 19.63^{Bb}	109.41 ± 1.91 ^{Bb}	119.34 ± 3.00 ^{Ab}
Springiness	**1**	0.99 ± 0.01 ^{Aa}	1.03 ± 0.01 ^{Aa}	0.971 ± 0.02 ^{Ba}	0.97 ± 0.02 ^{Ba}
	2	0.99 ± 0.11 ^{Aa}	1.01 ± 0.00 ^{Aa}	1.010 ± 0.00 ^{Aa}	0.98 ± 0.00 ^{Ba}
	3	0.99 ± 0.02 ^{Ba}	1.02 ± 0.00 ^{Aa}	1.02 ± 0.00 ^{Aa}	0.98 ± 0.01 ^{Ba}
	4	0.99 ± 0.02 ^{Aa}	1.01 ± 0.01 ^{Aa}	0.97 ± 0.02 ^{Ba}	0.98 ± 0.00 ^{Ba}
	5	0.99 ± 0.08 ^{Ba}	1.01 ± 0.00 ^{Aa}	0.98 ± 0.01 ^{Ba}	0.97 ± 0.00 ^{Ba}
	6	0.99 ± 0.01 ^{Ba}	1.01 ± 0.01 ^{Aa}	0.98 ± 0.00 ^{Ba}	0.98 ± 0.02 ^{Ba}
	7	0.99 ± 0.01 ^{Ba}	1.02 ± 0.01 ^{Aa}	0.99 ± 0.02 ^{Ba}	0.97 ± 0.01 ^{Ba}
	8	0.98 ± 0.03 ^{Ba}	1.02 ± 0.00 ^{Aa}	0.98 ± 0.00 ^{Ba}	0.98 ± 0.02 ^{Ba}
	9	0.96 ± 0.04 ^{Ba}	1.02 ± 0.00 ^{Aa}	1.01 ± 0.00 ^{Aa}	1.01 ± 0.01 ^{Aa}

^{a,b,c,d,e}: Means within a column and each category followed by the different letters are significantly differ ($p < 0.05$).
^{A,B,C,D}: Means within a row and each category followed by the different letters are significantly differ ($p < 0.05$).
1: Yogurt without pine honey and probiotics; 2: Yogurt with 5% pine honey without probiotics; 3: Yogurt with 7% pine honey without probiotics; 4: Yogurt without pine honey with *L. acidophilus* LA-5; 5: Yogurt with 5% pine honey and *L. acidophilus* LA-5; 6: Yogurt with 7% pine honey and *L. acidophilus* LA-5; 7: Yogurt without pine honey with *B. animalis* subsp. *lactis* BB-12; 8: Yogurt with 5% pine honey and *B. animalis* subsp. *lactis* BB-12; 9: Yogurt with 7% pine honey and *B. animalis* subsp. *lactis* BB-12.

The addition of pine honey and use of probiotic cultures did not have a statistically significant effect on the numbers of *Str. thermophilus* (Figure 4). At the end of storage, the lowest numbers of *Str. thermophilus* are found in samples containing pine honey and *B. animalis* subsp. *lactis* BB-12. In a study, it was determined that the number of *Str. thermophilus*, which was 9.23 in yogurts with 4% honey, decreased over time and was 6.01 on the 9th day (Metry and Owayss, 2009). After 28 days of storage, the counts of starter bacteria decreased in all goat milk yogurts both with (5, 10, and 15%) and without honey and the starter counts were close to each other (Machado et al., 2017).

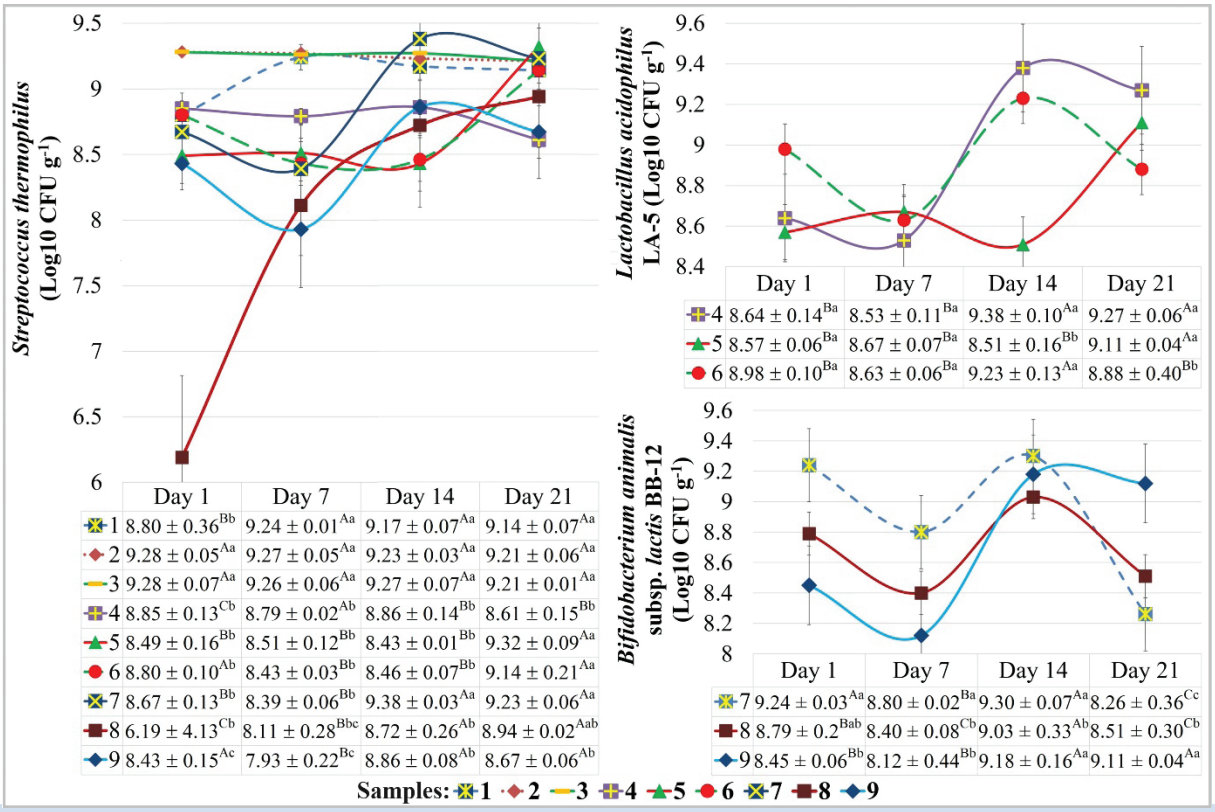

Figure 4. *Str. thermophilus*, *L. acidophilus* LA-5 and *B. animalis* subsp. *lactis* BB-12 counts of yogurt samples.

Different uppercase letters indicate a significant difference between the same samples at different storage times ($p < 0.05$).
Different lowercase letters indicate a significant difference between different samples at the same storage time ($p < 0.05$).
1: Yogurt without pine honey and probiotics; 2: Yogurt with 5% pine honey without probiotics; 3: Yogurt with 7% pine honey without probiotics; 4: Yogurt without pine honey with *L. acidophilus* LA-5; 5: Yogurt with 5% pine honey and *L. acidophilus* LA-5; 6: Yogurt with 7% pine honey and *L. acidophilus* LA-5; 7: Yogurt without pine honey with *B. animalis* subsp. *lactis* BB-12; 8: Yogurt with 5% pine honey and *B. animalis* subsp. *lactis* BB-12; 9: Yogurt with 7% pine honey and *B. animalis* subsp. *lactis* BB-12.

L. acidophilus LA-5 counts in both the control samples and the samples with 5% pine honey increased by 1 log compared to their initial counts at the end of storage while there was no statistically significant change in the *L. acidophilus* LA-5 numbers of the samples with 7% pine honey (Figure 4). In conclusion, it can be said that the addition of pine honey does not have a significant effect on the numbers of *L. acidophilus* LA-5 in yogurt, and any changes that occur are time-dependent.

Sohrabpour et al. (2021) reported that in the yogurts produced with honey (0% and 5%) and cinnamon extract, honey had no significant effect on viability of *L. acidophilus* LA-5, while cinnamon extract and time significantly affected *L. acidophilus* LA-5 viability. In this study, the *L. acidophilus* LA-5 counts at the end of storage were determined to be 10^6-10^7. In our study, however, the *L. acidophilus* LA-5 counts in the samples were over 10^8 in all periods of storage. These values are above 10^7, which is the critical limit for the beneficial effects of probiotic bacteria. In a study conducted with yogurt with Manuka honey, it was reported that the highest probiotic viability (7.0 log cfu/mL) was observed in yogurts with 5% honey (Mohan et al., 2020). The values obtained in both studies are lower than the values obtained in our study. It has been reported that both yogurt cultures and LA-5 counts decreased (1-2 log cycle) in yogurts with 5%, 10%, and 15% honey, but the decrease in *L. acidophilus* LA-5 counts was less in the honey-added samples than in the control group without honey (Machada et al., 2017).

Three samples in group C containing *B. animalis* subsp *lactis* BB-12, the counts of bifidobacteria increased on the 14th day of storage compared to their initial counts (Figure 4). After this period, there was a decrease in the *B. animalis* subsp *lactis* BB12 counts of both the without pine honey and 5% pine honey samples compared to the counts of the bifidobacteria at the beginning of storage. The decrease rates in bifidobacteria counts were statistically significant in the control samples ($p < 0.05$) but statistically insig-

nificant in samples with 5% pine honey. The highest *B. animalis* subsp *lactis* BB-12 counts in group C on days 14 and 21 of storage belong to samples with 7% pine honey and were 1 log cycle higher than the initial counts of these samples. Throughout the storage period, the counts of *B. animalis* subsp *lactis* BB-12 in all samples are above 10^8 CFU g^{-1}, which is the critical value for probiotic products. As a result, it was determined that the addition of 5% pine honey supported the viability of *B. animalis* subsp *lactis* BB-12 throughout storage, and the addition of 7% pine honey caused a 1 log cycle increase in BB-12 counts. In study of the impact of 5% clover honey, fructooligosaccharides (FOS), galactooligosaccharides (GOS), and inulin on *Bifidobacterium* spp. (*B. longum*, *B. adolescentis*, *B. breve*, *B. bifidum*, and *B. infantis*), clover honey was reported that like FOS, GOS, and inulin, promotes the growth of these bacteria (Kajiwara et al., 2002). The effect of 5% and 10% honey addition on the viability of *B. breve* and *B. longum* strains in fermented skim milk was investigated and it was determined that 10% honey enhanced the viability of bifidobacteria (Riazi and Ziar, 2012). In a study investigating the viability of bifidobacteria (ABT culture) in yogurts containing 2%, 4%, and 6% honey, it has been observed that the counts of bifidobacteria decreased during the storage period (Ammar et al., 2015). It was reported that the addition of fructooligosaccharide (2%) supported the viability of *B. animalis* more than *B. longum* in yogurt. Additionally, it has been determined that the rec-

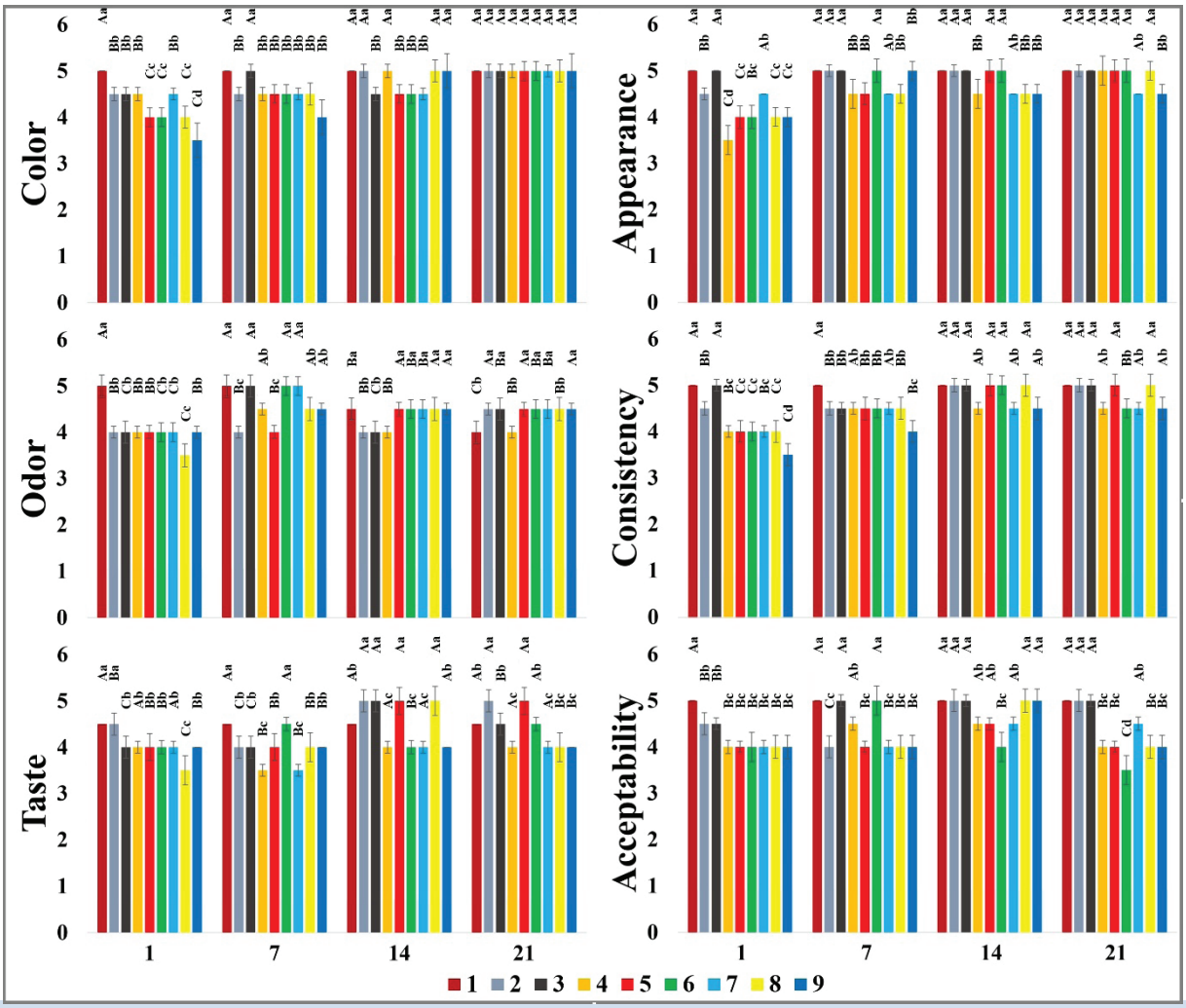

Figure 5. Sensorial characteristics of yogurt samples.

Different uppercase letters indicate a significant difference between the same samples at different storage times ($p < 0.05$).
Different lowercase letters indicate a significant difference between different samples at the same storage time ($p < 0.05$).
1: Yogurt without pine honey and probiotics; 2: Yogurt with 5% pine honey without probiotics; 3: Yogurt with 7% pine honey without probiotics; 4: Yogurt without pine honey with *L. acidophilus* LA-5; 5: Yogurt with 5% pine honey and *L. acidophilus* LA-5; 6: Yogurt with 7% pine honey and *L. acidophilus* LA-5; 7: Yogurt without pine honey with *B. animalis* subsp. *lactis* BB-12; 8: Yogurt with 5% pine honey and *B. animalis* subsp. *lactis* BB-12; 9: Yogurt with 7% pine honey and *B. animalis* subsp. *lactis* BB-12.

ommended level of 1 million cells for *B. animalis* is exceeded during storage, and in yogurts without fructooligosaccharide, probiotic counts decrease rapidly after the 7th day (Akalın et al., 2004).

The addition of pine honey and the use of probiotic cultures in yogurt have had a statistically significant impact on the appearance, consistency, odor, and taste attributes of the samples (Figure 5). The taste scores of yogurts containing probiotic cultures are lower than those of the control group yogurt. The odor, consistency and color scores of the samples are close to each other. In terms of overall acceptability, the highest scores generally belong to the control samples, while the lowest scores are attributed to samples with *L. acidophilus* LA-5 and 7% pine honey. It was reported that 2, 4 and 6% honey (Coskun and Karabulut-Dirican, 2019), 5, 10 and 15% honey (Zlatev et al., 2018) and 5% manuka honey and invert syrup (Mohan et al., 2020) improved sensory properties of yogurt.

CONCLUSION

Researchers and food manufacturers are continually working to develop strategies that can help maintain probiotic viability throughout the products' shelf life. One of the methods used to prevent reductions in probiotic counts is the use of prebiotics in the food formulation. Prebiotics can help maintain the viability of probiotics without causing undesirable changes in the food's composition. In the study, pine honey, which contains oligosaccharides and is a potential prebiotic, has been used. Honey is a prebiotic source that is structurally and sensorially compatible with yogurt. It has been determined that the addition of pine honey increases the viability of *B. animalis* subsp. *lactis* BB-12 without causing any negative changes in the structural and sensory qualities of yogurt. Pine honey (5% and 7%) had no effect on the viability of *L. acidophilus* LA-5. However, although prebiotics in honey may not directly enhance the viability of probiotic bacteria in the product, they can have an effect on the probiotic viability in the digestive system and may provide health benefits to consumers due to their functional properties. In conclusion, it can be said that 5% and 7% pine honey can be used as a prebiotic source in yogurt production.

CONFLICT OF INTEREST

The authors declare no competing interests.

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