



## ENGINEERING SCIENCES

# Viability of Probiotics, Rheological and the Sensorial Properties of Probiotic Yogurts Fortified with Aqueous Extracts of Some Plants

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**Abstract:** In this research, we aimed to evaluate the effects of various medicinal and aromatic plant aqueous extracts on physicochemical, rheological, sensorial properties of probiotic yogurts and the viability of yogurt bacteria and probiotic bacteria during the storage period. On the 28 day of storage, the maximum *Bifidobacterium longum* and *Lactocaseibacillus casei* counts were determined in thyme containing yogurt and mint and basil containing yogurt, respectively. On the 1<sup>st</sup> day of storage, the most favoured yogurt was garlic containing sample while on the 14<sup>th</sup> day, the control sample was the most favoured one. Hardness values were not showed statistically difference for all sample ( $p>0.05$ ). Especially the garlic and mint yogurt samples were more liked in terms of sensory properties. Consequently, aqueous extracts of some plants such as garlic, basil and thyme have successfully used in probiotic yogurt production and all yogurt samples containing plants have the enough probiotic counts to show therapeutic effect on human health.

**Key words:** Functional food, plant, probiotics, yogurt.

## INTRODUCTION

Yogurt is widely produced and consumed in the world as healthy dairy food because of its good taste and high nutritional and therapeutic characteristics. Yogurt consumption helps to strengthen the immune system, improves lactose digestion (Adolfsson et al. 2004), control allergic symptoms, prevention of diseases such as cardiovascular diseases, cancer, osteoporosis etc. (Freitas 2017). Probiotics are live microorganisms that benefit the health of the host when taken in adequate amounts (García-Burgos et al. 2020). As a result of many studies on probiotics, the use of these microorganisms in foods has become popular and people have become more interested in consuming products containing probiotics. In recent years, probiotic products have come to

the fore among the food products because of their positive health claims. Their effects on health have resulted in marketing efforts aimed at producing new functional food products (Grom et al. 2020, Zendeboodi et al. 2020). One of the most convenient ways to consume probiotics is the use of probiotic bacteria in the fermentation of dairy products (Rasika et al. 2021). Yogurt is consumed frequently around the world and is a good carrier of probiotics and bioactive compounds. Therefore, for many years, yogurt and yogurt-like fermented milk products have been enriched with herbal ingredients like medicinal and aromatic plants for different purposes. These plants have high antioxidant activity due to the polyphenols they contain, especially flavonoids. Studies have represented that phenolic compounds have high antimicrobial, and antioxidant activity

and certain therapeutic properties covering antidiabetic and antihypertensive activity (Amirdivani 2008). In literature, some researches have focused on using herbal ingredients in probiotic yogurt production. Hadjimbei et al. (2020) developed goat milk probiotic yogurt using *Pistacia atlantica* resin extract. Ozcan et al. (2020) produced red beetroot probiotic yogurt. Alizadeh et al. (2020) added microalgae and Zedo gum to probiotic yogurt. Alwazeer et al. (2020) fortified milk with thyme and grape seed extracts and fermented mixture with yogurt bacteria. El-Shafei et al. (2020) enriched goat milk yogurt with quinoa extract.

Antibiotics can be used inappropriately in the control of pathogenic and spoilage bacteria in foods, and this can cause antibiotic resistance in humans. Disadvantages such as antibiotic resistance in humans bring up the use of plants as natural antimicrobial agents. In some studies, it has been determined that the compounds obtained from plants have antimicrobial effects. In this respect, research to find new antimicrobial agents becomes attractive. To date highly little is known about the changes that can occur when medicinal and aromatic plants aqueous extracts are present during yogurt production. In this research, we used aqueous extracts of garlic, thyme, rosemary, mint, and basil in probiotic yogurt production. We tried to determine the usability and effect of different medicinal and aromatic plant aqueous extracts on physicochemical, textural, and sensorial properties and probiotic viability of yogurt samples during the 28 day of storage. It is aimed to use plant extracts to prevent or reduce competitive microorganism and fungus growth, which is one of the most important problems encountered during the shelf life of yogurt. Thus, it is thought to provide a functional product different from the yogurts available in the market for the consumer. Although there are

studies on plant extracts in dairy products in the literature, the number of studies on yogurt is few.

## MATERIALS AND METHODS

### Materials

The cow's milk obtained from the Menemen Research and Application Farm of Ege University Faculty of Agriculture, Izmir, Turkey was used in the production of yogurt. *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* containing yogurt culture (CSL Centro Sperimentale del Latte, Italy) and the *Lacticaseibacillus casei* L26 formerly known as *Lactobacillus casei* (Lafti L26, DSM, Germany) and B22 *Bifidobacterium longum* (Lafti B22, DSM, Germany) probiotic bacteria cultures were used. Among the medicinal and aromatic plants used in the study, basil; *Ocimum basilicum* L., garlic; *Allium sativum*, mint; *Mentha piperita* L., rosemary; *Rosmarinus officinalis*, thyme; *Origanum onites* L.) were obtained from the Aegean Agricultural Research Institute.

### Method

The yogurt samples were produced in two repetitions in the pilot plant of the Dairy Technology Department of Ege University Faculty of Agriculture, Izmir, Turkey. The analyses were carried out in three repetitions.

### Preparation of the yogurt starter culture and probiotics

The starter culture milk was prepared from 12% fat-free dry matter-containing fat-free milk powder and sterilized in an autoclave at 110°C for 15 minutes. During the production of the culture, yogurt culture and probiotics was added at recommended concentrations to the milk cooled to 42-43°C.

### **Preparation of the plant aqueous extracts**

After removing undesired materials from the plants that were used in production, the plants were rinsed and then, boiled for a minute to reduce their microbiological load. Later, the plants were cut to desired sizes and 10 g of each plant was weighed and incubated with 100 ml distilled water in a water bath at 70°C for a night to obtain the aqueous extracts of the plants.

### **Yogurt production**

The non-fat dry matter ratio of milk is thickened by using skimmed milk powder. The milk with increased dry matter used in yogurt production and it was pasteurized at 85-90°C for 5 minutes. Then, the milk was cooled to 37-40°C and divided into 6 groups. The aqueous extracts of the medicinal and aromatic plants were added to the milk groups at the rate of 10%. Then, the yogurt culture and probiotic bacteria were added to the milks according to producer advice and the milks were incubated at 37°C until the pH reached 4.6. After incubation, the yogurts were stored at 4°C and analyses were carried out on days 1, 14 and 28 of storage.

### **Physiochemical analyses**

In yogurts, pH meter was used to determine the pH values (Polychroniadou et al. 1999), the acidity was determined using the alkali titration method, total dry matter content was determined with the gravimetric method, fat content was determined with the Gerber method and nitrogen and protein contents were determined using the Kjeldahl method by following the methods specified in AOAC (2003).

### **Microbiological analyses**

The pour plate method was used for microbiological analyses. A total of 10-g yogurt sample was homogenized in 90 mL sterile

ringer solution and diluted at the appropriate dilution rates. Sequential decimal dilutions of the homogenate were plated in duplicates on specific media.

### **Viability of yogurt bacteria**

MRS agar medium was used to determine the *L. bulgaricus* count in the yogurt samples. After inoculations from the appropriate dilutions, the samples were incubated anaerobically at 42°C for 72h. M17 agar medium was used to determine the *S. thermophilus* count and after inoculations, the petri dishes were incubated under aerobic conditions at 37°C for 48h (Donkor et al. 2006).

### **Viability of probiotic bacteria**

#### *- Lactocaseibacillus casei counts*

Inoculations were made from the dilutions prepared using MRS-D-Sorbitol agar. The petri dishes were incubated anaerobically at 37°C for 48 hours. The colonies forming after incubation were counted (Tharmaraj & Shah 2003).

#### *- Bifidobacterium longum counts*

The colony count of bifidobacteria was done by following the ISO/DIS 29981 IDF 220 (2020) method. The TOS Propionate Agar was used for this purpose. The petri dishes were incubated under anaerobic conditions at 37°C for 48 hours.

### **Rheological analyses**

#### **Hardness**

The hardness values of samples were determined using a Texture Analyzer (CT3, Brookfield, USA) using a TA4/1000 (38.1mm diameter-20mm height) cylindrical probe. The analyses conditions are selected according to Yerlikaya et al. (2020). While determining the hardness properties of the yogurt samples, the sample temperature was ensured to be 4-8°C. The results were calculated using the Pro CT V 1.2 software program.

## Viscosity

Viscosity analyses were performed with a Brookfield viscometer (DV-II Pro) at 12 rpm at 4°C using a Spindle No: LV4. Measurements were taken at 4-8°C degrees after the yogurts were mixed equally on the right and left sides (Akpınar et al. 2020). The results were evaluated according to Rheocalc® program (Brookfield Eng. Lab., ABD) software.

## Sensorial properties

A scoring test (1: very bad, 9: very good) was used in the sensory evaluation of probiotic yogurt samples. The yogurt samples were analysed in terms of color, flavour, texture and overall attributes on the 1<sup>st</sup> and 14<sup>th</sup> day of storage. The scoring test was conducted with a group of 9 trained panellists with non-allergic reactions to milk and on a regular yogurt and/or fermented milk consumption from Ege University, Izmir, Turkey. Panellists had previous experience with sensory acceptance testing. Jury members consumed each yogurt in 100 ml of plastic containers at 4-8°C. During the evaluation of different yogurt formulations, a glass of water was given for palate cleansing (Costa et al. 2020, Lucatto et al. 2020).

## Statistical analysis

One Way ANOVA analysis was used to compare the properties of different medicinal and aromatic plant aqueous extracts-added yogurt samples during the storage period. The SPSS

version 25.0 (SPSS Inc. Chicago, Illinois) statistical analysis package program was used for the statistical analyses. The data that were revealed to be significant by variance analysis were tested using the Duncan multiple comparison test at the level of  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Physicochemical properties

Table I shows the chemical properties on day 1 after the production of the probiotic yogurts that were produced by adding the aqueous extracts of medicinal aromatic plants. The dry matter content of yogurt is important to obtain the desired textural and sensory properties. The dry matter content of the probiotic yogurts varied from 13.11% to 13.68% depending on the plant aqueous extract. According to the Turkish Food Codex Fermented Dairy Products Communiqué dated 16.02.2009 (Communiqué No: 2009/25), the milk protein of yogurt should be at least 3.0% by weight. The protein contents of the samples were in compliance with the values specified in the communiqué. The same also applies to the fat contents of the yogurts.

In this study, all pH values were close to each other at the beginning of storage (4.17-4.20) (Figure 1). The pH values of the yogurt samples during the storage period revealed that the use of different plant extracts was not statistically significant ( $p > 0.05$ ). Only the comparison of the samples within themselves during the storage

**Table I. Physicochemical properties of yogurt samples on the 1<sup>st</sup> day of storage period.**

Parameter/ Sample	C	B	T	M	R	G
Dry matter (%)	13.68±0.13	13.35±0.16	13.47±0.25	13.44±0.32	13.11±0.06	13.38±0.31
Protein (%)	3.72±0.18	3.86±0.15	3.52±0.32	4.29±0.28	3.47±0.15	3.71±0.31
Fat (%)	3.40±0.00	3.40±0.00	3.40±0.00	3.40±0.05	3.40±0.00	3.40±0.05

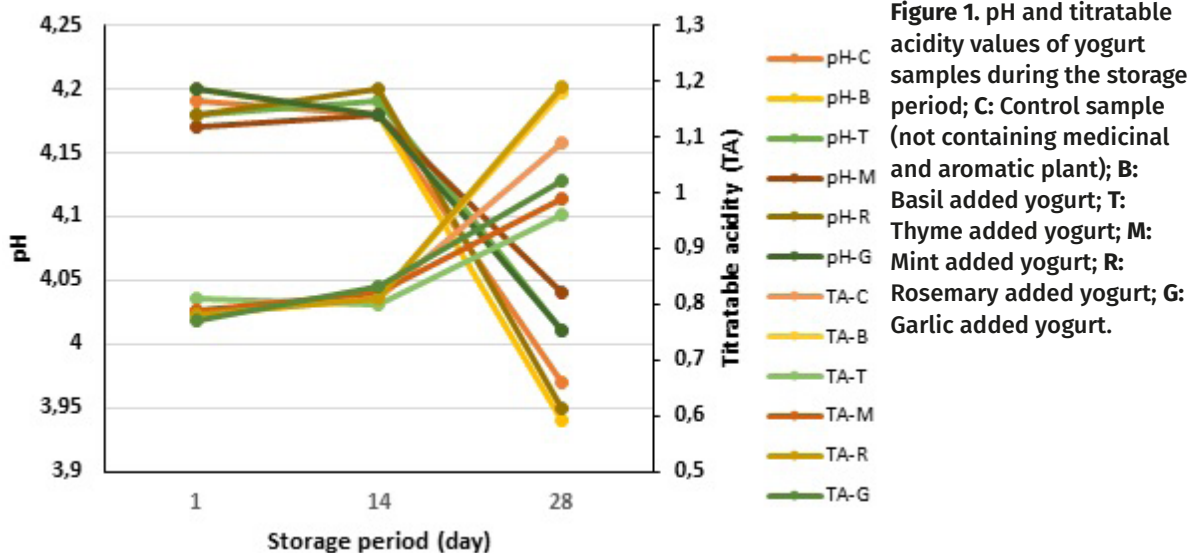
**C: Control sample (not containing medicinal and aromatic plant); B: Basil added yogurt; T: Thyme added yogurt; M: Mint added yogurt; R: Rosemary added yogurt; G: Garlic added yogurt.**

period showed that the pH value on day 28 of storage significantly changed compared with days 1 and 14 of storage ( $p < 0.05$ ). At the end of the storage sample M had the maximum pH value while sample B and R had the minimum pH values. In contrast with our study, Amirdivani & Baba (2011) added mint (*Mentha piperita*), dill (*Anethum graveolens*) and basil (*Ocimum basilicum*) to yogurt and found that, during fermentation, plant-containing yogurts had a more rapid decrease in pH compared with the control yogurts.

Similar to our study, Behrad et al. (2009) added cinnamon and licorice to probiotic yogurts and found that the pH values of the plain yogurts were almost the same as those of the plant-added yogurts. Again, similarly, Shori & Baba (2014) added garlic (*Allium sativum*) to the yogurts produced from cow and camel milks and reported that the addition of *A. sativum* did not cause a significant change in the pH value of the yogurt produced from cow milk. Total titratable acidity is a parameter considered in evaluating the fermentation capacity of microorganisms. Moreover, it plays a role in the taste-aroma balance and affects product quality by causing an acidic taste in yogurt. In this study, the lactic

acid levels of the yogurt samples were in the range of 0.78-1.18% (Figure 1).

Although there were no statistically significant changes in the lactic acid levels of the samples on days 1 and 14 of storage ( $p > 0.05$ ), there were significant differences between the samples on day 28 of storage ( $p < 0.05$ ). In parallel with the pH values, the lactic acid levels of the samples also changed compared among themselves on day 28 of storage ( $p < 0.05$ ). Shori & Baba (2011) determined that the titratable acidity of the cinnamon (*Cinnamomum verum*)-added yogurts increased during storage, but the addition of *C. verum* did not affect microbial fermentation. Yadav & Shukla (2014) found that the acidity% of the cinnamon extract-added yogurts was higher than that of the control yogurt. In their study, Shori & Baba (2014) determined that the addition of *Allium sativum* did not promote the formation of titratable acidity in the yogurts produced from cow milk both during fermentation and cold storage. In the study, in contrast with the data found in the literature, the addition of different medicinal aromatic plants to yogurt did not significantly affect the lactic acid levels compared with the control sample.

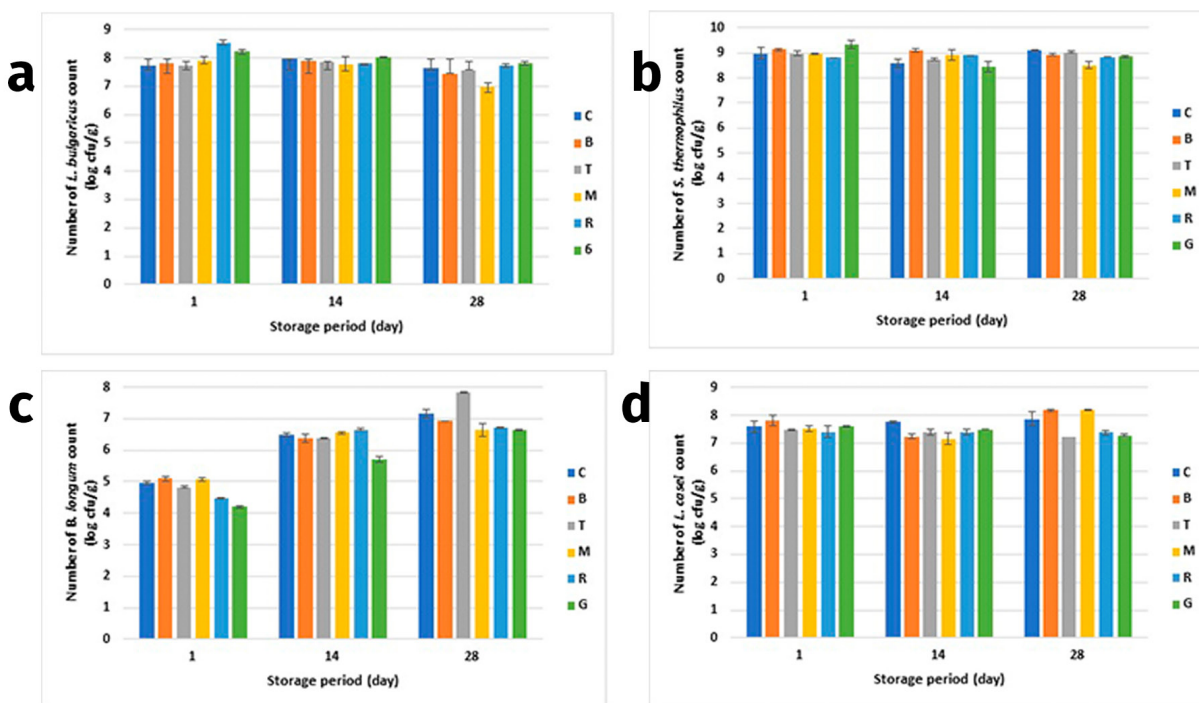


### Microbiological properties

Figure 2 shows the microbiological counts of the yogurt samples. The count of *L. bulgaricus*, a type of yogurt bacteria, on day 1 of storage was 7.74-8.54 log cfu/g and changed to 6.97-7.82 log cfu/g on day 28. The lowest *L. bulgaricus* count was obtained in the control sample on day 1, while the mint-added yogurt sample had the lowest count at the end of storage. The addition of these plants to the samples significantly affected the *L. bulgaricus* count ( $p < 0.05$ ). The *S. thermophilus* counts on the first day of storage ranged from 8.70 to 9.25 log cfu/g. The *S. thermophilus* counts of the samples fluctuated during the storage period. The control sample had the highest *S. thermophilus* count at the end of storage (9.10 log cfu/g). Therefore, we can assert that the growth of *S. thermophilus* was

somewhat inhibited by the medicinal aromatic plants.

When probiotic bacteria are considered, the *B. longum* count varied from 4.21 to 7.85 log cfu/g during the storage period. The garlic-added yogurt sample had the lowest *B. longum* count both at the beginning and end of storage. The *B. longum* counts of all samples increased during the storage period. The *L. casei* counts of the yogurt samples ranged from 7.40 (rosemary) to 7.82 (basil) on the first day of storage and from 7.24 (thyme) to 8.19 (mint) on day 28 of storage. Again, plant addition to yogurt caused statistically significant differences in the *L. casei* count ( $p < 0.05$ ). Moreover, the live organism counts determined for *L. casei* were within the acceptable levels for minimum therapeutic count.



**Figure 2.** a. *Lactobacillus bulgaricus* counts of yogurt samples during the storage period; b. *Streptococcus thermophilus* counts of yogurt samples during the storage period; c. *Bifidobacterium longum* counts of yogurt samples during the storage period; d. *Lactocaseibacillus casei* counts of yogurt samples during the storage period; C: Control sample (not containing medicinal and aromatic plant); B: Basil added yogurt; T: Thyme added yogurt; M: Mint added yogurt; R: Rosemary added yogurt; G: Garlic added yogurt.

The results revealed that the probiotic bacteria maintained and, even, improved their viability in all plant containing samples including the control sample during the storage period. Hussain et al. (2015) found that the use of plants (*Ferule* sp., *Thymus* sp., *Allium* sp., *Anhriscus* sp.) and in different concentrations (0.5, 1, 2 and 3% (w/w) promoted the acid production of *S. thermophilus* and *L. delbrueckii* and acid production increased with increasing plant concentration. Cimo et al. (2013) determined that the *Lactobacillus rhamnosus* GR-1 count of the ginseng extract-containing probiotic yogurt was higher than that of the plant-free yogurt during the 28-day storage period. In their study, Chaikham (2015) added Thai plant extract to encapsulated probiotic-containing yogurt and found that the Thai plant extract promoted probiotic stability during storage and the concentration of the extract was important. Similar to our study, Behrad et al. (2009) added cinnamon and licorice to yogurt and found that *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis* maintained their viability at the end of the 28-day storage period.

### Rheological properties

In this study, the rheological properties of yogurt samples were evaluated by determining their

hardness and viscosity values and are given in Table II. During the storage period of 28 days, the hardness values of the samples varied in the range of 80-98 g and it was determined that the storage time and the plant difference used did not have a statistically significant effect on the hardness values ( $p>0.05$ ). On the 28<sup>th</sup> day of storage, the structure of the yogurt was deteriorated due to the release of water from the R sample and therefore the hardness value could not be measured. Based on the results of the study, it was revealed that the use of various medicinal and aromatic plants extracts in probiotic yogurt had no significant effect on the hardness value ( $p>0.05$ ).

One of the factors determining the stability of yogurt curd is the viscosity value. At the end of storage period of 28 days, the viscosity value increased in samples containing control, garlic and medicinal mint, while it decreased in samples containing rosemary, thyme and basil. Viscosity values of B, T, R and G samples at the beginning of the storage period were significantly higher than the control sample ( $p<0.05$ ). The viscosity value of the mint containing sample did not differ from the control sample during the storage period ( $p>0.05$ ). On the 14<sup>th</sup> day of storage, the viscosity increased significantly in the control and M sample ( $p<0.05$ ). At the beginning of the

**Table II. Hardness and viscosity values of yogurt samples during the storage period.**

Parameter	Samples						
	Days	C	B	T	M	R	G
Hardness (g)	1	89.50±9.19 <sup>A</sup>	92.25±10.25 <sup>Aa</sup>	80.00±0.28 <sup>Aa</sup>	86.5±8.48 <sup>Aa</sup>	91.50±6.36 <sup>Aa</sup>	84.75±10.25 <sup>Aa</sup>
	14	96.75±11.66 <sup>Aa</sup>	98.00±3.43 <sup>Aa</sup>	91.75±18.38 <sup>Aa</sup>	92.25±8.34 <sup>Aa</sup>	93.5±11.31 <sup>Aa</sup>	91.50±7.78 <sup>Aa</sup>
	28	96.25±22.98 <sup>Aa</sup>	94.50±16.26 <sup>Aa</sup>	93.00±18.38 <sup>Aa</sup>	81.5±0.49 <sup>Aa</sup>	Not determined	90.50±10.60 <sup>Aa</sup>
Viscosity (cP)	1	5987±42 <sup>Aa</sup>	7511±268 <sup>Ba</sup>	7886.5±191.62 <sup>Ba</sup>	5891±175.36 <sup>Aa</sup>	9400±7.07 <sup>Ca</sup>	7459±340.82 <sup>Ba</sup>
	14	9725±709 <sup>Ab</sup>	6504±180 <sup>Db</sup>	8298±216.37 <sup>Bca</sup>	7182.5±53.03 <sup>Db</sup>	9391±192.33 <sup>ABa</sup>	7508±225.56 <sup>CDa</sup>
	28	7170±192 <sup>ABa</sup>	6288.5±243.95 <sup>Ab</sup>	4934.5±112.42 <sup>Cb</sup>	7723±55.15 <sup>ABb</sup>	8571±282.84 <sup>Da</sup>	7591±248.90 <sup>Ba</sup>

<sup>a, b</sup>: Refers to the changes occurring between the sample types during the storage period. The values shown with different letters in the same coloumn are important at the level of  $p < 0.05$ .

<sup>A, B, C, D</sup>: Refers to the change in the samples during the storage period. The values shown with different letters in the same line are important at the level of  $p < 0.05$ .

**C:** Control sample (not containing medicinal and aromatic plant); **B:** Basil added yogurt; **T:** Thyme added yogurt; **M:** Mint added yogurt; **R:** Rosemary added yogurt; **G:** Garlic added yogurt.

storage period, the R sample had the highest viscosity value and the control sample had the lowest viscosity. It was determined that the use of different herbs and storage time in probiotic yogurt caused a significant change in viscosity values ( $p < 0.05$ ).

### Sensorial properties

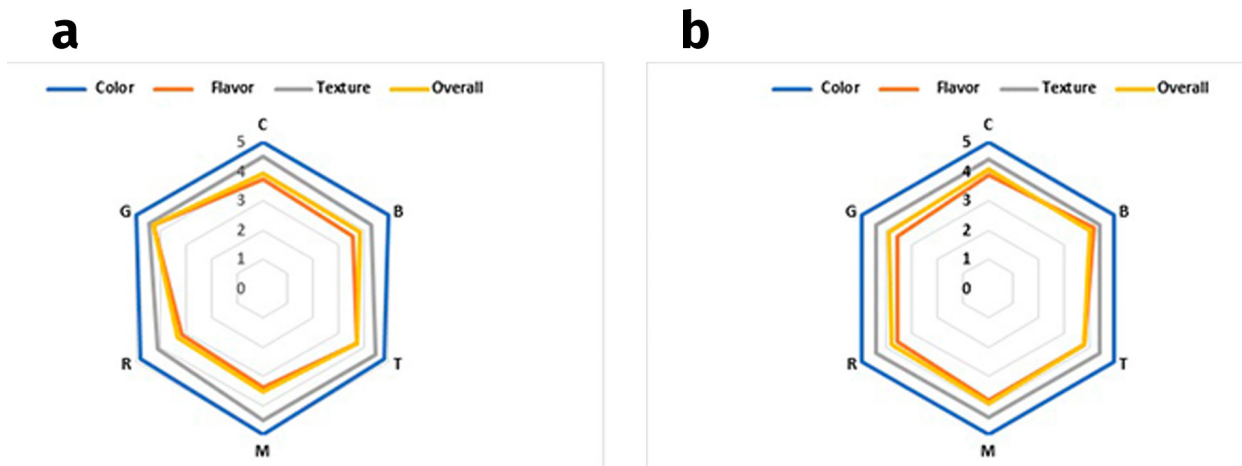
The main reason for the consumption of probiotic products is that although these products have positive effects on health, the sensorial characteristics are important in terms of daily consumption. In our study, probiotic yogurt samples were evaluated according to their colour, flavour, texture and general impression characteristics. The sensory evaluation results of probiotic yogurts were obtained by using the sensory evaluation scale for scoring and shown in Figure 3. On the 1<sup>st</sup> day of storage, yogurt containing garlic was the most preferred (G) in terms of taste, while the sample containing basil on the 14<sup>th</sup> day of storage was (B). According to Turkish palate culture, garlic is added to yogurt and consumed in many dishes. Although it is not preferred by some consumers because of

the smell of garlic, many consumers believe that it has a flavour compatible with yogurt.

There was no significant difference between the texture scores of the samples during the storage period ( $p > 0.05$ ). While G was the most popular sample at the beginning of storage, samples C and B were the most popular samples on the 14<sup>th</sup> day of storage. After the 14<sup>th</sup> day of storage, sensory evaluation could not be made due to bitterness and foreign taste in the samples. Garlic, basil, mint and rosemary did not negatively affect the sensory properties of yogurts, and mint in particular had a positive sensory response.

### CONCLUSION

In this study, the effect of the aqueous extracts of various medicinal and aromatic plants in the production of probiotic yogurt on the physicochemical, rheological, sensory properties and probiotic viability of yogurt was tried to be determined. The pH values of all samples including the control sample on days 1 and 14 of storage were similar. At the end of the storage period, samples containing basil and rosemary had the



**Figure 3. a.** Sensorial properties of yogurt samples on the 1<sup>st</sup> day of storage period; **b.** Sensorial properties of yogurt samples on the 14<sup>th</sup> day of storage period; **C:** Control sample (not containing medicinal and aromatic plant); **B:** Basil added yogurt; **T:** Thyme added yogurt; **M:** Mint added yogurt; **R:** Rosemary added yogurt; **G:** Garlic added yogurt.



lowest pH value ( $p < 0.05$ ). It was determined that all samples containing medicinal and aromatic plants maintained their probiotic viability above  $10^6$  log cfu/g during the 28-day storage period and using of these plant did not adversely affect the viability of starter culture and probiotic bacteria. While the yogurt sample containing garlic was the most preferred yogurt sample at the beginning of storage, it was determined that the favoured level of yogurt samples containing plants decreased as the storage period progressed. As a result of this study, it was revealed that aqueous extracts of various medicinal and aromatic plants can be used successfully in the production of probiotic yogurt without negatively affecting the rheological and microbiological properties of yogurts. However, it is clear that the sensory properties of the products should be improved considering that the sensory taste of the plant-added yogurts decreases as the storage period progresses. It is recommended to elaborate the studies by making different concentration trials.

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The design of study was done by Ozer Kinik. The analysis and interpretation of data were performed by Ecem Akan, Oktay Yerlikaya, and Ozge Yildiz Bayram. The manuscript was drafted by Oktay Yerlikaya. All authors read and approved the final manuscript.

