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Physicochemical, rheological, and microbiological properties of honey-fortified probiotic drinkable yogurt

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Abstract:
This study aimed to investigate the physicochemical, rheological, and microbiological attributes of drinkable yogurts prepared with three distinct types of honey (flower, pine, and thyme) in amounts of 10 and 20% and probiotic cultures (*Lactobacillus acidophilus* and *Bifidobacterium* spp.).
The control sample was brighter while the yogurt containing 20% pine honey was more yellow during storage (21 days). The samples' serum separation quantities rose together with the honey ratio. All the honey-fortified drinkable yogurts were found to be non-Newtonian pseudoplastic liquids that are thixotropic. However, as the honey ratio increased, the apparent viscosity and consistency coefficients increased, too. After 21 days of storage, *L. acidophilus* and *Bifidobacterium* spp. counts rose to more than 5.0 log CFU/mL in the experimental yogurts containing honey (except for the sample with 20% flower honey). The panelists preferred the 10% honey-fortified drinkable yogurts over the others. The yogurts with flower honey were mostly favored, followed by pine and thyme honeys. Although honey contributed to the properties of drinkable yogurt, adding more than 10% of honey degraded the product's quality and acceptability.
In conclusion, 10% is an optimal amount for flower and pine honey, with a smaller amount recommended for thyme honey. More research is needed on honey-fortified drinkable yogurt for its commercial production.

Keywords: Drinkable yogurt, flower honey, pine honey, thyme honey, functional foods, dairy drinks

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INTRODUCTION

Functional foods such as yogurt and honey are crucial to human health. Yogurt has already demonstrated its functional efficacy against a variety of human illnesses, including diabetes, chronic diseases, and metabolic syndrome risk factors such as hyperglycemia [1, 2]. Recently, a few highly diverse dairy drinks, such as drinkable yogurt, fermented milk, and other milk-based beverages, have been added to the dairy production program to increase dairy consumption [3]. Drinkable yogurt is a non-alcoholic fermented milk product. Traditionally, it is produced by adding water (30–50%) and salt (0.5–1%) to yogurt. Starter bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) are used to facilitate the fermentation process in industrial production [4, 5]. Probiotic bacteria have recently become more prevalent in fermented milk products such as drinkable yogurt. *Lactobacilli* and *Bifidobacteria* are the most popular strains of the microbial genera linked to drinkable probiotic yogurts. Probiotics are live microorganisms that have health benefits for the host and regulate microbial activity in the gastrointestinal system [6, 7]. They help restore the balance of beneficial intestinal microflora while also preventing dangerous enteropathogens. Probiotics lower blood cholesterol, boost the body immunity, and have antimutagenic and anticarcinogenic properties. They also regulate lactose intolerance symptoms, reduce antibiotic side effects, and prevent gut infections by creating organic acids and antibacterial compounds. In addition to their health benefits, probiotics help dairy products last longer and have better sensory characteristics. To produce health-benefitting effects, dairy products must contain at least 6–7 log CFU/g of live probiotic bacteria. Their growth and activity are usually improved by prebiotics. In particular, prebiotics selectively stimulate the growth of bacterial species such as *Bifidobacterium* spp. and *Lactobacilli* spp. while inhibiting the proliferation of bacteria such as *Salmonella* spp. and *Escherichia coli* [8].

Recent years have seen a widespread intake of dairy drinks containing flavorings, sugar syrup, and water. Various ingredients, including chocolate, honey, and strawberries, are used to enhance the flavor of dairy-based healthy drinks [3]. Honey has been regarded a better alternative to artificial sweeteners in new dairy products [9]. This natural nutritious sweetener is one of the most popular foods around the world. In 2019, the global output of honey was 1.9 million tons, with China accounting for 24% of total production (444 100 tons), followed by Türkiye (109 330 tons), Canada (80 345 tons), Argentina (78 927 tons), Iran (75 463 tons), and the United States (71 179 tons) [1]. Honey has traditionally played an important role in diets due to its superior flavor and many other beneficial characteristics.

There is a wide variety of natural bee honeys, depending on many factors such as botanical and geographical (regional or local) origins and bee species [10, 11]. The botanical origin (honey harvest) distinguishes between flower and honeydew honeys [10]. Furthermore, the honey's botanical and geographical origins determine its quality criteria, such as color, moisture, acidity, and phenolic content. Other important factors include the climate, environmental conditions, and the processes that honey goes through [11]. Additionally, two types of honey are generally defined: multifloral or monofloral, which are made from a combination of numerous botanical species or a single flower variety, respectively. Monofloral honey has a higher market value due to its physicochemical properties [12]. Türkiye boasts a large variety of monofloral honeys because of its geographic position [11].

The natural organic material known as honey is created by honeybees (*Apis mellifera* L.) from flower nectar [1]. Pine honey is made by honeybees processing the secretions of *Marchalina hellenica*. This insect lives on *Pinus brutia*, which grows in Türkiye, particularly in the Aegean, Western Mediterranean, and Southern Marmara regions [9]. Thyme honey, on the other hand, is produced by bees from thyme (*Thymus* spp.) blossoms and has a high sensory value [12]. Honey provides body cells with a significant amount of energy [11]. It generally consists of 70–80% sugar, 10–20% water, and such components as proteins, free amino acids, vitamins, mineral salts, phenolics, and organic acids. Monosaccharides, glucose, and fructose are the main sugars present in honey [13]. In addition, honey contains 25 oligosaccharides, including hybridose, panose, and raffinose. These oligosaccharides are reported to have similar effects to those of fructooligosaccharides and glucooligosaccharides. Honey also contains antioxidants (such as carotenoids, flavonoids, and phenolics) and Maillard reaction products. Together with its acidity and sugar profile, they provide honey with special sensory qualities [14]. Non-peroxide substances (such as glucose oxidase, catalase, hydrogen peroxide, and lysozyme) and phenolic substances found in honey exhibit antimicrobial properties [15]. Furthermore, honey contains probiotics and prebiotics, has immunomodulatory and antiviral properties, and is used to treat cancer and neurological illnesses. Honey has always held a unique position in the human diet due to its functional and therapeutic qualities [10].

In this study, honey was added to drinkable yogurts, which have a slightly salty and sour flavor, to improve their functional characteristics. We aimed to see how different types (flower, pine, and thyme) and ratios (10 and 20%) of honey added to drinkable yogurts containing probiotic culture (*Lactobacillus acidophilus* and *Bifidobacterium* spp.) affected their physicochemical, rheological, and microbiological properties. We expect this study to benefit industry, science, and consumers.

STUDY OBJECTS AND METHODS

Materials. In this study, floral, pine, and thyme honeys were added to drinkable probiotic yogurts at two different ratios (10 and 20%). Raw cow milk (4 ± 1°C) was provided by the Ünsüt Dairy Products Plant (Isparta, Türkiye) and the Isparta Cattle Breeders' Association. To prepare drinkable yogurt, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were mixed with the yogurt culture (YC380) and the probiotic cultures *Lactobacillus acidophilus* and *Bifidobacterium* spp. (LA-5 and BB-12) obtained from Peyma-Chr. Hansen (Istanbul, Türkiye). Thyme honey was acquired from the regional honey producers in Isparta, while the honeys sold in the market (Anavarza Honey) were supplied by Sezen Gıda Ltd. Şti. (Istanbul, Türkiye).

Drinkable yogurt production. Homogenized cow's milk was pasteurized at 90°C for 15 min and then cooled to 43 ± 2°C. The yogurt culture (1%) and the probiotic culture (2%) were used as inoculants. The drinkable yogurts were normalized with water once the pH level was 4.6 and the dry matter was 7.5%. Then, 0.3% of table salt was added to the samples. The yogurts were divided into seven groups, namely two samples with flower honey (10 and 20%), two samples with pine honey (10 and 20%), two samples with thyme honey (10 and 20%), and the control yogurt without any honey. The yogurts were placed in sterilized glass jars and kept chilled (4 ± 1°C) for storage. Physicochemical, microbiological, and rheological analyses were performed on days 1, 10, and 21 of storage.

Applied analyses. ***Raw milk and honey analysis.*** Raw milk's dry matter, fat, titration acidity, and total nitrogen were calculated according to AOAC [16]. A WTW pH 315 digital meter (Weilheim, Germany) was used to monitor pH readings. A Hanna HI 96801 digital refractometer (Hanna Instruments Inc., USA) was used to measure the total soluble solids content (Brix) in the honeys.

Physicochemical analysis. Dry matter, fat, titration acidity (ISO/TS 11869:2012) and salt analyses were carried out. The micro-Kjeldahl method was employed to measure protein contents [16]. Serum separation was performed as described in [17]. A WTW pH 315 digital meter (Weilheim, Germany) was used to monitor pH readings.

Color properties. The Hunter method was employed to analyze the color properties of the honey-fortified drinkable yogurts [18]. A CR-400 Minolta chroma meter (Konica Minolta, Inc., Japan) was used to determine the L^*, a^*, and b^* values representing bright/dark (0 black, 100 white), green/red (–60 green, 60 red), and blue/yellow (–60 blue, 60 yellow), respectively. The colors were examined by utilizing cells of 9 cm in diameter and 4 cm in height. Calibration was performed on a white plate ($Y = 92.7$, $x = 0.3160$, $y = 0.3321$). The L^*, a^*, and b^* values were measured in triplicate.

Rheological properties. A Brookfield DV-II+Pro Extra viscometer (Brookfield Engineering Laboratories Inc., USA) was employed to determine the rheological characteristics of the drinkable yogurt and honey samples. The yogurt's flow type was ascertained by using a tiny sample adaptor, and the honey's viscosity was assessed by using a 0.6-mm spindle tip. The samples were recorded and plotted using the RHEOCAL® application software (Brookfield Engineering Laboratories Inc., USA). The yogurt samples were stored at 4°C and examined on days 1, 10, and 21 of storage.

Microbiological analysis. Under aseptic conditions, 10 mL of a drinkable yogurt sample was added to 90 mL of a sterile Ringer's solution (1/10), and 1 mL of this dilution was transferred to 9 mL of a sterile Ringer's solution. Then, serial dilutions were carried out. The materials were microbiologically analyzed using the spread plate method. During storage, the contents of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* in the yogurts were detected on MRS (de Man, Rogosa, and Sharpe) and M17 agars, respectively (Merck, Germany) [19]. Petri dishes were incubated at 37°C for 48 and 72 h to enumerate *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* counts, respectively.

The Plate Count Agar was used to determine the total number of mesophilic bacteria. Petri dishes were incubated for 72 h at 30°C under aerobic conditions [20]. The numbers of *Bifidobacterium* spp. and *L. acidophilus* were determined on MRS-NNLP and MRS-sorbitol agars, respectively [21, 22]. The MRS-NNLP agar medium contained nalidixic acid (50 mg/L), neomycin sulphate (100 mg/L), lithium chloride (3000 mg/L), and paronomycin sulphate (200 mg/L). The NNLP was mixed with the MRS agar medium, which had been sterilized with a 0.45-μm disposable sterile filter just before pouring into Petri plates. For *L. acidophilus* enumeration, the MRS agar (90 mL) was sterilized and mixed with 10% (w/v) D-sorbitol solution (10 mL) using a sterile 0.45-μm filter. For the enumeration of both probiotics, the Petri dishes were incubated for 72 h at 37°C in anaerobic jars (Merck, Germany). For yeast-mold counting, 1 mL of the prepared 1:10 dilution was inoculated into the PDA (Potato Dextrose Agar) medium (Merck, Germany). The cultivated petri dishes were incubated at 25°C for 4–5 days [23]. Then, 1 mL of a 1:10 dilution was obtained and put into the EMB (Eosin Methylene-Blue Lactose Sucrose) medium (Merck, Germany) for coliform bacteria detection. The Petri dishes were incubated at 37°C for 24–48 h [24]. The results were expressed as log transformed data in CFU/g.

Sensory analysis. Until their sensory evaluation, the drinkable yogurts were kept in sterile glass jars at 4 ± 1°C in the refrigerator. On days 1, 10, and 21 of storage, they were evaluated by 10 panelists (6 women and 4 men aged 20–40). Although the panelists had a prior experience with sensory analysis, they were given two two-hour training sessions on evaluating drinkable yogurts. Three-digit numbers were chosen at random to code the samples. The panelists tasted the samples that were very good and very bad in terms of the indicated sensory qualities, and they were instructed to use those samples as a benchmark for evaluating the test samples. With the use of a less-to-more indicator across 10 points, the sensory qualities of the drinkable yogurts were assessed in accordance with five criteria: appearance (yellowness, general color, etc.), texture (fluidity, consistency, etc.), taste (sweetness, saltiness, etc.), odor (aroma, honey-like odor, etc.), and general acceptance [25].

Statistical analysis. Three parallel analyses were set up in triplicate. The SPSS 17.0 program was used to statistically examine the analysis outcomes. The Duncan multiple comparison test ($p < 0.05$) was used to interpret the samples where there was a statistically significant variation in storage times for the analysis results and differences between the samples [26].

RESULTS AND DISCUSSION

Raw milk and honey analysis. The average specific gravity, pH, titration acidity (% lactic acid), dry matter, fat, and total nitrogen values of raw cow's milk used in drinkable yogurt production were found as 1.031 g/cm^3, 6.72, 0.18, 11.99, 3.75, and 3.35%, respectively.

The average L^*, a^* and b^* values were 23.63, 2.64, and 8.29, respectively, in the flower honey; 22.91, 3.23, and 6.74, respectively, in the pine honey; and 25.29, 1.16, and 9.42, respectively, in the thyme honey. These results were different from those found in other studies [27, 28]. The color differences may be due to the type of honey used. In general, the color is light in honeys with a low value and dark in honeys with a high value. The color of honey is essentially related to the total mineral content. It is derived from plant pigments that include unknown amounts of chlorophyll, carotene, xanthophyll, as well as yellow and green hues [27]. In our study, the total soluble solid contents (brix) in the flower, pine, and thyme honeys were 79.533 ± 1.357, 78.633 ± 1.422, and 79.500 ± 0.264, respectively (n = 3). Since there are few studies on thyme honey, we compared the brix values for flower, pine, and thyme honeys with those for different types of honey. We found that our results were similar to those reported by some other studies [13, 27].

Physicochemical analysis. Table 1 shows the results of the physicochemical and color analyses of the probiotic drinkable yogurts on days 1, 10, and 21. We found a statistically significant ($p < 0.05$) difference in the samples' pH values but no statistical difference between the storage times and the samples in terms of titration acidity (% lactic acid). On day 1, the control group had the lowest pH levels (4.19) compared to the other samples. After 21 days, however, the pH values of the samples became close to each other. The control group had the highest (0.60) lactic acid content among the samples. In a study by Özünlü on drinkable yogurt, lactic acid levels were found between 0.495 and 0.817% [29]. In another study, yogurts made with flower honey had the lowest pH value (4.13), while yogurts made with chestnut honey had the highest pH value (4.20) [30]. Since there are few studies on honey-fortified drinkable yogurt, we compared our results with those for different types of honey. Coskun and Dirican reported that the titration acidity of yogurts with pine honey increased during storage, while their pH values decreased [9]. The titration acidity of honey yogurts is believed to be affected by the organic acids that honey contains [30]. These acids include formic, acetic, butyric, lactic, oxalic, succinic, tartaric, maleic, pyruvic, pyroglutamic, alpha-ketoglutaric, glycolic, citric, malic, 2- or 3-phosphoglyceric, α- or β-glycerophosphate, glucose-6-phosphate, and others.

The serum separation values showed a statistically significant difference between the samples under study ($p < 0.05$). Later during storage, more serum separated from all the samples (Table 1). The highest rise in serum separation (from 3.70 to 14.12 mL/25 g) was recorded in the sample containing 10% flower honey. We also found that the yogurts with larger amounts of honey had higher serum separation values. Özünlü determined that the serum separation values of drinkable yogurts increased gradually during 14 days [29].

In our study, the average dry matter content in the control group was 7.22% (Table 2), which was lower than in the yogurts with different types and amounts of honey added. The samples with larger amounts of honey (20%) had higher dry matter values. We compared our results with those found in some honey-supplemented kefir studies since kefir is a probiotic-containing drink. For example, in a study by Dogan, the dry matter contents in the kefir samples were directly proportionate to

Table 1 Physicochemical and color characteristics of drinkable yogurts (n = 3)

Parameter	Storage day	Samples						
		Control	Flower honey (10%)	Flower honey (20%)	Pine honey (10%)	Pine honey (20%)	Thyme honey (10%)	Thyme honey (20%)
pH	1	4.19 ± 0.03^{bcd}	4.27 ± 0.03$^{a–d}$	4.32 ± 0.09^{ab}	4.25 ± 0.03$^{a–d}$	4.31 ± 0.06^{abc}	4.24 ± 0.01$^{a–d}$	4.30 ± 0.09$^{a–d}$
	10	4.10 ± 0.02^{bcd}	4.11 ± 0.05^{bcd}	4.15 ± 0.01$^{a–d}$	4.11 ± 0.08^{bcd}	4.14 ± 0.11$^{a–d}$	4.14 ± 0.08$^{a–d}$	4.17 ± 0.09$^{a–d}$
	21	4.05 ± 0.05^{d}	4.06 ± 0.06^{d}	4.10 ± 0.01^{bcd}	4.10 ± 0.08^{bcd}	4.11 ± 0.11^{bcd}	4.06 ± 0.09^{cd}	4.08 ± 0.11^{bcd}
Lactic acid, %	1	0.56 ± 0.07	0.55 ± 0.04	0.53 ± 0.09	0.54 ± 0.03	0.53 ± 0.02	0.54 ± 0.03	0.53 ± 0.04
	10	0.60 ± 0.03	0.58 ± 0.02	0.57 ± 0.07	0.59 ± 0.06	0.58 ± 0.05	0.59 ± 0.04	0.58 ± 0.06
	21	0.59 ± 0.02	0.57 ± 0.01	0.56 ± 0.03	0.57 ± 0.05	0.54 ± 0.06	0.58 ± 0.05	0.57 ± 0.07
Serum separation, mL/25 g	1	2.72 ± 0.32^{fg}	3.70 ± 0.80^{ef}	4.27 ± 0.27^{def}	3.35 ± 0.20^{efg}	4.25 ± 0.40^{def}	3.47 ± 0.97^{efg}	4.40 ± 0.50^{def}
	10	3.25 ± 0.75^{efg}	5.15 ± 0.10^{cde}	6.20 ± 0.05^{cd}	4.90 ± 0.30^{def}	5.75 ± 0.10^{cde}	6.67 ± 0.37^{cd}	7.15 ± 0.60^{cd}
	21	6.02 ± 0.42^{cd}	14.12 ± 0.12^{a}	14.55 ± 0.60^{a}	12.67 ± 0.27^{abc}	13.00 ± 0.50^{ab}	12.70 ± 0.25^{abc}	13.47 ± 0.75^{ab}
L^*	1	82.08 ± 0.06^{a}	77.92 ± 0.50^{bc}	74.47 ± 0.27^{cd}	77.01 ± 0.11^{bcd}	72.89 ± 0.09^{gh}	78.10 ± 0.03^{b}	74.94 ± 0.14^{ef}
	10	81.50 ± 0.16^{a}	76.82 ± 0.74^{bcd}	74.32 ± 0.42^{d}	76.40 ± 0.23^{d}	72.75 ± 0.31^{gh}	77.99 ± 0.22^{bc}	74.77 ± 0.17^{ef}
	21	80.83 ± 0.82^{a}	76.68 ± 0.60$^{a–d}$	73.64 ± 0.71^{de}	75.77 ± 0.61^{de}	72.01 ± 0.61^{h}	76.82 ± 0.64^{bcd}	74.09 ± 0.44^{fg}
a^*	1	–2.80 ± 0.27^{d}	–2.03 ± 0.29^{bc}	–1.73 ± 0.23^{ab}	–1.74 ± 0.39^{ab}	–1.21 ± 0.35^{a}	⁻2.14 ± 0.27^{bcd}	1.77 ± 0.26^{ab}
	10	–2.80 ± 0.03^{d}	–2.04 ± 0.20^{bc}	–1.74 ± 0.12^{ab}	–1.71 ± 0.28^{ab}	–1.13 ± 0.13^{a}	⁻2.04 ± 0.28^{bcd}	–1.70 ± 0.27^{ab}
	21	–2.74 ± 0.35^{d}	–1.98 ± 0.35^{bc}	–1.66 ± 0.03^{ab}	–1.65 ± 0.08^{ab}	–1.14 ± 0.22^{a}	⁻2.01 ± 0.08^{bcd}	–1.66 ± 0.10^{ab}
b^*	1	3.69 ± 0.08^{h}	7.69 ± 0.25^{f}	10.38 ± 0.05^{b}	9.20 ± 0.14^{c}	12.40 ± 0.04^{a}	6.45 ± 0.10^{g}	8.34 ± 0.04^{de}
	10	3.72 ± 0.03^{h}	7.46 ± 0.78^{f}	10.49 ± 0.01^{b}	9.41 ± 0.01^{c}	12.42 ± 0.05^{a}	6.52 ± 0.11^{g}	8.38 ± 0.18^{de}
	21	3.71 ± 0.15^{h}	7.67 ± 0.02^{f}	10.07 ± 0.17^{b}	9.32 ± 0.03^{c}	12.10 ± 0.03^{a}	6.03 ± 0.04^{g}	8.27 ± 0.05^{de}

*$^{a–h}$ – Different letters indicate statistical significance between the groups ($p < 0.05$)

Table 2 Physicochemical parameters of drinkable yogurts (n = 3)

Parameter, %	Samples						
	Control	Flower honey (10%)	Flower honey (20%)	Pine honey (10%)	Pine honey (20%)	Thyme honey (10%)	Thyme honey (20%)
Dry matter	7.22 ± 0.02^{c}	13.61 ± 0.03^{ab}	19.73 ± 0.03^{a}	13.33 ± 0.01^{ab}	19.32 ± 0.08^{a}	13.82 ± 0.05^{ab}	19.23 ± 0.03^{a}
Fat	1.25 ± 0.05^{a}	1.15 ± 0.00^{ab}	1.00 ± 0.00^{b}	1.15 ± 0.05^{ab}	1.00 ± 0.00^{b}	1.15 ± 0.02^{ab}	1.10 ± 0.00^{b}
Salt	0.51 ± 0.13^{a}	0.47 ± 0.13^{ab}	0.45 ± 0.12^{ab}	0.47 ± 0.11^{ab}	0.45 ± 0.12^{ab}	0.48 ± 0.11^{ab}	0.44 ± 0.11^{b}
Protein	2.35 ± 0.26^{a}	2.11 ± 0.16^{a}	1.95 ± 0.11^{a}	2.12 ± 0.14^{a}	2.04 ± 0.11^{a}	2.10 ± 0.12^{a}	1.98 ± 0.14^{a}

*$^{a–c}$ – Different letters indicate statistical significance between the groups ($p < 0.05$)

the amount of honey added [31]. This was because honey contains a large amount of total soluble solids, most of which are sugars.

Our study revealed that the fat content was the highest (1.25) in the control group and the lowest (1.00) in the samples with 20% flower honey and 20% pine honey. The fat content decreased as the honey concentration increased. The fat content in our yogurts was lower than that reported by Şanlı [32].

The salt content was the highest in the control group (Table 2). In the study by Şanlı, salt ranged from 0.74 to 0.79% [32]. Salt is known to enhance flavor and increase sweetness, hide any metallic or chemical flaws, and speed up the product's processing [33]. Therefore, the honey-fortified samples in our study were minimally salted, resulting in lower salt values compared to other studies.

The protein contents in the yogurt samples decreased with larger amounts of honey added. The lowest protein content was detected in the yogurt with 20% flower honey. Chapagain *et al.* reported that a yogurt beverage with 7.5% honey had a protein content of 2.25% [34]. Our samples containing 10% honey had slightly higher protein contents. Protein production is stimulated by proline, lysine, phenylalanine, GABA, glutamine, serine, as well as glutamic and aspartic acids present in honey. However, storing honey for a long time in unfavorable conditions significantly decreases the number of amino acids [35].

The L^* values showed a statistically significant ($p < 0.05$) difference between the drinkable yogurt samples (Table 1). The control group had the highest brightness (L^*) value. The samples with 20% honey had a lower L^* value than those with 10% honey, and these values dropped throughout the storage time.

The a^* values increased with larger amounts of honey in all the samples. The samples with 10% thyme and 10% floral honey had the closest a^* values to those in the control group. Also, we found no statistically significant changes in the a^* values between the control and any other samples throughout the storage time.

The samples with pine honey had higher b^* values than those with floral and thyme honeys, while the samples with thyme honey had the lowest b^* values (apart from the control group). The b^* values partially dropped during storage, with no statistically significant changes.

In the study by İnce, the samples with flower and pine honeys had their L^* values ranging between 74.57 and 81.14, a^* values of –2.17 to –2.88, and b^* values ranging from 4.54 to 8.58 during storage [36]. Similarly, Machado *et al.* found that the L^* values of yogurt decreased with larger amounts of honey, but the a^* and b^* values increased [14]. They noted that the bright white color of goat milk combined with high gloss values made the honey appear brighter in the honey-containing samples. The L^*, a^*, and b^* values found in our study differed from those in other studies due to the coloring properties of honey, the natural proteolytic activity in yogurt or drinkable yogurt, and the oxidation of fatty acids [14].

Rheological properties. Thyme honey had the maximum viscosity (970.5 mPa·s) at various rotational speeds, followed by pine and flower honeys (Fig. 1). Durmuş found the greatest viscosity values at 50 rpm, namely 11.50 Pa·s in flower honey and 9.15 Pa·s in pine honey [28]. In another study, the viscosity values measured at 25°C at 5 rpm ranged between 1866 and 31 600 mPa·s for floral honeys and between 3033 and 40 600 mPa·s for pine honeys [27]. The honey samples were found to exhibit Newtonian behavior over the whole shear rate range, and their viscosity reduced as the temperature rose [13]. The viscosity of honey is affected by the brix value, the types of sugars, as well as their amounts and ratios in honey [27].

The Power Law model was selected because the threshold shear stress (τ_0) was zero for probiotic drinkable yogurt fortified with honey. The graph in Fig. 2 displays the apparent viscosity measurements for the probiotic yogurts taken at 100 rpm. The results from different storage times were found to be statistically similar. The

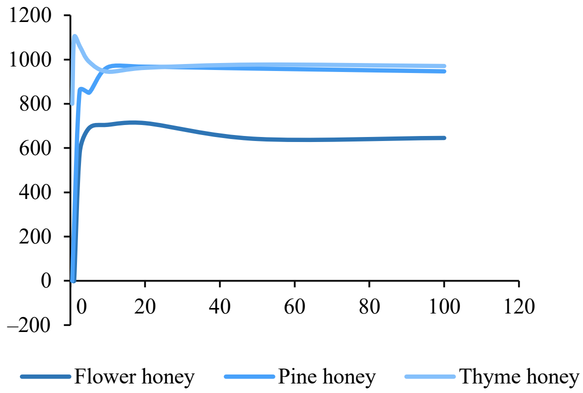
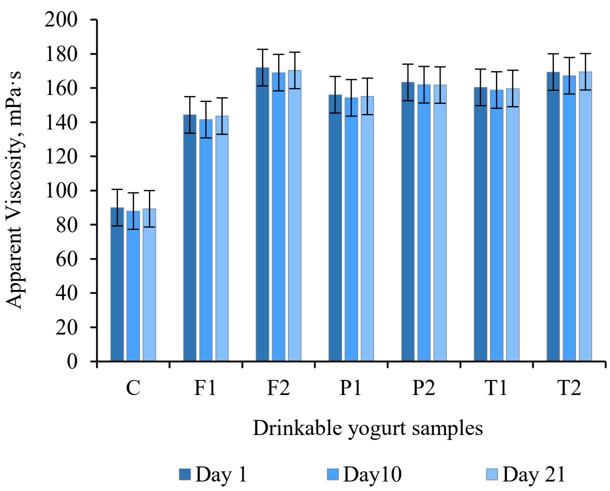


Figure 1 Viscosity values of honey samples (cP) (n = 3)



C – Probiotic drinkable yogurt without honey (Control group); F1 – Probiotic drinkable yogurt with 10% flower honey, F2 – Probiotic drinkable yogurt with 20% flower honey, P1 – Probiotic drinkable yogurt with 10% pine honey, P2 – Probiotic drinkable yogurt with 20% pine honey, T1 – Probiotic drinkable yogurt with 10% thyme honey, T2 – Probiotic drinkable yogurt with 20% thyme honey

Figure 2 Apparent viscosity values of drinkable yogurts at 100 rpm sample with 10% flower honey had the closest apparent viscosity value to that of the control.

According to İnce, the apparent viscosity of drinkable yogurt with 20% flower honey was 247.5 ± 10 mPa·s on the 10th day and that of the sample with 20% pine honey was 170 ± 30 mPa·s on the first day [36]. Another study found that goat yogurt without honey showed a decrease in perceived viscosity during storage ($p \leq 0.05$), whereas all the formulations with various honey contents showed an increase in perceived viscosity over time ($p \leq 0.05$) [14]. The addition of honey increased the yogurt's dry matter content and consistency, causing an initial rise in apparent viscosity proportional to the amount added. However, the viscosity values of yogurt formulations with honey became more unstable during storage, possibly due to honey's ability to act as a pseudoplastic liquid and resist the force applied to fluids.

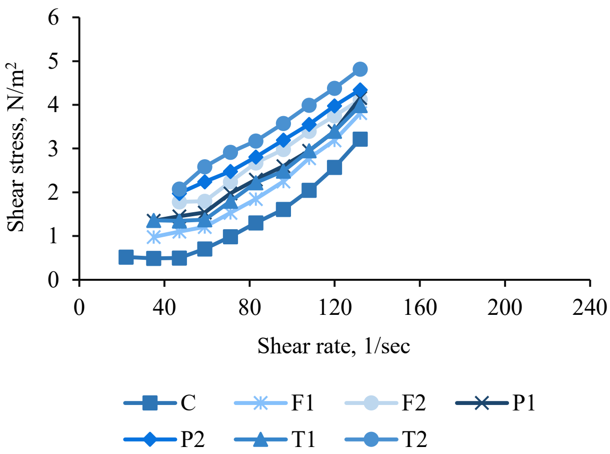
According to Table 3, the flow indexes of the samples decreased during storage. The consistency coefficients were higher in the samples with larger amounts of honey (20%). Additionally, the yogurt fortified with thyme honey had the highest consistency coefficient. The confidence coefficients of the samples ranged from 97.1 to 99.3.

Figures 3 and 4 illustrate the relationship between the shear stress (N/m^2) and the shear rate (1/s) for the drinkable yogurt samples (first day). In both figures, the flow of all the samples was identified as pseudoplastic because the shear stress increased with the shear rate in response to the form. The apparent viscosity of the samples was found to decrease as the shear rate increased (Fig. 4). This decrease in viscosity characterizes the flow as both thixotropic and non-Newtonian.

Microbiological analysis. Not all the samples were found to have coliform bacteria or yeast-mold during storage (Table 4). The control yogurt had the lowest number of mesophilic bacteria (7.39 log CFU/mL), while the sample containing 20% pine honey had the highest number (8.35 log CFU/mL) on the first day of storage. The total mesophilic bacteria count decreased over time for all the samples. On day 21, the honey yogurts had a lower

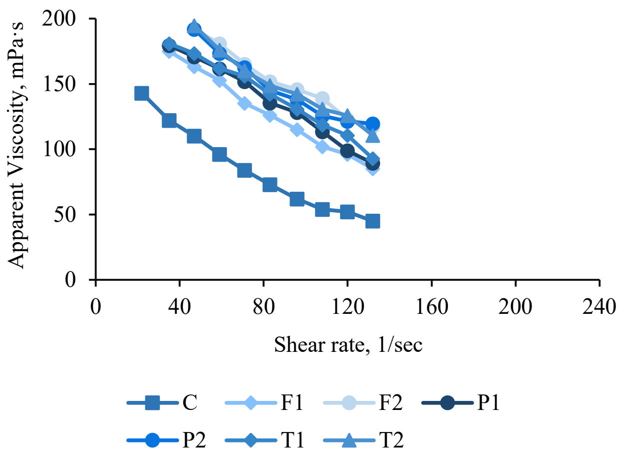
Table 3 Flow indexes, consistency and confidence coefficients of drinkable yogurts according to the Power Law model (n = 3)

Samples	Storage, day								
	1			10			21		
	Flow index (n)	Consistency coefficient, mPa·s	Confidence coefficient, %	Flow index (n)	Consistency coefficient, mPa·s	Confidence coefficient, %	Flow index (n)	Consistency coefficient, mPa·s	Confidence coefficient, %
Control	0.52	1670	98.4	0.29	1705	99.2	0.28	1820	98.2
Flower honey (10%)	0.44	1703	97.2	0.36	1785	98.7	0.21	1975	98.7
Flower honey (20%)	0.42	1888	99.0	0.38	1956	99.1	0.40	2125	99.2
Pine honey (10%)	0.35	2189	98.4	0.40	2293	98.4	0.39	2498	98.4
Pine honey (20%)	0.41	2571	99.3	0.33	2746	97.2	0.30	2869	99.2
Thyme honey (10%)	0.42	2193	97.1	0.37	2256	98.8	0.38	2787	98.9
Thyme honey (20%)	0.47	2557	98.2	0.43	2673	99.1	0.33	2917	97.3



C – Probiotic drinkable yogurt without honey (Control group); F1 – Probiotic drinkable yogurt with 10% flower honey, F2 – Probiotic drinkable yogurt with 20% flower honey, P1 – Probiotic drinkable yogurt with 10% pine honey, P2 – Probiotic drinkable yogurt with 20% pine honey, T1 – Probiotic drinkable yogurt with 10% thyme honey, T2 – Probiotic drinkable yogurt with 20% thyme honey

Figure 3 Shear stress/shear rate graph for drinkable yogurt samples (First day) (n = 3)



C – Probiotic drinkable yogurt without honey (Control group); F1 – Probiotic drinkable yogurt with 10% flower honey, F2 – Probiotic drinkable yogurt with 20% flower honey, P1 – Probiotic drinkable yogurt with 10% pine honey, P2 – Probiotic drinkable yogurt with 20% pine honey, T1 – Probiotic drinkable yogurt with 10% thyme honey, T2 – Probiotic drinkable yogurt with 20% thyme honey

Figure 4 Apparent viscosity/shear rate graph for drinkable yogurt samples (First day) (n = 3) count than the control group. There was a significant variation in the total mesophilic bacteria count between the samples and over time ($p < 0.05$). İnce *et al.* reported the total counts of 7.13 to 8.51 log CFU/mL for the samples with pine and floral honeys [37].

On day 1, the highest amount of *Lactobacillus delbrueckii* subsp. *bulgaricus* was found in the control sample, while the lowest was registered in the yogurt with 20% thyme honey (Table 4). According to the results, storage decreased the amount of *L. delbrueckii* subsp. *bulgaricus* in all the samples. The samples with 20% honey had lower concentrations of these bacteria compared to those with 10% honey. According to Mercan, drinkable yogurts with karakovan honey (100% raw honey unique to Anatolia) had the highest concentration of *L. delbrueckii* subsp. *bulgaricus* [30]. The decrease in these bacteria was also significant in all the samples after storage.

An increase in the amount of *Streptococcus thermophilus* was seen in all the samples except for the control group at the end of storage. Thus, adding honey promoted the growth of these bacteria. According to Coskun and Dirican, probiotic drinkable yogurts with pine honey contained more *S. thermophilus* than the control sample during storage [9].

Our results revealed the presence of *Lactobacillus acidophilus* in the samples containing probiotic culture (Table 4). On day 1, the control sample had 6.95 log CFU/mL of *L. acidophilus*. The lowest and highest concentrations of these bacteria were 6.02 and 7.65 log CFU/mL, respectively, in the sample with 20% flower honey. After 21 days of storage, the quantity of *L. acidophilus* decreased in all the samples. Coskun and Dirican reported that adding and storing honey had a significant impact on *L. acidophilus* populations [9]. In the study by Machado *et al.*, goat yogurt fortified with honey (*Melipona scutellaris Latrelle*-Urucu) had 6.0 log CFU/g of *L. acidophilus* La-05 during 28 days of storage, and the addition of honey maintained their count and the quantity of yogurt starter germs [14].

Bifidobacterium spp. counts ranged from 5.22 to 6.45 log CFU/mL on day 1 and from 4.92 to 6.04 log CFU/mL on day 21. They were higher at the start of storage in the samples fortified with 10% of thyme honey and 20% of each honey, but decreased throughout storage. Fiorda *et al.* found that honey has good potential as a stimulating ingredient to produce probiotic beverages and that honey-based kefir can prevent microbial DNA damage [38]. We believe that adding honey to drinkable yogurt during its manufacture can enhance the product's functional qualities.

Sensory analysis. The control sample was rated the highest in appearance, texture, taste, odor, and general acceptability (Table 5). After 21 days of storage, the sample with 10% honey (flower, thyme) had higher sensory scores, and was more similar in appearance to the control, than the sample with 20% honey. The structural scores of the samples with 20% flower honey and 10% flower, pine, and thyme honeys were like those of the control. The samples with 10% honey (flower, pine, and thyme) were rated higher in taste than those with 20% honey. The general acceptability scores during storage were between 2.6 to 9.17, with higher scores given to the samples with 10% honey (flower, pine, and thyme). This might suggest that a honey concentration of 20% is too high for drinkable yoghurts. Additionally, the adult panelists might prefer yogurt with the usual salty taste or a less sweet taste than that of honey yogurt. Studies show that fruit yogurts appeal more to children than adults. Therefore, we think that honey-fortified drinkable yogurt may be more suitable for children.

In Mercan's study, the yogurts with karakovan honey had the highest scores, while those with thyme

Table 4 Microbiological parameters of drinkable yogurt samples (n = 3)

Parameter, CFU/mL	Day	Samples						
		Control	Flower honey (10%)	Flower honey (20%)	Pine honey (10%)	Pine honey (20%)	Thyme honey (10%)	Thyme honey (20%)
Total mesophilic bacteria	1	7.39 ± 0.02$^{a–e}$	8.16 ± 0.68^{abc}	8.28 ± 0.68^{ab}	7.68 ± 1.06$^{a–d}$	8.35 ± 0.78^{a}	7.46 ± 1.46$^{a–e}$	8.15 ± 0.75^{abc}
	10	6.37 ± 0.28$^{c–f}$	5.80 ± 0.58^{efg}	5.80 ± 0.90^{efg}	6.59 ± 0.60$^{c–f}$	6.47 ± 0.15$^{b–f}$	6.06 ± 0.25$^{d–g}$	6.06 ± 0.28$^{d–g}$
	21	6.07 ± 0.40$^{d–g}$	4.41 ± 0.29^{g}	4.29 ± 0.37^{g}	5.10 ± 0.02^{fg}	4.72 ± 0.03^{fg}	5.03 ± 0.10^{fg}	4.45 ± 0.11^{g}
Lactobacillus delbrueckii subsp. *bulgaricus*	1	6.99 ± 0.08^{a}	6.19 ± 0.07^{abc}	6.17 ± 0.42^{abc}	6.31 ± 0.09^{abc}	5.94 ± 0.35^{abc}	6.07 ± 0.04^{abc}	5.91 ± 0.81^{abc}
	10	6.92 ± 0.09^{ab}	6.04 ± 0.08^{abc}	6.12 ± 0.43^{abc}	6.22 ± 0.16^{abc}	5.76 ± 0.40^{c}	6.04 ± 0.03^{abc}	5.41 ± 0.64^{c}
	21	5.80 ± 0.80^{abc}	5.98 ± 0.12^{abc}	5.91 ± 0.35^{abc}	6.01 ± 0.04^{abc}	5.67 ± 0.34^{abc}	6.03 ± 0.01^{abc}	5.39 ± 0.82^{abc}
Streptococcus thermophilus	1	7.07 ± 0.17$^{a–d}$	7.63 ± 0.13$^{a–d}$	8.08 ± 0.42^{abc}	7.66 ± 0.84$^{a–d}$	6.91 ± 0.95$^{a–d}$	6.49 ± 0.60^{cd}	6.60 ± 0.53^{bcd}
	10	6.81 ± 0.25$^{a–d}$	8.04 ± 0.05^{abc}	8.13 ± 0.09^{abc}	7.83 ± 1.35$^{a–d}$	6.96 ± 0.77$^{a–d}$	6.66 ± 0.39^{bcd}	6.45 ± 0.30^{cd}
	21	7.02 ± 0.18$^{a–d}$	8.08 ± 0.01^{abc}	8.19 ± 0.23^{abc}	8.42 ± 0.19^{ab}	7.40 ± 1.00$^{a–d}$	7.38 ± 1.08$^{a–d}$	7.36 ± 0.24$^{a–d}$
Lactobacillus acidophilus	1	6.95 ± 0.23^{bc}	6.90 ± 0.03^{c}	7.65 ± 0.14^{a}	7.01 ± 0.02^{bc}	7.14 ± 0.12^{ab}	6.02 ± 0.01^{efg}	6.88 ± 0.01^{c}
	10	6.44 ± 0.58$^{c–f}$	6.18 ± 0.43$^{d–g}$	6.56 ± 0.35^{cde}	6.84 ± 0.04^{c}	6.92 ± 0.14^{bc}	5.85 ± 0.06^{fg}	6.75 ± 0.04^{cd}
	21	5.59 ± 0.03^{g}	5.63 ± 0.02^{g}	4.68 ± 0.03^{h}	5.69 ± 0.10^{g}	5.87 ± 0.10^{fg}	5.71 ± 0.03^{g}	5.80 ± 0.04^{g}
Bifidobacterium spp.	1	5.41 ± 0.02$^{c–f}$	5.22 ± 0.07^{efg}	6.40 ± 0.08^{a}	5.24 ± 0.12^{ef}	5.23 ± 0.07^{efg}	6.45 ± 0.06^{a}	5.81 ± 0.01^{bcd}
	10	5.52 ± 0.15^{cde}	5.06 ± 0.10^{efg}	5.22 ± 0.26^{efg}	4.71 ± 0.58^{g}	4.92 ± 0.13^{fg}	5.88 ± 0.08^{bc}	5.13 ± 0.17^{efg}
	21	6.04 ± 0.03^{ab}	5.04 ± 0.05^{efg}	4.92 ± 0.13^{fg}	5.00 ± 0.10^{fg}	5.09 ± 0.13^{efg}	5.44 ± 0.01$^{c–f}$	5.19 ± 0.07^{efg}

*$^{a–h}$ – Different letters indicate statistical significance between the groups ($p < 0.05$)

Table 5 Sensory evaluation of drinkable yogurt samples (n = 3)

Parameter	Day	Samples						
		Control	Flower honey (10%)	Flower honey (20%)	Pine honey (10%)	Pine honey (20%)	Thyme honey (10%)	Thyme honey (20%)
Appearance	1	9.52 ± 0.19^{ab}	7.68 ± 0.18^{bc}	5.05 ± 0.38^{e-i}	5.95 ± 0.38^{c-g}	4.05 ± 0.38^{g-j}	7.06 ± 0.77^{cde}	4.57 ± 0.57^{f-j}
	10	9.75 ± 0.25^{ab}	7.17 ± 0.50^{cd}	5.33 ± 1.00^{d-h}	5.42 ± 0.75^{d-h}	3.42 ± 0.58^{hij}	6.42 ± 0.58^{c-f}	4.42 ± 0.42^{f-j}
	21	9.83 ± 0.03^{a}	7.37 ± 1.23^{cd}	3.76 ± 0.04^{hij}	4.46 ± 0.26^{f-j}	2.91 ± 0.51^{j}	6.17 ± 0.97^{c-f}	2.99 ± 1.59^{ij}
Texture	1	8.68 ± 0.18^{ab}	7.80 ± 0.37^{a-e}	6.26 ± 0.40^{d-g}	7.08 ± 0.25^{b-g}	6.01 ± 0.15^{d-h}	5.98 ± 0.31^{d-h}	5.08 ± 0.08^{gh}
	10	9.17 ± 0.50^{a}	7.92 ± 0.25^{a-d}	7.33 ± 0.33^{a-f}	6.33 ± 0.67^{c-g}	5.89 ± 0.61^{e-h}	7.33 ± 0.33^{a-f}	6.08 ± 0.58^{d-g}
	21	8.31 ± 0.11^{abc}	7.50 ± 0.50^{a-f}	7.04 ± 0.24^{b-g}	6.34 ± 0.94^{c-g}	5.56 ± 1.16^{fgh}	6.46 ± 0.26^{c-g}	4.00 ± 2.00^{h}
Taste	1	8.78 ± 0.19^{a}	6.93 ± 0.54^{abc}	4.73 ± 0.44^{cde}	4.90 ± 0.76^{cde}	4.25 ± 0.25^{cde}	4.33 ± 0.33^{cde}	3.17 ± 1.97^{e}
	10	8.83 ± 0.33^{a}	6.25 ± 0.75^{a-d}	4.92 ± 0.42^{cde}	4.67 ± 0.50^{cde}	4.08 ± 0.25^{cde}	4.37 ± 0.17^{cde}	2.96 ± 0.08^{e}
	21	8.93 ± 1.07^{a}	6.96 ± 1.19^{abc}	5.19 ± 0.79^{cde}	5.16 ± 0.56^{cde}	5.01 ± 2.41^{cde}	4.36 ± 1.36^{cde}	2.92 ± 0.54^{e}
Odor	1	9.06 ± 0.23^{a}	7.77 ± 0.06^{ab}	4.96 ± 0.53^{cd}	5.11 ± 0.39^{cd}	4.61 ± 0.11^{d}	4.88 ± 0.55^{cd}	3.85 ± 0.01^{def}
	10	9.25 ± 0.25^{a}	6.50 ± 0.50^{bc}	5.58 ± 0.42^{cd}	5.50 ± 0.17^{cd}	4.75 ± 0.25^{d}	3.92 ± 0.08^{def}	2.83 ± 0.01^{ef}
	21	8.71 ± 1.29^{a}	5.57 ± 0.57^{cd}	5.13 ± 0.73^{cd}	4.54 ± 0.74^{de}	4.30 ± 0.70^{de}	4.13 ± 0.27^{def}	2.46 ± 1.26^{f}
General acceptability	1	9.13 ± 1.82^{a}	7.50 ± 1.75^{abc}	4.90 ± 0.76^{d-g}	5.00 ± 0.67^{d-g}	4.09 ± 1.48^{fg}	4.46 ± 0.04^{efg}	3.14 ± 0.14^{fg}
	10	9.17 ± 0.50^{a}	6.86 ± 0.14^{a-d}	5.33 ± 1.00^{c-f}	4.69 ± 0.89^{d-g}	4.08 ± 0.75^{fg}	4.39 ± 0.19^{fg}	2.92 ± 0.08^{g}
	21	8.73 ± 0.87^{ab}	6.75 ± 0.75^{b-e}	4.80 ± 1.20^{d-g}	4.45 ± 0.88^{efg}	3.86 ± 0.14^{fg}	3.83 ± 0.17^{fg}	2.67 ± 1.47^{g}

*$^{a-j}$ – Different letters indicate statistical significance between the groups ($p < 0.05$)

honey were disliked the most. The general acceptability ratings reportedly dropped at the end of storage [30].

CONCLUSION

Our results showed a significant difference ($p < 0.05$) in the total dry matter, fat, salt, pH, and serum separation values among the drinkable yogurts. The samples with floral and thyme honeys had similar serum separation values. The samples with 20% honey had a lower L^* value than those with 10% honey. The samples' viscosity decreased throughout storage, assigning drinkable yogurts to the class of thixotropic and non-Newtonian pseudoplastic liquids. As the honey content increased, so did the apparent viscosity and consistency coefficients. The samples fortified with 20% thyme honey had the greatest consistency coefficient on day 21 of storage.

We found that *Lactobacillus delbrueckii* subsp. *bulgaricus* were more abundant in the yogurts containing 10% honey compared to those with 20% honey at the end of 21 days of storage. However, the count of *Streptococcus thermophilus* increased only with a higher flower honey ratio at the end of storage. After 21 days of storage, the probiotic bacteria levels in the honey-containing samples were adequate (> 5 log CFU/mL) for consumer health, except for the samples with 20% floral honey. The sensory evaluation showed a preference for the yogurts with 10% honey (flower, pine, and thyme) over those with 20% honey. Based on the probiotic levels at the end of storage and consumer preference, 10% was determined as an optimal amount of flower or pine honey for drinkable yogurt, with 10% flower honey being particularly favored. We also expect that limiting the amount of thyme honey to less than 10% will produce drinkable yogurt with more desirable sensory characteristics. Our study showed that honey improves the functional properties of drinkable yogurt by promoting the growth of probiotic bacteria.

CONTRIBUTION

The authors contributed equally to this work.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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