GIDA
THE JOURNAL OF FOOD
E-ISSN 1309-6273, ISSN 1300-3070

Araştırma / *Research*
GIDA (2020) 45(4) 814-824
doi: 10.15237/gida.GD20028

INVESTIGATION OF PRODUCTION AND CHARACTERISTICS OF HOMEMADE YOGURT BY USING A COMMERCIAL PROBIOTIC CULTURE

Özüm Özoğlu[1*], Rukiye Çolak Şaşmazer[1], Zerrin Erginkaya[2], Mihriban Korukluoğlu[1]
[1]Department of Food Engineering, Faculty of Agriculture, Bursa Uludağ University, Bursa, Turkey
[2]Department of Food Engineering, Faculty of Agriculture, Çukurova University, Adana, Turkey

Received /Geliş: 12.02.2020; *Accepted*/ Kabul: 29.06.2020; *Published online* /Online baskı: 11.08.2020

Özoğlu, Ö., Çolak Şaşmazer, R., Erginkaya, Z., Korukluoğlu, M. (2020). Surveying production and characteristics of homemade yogurt by using a commercial probiotic culture. GIDA (2020) 45(4) 814-824 doi: 10.15237/gida.GD20028

Özoğlu, Ö., Çolak Şaşmazer, R., Erginkaya, Z., Korukluoğlu, M. (2020). Ticari probiyotik kültür kullanılan ev yapımı yoğurt üretiminin ve özelliklerinin araştırılması. *GIDA* (2020) 45(4) 814-824 doi: 10.15237/gida.GD20028

ABSTRACT
In this study, probiotic homemade yogurt was made by using a commercial probiotic culture and its quality parameters were investigated on the 1st, 5th, 10th and 15th days of storage. For this purpose, probiotic and non-probiotic commercial yogurts were also investigated as control groups. According to the results obtained; level of total lactic acid bacteria, *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. l*actis* and pH values were not significantly changed in storage time for all samples (P >0.05). Any significant number of coliforms, mould or/and yeast were not observed in all samples (<10 CFU/g). Dry matter values were found similar among the milks used in yogurt makings, while fat and protein values showed difference. The homemade yogurt was not highly preferred by the panellists; however, it did not show any significant difference in the evaluation compared to the other yogurts (P >0.05).
Keywords: Homemade yogurt, probiotic, starter culture

TİCARİ PROBİYOTİK KÜLTÜR KULLANILAN EV YAPIMI YOĞURT ÜRETİMİNİN VE ÖZELLİKLERİNİN ARAŞTIRILMASI

ÖZ
Bu çalışmada ticari probiyotik kültür kullanılarak probiyotik ev yapımı yoğurt yapılmış ve kalite parametreleri depolamanın 1., 5., 10. ve 15. günlerinde araştırılmıştır. Bu amaçla probiyotik ve probiyotik olmayan ticari yoğurtlar da kontrol grubu olarak araştırılmıştır. Elde edilen sonuçlara göre; toplam laktik asit bakterisi, *Lactobacillus acidophilus* ve *Bifidobacterium animalis* ssp. *lactis* ve pH değerleri, tüm numuneler için saklama süresinde önemli ölçüde değişmemiştir (P >0.05). Tüm örneklerde önemli sayıda koliform, küf ve/veya maya gözlenmemiştir (<10 KOB/g). Yoğurt yapımında kullanılan sütler arasında kuru madde değerleri benzer bulunurken; yağ ve protein değerleri farklılık göstermiştir. Ev yapımı yoğurt, panellistler tarafından çok tercih edilen olmamıştır, ancak değerlendirmede diğer yoğurtlarla kıyaslandığında önemli ölçüde farklılık göstermemiştir (P >0.05).
Anahtar kelimeler: Ev yapımı yoğurt, probiyotik, starter kültür

[*] *Corresponding author* / Yazışmalardan sorumlu yazar:
✉ : ozoglu@uludag.edu.tr 🖷 (+90) 224 294 1402 ✆ (+90) 224 294 1509

Özüm Özoğlu ORCID ID: 0000-0003-3600-142X
Rukiye Çolak Şaşmazer ORCID ID: 0000-0002-1828-1205
Zerrin Erginkaya ORCID ID: 0000-0001-6208-2927
Mihriban Korukluoğlu ORCID ID: 0000-0003-3043-1904

INTRODUCTION

Yogurt is a traditional fermented dairy product that has been widely consumed for a long time (Herdem, 2006; Durak et al., 2008; Demirkaya and Ceylan, 2013; Çelik et al., 2016). Traditionally, yogurt is made by adding a part of the previous yogurt to milk (Durak et al., 2008). On the other hand, in commercial production of yogurt is made with milk fermentation by using two major lactic acid bacteria (LAB) symbiotic cultures which are *Lactobacillus bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* (*St. thermophilus*). Fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein to give texture and characteristic flavour of the yogurt (Aslim et al., 2006; Çayır and Şahan, 2007; Anonymous, 2009; Akpinar et al., 2011; Shima et al., 2012; Hakimi et al., 2013). Besides, yogurt is the most consumed dairy product due to the fact that it is suitable for lactose-intolerance people because of fermentation, desirable taste, wealthy nutrition contents and being more resistant to pathogen microorganisms (Herdem, 2006; Durak et al., 2008; Akpinar et al., 2011; Shima et al., 2012; Demirkaya and Ceylan, 2013; Çelik et al., 2016). Additionally, yogurt is a significant vehicle for probiotic microorganisms (Lourens-Hattingh and Viljoen, 2001; Shima et al., 2012; Batista et al., 2015; Shori, 2015). Probiotic microorganisms are living microorganisms containing *Bifidobacterium*, *Lactobacillus* and non-pathogenic yeasts which have beneficial effects on the host, especially the gastrointestinal system. Also, they should be found at least10^6 CFU/g-mL in the products for admitting of the products as probiotics (Isolauri et al., 2001; Reid et al., 2003; Çayır and Şahan, 2007; Quigley, 2010; Lee et al., 2013; Quin et al., 2018). People tend to consume probiotic products because of their benefits on health. Thus, there has been an important increase in the production and consumption of probiotic food products especially dairy probiotic products like probiotic yogurts (Reid, 2015; Shori, 2015).

Consumers prefer to make their yogurt at home instead of buying commercial ones due to the admirable taste of homemade yogurt and the common thought that homemade yogurt is healthier than commercial yogurts (Herdem, 2006; Kaya et al., 2016). As a consequence of eating habits and trends on probiotic foods and homemade yogurts, commercial probiotic cultures used for making homemade yogurts have been started to be produced. In view of these considerations, the purpose of this study was to make homemade probiotic yogurt by using one of these cultures at home conditions and to compare some microbiological, physicochemical and sensory properties at 5-day intervals from 1st day to 15th day with commercial probiotic and without probiotic content (non-probiotic) yogurts which were obtained by the producer company (Bursa, TURKEY) in order to determine characteristics of the homemade probiotic yogurt.

MATERIAL AND METHODS

Preparation of yogurt samples

Homemade probiotic yogurt was made under home conditions by using starter culture which was bought from a supermarket in Bursa-TURKEY based on the directions written on the package. Then, the milk (1 Lt) to be used for yogurt preparation were boiled for 10-15 minutes. Furthermore, to activate the culture contents in the sachet, it was emptied into one glass of the warm milk, mixed well and then left for activation for 15-20 min. Then, the activated starter culture was added to the milk cooled down to 45°C and mixed well. Following this, the milk was left for incubation at room temperature for 7-8 hours. Then, the prepared yogurt was stored at 4°C. Regarding to label information of the commercial starter culture; it was contained *St. thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (*L. bulgaricus*), *Lactobacillus acidophilus* (*L. acidophilus*) and *Bifidobacterium animalis* ssp. *lactis* (*B. animalis* ssp. l*actis*) and lactose.

Commercial probiotic and non-supplement probiotic yogurts were supplied from production lines of a company in Bursa, TURKEY. According to informing of the company, the yogurt samples were prepared based on the following instructions. After pasteurization of milk at 90°C for 7-8 minutes, the cultures (in the ratio of 1.5-2 %) were added to milk cooled down up to 42°C and mixed well. After that, they were left for incubation for 4 hours. Then, the prepared

yogurts were stored at 4°C. Besides, *B. animalis* subsp. *lactis*, *L. acidophilus*, *L. bulgaricus*, *St. thermophilus* culture composition was used as probiotic microorganisms on the commercial probiotic yogurt sample.

All yogurt samples were prepared by using commercial homogenized and UHT (Ultra High Temperature) milks in the same amount (1 Lt). Although all milk samples belonged to the same company, they had different lot numbers due to the production process of the company. In addition, all yogurt samples were stored at 4˚C after production during the 15-day period.

Microbiological analyses

Microbiological analyses were performed by using some reference methods for milk products which were detailed below. Inoculations were performed by Pour Plate Technique for all protocols in proper incubation conditions for each of them. Besides, all analyses were done in triplicate. Furthermore, all samples were checked every 5 days during the 15-day period (ISO 6611:2004; ISO 20128:2006; ISO 4832:2006; Moreno et al., 2006; ISO 29981:2010).

Number of *St. thermophilus* and *L. bulgaricus* were calculated and gathered for determination of total LAB. Inoculation was done from the 3rd, 4th and 5th dilutions of five-fold serial dilutions at a sterile UV cabinet. In line with this purpose, M17 Agar (Oxoid) and MRS (De Man, Rogosa, Sharpe) Agar (Oxoid) were used as media for *St. thermophilus* and *L. bulgaricus*, respectively. Meanwhile Petri Dishes containing M17 Agar were incubated at 37°C for 48 hours and opaque colonies in diameter of 3-4mm were calculated. Similarly, Petri Dishes containing MRS Agar were incubated at 37˚C for 72 hours and opaque colonies were calculated (Moreno et al., 2006).

With the addition of 5 ml of CyHCl (Merck 2839) stock solution and 2.5 ml Mupirocin (LGC promochem, art no. EPM3806000) stock solution per litter of the medium, MRS (Difco 288210) Agar was prepared as a medium for enumeration of *Bifidobacterium animalis* ssp. *lactis*. Inoculation was performed from 3rd, 4th and 5th dilutions of five-fold serial dilutions. Then, incubation was ensured at 37˚C for 72 hours under anaerobic conditions. At the end, all colonies were counted as *B. animalis* ssp. *lactis* (ISO 29981:2010).

With addition of 0.5 ml of clindamycine (Sigma C5269) stock solution per litter of the medium, MRS (Difco 288210) Agar was prepared as a medium for enumeration of *Lactobacillus acidophilus*. Inoculation was done from 3rd, 4th and 5th dilutions of five-fold serial dilutions. Moreover, anaerobic incubation was performed at 37˚C for 72 hours. Finally, all colonies are counted as *L. acidophilus* (ISO 20128:2006).

To determine the number of coliforms, samples were diluted in ways that been five-fold serial dilutions and inoculation was done from all dilutions to Petri Dishes containing VRBL (Violet Red Bile Lactose) Agar (Oxoid). Then, incubation was performed at 30˚C for 24 hours. After the incubation process, dark red coloured colonies, which were minimum 0.5 mm in diameter, were counted (ISO 4832:2006).

For the enumeration of yeasts and/or moulds, samples were inoculated to Malt Extract Agar (Oxoid) and aerobically incubated at 25˚C for 5 days. Then, colonies were counted. Also, samples were renewed in each analysis day (ISO 6611:2004).

Physico-chemical analyses

Determination of dry matter, fat and protein in the milk sample used to make the homemade probiotic yogurt were done by some reference methods which were the same as the methods applied on milks using for commercial yogurts. Determination of dry matter on milks was done according to AOAC 1990 Methods by using a drying oven (Nüve KD 200) (Helrich, 1990). Fat and protein in milk were determined regarding to Gerber Method and Kjeldahl Method (Foss Kjeltec 8000), respectively (Helrich, 1990; Kleyn et al., 2001). pH values were measured in triplicate directly using hand type pH meter with a standard pH probe (Mettler Toledo- SG2-FK).

Sensory analysis
Sensory analysis of the yogurt samples were done with 9 trained panellists by using the 9-point hedonic scale (Lawless and Heymann, 2003). The panellists scored the yogurt samples in terms of flavour, odour, colour and consistency properties.

Statistical analysis
Data that collected in the context of the study were statistically analysed by IBM SPSS Software (Version 2.1.) and Minitab ANOVA. Results were double checked with these programmes. Additionally, the statistically significant differences between the values were determined by Tukey's test ($P < 0.05$).

RESULTS AND DISCUSSION

Microbiological evaluations
Number of total LAB, *B. animalis* ssp. l*actis* and *L. acidophilus* in the yogurt samples, which were stored at 4°C, on the 1st, 5th, 10th and 15th days were shown in Figures 1, 2 and 3, respectively.

As seen in the figures, level of the LAB in the homemade probiotic, commercial probiotic and commercial non-probiotic yogurt samples were found roughly 9, 8 and 7 log CFU/g, respectively. Similar results were obtained by the studies of Moreno et al. (2006) and Sarıca et al. (2019). Similar to other fermented samples in the study of Moreno et al. (2006), number of LAB was observed higher than 7 log CFU/g in probiotic and traditional yogurt samples in this study. In the study of Sarıca et al. (2019), numbers of LAB (*Lactococcus* and *Lactobacillus*) were found approximately 9 and 8 log CFU/g in homemade yogurts made by buffalo and cow milks during 28-day storage (Sarıca et al., 2019). Then, the level of *B. animalis* ssp. l*actis* in the homemade probiotic and commercial probiotic yogurt samples were almost 8 and 6, respectively. Besides, the level of *L. acidophilus* in the samples were roughly 7 log CFU/g. Also, enumeration of bifidobacteria and *L. acidophilus* was not needed for commercial non-probiotic yogurt due to the fact that no supplemented probiotic microorganisms, when the yogurt was produced. Additionally, in terms of bacterial counts there was not any significant difference ($P > 0.05$) among the storage periods of the samples.

According to Figure 2, the level of *B. animalis* ssp. l*actis* in the homemade probiotic and commercial probiotic yogurt samples changed from 8.21 to 8.04 and from 6.52 to 6.29 between 1st and 15th storage days respectively. Also, as seen in Figure 3, the level of *L. acidophilus* in the homemade probiotic and commercial probiotic yogurt samples decreased from 7.36 to 7.17 and from7.79 to 7.62 between the 1st and 15th storage days respectively. Furthermore, a food matrix should contain a minimum of 10^6 CFU/g-mL (≥6 log CFU/g) viable probiotic microorganism in order for it to be accepted as a probiotic food (Yerlikaya, 2014; Mohammadi et al., 2017). Thus, in the study it was observed that amounts of probiotic cultures protected their 6, 7 and 8 log CFU/g level during the 15-day investigation period. Therefore, the probiotic yogurt products in this studycertainly showed probiotic characteristics. In the study of Cruz et al. (2012), probiotic yogurts that containing different ratios of glucose oxidase (0 to 1000 mg/kg) were investigated during 30 days with 15-day intervals. Levels of *St. thermophilus*, *L. bulgaricus*, *B. longum*, and *L. acidophilus* which changed from 9 to 6 log CFU/g were saved during storage time (Cruz et al., 2012). Their results were similar to the current study.

In the current study, number of coliforms, mould and yeast were found below 1 log CFU/g (<10 CFU/g) on the 1st, 5th, 10th and 15th days in all yogurt samples. Even though homemade probiotic yogurt was prepared under home conditions, making point of working sterile and using UHT milk should affect that not to seen any coliforms and mould or/and yeast growth as well as the commercial yogurts. Besides, probiotic microorganisms and some LAB have inhibition effects on pathogen microorganisms including coliforms, mould and yeast (Dias et al., 2013; Simone et al., 2014; Tripathi and Giri, 2014; Tatsadjieu et al., 2016; Abhisingha et al., 2018).

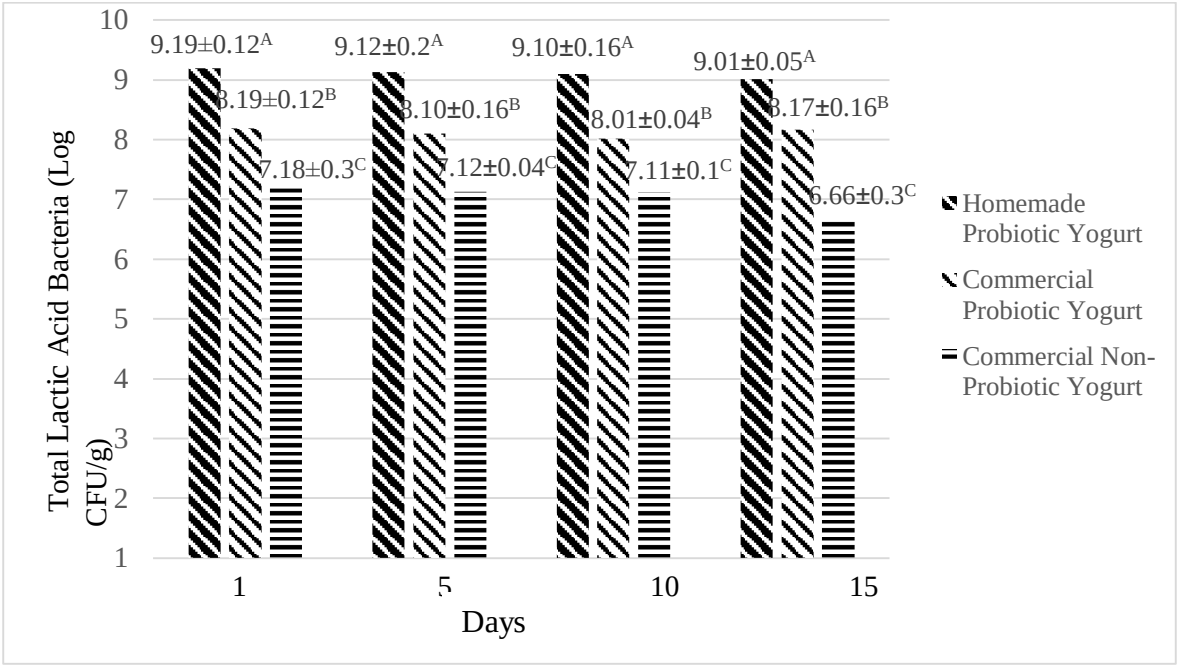

Figure 1. The number of total LAB in homemade probiotic, commercial probiotic and non-probiotic yogurts. (Values followed by the same capital letters in bars are not significantly different (P <0.05).)

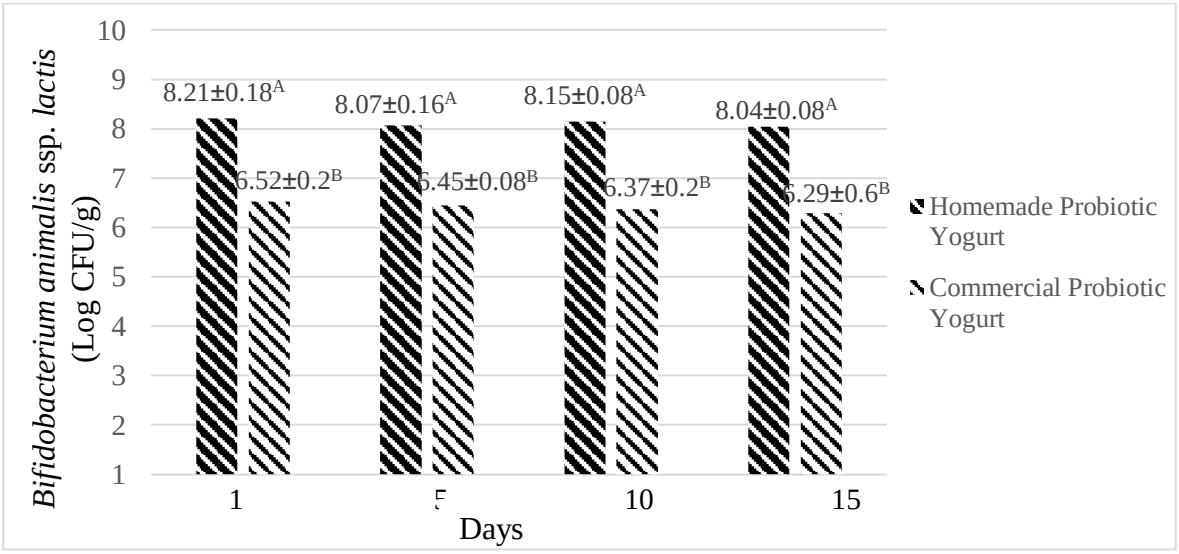

Figure 2. The number of *B. animalis* ssp. l*actis* in homemade probiotic and commercial probiotic yogurts. (Values followed by the same capital letters in bars are not significantly different (P <0.05).)

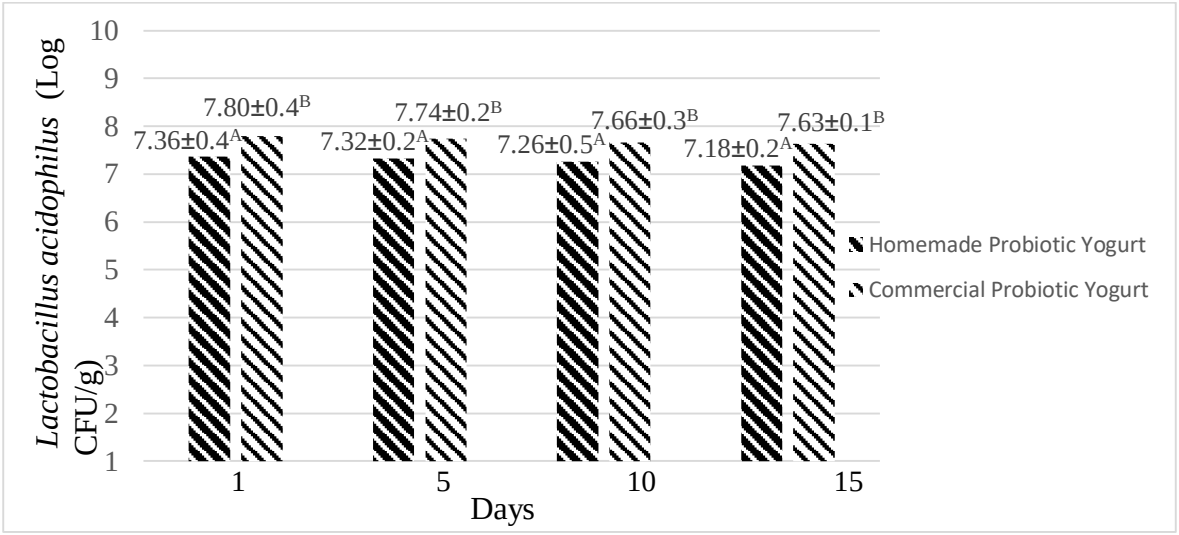

Figure 3. The number of *L. acidophilus* in homemade probiotic and commercial probiotic yogurts. (Values followed by the same capital letters in bars are not significantly different (P <0.05).)

Similar to this study, in the study of Dias et al. (2013) done with symbiotic fermented drink; growth of any coliforms and *Salmonella* spp. was not observed as a result of sterile working and inhibition effect of symbiotic.

Physico-chemical changes

Amounts of dry matter, protein and fat in milk samples used for producing probiotic homemade, commercial non-probiotic and commercial probiotic yogurts were demonstrated at Table 1 in percentages. The values for the commercial yogurts were taken from the producing company.

As seen in Table 1, dry matter contents of all milks were similar. Also, protein and fat contents of milks used for homemade probiotic yogurt and commercial non-probiotic yogurt were close to each other. On the other hand, these values were higher than that of the milk used for the commercial probiotic yogurt. However, the differences among the milks did not affect the pH values and sensory properties of the yogurt samples (as seen in Table 2 and Table 3 respectively). In addition, contents of dry matter, fat and protein of the milk samples were similar to the milks used for producing yogurts in the studies of Çayır and Şahan (2007) and Sarıca et al. (2019).

pH values of all three yogurt samples were measured in triplicate at 5-day intervals between the 1st day and 15th day. Changes on the values were indicated with mean values and standard deviations at Table 2.

It is obviously seen in Table 2 that, any significant decrease in pH values ofall yogurt samples was not observed during the 15-day study period. The result was similar to the study of Mohammadi et al. (2017) focusing on probiotic fermented composite drink; in that study, major decreases were not observed on pH values of the fermented probiotic drink samples (Mohammadi et al., 2017). Besides, there was not any statistical difference between pH properties of yogurt samples. Additionally, pH values of the yogurts slightly decreased during storage due to the higher amount of LAB that were produced acids (Cruz et al., 2012; Dias et al., 2013; Turgut and Cakmakci, 2018). In terms of the 1st day, pH value results were similar to results of the study of Turgut and Cakmakci (2018); however, compared to the current study, much more decrease were observed in pH values (from 4.415 to 4.180) on the other 14-day period in that study. The reason of that, probiotic strawberry yogurt was investigated in the mentioned study. That is, fruits could have an effect on the dropping of pH values because of the acid content of them (Kandylis et al., 2016).

Table 1. Amounts of dry matter, protein and fat in milks used making the yogurt samples.

Samples	Dry Matter (%)	Protein (%)	Fat (%)
Homemade Probiotic Yogurt	11.25	2.95	3.05
Commercial Probiotic Yogurt	11.15	4.00	3.70
Commercial Non-Probiotic Yogurt	11.45	3.01	3.01

Table 2. pH values of the yogurt samples at the 1st, 5th, 10th and 15th days.

Sample/ Days	Homemade Probiotic Yogurt	Commercial Probiotic Yogurt	Commercial Non-Probiotic Yogurt
1	$4.45\pm0.005^{A,a}$	$4.48\pm0.005^{A,a}$	$4.48\pm0.009^{A,a}$
5	$4.36\pm0.005^{B,a}$	$4.36\pm0.005^{B,a}$	$4.41\pm0.017^{B,a}$
10	$4.34\pm0.008^{B,a}$	$4.34\pm0.008^{B,a}$	$4.33\pm0.012^{C,a}$
15	$4.30\pm0.005^{C,a}$	$4.30\pm0.012^{C,a}$	$4.32\pm0.005^{C,a}$

* Values followed by the same capital letters in columns are not significantly different ($P < 0.05$).
*Values followed by the same small letters in rows are not significantly different ($P < 0.05$).

Evaluation of sensory analysis

The yogurt samples were evaluated by 9 trained panellists in terms of four different sensory characteristics (flavour, odour, colour and consistency). The results related to the panellists' evaluation scores which were ranked from 1 to 9 (1: extremely dislike and 9: extremely like) were given in Table 3.

Table 3. Sensory analysis results of the yogurt samples based on the evaluation scores of the 9 panellists (mean and standard deviation).

Samples	Days	Flavour	Odour	Colour	Consistency
Homemade Probiotic Yogurt	1	7.00 ± 1.25^{AB}	7.56 ± 1.06^{AB}	7.56 ± 1.16^{A}	6.44 ± 1.71^{B}
	5	7.56 ± 0.68^{AB}	7.89 ± 0.73^{AB}	7.78 ± 0.79^{A}	7.22 ± 1.31^{B}
	10	7.44 ± 0.68^{AB}	7.78 ± 0.63^{AB}	7.78 ± 0.63^{A}	7.00 ± 1.15^{B}
	15	6.80 ± 0.98^{AB}	7.00 ± 1.55^{AB}	7.60 ± 0.49^{A}	7.20 ± 1.83^{B}
Commercial Probiotic Yogurt	1	7.30 ± 1.10^{AB}	7.85 ± 0.98^{AB}	7.72 ± 1.07^{A}	7.30 ± 1.06^{B}
	5	7.82 ± 0.76^{AB}	7.80 ± 0.77^{AB}	7.80 ± 0.83^{A}	7.15 ± 1.21^{B}
	10	7.73 ± 0.89^{AB}	7.69 ± 1.38^{AB}	7.80 ± 0.81^{A}	7.43 ± 1.29^{B}
	15	6.80 ± 1.23^{AB}	6.80 ± 1.15^{AB}	7.53 ± 0.80^{A}	7.22 ± 1.43^{B}
Commercial Non-Probiotic Yogurt	1	8.11 ± 0.87^{AB}	8.00 ± 0.82^{AB}	8.11 ± 0.88^{A}	7.78 ± 1.03^{B}
	5	8.22 ± 1.23^{AB}	8.00 ± 0.82^{AB}	8.11 ± 0.88^{A}	8.11 ± 1.10^{B}
	10	7.22 ± 1.40^{AB}	7.67 ± 1.05^{AB}	8.11 ± 0.88^{A}	7.56 ± 1.34^{B}
	15	6.80 ± 1.47^{AB}	8.33 ± 0.47^{AB}	7.40 ± 0.80^{A}	6.80 ± 0.98^{B}

* Values followed by the same capital letters in columns are not significantly different ($P < 0.05$).

According to the results evaluating general admiration for the 15-day period, while commercial non-probiotic yogurt was the yogurt most liked by the panellists, homemade probiotic yogurt was the least desired. Also, when the evaluation results of 9 panellists were compared statistically with each other for three replicates; it was observed that variations were not significant among the panellists' surveys ($P > 0.05$) although the properties were changed day by day ($P < 0.05$).

Nevertheless, when the sensory characteristics of the yogurt samples were tested by untrained panellists (the results were not given), it was seen that homemade commercial probiotic yogurt was highly preferred.

According to the table, the overall acceptability of the yogurt samples was higher than 7.0 (moderately like) which means all of the samples have a good overall acceptability. Also, it could be deduced that to guarantee consumers' admiration, a 10-day storage period was better for the yogurt samples.

When the results were compared with the study of Akalın et al. (2012) investigating probiotic yogurts fortified with sodium calcium caseinate or whey protein concentrate, it was seen that alterations of the scores were roughly the same as the current study. Furthermore, sensory characteristics of the sample were evaluated by every two weeks in a 28-day period and as in the current study, scores of admiration were observed to decrease slowly by time as roughly from 5 to 4 on the basis of 5 like the recent study. Moreover, the yogurts were generally found desirable as in the current study. Besides, according to the study of Kailasapathy (2006) conducted with probiotic yogurts that were included free and encapsulated probiotics, the panellists found the yogurt samples moderately desirable. The reason for this was that consuming probiotic products was not as common as known and people could not like the taste of these yogurts because they did not familiar with the taste of them. Moreover, it might also have been related to using different probiotic culture (*L. acidophilus* and *B. lactis*) instead of common substrain (*B. animalis* ssp. l*actis* and *L. acidophilus*).

CONCLUSION

The results of this study indicated that the homemade probiotic yogurt sample made by using commercial probiotic culture did not show any significant variation ($P > 0.05$) in terms of microbiological, physicochemical and sensory properties compared to the commercial yogurt samples (probiotic and non-probiotic). The level of probiotic microorganisms was appropriate for the product to be accepted as probiotic and was stable during storage. Besides, any growth of mould, yeast and coliforms was not observed. Also, the yogurt maintained its pH and sensory characteristics during 15-day storage however, it was concluded that for a better acceptable taste, the yogurt should be consumed within 10 days. Recently, people tend to consume either homemade or probiotic products due to health benefits of probiotics and popular thought that homemade yogurt was healthier than commercial ones. Therefore, consuming commercial products are safety by taking into consideration however, homemade probiotic yogurts are prepared with commercial probiotic cultures can be a good alternative to consume probiotic products.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTION

ÖÖ and RÇŞ performed all of the analyses. ÖÖ wrote the paper. RÇŞ provided the milk and yogurt samples. MK designed the research. MK and ZE evaluated the results. All authors have read and agreed to the published version of the paper.

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