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Effect of banana and ABT-2 probiotic culture use on quality parameters and microstructure of buffalo yoghurt

Auswirkung der Verwendung von Bananen und der probiotischen Kultur ABT-2 auf Qualitätsparameter und Mikrostruktur von Büffeljoghurt

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Summary

Yoghurt, whose positive health benefits have been proven and whose consumption rate is increasing day by day, is one of the most popular dairy products consumed by consumers. Although cow's milk is often preferred in yoghurt production, buffalo milk, whose consumption as drinking milk is quite limited, is very suitable for yoghurt production due to its unique composition and textural properties. However, in order to relatively suppress the unique organoleptic properties of buffalo milk and present it to the consumer as a new product, the addition of fruit is a suitable method. Among the fruits to be added to yoghurt, banana stands out especially with its prebiotic content and desirable sensory properties. In this context, in the present study, the changes in some quality characteristics and microstructures of experimental yoghurts, produced with ABT-2 and YC-350 starter cultures and five different banana-sugar combinations, were researched during storage period. According to the results, it was seen that the use of different starter cultures had a very significant effect ($p<0.01$) on viscosity, syneresis, titratable acidity, pH, and all sensory parameters, and had a significant effect ($p<0.05$) on ash content. It was determined that the fruit ratio had a very significant effect ($p<0.01$) on all physicochemical, microbiological, and sensory analyses. It was observed that sugar ratio had a very significant effect ($p<0.01$) on total solids, fat, solids nonfat, protein, viscosity, syneresis, pH, and all sensory parameters, while it had a significant effect ($p<0.05$) on the counts of *S. thermophilus, L. delbrueckii* subsp. *bulgaricus* and *B. animalis* subsp. *lactis* BB-12. It was observed that the storage period had a very significant effect ($p<0.01$) on all analyses except solids nonfat ($p<0.05$). In the SEM investigations, different banana-sugar ratios, storage times, and starter cultures caused the formation of different levels of microstructures in experimental yoghurts. As a result of the study, it was observed that the addition of banana and sugar caused larger gap diameter and number due to increased syneresis. In this context, it was revealed that the addition of banana and sugar to yoghurts produced from buffalo milk supported the nutritional value and probiotics, while causing partial deterioration in microstructure. It was observed that the storage period had negative effects on nutritional value and probiotic microorganisms.

Keywords: buffalo yoghurt, banana, yoghurt culture, probiotic culture, microstructure

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Introduction

Buffalo milk, which comes after cow's milk in terms of its production worldwide, stands out as an important alternative in yoghurt production due to its higher total solids and fat contents (Akgun et al., 2016; Ehsani et al., 2016; Gupta et al., 2022). Compared to cow's milk, the higher sensory properties, total solids, and fat content, as well as higher protein, lactose, vitamin, and mineral substances increase the demand for buffalo milk and its products. These differences in it's composition affect the textural and microbiological properties of the final product, especially the yoghurt production process steps (Nguyen et al., 2014; Ehsani et al., 2016). In addition, buffalo yoghurt, which is a food with a higher nutritional value compared to yoghurts obtained from other milk, is a more viscous and aromatic product and is digested more easily in the body (Ertaş et al., 2014).

It is recommended to use probiotics, prebiotics, or their combinations to improve the functional properties of yoghurts (nutritional value, sensory profile, physicochemical and rheological properties) and enable them to be processed in an innovative way (Fazilaha et al., 2018). Today, in addition to the classical yoghurt culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) in yoghurt production, probiotic cultures, which are defined as live microbial food supplements, can be preferred due to their positive effects on the immune system, lactose digestion, intestinal infections, and cholesterol control in the host organism, when taken into the body (Kavaz Yüksel and Bakırcı, 2014; Nguyen et al., 2014). In addition, *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus acidophilus* LA-5 which belongs to probiotic cultures, are used together with classical yoghurt cultures in yoghurt production due to their slow growth during milk fermentation (Dave and Shah, 1996).

The use of buffalo milk as a raw material in the production of various dairy products, especially yoghurt, is an important alternative for both the consumer and the dairy industry, due to the rich nutritional content and textural properties it will provide to the product. On the other hand, in the literature, it has been seen that there are not enough studies on yoghurt production using buffalo milk and probiotic culture. On the other hand, for the growth and long-term survival of probiotic microorganisms, it is known that the prebiotics obtained from fruits and the appropriate buffering capacity and pH value sourced the nutritional content of milk have a supporting effect (Terzioğlu et al., 2023).

In the present study, buffalo yoghurts produced using classic yoghurt culture and buffalo yoghurts produced using probiotic culture were compared in detail in terms of both nutritional value and textural and sensory properties during the storage period. In addition, the effect of different ratios of banana and sugar addition in yoghurt samples on the aforementioned properties was examined. As a result, it was aimed to produce a superior product in terms of nutritional value and to offer a new product to the consumer with the production of probiotic fruit yoghurts, which are characterized as functional foods due to their numerous effects on health. For this purpose, physicochemical, microbiological and sensory analyses as well as microstructure analyses were performed on yoghurt samples during a 14-day storage period.

Materials and methods

Raw milk and starter cultures

Raw buffalo milk used in the experimental yoghurt production was obtained approximately 100 L from 10 different animals from 4 different local producers through Atatürk University Food and Livestock Application and Research Center (Project no: FBA-2019-7335). The buffalo milk was stored at –18ºC until yoghurt production.

In buffalo milk processed into yoghurt, the total solids (by gravimetric method), fat (by Gerber method), solids nonfat, protein (by the Kjeldahl method), ash (by the gravimetric method), specific gravity (by lactodensimeter), titratable acidity (in % lactic acid), and pH (by digital pH-meter with the combined electrode) analyses were realized according to the methods specified by Kurt et al. (2015). Total solids, solids nonfat, fat, ash, protein, density, titratable acidity and pH values of buffalo milk were determined as 17.34%, 10.55%, 6.79%, 0.80%, 6.60%, 1.034 g/mL, 0.23% lactic acid, and 6.85, respectively. Classic yoghurt culture (YC-350 *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*) and ABT-2 probiotic starter culture (*S. thermophilus, B. animalis* subsp. *lactis* BB-12, and *L. acidophilus* LA-5) were obtained from CHR Hansen (Süt-Sa, Adapazarı/Türkiye).

Preparation of banana puree

Fresh and ripe fruits (bananas) were peeled and pureed and then divided into 8 different groups according to the ratios to be added to yoghurt. Then, 5% and 7.5% sugar was added to the grouped banana purees. Each group was then heat treated at 90±1ºC for 5–10 min and kept in glass jars at room temperature until added to the experimental yoghurt samples.

Production of experimental yoghurts

Production of experimental yoghurt samples was realized according to the method given by Yıldız and Bakırcı (2019) and Terzioğlu et al. (2022). After being taken into the pasteurization tank, the buffalo milk, which was applied to heat treatment at 90°C for 10 minutes, was divided into 2 separate groups and ABT-2 probiotic culture at the level of 30 g/100 L (w/v) was added to one group on the basis of Direct Vat Inoculation (DVI) at 37–38ºC, and the classical yoghurt culture at the level of 20 g/100 L (w/v) was added to the other group according to the DVI basis at 43–45ºC and they were left to incubate separately. At the end of incubation (pH 4.6±0.1), different banana-sugar combinations prepared earlier were added to the yoghurt samples kept at +4ºC for 24 hours, placed in jars of 200 mL and subjected to analysis (1st, 7th and 14th days). Yoghurt combinations were named and grouped as A1: classic yoghurt culture, without banana and sugar; B1: classic yoghurt culture+%5 banana+%5 sugar; C1: classic yoghurt culture+%5 banana+%7.5 sugar; D1: classic yoghurt culture+%10 banana+%5 sugar; E1: classic yoghurt culture+%10 banana+%7.5 sugar; A2: probiotic culture, without banana and sugar; B2: probiotic culture+%5 banana+%5 sugar; C1: probiotic culture+%5 banana+%7.5 sugar; D1: probiotic culture+%10 banana+%5 sugar and E1: probiotic culture+%10 banana+%7.5 sugar. In the study, yoghurt production and analyses were applied in 2 parallel and 2 replicates.

Physicochemical analyses of the experimental yoghurts

The physicochemical analyses (total solids, solids nonfat, fat, ash, protein, viscosity, syneresis, titratable acidity and pH) of the experimental yoghurt samples produced by using different starter culture and fruit-sugar combinations were carried out by using the methods put forward by Kavaz (2012) and Kurt et al. (2015).

Microbiological analyses of the experimental yoghurts

The microbiological analyses were carried out by the references to Dave and Shah (1997) and Kavaz (2012) in the experimental yoghurt samples. For the preparation of the samples, 10 g yoghurt sample was homogenized with 90 mL sterile physiological saline solution (0.85% NaCl). Different dilutions were prepared by taking 1 mL of each of the dilutions obtained and inoculations were performed. The details of the microbiological analyses performed in the current research are given in Table 1.

Microstructure of the experimental yoghurts

The microstructure analysis of the buffalo yoghurt samples produced by using different starter cultures and fruit-sugar combinations was determined by using scanning electron microscopy (SEM; Quanta-Feg 250 FEI) on the 1st and 14th days of the storage by modifying the method introduced by Akalın et al. (2012). A magnification degree of 2000x was used to obtain SEM images of the samples.

Sensory analyses of the experimental yoghurts

The sensory analyses of the control group and buffalo yoghurt with banana were carried out on the 1st, 7th, and 14th days of the storage by a panelist group of 8, consisting of the academic staff of the Department of Food Engineering, by modifying the criteria given by Anonymous (2006) for fruit probiotic yoghurt.

Statistical analysis

The research was conducted according to the Randomized Complete Block Designs in factorial arrangement and 2 parallel and 2 replicates. Duncan's Multiple Range Test was used with SPSS 20 package program on a computer and the data were interpreted in tables. PCA (Principal Component Analysis) SIMCA-P + 14.1 (UMETRICS, Umea, Sweden) was used to differentiate the samples and analyses.

Results and discussion

Physicochemical analyses results of the buffalo yoghurts

The physicochemical analyses results of the control group and buffalo yoghurt with banana are given in Table 2.

In the study, it was observed that the use of different starter cultures in the production of buffalo yoghurt had a statistically very significant effect ($p<0.01$) on viscosity, syneresis, titratable acidity, and pH values, and a significant effect ($p<0.05$) on ash content. On the other hand, it was detected that the use of different starter cultures had no significant effect ($p>0.05$) on total solids, fat, solids nonfat, and protein content. It was observed that the fruit ratio had a very significant effect ($p<0.01$) on all physicochemical analyses. While it was seen that the sugar content had no significant effect ($p>0.05$) on ash and titratable acidity, it was found that it had a very significant effect ($p<0.01$) on other physicochemical analyses. It was determined that the storage period had a significant effect ($p<0.05$) on the solids nonfat and a very significant effect ($p<0.01$) on all other physicochemical analyses.

TABLE 1: *The details of the microbiological analyses.*

Microorganisms	Agar	Incubation duration and temperature
S. thermophilus	M17 Agar (Oxoid Ltd.)	at 42°C for 24 h
L. delbrueckii subsp. *bulgaricus*	MRS Agar (Oxoid Ltd.)	at 42°C for 48 h
B. animalis subsp. *lactis* BB-12	MRS Agar (Oxoid Ltd.) + 0.5 g/L cysteine (L-cysteine, Sigma-Aldrich)	at 37°C for 48 h
L. acidophilus LA-5	MRS Agar (Oxoid Ltd.) + 1.5 g/L bile (Bile salts, Sigma-Aldrich)	at 37°C for 48 h
L. acidophilus LA-5+*B. animalis* subsp. *lactis* BB-12	MRS Agar (Oxoid Ltd.)	at 37°C for 48 h

Depending on the addition of fruit and sugar, an increase in total solids and solids nonfat content was determined, as expected. On the other hand, a proportional decrease was detected in protein and fat content compared to total solids content. As a matter of fact, the highest total solids and solids nonfat contents were observed in E1 and E2 samples, while the highest fat and protein contents were found in A1 and A2 samples. The total solids content (Mukisa and Birungi, 2018; Abdel-Hamid et al., 2020), fat content (Abdel-Hamid et al., 2020; Barakat et al., 2021), solids nonfat content (Bakırcı and Kavaz, 2008; Petridis et al., 2014), and protein content (Hameed et al., 2020) detected in the experimental yoghurt samples are in accordance with the previous studies. Compared to the control group yoghurt samples (A1 and A2), there was an increase in the ash content of the yoghurt samples with banana. Mukisa and Birungi (2018) emphasized that the ash content of yoghurt samples increased due to the high ash content of bananas.

An increase in viscosity and syneresis values was determined due to the increase in the fruit-sugar ratio and the progression of the storage period. On the other hand, the use of ABT-2 probiotic culture caused a decrease in the viscosity value and an increase in the syneresis value. As a matter of fact, the highest viscosity value was seen in the E1 sample, which was produced by using yoghurt culture and had the highest fruit-sugar ratio, while the highest syneresis value was observed in the E2 sample, which was produced by using ABT-2 probiotic culture and had the highest fruit-sugar ratio. It is thought that this increase in the viscosity and syneresis values is due to the fibrous structure of the fruit.

While exopolysaccharides formed as a result of the metabolic activity of lactic acid bacteria and milk components are reported to have an effect on viscosity by forming gels (Korcz and Varga, 2021; Parvarei et al., 2021), it has also been shown that the bonds formed during protein aggregation, protein ratio, fat ratio and curd tension affect the viscosity of yoghurt (Farnsworth et al., 2006; Erkaya, 2009). The increase in viscosity values during the storage period is explained by protein aggregation and fat agglutination, as well as the thixotropic flow and non-Newtonian gel properties of the yoghurt samples (Kavaz, 2012; Al Mijan et al., 2014). Pasteurization process, homogenization process, total solids, acidity, starter culture type, protein denaturation and proteolytic activity of starter cultures are effective on syneresis (Lucey and Singh, 1998; Lı and Guo, 2006).

In the control and buffalo yoghurt with banana, it was revealed that the use of the yoghurt culture, the increase of the fruit ratio, the increase of the sugar ratio, and the progression of the storage time caused an increase in the titratable acidity value, but the pH value naturally decreased. It is thought that the low acid production ability of the probio-

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TABLE 2: *Physicochemical analyses of the experimental yoghurts.*

	Total Solids (%)	Fat (%)	Solids Nonfat (%)	Protein (%)	Ash (%)	Viscosity (cP)	Syneresis (mL/25 g)	Titratable Acidity (% LA)	pH
Culture									
Yoghurt Culture	23.14±1.89^{a}	5.94±0.50^{a}	17.20±2.38^{a}	4.54±0.36^{a}	0.87±0.05^{b}	12915.50±2375.64^{a}	1.10±0.49^{b}	1.10±0.14^{a}	4.24±0.13^{b}
Probiotic Culture	23.12±1.91^{a}	5.93±0.48^{a}	17.19±2.39^{a}	4.54±0.34^{a}	0.88±0.06^{a}	10654.57±2307.37^{b}	1.81±0.64^{a}	0.97±0.11^{b}	4.37±0.07^{a}
Sign.	ns	ns	ns	ns	*	**	**	**	**
Fruit Ratio (%)									
0	19.55±0.06^{c}	6.84±0.03^{a}	12.71±0.04^{c}	5.05±0.04^{a}	0.79±0.02^{c}	8885.25±994.65^{c}	0.93±0.33^{c}	0.95±0.11^{c}	4.39±0.10^{a}
5	23.43±0.11^{b}	5.87±0.07^{b}	17.55±0.11^{b}	4.66±0.08^{b}	0.86±0.02^{b}	11590.83±2065.93^{b}	1.36±0.55^{b}	1.03±0.14^{b}	4.31±0.11^{b}
10	24.62±0.13^{a}	5.54±0.08^{c}	19.08±0.16^{a}	4.17±0.08^{c}	0.93±0.02^{a}	13429.13±2274.68^{a}	1.81±0.71^{a}	1.08±0.15^{a}	4.26±0.12^{c}
Sign.	**	**	**	**	**	**	**	**	**
Sugar Ratio (%)									
0	19.55±0.06^{c}	6.84±0.03^{a}	12.71±0.04^{c}	5.05±0.04^{a}	0.79±0.02^{a}	8885.25±994.65^{c}	0.93±0.33^{c}	0.95±0.11^{a}	4.39±0.10^{a}
5	23.95±0.60^{b}	5.76±0.16^{b}	18.19±0.75^{b}	4.47±0.26^{b}	0.89±0.05^{a}	12227.96±2298.12^{b}	1.51±0.64^{b}	1.05±0.15^{a}	4.29±0.12^{b}
7.5	24.10±0.63^{a}	5.66±0.19^{c}	18.44±0.81^{a}	4.36±0.26^{c}	0.89±0.04^{a}	12792.00±2399.59^{a}	1.67±0.70^{a}	1.06±0.14^{a}	4.28±0.11^{c}
Sign.	**	**	**	**	ns	**	**	ns	**
Storage Period (days)									
1	23.17±1.93^{a}	5.96±0.49^{a}	17.21±2.41^{a}	4.56±0.34^{a}	0.88±0.06^{a}	9925.65±1874.15^{c}	0.94±0.47^{c}	0.91±0.06^{c}	4.40±0.06^{a}
7	23.14±1.92^{a}	5.94±0.49^{a}	17.20±2.41^{a}	4.55±0.35^{a}	0.88±0.06^{a}	12028.40±2294.09^{b}	1.47±0.57^{b}	1.01±0.11^{b}	4.32±0.07^{b}
14	23.07±1.90^{b}	5.91±0.50^{b}	17.17±2.40^{b}	4.51±0.37^{b}	0.86±0.06^{b}	13401.05±2355.79^{a}	1.95±0.56^{a}	1.18±0.11^{a}	4.19±0.11^{c}
Sign.	**	**	*	**	**	**	**	**	**

a–c: Different letters indicate significant differences in column. Sign.: significant; *: p<0.05, **: p<0.01, ns: p>0.05

tic cultures may be effective in the lower titratable acidity and higher pH value of the buffalo yoghurts using ABT-2 probiotic culture compared to the buffalo yoghurts using yoghurt culture. In addition, it has been revealed by various studies that the total solids, lactose, protein, phosphate and citrate contents and the organic acid production (especially lactic acid) of lactic acid bacteria play an important role on the acidity values of yoghurt samples as well as the culture type (Dayısoylu, 1997; Tamime and Robinson, 1999; Nagaoka, 2019). Similar to the present study, Akgun et al. (2016) reported that pH values of buffalo yoghurts decreased and titratable acidity, viscosity and syneresis values increased with storage period. Abdel-Hamid et al. (2020) stated that the total solids content of probiotic buffalo yoghurts produced with *Siraitia grosvenorii* fruit extract increased with the addition of the extract, while the pH values decreased. Hamdy et al. (2021) reported that total solids, protein, ash, fat, pH, and titratable acidity values of buffalo yoghurt were 4.65, 0.83%, 13.53%, 5.41%, 3.10% and 0.71%, respectively.

Microbiological analyses results of the buffalo yoghurts

The microbiological analyses results of the control group and buffalo yoghurt with banana are given in Table 3.

It was determined that the use of different starter cultures, fruit ratio, and storage period had a very significant effect (p<0.01) on *S. thermophilus,* while sugar ratio had a significant effect (p<0.05). While the increase at the fruit-sugar ratio in yoghurt samples positively affected the development of *S. thermophilus,* it was observed that there was a certain decrease in the count of *S. thermophilus* as expected with the progression of the storage period. It was observed that the fruit ratio and storage period had a very significant effect (p<0.01) on *L. delbrueckii* subsp. *bulgaricus,* while the sugar ratio had a significant effect (p<0.05). Compared to *S. thermophilus,* the count of *L. delbrueckii* subsp. *bulgaricus* in yoghurt samples was higher as expected. While the increase at the fruit-sugar ratio positively affected the development of *L. delbrueckii* subsp. *bulgaricus,* it was observed that a very significant decrease in the count of *L. delbrueckii* subsp. *bulgaricus* occurred with the progression of the storage period. Decreased *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* numbers during the storage period are thought to be influenced by increased acidity through the formation of lactic acid and other organic acids (McKevith and Shortt, 2003). There are similar results to the studies conducted by Bakırcı and Kavaz (2008), Abdel-Hamid et al. (2020) and Hamdy et al. (2021).

While it was determined that fruit ratio and storage period had a very significant effect (p<0.01) on the counts of *L. acidophilus* LA-5+*B. animalis* subsp. *lactis* BB-12 and *L. acidophilus* LA-5, sugar ratio did not have a significant effect (p>0.05). On the other hand, the sugar ratio was found to have a statistically significant effect (p<0.05) on the count of *B. animalis* subsp. *lactis* BB-12. Probiotic microorganisms must be at least 10^6 cfu/g in the product in order to exhibit positive effects on health (Cho et al., 2013) and in the current research, the count of probiotic microorganisms, including the control group, is higher than this level. In the experimental yoghurts in which probiotic cultures were used, an increase in the counts of *L. acidophilus* LA-5+*B. animalis* subsp. *lactis* BB-12, *L. acidophilus* LA-5, and *B. animalis* subsp. *lactis* BB-12 was observed in parallel with the addition of banana. As a matter of fact, it was determined that the counts of probiotic microorganisms were the highest in the E2 sample with the highest fruit and sugar ratio, while the counts of probiotic microorganisms were the lowest in the A2 sample with no fruit and sugar. It is thought that inulin and fructooligosaccharides, which are found in the composition of bananas and have a prebiotic effect, contribute to this increase (Özyurt and Ötleş, 2014). On the other hand, with the progression of the storage period, it was observed that the count of these probiotic bacteria decreased at a very significant level. It is known that the decrease in the numbers of *B. animalis* subsp. *lactis* BB-12 and *L. acidophilus* LA-5 during the storage period are due to the decrease in the amount of nutrients needed by microorganisms for their development. In addition, this decrease during the storage period is thought to be due to a parallel decrease in the number of *S. thermophilus,* which provides the anaerobic environment for the development of *B. animalis* subsp. *lactis* BB-12 and *L. acidophilus* LA-5 (Lourens-Hattingh and Viljoen, 2001). Indeed, Kavaz (2012), and Ehsani et al. (2016) reported similar results.

TABLE 3: *Microbiological analyses of the experimental yoghurts.*

	S. thermophilus count (log cfu/g)	*L. delbrueckii* subsp. *bulgaricus* count (log cfu/g)	*L. acidophilus* LA-5 + *B. animalis* subsp. *lactis* BB-12 count (log cfu/g)	*L. acidophilus* LA-5 count (log cfu/g)	*B. animalis* subsp. *lactis* BB-12 count (log cfu/g)	Yeast and Mold count (log cfu/g)
Culture						
Yoghurt Culture	7.43±0.14^{b}	7.64±0.13	–	–	–	1.53±1.28^{a}
Probiotic Culture	7.47±0.15^{a}	–	7.85±0.19	7.71±0.17	7.73±0.20	1.54±1.14^{a}
Sign.	**	–	–	–	–	ns
Fruit Ratio (%)						
0	7.27±0.08^{c}	7.48±0.08^{c}	7.51±0.09^{c}	7.45±0.07^{c}	7.39±0.11^{c}	<1^{c}
5	7.46±0.12^{b}	7.62±0.09^{b}	7.87±0.06^{b}	7.72±0.10^{b}	7.75±0.08^{b}	1.42±1.12^{b}
10	7.58±0.10^{a}	7.76±0.06^{a}	7.99±0.07^{a}	7.84±0.10^{a}	7.87±0.07^{a}	2.41±0.62^{a}
Sign.	**	**	**	**	**	**
Sugar Ratio (%)						
0	7.27±0.08^{b}	7.48±0.08^{b}	7.51±0.09^{a}	7.45±0.07^{a}	7.39±0.10^{c}	<1^{a}
5	7.50±0.13^{a}	7.67±0.10^{a}	7.91±0.09^{a}	7.79±0.11^{a}	7.79±0.09^{b}	1.87±1.09^{a}
7.5	7.54±0.12^{a}	7.70±0.11^{a}	7.95±0.09^{a}	7.77±0.12^{a}	7.84±0.10^{a}	1.96±0.99^{a}
Sign.	*	*	ns	ns	*	ns
Storage Period (days)						
1	7.56±0.13^{a}	7.73±0.10^{a}	7.91±0.19^{a}	7.82±0.17^{a}	7.81±0.18^{a}	0.80±0.85^{c}
7	7.47±0.14^{b}	7.63±0.11^{b}	7.84±0.17^{b}	7.70±0.14^{b}	7.72±0.18^{b}	1.53±0.97^{b}
14	7.37±0.13^{c}	7.57±0.13^{c}	7.79±0.21^{c}	7.63±0.16^{c}	7.65±0.21^{c}	2.26±1.29^{a}
Sign.	**	**	**	**	**	**

$^{a-c}$: Different letters indicate significant differences in column. Sign.: significant; *: p<0.05, **: p<0.01, ns: p>0.05

While it was observed that the fruit ratio and storage period had a very significant effect (p<0.01) on yeast and mold, it was observed that the use of different starter cultures and the sugar ratio did not have a significant effect (p>0.05). As expected, there was a partial increase in the count of yeast and molds in parallel with the increase at fruit and sugar ratios and the advancement of the storage time in the experimental yoghurt samples. It is thought that this situation is mostly caused by the addition of bananas and sugar. Akgun et al. (2016) reported that the increase in yeast count in buffalo yoghurts with the storage period may be related to the decrease in pH value. de Toledo et al. (2018) reported that yeast and mold counts increased in fruit drinkable yoghurts depending on the storage time and fruit addition. The researchers emphasized that a maximum of 10 yeast cells can be found in yoghurts. Çakmakçı et al. (2012) reported that the counts of *B. bifidum* and *L. acidophilus* in banana probiotic buffalo yoghurts were above 10^{6} cfu/g, although the counts of *B. bifidum* and *L. acidophilus* decreased with the storage period, while the counts of yeast and molds increased. Hamdy et al. (2021) reported that yeast and mold counts in buffalo yoghurt samples increased during the storage time and ranged between 2.23–4.83 log cfu/g.

Microstructure analysis results of the buffalo yoghurts

The SEM (Scanning Electron Microscopy) images of the control group and buffalo yoghurt with banana are given in Figure 1.

In the microstructure analysis of the control group and buffalo yoghurt with banana, 2000x magnification degree was used to obtain SEM images. As a result of the analysis, it was observed that the use of two different cultures, the use of different fruit-sugar combinations, and the effect of the storage period had different microstructures. Similarly, it was determined by SEM images that the microstructures of the 1st and 14th days were different. Prasanna et al. (2018) stated that the differences in the microstructure of stirred type buffalo yoghurt produced with probiotic culture and added banana-sugar compared to set type cow yoghurt produced with yoghurt culture and no fruit were due to reasons such as milk type, starter culture type, starter culture ratio, incubation temperature, fibers, pH, production method (set type, stirred type), and fat globule diameter.

Once the microstructure of the yoghurt samples was examined, it was observed that the gaps in the structure of fruit-sugar added yoghurt samples increased with the progress of the storage period, the diameter of the gaps increa-

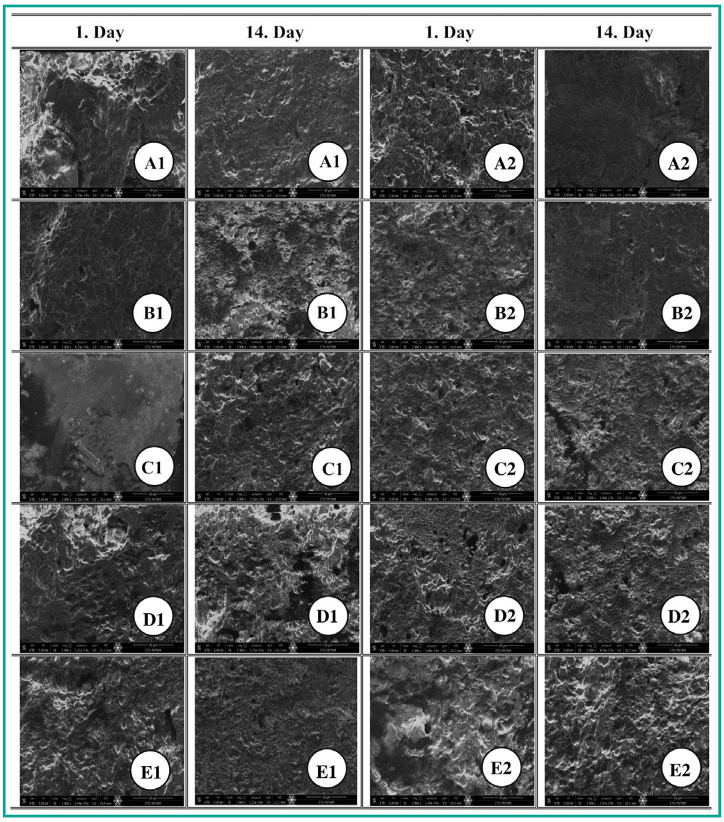

FIGURE 1: *SEM micrographs of the experimental yoghurts.*

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TABLE 4: *Sensory analyses of the experimental yoghurts.*

	Appearance	Consistency (by spoon)	Consistency (by mouth)	Smell	Taste Intensity	Fruit Ratio	Sugar Ratio	Overall Acceptability
Culture								
Yoghurt Culture	3.52±0.22[b]	4.08±0.34[b]	4.08±0.36[b]	3.42±0.15[b]	3.43±0.17[b]	4.33±0.20[b]	4.32±0.11[b]	25.45±4.25[b]
Probiotic Culture	4.18±0.34[a]	4.16±0.35[a]	4.16±0.32[a]	4.11±0.38[a]	4.21±0.38[a]	4.37±0.23[a]	4.37±0.11[a]	27.80±5.15[a]
Sign.	**	**	**	**	**	**	**	**
Fruit Ratio (%)								
0	3.44±0.20[c]	3.56±0.13[c]	3.55±0.17[c]	3.36±0.16[c]	3.52±0.15[c]	–	–	17.44±0.74[c]
5	3.80±0.37[b]	4.23±0.15[b]	4.22±0.16[b]	3.74±0.37[b]	3.73±0.43[b]	4.16±0.07[b]	4.30±0.10[b]	28.18±1.05[b]
10	4.11±0.41[a]	4.28±0.25[a]	4.30±0.22[a]	3.98±0.50[a]	4.06±0.54[a]	4.54±0.12[a]	4.40±0.11[a]	29.67±2.02[a]
Sign.	**	**	**	**	**	**	**	**
Sugar Ratio (%)								
0	3.44±0.20[c]	3.56±0.13[c]	3.55±0.17[c]	3.36±0.16[c]	3.52±0.15[c]	–	–	17.44±0.74[c]
5	3.88±0.41[b]	4.19±0.12[b]	4.20±0.12[b]	3.78±0.36[b]	3.81±0.46[b]	4.32±0.22[b]	4.27±0.08[b]	28.45±1.45[b]
7.5	4.02±0.42[a]	4.33±0.25[a]	4.32±0.22[a]	3.94±0.52[a]	3.98±0.55[a]	4.38±0.22[a]	4.42±0.10[a]	29.40±1.95[a]
Sign.	**	**	**	**	**	**	**	**
Storage Period (days)								
1	3.92±0.43[a]	4.20±0.34[a]	4.19±0.33[a]	3.83±0.44[a]	3.89±0.49[a]	4.43±0.24[a]	4.42±0.11[a]	27.12±5.00[a]
7	3.86±0.43[b]	4.12±0.33[b]	4.12±0.34[b]	3.78±0.46[b]	3.83±0.48[b]	4.36±0.21[b]	4.34±0.09[b]	26.67±4.97[b]
14	3.77±0.45[c]	4.04±0.36[c]	4.04±0.35[c]	3.68±0.47[c]	3.74±0.50[c]	4.26±0.18[c]	4.28±0.10[c]	26.10±4.94[c]
Sign.	**	**	**	**	**	**	**	**

[a–c]: Different letters indicate significant differences in column. Sign.: significant; **: p<0.01

sed and the homogeneous structure deteriorated. Buffalo yoghurts produced with ABT-2 probiotic culture showed a more homogeneous structure compared to the samples produced with yoghurt culture, and the homogeneous structure was disrupted with the increase at fruit ratio in all yoghurts. It is thought that this situation is caused by an increase in the amount of syneresis due to the decrease in the protein ratio. In addition, in the present study, it is thought that high fat content is also effective in the disruption of the protein network in the microstructure of buffalo yoghurts, the disintegration of the homogeneous structure and the formation of gaps. As a matter of fact, it has been reported that the fat globules in buffalo yoghurt disrupt the protein network and generally cause irregular protein network and serum pores in the structure (Nguyen et al., 2014; Khubber et al., 2021).

Sensory analyses results of the buffalo yoghurts

The sensory analyses results of the control group and buffalo yoghurt with banana are given in Table 4.

In the experimental yoghurt samples, it was found that the use of different starter cultures, fruit ratio, sugar ratio, and storage period had a very significant effect ($p<0.01$) on appearance, consistency (spoon), consistency (mouth), smell, taste, fruit ratio, sugar ratio, and overall acceptability parameters. It was determined that all sensory parameters increased with the use of ABT-2 probiotic culture, fruit rate increase, and sugar rate increase, while a decrease in these values occurred due to the progression of the storage period. In terms of sensory evaluations of the experimental yoghurt samples, the E2 sample received the highest total score (32.45) by the panelists, while the A1 sample received the lowest score (16.85). As a matter of fact, similar results were found by Abdel-Hamid et al. (2020), Hameed et al. (2020), Yıldız and Bakırcı (2019), and Hovjecki et al. (2021).

Correlation analysis of the buffalo yoghurts

The correlation analysis of the physicochemical analyses, the microbiological analyses and the sensory analyses of the control group and buffalo yoghurt with banana are given in Figure 2.

As expected, while it was seen that there was a very important strong positive correlation between total solids and solids nonfat ($r=0.999$, $p<0.01$), it was determined that there was a very significant strong negative correlation between total solids and fat ($r=-0.988$, $p<0.01$), and between fat and solids nonfat ($r=-0.992$, $p<0.01$). As a matter of fact, the fact that the fat content of buffalo milk used as a raw material in the current study was higher compared to other nutrients and the addition of fruit-sugar in yoghurt samples, and the change of fat content during the storage period affected the total solids and solids nonfat content in parallel.

It was observed that there was a very important strong positive correlation ($r=1.000$, $p<0.01$) between probiotic microorganisms (*L. acidophilus* LA-5+*B. animalis* subsp. *lactis* BB-12, *B. animalis* subsp. *lactis* BB-12, and *L. acidophilus* LA-5). It was observed that there was a very significant strong negative correlation ($r=-0.999$, $p<0.01$) between the count of *L. delbrueckii* subsp. *bulgaricus* and probiotic microorganisms. In addition, it was determined that there was a very significant strong positive correlation between the fruit ratio and the total solids ($r=0.980$, $p<0.01$), between the fruit ratio and solids nonfat ($r=0.979$, $p<0.01$), and between the fruit ratio and sugar ratio ($r=0.997$, $p<0.01$).

It is known that the addition of fruit-sugar at different rates in yoghurt production contributes to the total solids content, and this contribution has also been proven with the results of current research (Abdel-Hamid et al., 2020). Indeed, Çakmakçı et al. (2012) identified that microbial growth was supported by the addition of banana to probiotic cow yoghurt. Jaster et al. (2018) identified that the addition of strawberry puree to cow yoghurt increased the total solids and titratable acidity values correlatively. Terzioglu and Bakirci (2023) reported that the total solids, ash, viscosity, syneresis and titratable acidity values increased with the addition of banana-kiwi to sheep yoghurt. In addition, very significant and significant correlations between other analyses are shown in Figure 2.

Discrimination of the buffalo yoghurts by using PCA

PCA was used to show the differences between all analyses of the experimental yoghurt samples produced with

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
2	-0.988**	1																				
3	0.999**	-0.992**	1																			
4	-0.885**	0.915**	-0.893**	1																		
5	0.865**	-0.839**	0.862**	-0.889**	1																	
6	0.629**	-0.656**	0.635**	-0.666**	0.479**	1																
7	0.457**	-0.514**	0.469**	-0.548**	0.445**	0.436**	1															
8	0.298*	-0.350**	0.309*	-0.357**	0.096	0.837**	0.393**	1														
9	-0.385**	0.425**	-0.394**	0.430**	-0.204	-0.855**	-0.360**	-0.950**	1													
10	0.739**	-0.716**	0.736**	-0.683**	0.790**	0.061	0.233	-0.293*	0.201	1												
11	0.018	0.001	0.014	-0.009	-0.093	0.447**	-0.534**	0.457**	-0.507**	-0.274*	1											
12	0.018	-0.035	0.021	-0.024	0.127	-0.433**	0.544**	-0.456**	0.504**	0.310*	-0.999**	1										
13	0.014	-0.031	0.018	-0.020	0.125	-0.437**	0.539**	-0.461**	0.507**	0.309*	-0.999**	1.000**	1									
14	0.018	-0.036	0.022	-0.024	0.128	-0.434**	0.543**	-0.458**	0.504**	0.312*	-0.999**	1.000**	1.000**	1								
15	0.745**	-0.783**	0.754**	-0.802**	0.673**	0.788**	0.752**	0.648**	-0.658**	0.363**	-0.029	0.051	0.045	0.05	1							
16	0.551**	-0.566**	0.555**	-0.573**	0.641**	-0.047	0.654**	-0.286*	0.268*	0.742**	-0.751**	0.775**	0.773**	0.776**	0.418**	1						
17	0.820**	-0.798**	0.817**	-0.643**	0.640**	0.373**	0.366**	0.084	-0.119	0.693**	-0.104	0.134	0.131	0.135	0.503**	0.571**	1					
18	0.845**	-0.822**	0.842**	-0.674**	0.671**	0.405**	0.373**	0.096	-0.132	0.701**	-0.109	0.139	0.137	0.140	0.534**	0.572**	0.987**	1				
19	0.498**	-0.509**	0.501**	-0.497**	0.569**	-0.097	0.639**	-0.297*	0.302*	0.708**	-0.762**	0.785**	0.782**	0.786**	0.370**	0.968**	0.597**	0.579**	1			
20	0.389**	-0.401**	0.392**	-0.437**	0.530**	-0.168	0.630**	-0.351**	0.356**	0.661**	-0.797**	0.816**	0.814**	0.817**	0.320*	0.956**	0.470**	0.454**	0.972**	1		
21	0.980**	-0.966**	0.979**	-0.792**	0.775**	0.570**	0.412**	0.271*	-0.350**	0.711**	0.003	0.031	0.028	0.032	0.681**	0.519**	0.839**	0.858**	0.482**	0.354**	1	
22	0.964**	-0.950**	0.963**	-0.752**	0.735**	0.554**	0.399**	0.267*	-0.342**	0.691**	-0.002	0.035	0.031	0.036	0.658**	0.503**	0.838**	0.855**	0.471**	0.337**	0.997**	1
23	0.948**	-0.938**	0.948**	-0.786**	0.796**	0.428**	0.522**	0.118	-0.179	0.797**	-0.231	0.265*	0.261*	0.266*	0.656**	0.719**	0.893**	0.903**	0.699**	0.586**	0.961**	0.956**

1: Total Solids; **2**: Fat; **3**: Solids Nonfat; **4**: Protein; **5**: Ash; **6**: Viscosity; **7**: Syneresis; **8**: Titratable Acidity; **9**: pH; **10**: *S. thermophilus*; **11**: *L. delbrueckii* subsp. *bulgaricus*; **12**: *L. acidophilus* LA-5+ *B. animalis* subsp. *lactis* BB-12; **13**: *L. acidophilus* LA-5; **14**: *B. animalis* subsp. *lactis* BB-12; **15**: Yeast and Mold; **16**: Appearance; **17**: Consistency (by spoon); **18**: Consistency (by mouth); **19**: Smell; **20**: Taste Intensity; **21**: Fruit Ratio; **22**: Sugar Ratio; **23**: Overall Acceptability; *: p<0.05; **: p<0.01

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FIGURE 2: *Correlation between physicochemical, microbiological, and sensory analyses.*

different cultures and with the addition of fruit-sugar at different rates and stored during different storage periods. Score Scatter Plot, Loading Scatter Plot, Biplot, Dendrogram, and Scatter 3D Plot are shown in Figure 3. The first two principal components (PC1=53.4% and PC2=30.2%) accounted for 83.6% of the variance. The buffalo yoghurts with plain classic cultured, plain probiotic cultured, fruit classic cultured, and fruit probiotic cultured were well divided into four different groups and this situation indicated that there were some differences. In addition, significant differences were observed between plain classical cultured yoghurt samples and probiotic cultured yoghurts with the highest fruit-sugar ratio (Figure 3-Score Scatter Plot, Figure 3-Dendogram, and Figure 3-Scatter 3D Plot). Fat, protein, pH, and *L. delbrueckii* subsp. *bulgaricus* were clustered on the left side of the graph, while all the remaining analyses were collected on the right side of the graph. It was determined that there was a negative correlation between probiotic microorganisms and *L. delbrueckii* subsp. *bulgaricus* count and titratable acidity, and a positive correlation between *S. thermophilus* count, pH, and overall acceptability. In addition, a negative correlation was found between fat and protein and syneresis (Figure 3-Loading Scatter Plot). During the storage period, the highest fat and protein content was determined in plain classical culture and plain probiotic culture yoghurts, the highest syneresis was detected in the E2 sample on the 14th day of the storage, and the overall acceptability was found in the E2 sample on the 1st and 7th days of the storage (Figure 3-Biplot).

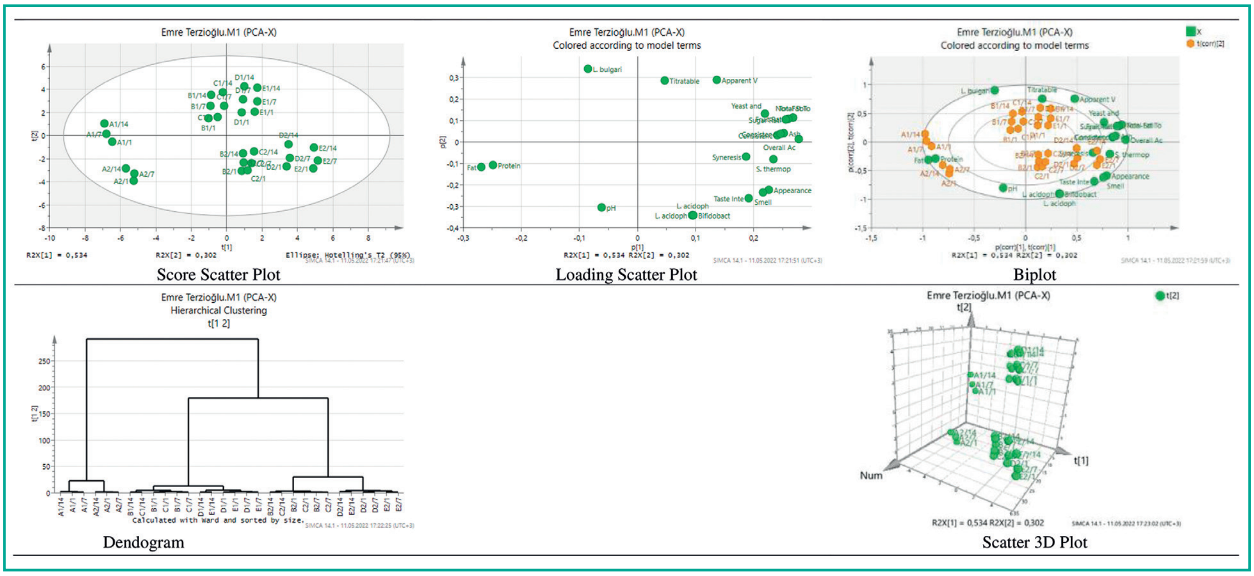

FIGURE 3: *PCA between physicochemical, microbiological, and sensory analyses.*

Conclusion

The use of buffalo milk as a raw material in yoghurt production and the addition of banana-sugar at different proportions to the samples made significant contributions to the nutritional value and textural properties. In addition, it was determined that the buffalo milk and the addition of banana-sugar increased the total solids content, ash content, and viscosity values in the yoghurt samples. It has been observed that the use of buffalo milk has a significant effect on the counts of probiotic microorganisms in the experimental yoghurt samples being higher than the minimum level (10^6 cfu/g) that should be present in the product, as well as the development of probiotic microorganisms is also supported by the increase at the ratio of banana and sugar. In the studies conducted by using SEM, it was observed that different starter cultures used in the production of yoghurt, different fruit-sugar ratios, and storage period affected the number of gaps, the diameter of the gap, and homogenity in the structure of yoghurt samples at different levels. In the current study, once the physicochemical, microbiological, sensory, and microstructure properties were examined, it was determined that the E2 sample, which was produced by adding ABT-2 probiotic culture, contained 10% banana and 7.5% sugar, had superior properties.

As a matter of fact, in line with the data obtained, it is thought that banana probiotic yoghurts produced from buffalo milk can be very beneficial in terms of nutrition and health through the functional properties gained. It is thought that this study will provide scientific guidance and contribution to the researchers who want to research the production of fruit probiotic yoghurt from buffalo milk.

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Authors' contributions

Murat Emre Terzioğlu: Conceptualization, Methodology, Investigation, Formal analysis, Writing-original draft, Writing-review & editing. İhsan Bakirci: Conceptualization, Methodology, Investigation, Writing-original draft, Writing-review & editing, Supervision.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors have declared no conflict of interest.

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