Erzincan Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
2022, 15(ÖZEL SAYI I), 84-97
ISSN: 1307-9085, e-ISSN: 2149-4584
Araştırma Makalesi

Erzincan University
Journal of Science and Technology
2022, 15(SPECIAL ISSUE I), 84-97
DOI: 10.18185/erzifbed1.199636
Research Article

Effect of Pineapple Addition at Different Rates to Probiotic Sheep Yoghurt on Antioxidant Activity, 5-Hydroxymethylfurfural (HMF) Content, and ABT-2 Probiotic Culture Growth

Murat Emre TERZİOĞLU[1]*, Neslihan YILDIZ KÜÇÜK[2], İhsan BAKIRCI[1]

[1]Atatürk University, Faculty of Agriculture, Food Engineering Dep., 25240, Erzurum, Türkiye
[2]Muş Alparslan University, Vocational School of Social Sciences, Restaurant and Catering Services Dep., 49250, Muş, Türkiye

Geliş / Received: 04/11/2022, Kabul / Accepted: 09/12/2022

Abstract

In the present study, sheep milk was used for production of yoghurts with addition of different concentrations of pineapple, using ABT-2 cultures and its effect on antioxidant activity, total phenolic compound and 5-hydroxymethylfurfural (HMF) contents were investigated. During storage the experimental sheep yoghurt samples were analyzed for physicochemical parameters (total solids, fat, protein, ash, viscosity, pH, and titratable acidity values), growth of ABT-2 probiotic cultures (*Streptococcus salivarius* subsp. *thermophilus*, *Bifidobacterium* sp., *Lactobacillus acidophilus*), and sensory evaluation. It was determined that the addition of pineapple to sheep yoghurt samples produced with ABT-2 probiotic culture had a very significant effect ($p<0.01$) on all physicochemical analyses, antioxidant activity, total phenolic compound, HMF content, microbiological analyses, and sensory evaluation. It was determined that the storage period had a very significant effect ($p<0.01$) on viscosity, titratable acidity, pH value, microbiological analyses, appearance, and overall acceptability. It was found that the antioxidant activity and the total amount of phenolic compounds increased with the addition of fruit to the yoghurt samples. On the other hand, it was determined that HMF, which is a toxic component, increased with the addition of fruit. As a result of the research, it was revealed that by adding pineapple at different rates to yoghurt, the growth of ABT-2 probiotic cultures was supported and the functionality increased.

Keywords: Sheep yoghurt, ABT-2 probiotic culture, pineapple, antioxidant activity, HMF content

Probiyotik Koyun Yoğurduna Farklı Oranlarda Ananas İlavesinin Antioksidan Aktivite, 5-Hidroksimetilfurfural (HMF) İçeriği ve ABT-2 Probiyotik Kültür Gelişimi Üzerine Etkisi

Öz

Mevcut araştırmada, ABT-2 kültürleri kullanılarak farklı konsantrasyonlarda ananas ilavesi ile yoğurt üretiminde koyun sütü kullanılmış ve bunların antioksidan aktivite, toplam fenolik bileşik ve 5-hidroksimetilfurfural (HMF) içerikleri üzerine etkisi incelenmiştir. Depolama süresince deneme koyun yoğurdu örnekleri fizikokimyasal parametreler (toplam kurumadde, yağ, protein, kül, viskozite, pH ve titrasyon asitliği değerleri), ABT-2 probiyotik kültürlerin (*Streptococcus salivarius subsp. thermophilus*, *Bifidobacterium* sp., *Lactobacillus acidophilus*) gelişimi ve duyusal değerlendirme bakımından analiz edilmiştir. ABT-2 probiyotik kültürle üretilen koyun yoğurdu örneklerinde ananas ilavesinin tüm fizikokimyasal analizler, antioksidan aktivite, toplam fenolik bileşik miktarı, HMF içeriği, mikrobiyolojik analizler ve duyusal değerlendirme üzerine istatistiksel olarak çok önemli ($p<0.01$) etkiye sahip olduğu saptanmıştır. Depolama periyodunun ise viskozite, titrasyon asitliği, pH değeri, mikrobiyolojik analizler, görünüş ve genel kabul edilebilirlik üzerine istatistiksel olarak çok önemli ($p<0.01$) etkiye sahip olduğu saptanmıştır. Yoğurt örneklerine meyve ilavesiyle antioksidan aktivite ve toplam fenolik bileşik miktarının arttığı tespit edilmiştir. Diğer taraftan toksik bir bileşen olan HMF'nin de meyve ilavesiyle arttığı belirlenmiştir. Araştırma sonucunda yoğurda farklı oranlarda ananas ilavesiyle ABT-2 probiyotik kültürlerin gelişiminin desteklendiği ve fonksiyonelliğin arttığı ortaya konulmuştur.

Anahtar Kelimeler: Koyun yoğurdu, ABT-2 probiyotik kültür, ananas, antioksidan aktivite, HMF içeriği

*Corresponding Author: murat.terzioglu@atauni.edu.tr
Murat Emre Terzioğlu, https://orcid.org/0000-0001-6370-0694
Neslihan Yıldız Küçük, https://orcid.org/0000-0002-2467-5454
İhsan Bakırcı, https://orcid.org/0000-0002-3744-3863

1 Introduction

Yoghurt, a better alternative for individuals who have a number of health problems (milk allergy and lactose intolerance) in milk consumption and contains some important components in nutrition (such as calcium, magnesium, phosphorus, cobalamin, niacin, riboflavin, and thiamine) at a higher rate compared to milk, is a fermented dairy product produced by adding *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* starter cultures at different rates to milk [1-3]. Although cow milk is generally preferred as a raw material for yoghurt production, but due to high nutrients (total solids, fat, protein, mineral substances, and vitamins) in sheep milk is more suitable for yoghurt production compared to cow milk, since it is not subjected to any total solids increase process [4-6]. Sheep milk is an important option, especially for consumers with milk allergies, due to its different micellar-structured proteins available [7,8]. With the use of fruits and probiotic cultures in the production of sheep yoghurt, a functional food with typical fatty acids and bioactive peptides, its functional properties have been increased and consumer preference has been improved [9,10].

Pineapple, a rich source of vitamins A and C as well as mineral substances such as calcium, potassium, and phosphorus, owns an important place in terms of health due to its low cholesterol and fat contents [11]. Probiotic microorganisms, which are defined as organisms resistant to stomach acid, strengthen the immune system, increase intestinal absorption, reduce cholesterol levels and protect intestinal flora when taken into the body with food [12,13]. Fruits such as pineapple added to yoghurt support the growth of probiotic organisms and increase their survival time by creating a prebiotic effect while, enriching the yoghurt in terms of vitamins and minerals [14,15]. In addition, it is reported that yoghurt, which already has various functional properties, has a remarkable functional food potential in the diet as an important antioxidant source with the addition of fruit. Today, it has become more and more important to include antioxidant substances in the diet to protect against the effects of oxidative damage caused by free radicals or reactive oxygen species [16, 17]. On the other hand, 5-hydroxymethylfurfural (HMF), which is the product of thermal decomposition of sugars, is formed as a result of heat treatment or storage applied to milk in order to extend its shelf life. It has been proven that HMF, which is formed in the advanced stages of the Maillard reaction and belongs to the furfurals group, has genotoxic, tumoral, and cytotoxic effects [18]. The amount of Maillard reaction products with toxic properties such as HMF above a certain level in milk and dairy products is important both in terms of human health and as a quality parameter [19]. For this purpose, in the present study, antioxidant activity, total phenolic and HMF contents were investigated in the experimental sheep yoghurt samples containing different ratios of pineapple (5%, 7.5%, and 10%) and 7.5% sugar added. In addition, their effect on physicochemical properties, growth of ABT-2 probiotic cultures (*S. thermophilus*, *Bifidobacterium* sp. and *Lactobacillus acidophilus*), and sensory properties of the experimental sheep yoghurt samples were examined during the storage period (1st and 10th days).

2 Material and Methods

2.1 Material

In the present study, sheep milk was used as a raw material in the yoghurt production, obtained from local farms in Erzurum (Turkey); ABT-2 probiotic culture was obtained from a commercial company (CHR Hansen/Süt-Sa Süt Sanayii İht. Malz. Tic. Sakarya/Turkey), and pineapple fruits were obtained from the local markets in Erzurum (Turkey).

2.2 Method

2.2.1 Preparation of pineapple puree

Pineapple fruits were washed, peeled and cut in to slices to make puree, sugar was added and mixed thoroughly pasteurized at 90±1ºC for 10 minutes, and then cooled to the room temperature and added to the experimental yoghurt samples at the rates of 5%, 7.5%, and 10%.

2.2.2 Experimental yoghurt production

Experimental yoghurt production was carried out following the flow chart given in Figure 1. ABT-2 probiotic culture used in yoghurt production was added at 37-38ºC and inoculated samples were incubated until their pH reached to 4.6±0.1. After incubation, the samples were left to cool at 4±1ºC for 24 hours, and mashed pineapples were added at different concentration to the yoghurt samples.

2.2.3 Physicochemical analyses of raw sheep milk and experimental yoghurt samples

Total solids, fat, protein, ash, titratable acidity, and pH values were determined in raw sheep milk and sheep yoghurt samples by the methods given by Kavaz [20], and viscosity analysis was performed in the experimental sheep yoghurt samples as well.

2.2.4 Determination of antioxidant activity in experimental yoghurt samples

DPPH (2,2-diphenyl-1-picrylhydrazil) free radical scavenging activity method was used to determine the antioxidant activity of experimental yoghurt samples. For this purpose, 50 µL yoghurt was weighed and 0.004% DPPH solution and 5 mL methanol were added to it and mixed. After the mixture was kept at room temperature for 30 minutes, absorbance measurement was performed on a spectrophotometer (Optizen POP) at a wavelength of 517 nm. In the determination of DPPH free radical scavenging activity, a curve was drawn with reference to Trolox and the results were given as mg TE/100 g [21].

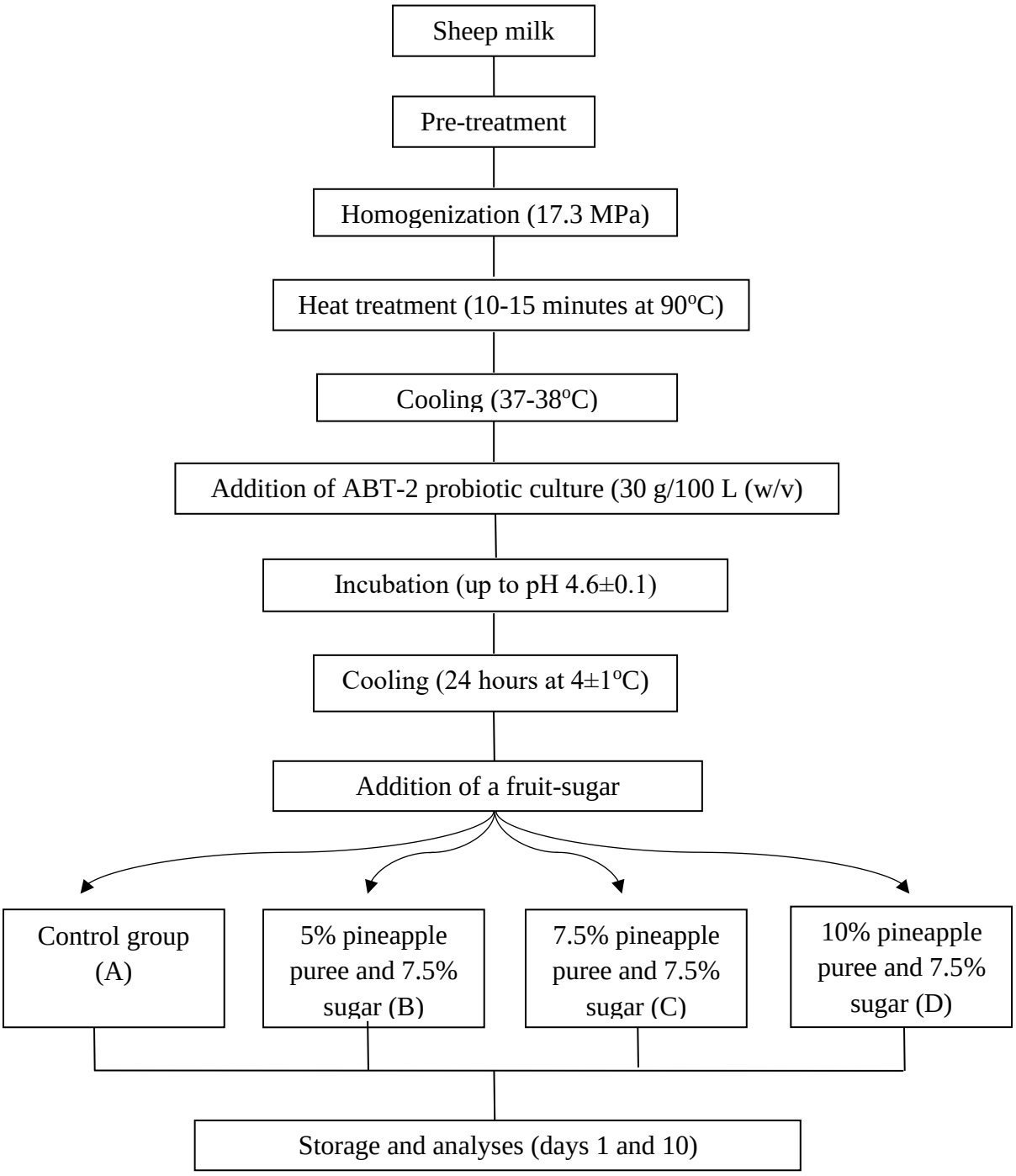

Figure 1. The flow chart of the experimental probiotic sheep yoghurt production

2.2.5 Determination of total amount of phenolic compounds of the experimental yoghurt samples

The method given by Arslaner et al. [21] was taken as a reference in determining the total phenolic compounds of the experimental yoghurt samples. To determine the total phenolic compounds, 1 mL of Folin-Ciocalteu reagent and 46 mL of distilled water were added to 0.1 mL of the yoghurt sample. Afterward, the mixture was stirred for 3 minutes and then 3 mL of 2% Na_2CO_3 was added to this mixture and stirred for 2 hours. All the processes applied to the

yoghurt sample were also applied to the gallic acid solution and absorbance measurements were made at 760 nm using a spectrophotometer (Optizen POP).

2.2.6 Determination of HMF content of the experimental yoghurt samples

To determine the HMF content of the experimental yoghurt samples, the method given by Urgu et al. [22] was used with some modifications. In this context, 5 mL of yoghurt sample was weighed and 5 mL of oxalic acid was added to it. After mixing, it was kept in a water bath for 1 hour and then cooled to the room temperature. After the mixture was filtered, 4 mL of filtrate was taken and 1 mL of 0.05 M thiobarbituric acid solution was added to it. The mixture was kept in a water bath again and then the absorbance was measured at a wavelength of 443 nm using a spectrophotometer (Optizen POP).

2.2.7 Microbiological analyses of the experimental yoghurt samples

Microbiological analyses of the experimental sheep yoghurt samples were carried out using the methods given by Kavaz [20]. The agars and incubation conditions used in microbiological analyses are given in Figure 2.

Microorganisms	**Agar**	**Incubation duration and temperature**
S. thermophilus	M17 Agar (Oxoid Ltd.)	at 42°C for 24 h
L. acidophilus + *Bifidobacterium* sp.	MRS Agar (Oxoid Ltd.)	at 37°C for 48 h
L. acidophilus	MRS Agar (Oxoid Ltd.) + 1.5 g/L bile (Bile Salts, Sigma-Aldrich)	at 37°C for 48 h
Bifidobacterium sp.	MRS Agar (Oxoid Ltd.) + 0.5 g/L sistein (L-Cysteine, Sigma-Aldrich)	at 37°C for 48 h

Figure 2. Microbiological analysis conditions

2.2.8 Sensory evaluation of the experimental yoghurt samples

Sensory evaluations of the experimental yoghurt samples were carried out over 5 points by 8 laboratory staff panellists familiar with yoghurt by evaluating the parameters of consistency, taste, smell, appearance, and overall acceptability [23].

2.2.9 Statistical analysis

The current study was carried out according to a completely randomized block design. The statistical analyses were interpreted with 2 replications on the 1st and 10th days of the storage using Duncan Multiple Comparison Test through the SPSS 20 package program.

3 Results and Discussion

3.1 Physicochemical analysis results of raw sheep milk

The physicochemical analysis results of raw sheep milk used as raw material in the production of experimental yoghurt samples are given in Table 1. It was determined that the total solids, fat, protein, ash, titratable acidity, and pH values of sheep milk were compatible with the literature [24].

3.2 Physicochemical analysis results of experimental yoghurt samples

The physicochemical analysis results of both control group sheep yoghurts and the sheep yoghurts with pineapple puree at different proportions are given in Table 1.

It was found that the addition of pineapple fruit puree to the experimental yoghurt samples had a very significant effect ($p<0.01$) on all physicochemical properties. Compared to the control group, an increase was observed in total solids, ash, viscosity, and titratable acidity values, while a decrease was observed in fat, protein, and pH values in the experimental yoghurt samples to which pineapple puree was added at different rates. The fact that sources rich in polyphenols, such as fruits and spices, have better physicochemical properties as a result of using them in products such as yoghurt has been expressed by various mechanisms such as the complexes they form with proteins and increasing the amount of the total solids [25].

It was determined that the storage period of the experimental yoghurt samples had a very significant effect ($p<0.01$) on viscosity, titratable acidity, and pH value, while it had no significant effect ($p>0.05$) on other physicochemical properties. While the viscosity and pH values of the experimental yoghurt samples decreased with the progression of the storage period, an increase was observed in the titratable acidity value. As a matter of fact, there are studies in which the same effects have been observed on physicochemical analyses with the addition of fruit to yoghurt [26].

3.3 Antioxidant activity

The DPPH free radical scavenging activity results used to determine the antioxidant activity of the experimental yoghurt samples are given in Table 2. It was determined that the addition of fruit had a very significant effect ($p<0.01$) on the DPPH free radical scavenging activity of the experimental yoghurt samples. The DPPH free radical scavenging activity, which was 9.71 mg TE/100 g in the control group, increased in the samples to which pineapple puree was added at different rates and varied between 19.31-36.37 mg TE/100 g. During the fermentation in yoghurt, which is an important source for transporting and using functional food components to the body, lactic acid bacteria break down milk protein and provide the formation of peptides

Table 1. Physicochemical analysis results of raw sheep milk and experimental probiotic sheep yoghurts

		Total Solids (%)	Fat (%)	Protein (%)	Ash (%)	Apparent Viscosity (cP)	Titratable Acidity (Lactic Acid %)	pH
Sheep Milk		17.30±0.06	6.58±0.06	5.04±0.02	0.87±0.02	-	0.21±0.02	6.65±0.01
Experimental Probiotic Sheep Yoghurts	**A**	19.17±0.06^{d}	7.62±0.05^{a}	5.84±0.04^{a}	0.93±0.02^{d}	9487.75±374.49^{d}	0.94±0.05^{d}	4.33±0.05^{a}
	B	22.49±0.11^{c}	6.47±0.10^{b}	5.46±0.06^{b}	1.03±0.02^{c}	11682.75±267.76^{c}	1.06±0.07^{c}	4.22±0.07^{b}
	C	23.15±0.12^{b}	6.16±0.03^{c}	5.22±0.03^{c}	1.06±0.01^{b}	12330.50±335.25^{b}	1.12±0.06^{b}	4.15±0.06^{c}
	D	24.65±0.03^{a}	6.03±0.00^{d}	5.08±0.04^{d}	1.13±0.01^{a}	14562.25±302.65^{a}	1.19±0.07^{a}	4.06±0.09^{d}
	Sign.	**	**	**	**	**	**	**
Storage Time (days)	**1.**	22.38±2.15^{a}	6.56±0.69^{a}	5.41±0.30^{a}	1.04±0.08^{a}	12288.25±1915.85^{a}	1.03±0.09^{b}	4.24±0.10^{a}
	10.	22.34±2.14^{a}	6.58±0.65^{a}	5.39±0.32^{a}	1.04±0.08^{a}	11743.38±1953.33^{b}	1.12±0.11^{a}	4.14±0.12^{b}
	Sign.	ns	ns	ns	ns	**	**	**

a-d: Different letters indicate significant differences in column
**: p<0.01; ns: p>0.05
A: control sheep yoghurt; B: %5 pineapple puree + %7.5 sugar; C: %7.5 pineapple puree + %7.5 sugar; D: %10 pineapple puree + %7.5 sugar

with antioxidant properties [27]. In addition, it has been reported that the antioxidant activity of yoghurt is mainly due to the high electron/atom donor or metal chelating activities of whey proteins and casein [16]. On the other hand, the addition of plant or extract to yoghurt significantly affects the existing antioxidant activity by increasing the amount of bioactive compounds and releasing peptides [28]. In addition, another parameter contributing to the antioxidant activity of experimental yoghurt samples is that pineapple contains high amounts of compounds with antioxidant properties such as carotenoids, phenolic compounds, and vitamin C [29]. As a matter of fact, a significant increase in the antioxidant activity was determined depending on the ratio in the pineapple-added yoghurt samples. Similar antioxidant activity results have been reported in fruit-added yoghurt samples in the literature [30].

activity results have been reported in fruit-added yoghurt samples in the literature [30].

3.4 Total phenolic compound amount

The results of the total amount of phenolic compounds of the experimental yoghurt samples are given in Table 2.

Table 2. DPPH free radical scavenging activity, total phenolic compound amount and HMF contents of experimental probiotic sheep yoghurts

		DPPH (mg TE/100 g)	Total Phenolic Compound (mg GAE/100 g)	HMF (µmol/L)
Experimental Probiotic Sheep Yoghurts	A	9.71±0.06^{d}	8.12±0.10^{d}	17.16±0.17^{d}
	B	19.31±0.25^{c}	15.55±0.47^{c}	33.67±0.25^{c}
	C	25.39±0.83^{b}	23.68±0.06^{b}	35.51±0.57^{b}
	D	36.37±0.40^{a}	30.53±0.63^{a}	39.04±0.88^{a}
	Sign.	**	**	**

a-d: Different letters indicate significant differences in column
**: p<0.01
A: control sheep yoghurt; B: %5 pineapple puree + %7.5 sugar; C: %7.5 pineapple puree + %7.5 sugar; D: %10 pineapple puree + %7.5 sugar

It was determined that the addition of pineapple fruit puree had a very significant effect ($p<0.01$) on the total amount of phenolic compounds of the experimental yoghurt samples. As expected, the addition of pineapple, which is a rich source of phenolic compounds, to the experimental sheep yoghurts increased the total amount of phenolic compounds. The total amount of phenolic compounds, which was 8.12 mg GAE/100 g in the control group, increased and ranged from 15.55-30.53 mg GAE/100 g. On the other hand, the total phenolic compounds contained in the control group are thought to be feed origin and it has been reported that various factors such as nutrition, season, heat treatment, and production method are effective on the amount of phenolic compounds in milk and dairy products [31]. The fact that phenolic compounds have a significant level of free radical scavenging activity is observed by the fact that the total amount of phenolic compounds and DPPH free radical scavenging activities have similar increases in yoghurt samples [25].

3.5 5-Hydroxymethylfurfural (HMF) content

One of the important quality parameters of milk and dairy products is HMF, which formed during the advanced stages of the Maillard reaction, which is the reaction of reducing sugars and amino acids (group) during heat treatment. It has been reported that the most important parameters affecting the formation of furfural in milk and dairy products are temperature and duration, but fermentation process and additional ingredients are also effective [19].

The HMF contents of the experimental sheep yoghurt samples are given in Table 2. It was determined that the addition of fruit had a very significant effect ($p<0.01$) on the HMF content of the experimental yoghurt samples. The HMF contents of the control group and the samples with different amounts of pineapple added were determined as: the control group (17.16 μmol/L) < 5% pineapple added yoghurt sample (33.67 μmol/L) < 7.5% pineapple added yoghurt sample (35.51 μmol/L) < 10% pineapple added yoghurt sample (39.04 μmol/L), respectively. Studies investigating HMF content in yoghurt samples are quite limited in the literature, and Cui et al. [32] determined the HMF content in yoghurt samples up to 3.43 mg/kg. The European Food Safety Authority (EFSA) has reported the maximum detectable limit of 15 mg/kg in milk and dairy products for HMF with proven toxicity [33]. Accordingly, in the present study, it was determined that the HMF content of all experimental yoghurt samples was below this limit.

3.6 Microbiological analysis

The counts of *S. thermophilus*, *Bifidobacterium* sp., *L. acidophilus* and *Bifidobacterium* sp. + *L. acidophilus* of the experimental yoghurts during the storage are given in Table 3.

Table 3. Microbiological analysis of the experimental probiotic sheep yoghurts

		S. thermophilus	***Bifidobacterium* sp.**	***L. acidophilus***	***Bifidobacterium* sp. + *L. acidophilus***
Experimental Probiotic Sheep Yoghurts	**A**	7.37±0.08^{c}	7.12±0.08^{d}	7.18±0.08^{d}	7.29±0.08^{c}
	B	7.43±0.10^{c}	7.28±0.08^{c}	7.32±0.13^{c}	7.49±0.08^{b}
	C	7.60±0.10^{b}	7.42±0.10^{b}	7.53±0.11^{b}	7.66±0.12^{a}
	D	7.76±0.11^{a}	7.54±0.13^{a}	7.63±0.11^{a}	7.73±0.09^{a}
	Sign.	**	**	**	**
Storage Time (days)	**1.**	7.62±0.18^{a}	7.42±0.19^{a}	7.50±0.20^{a}	7.61±0.19^{a}
	10.	7.47±0.16^{b}	7.26±0.16^{b}	7.32±0.18^{b}	7.47±0.17^{b}
	Sign.	**	**	**	**

a-d: Different letters indicate significant differences in column
**: $p<0.01$; ns: $p>0.05$
A: control sheep yoghurt; B: %5 pineapple puree + %7.5 sugar; C: %7.5 pineapple puree + %7.5 sugar; D: %10 pineapple puree + %7.5 sugar

It was determined that fruit addition and storage period had a very significant effect ($p<0.01$) on the *S. thermophilus*, *Bifidobacterium* sp., *L. acidophilus*, and *Bifidobacterium* sp. + *L. acidophilus* counts of the experimental yoghurt samples. It was determined that microbial growth was supported by increasing the addition of pineapple puree at different rates. As a matter of fact, in the D sample, which has the highest fruit ratio, the counts of *S. thermophilus*, *Bifidobacterium* sp., *L. acidophilus*, and *Bifidobacterium* sp. + *L. acidophilus* were found to be at the highest level. Considering the results of the analysis, it is seen that the addition of pineapple to the yoghurt samples creates a prebiotic effect for the ABT-2 probiotic cultures. It was determined that the acidity increased and the counts of *S. thermophilus*, *Bifidobacterium*

sp., *L. acidophilus*, and *Bifidobacterium* sp. + *L. acidophilus* decreased depending on the progression of the storage period. However, it was determined that the count of probiotic microorganisms in the experimental yoghurt samples during the storage was higher than 10^6 cfu/g, which is the lower limit assigned for probiotic products [12]. The present study results are in agreement with the literature [34-37].

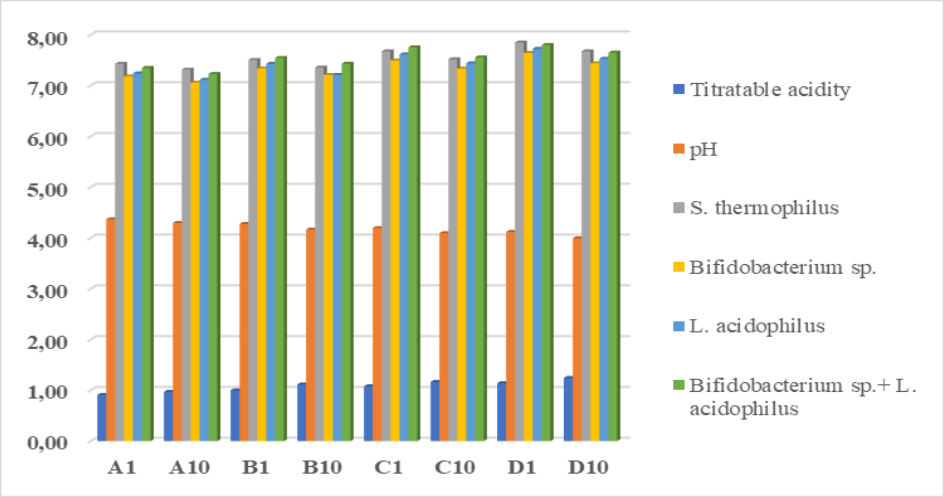

Figure 3. The relationship of pH and titratable acidity value with microbiological analyses during the storage period in the yoghurt samples

3.7 Sensory evaluation

Sensory evaluations of the experimental yoghurt samples are given in Table 4.

Table 4. Sensory evaluations of experimental probiotic sheep yoghurts

		Consistency	Taste	Smell	Appearance	Overall acceptability
Experimental Probiotic Sheep Yoghurts	**A**	3.23±0.34^{b}	3.12±0.31^{b}	2.89±0.44^{b}	2.86±0.61^{b}	12.11±1.13^{c}
	B	3.46±0.31^{b}	3.63±0.46^{b}	3.39±0.27^{b}	3.26±0.39^{b}	13.74±0.69^{b}
	C	4.27±0.29^{a}	4.43±0.27^{a}	4.31±0.33^{a}	3.97±0.20^{a}	16.97±0.39^{a}
	D	3.10±0.20^{b}	3.12±0.52^{b}	3.07±0.39^{b}	3.96±0.51^{a}	13.24±1.12^{b}
	Sign.	**	**	**	**	**
Storage Time (days)	**1.**	3.67±0.52^{a}	3.53±0.71^{a}	3.61±0.57^{a}	3.80±0.48^{a}	14.61±1.71^{a}
	10.	3.36±0.54^{b}	3.62±0.65^{a}	3.22±0.70^{b}	3.22±0.66^{b}	13.42±2.26^{b}
	Sign.	*	ns	*	**	**

a-c: Different letters indicate significant differences in column
**: p<0.01; *: p<0.05; ns: p>0.05
A: control sheep yoghurt; B: %5 pineapple puree + %7.5 sugar; C: %7.5 pineapple puree + %7.5 sugar; D: %10 pineapple puree + %7.5 sugar

Sensory evaluation was examined in terms of consistency, taste, smell, appearance, and overall acceptability parameters. It was determined that the addition of pineapple fruit puree to the experimental yoghurt samples had a very significant effect (p<0.01) on the sensory evaluation, while the storage period had a significant effect (p<0.05) on the consistency and smell, and a very significant effect (p<0.01) on the appearance and overall acceptability. While the addition of pineapple at different rates to the yoghurt enhanced all parameters, the yoghurt sample of group C (7.5% pineapple puree + 7.5% sugar) received the highest score in the evaluations.

During the storage period, a decrease in the other parameters except for taste was determined. In the literature, there are similar sensory evaluation results in the yoghurt samples with fruit [38].

4 Conclusion

It has been concluded that pineapple, which is a rich source of phenolic compounds, provides positive contributions to the probiotic sheep yoghurt in terms of the antioxidant activity and total amount of phenolic compounds, while it has been determined that it increases the HMF content, which is one of the heat treatment toxicants. On the other hand, HMF contents of all yoghurt samples were found below the maximum limit (15 mg/kg) given by EFSA. In addition, it has been determined that pineapple addition has a prebiotic effect on ABT-2 probiotic cultures and the yoghurt sample with pineapple at the rate of 7.5% comes to the fore in the sensory evaluation. In this context, as a result of the research, it has been seen that probiotic sheep yoghurt with pineapple can be used as a functional food in diets and can provide positive contributions.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

Murat Emre TERZİOĞLU: Conceptualization, Methodology, Investigation, Formal analysis, Writing-original draft, Writing-review & editing. **Neslihan YILDIZ KÜÇÜK:** Methodology, Investigation, Formal analysis, Writing-original draft, Writing-review & editing. **İhsan BAKIRCI:** Conceptualization, Methodology, Investigation, Writing-original draft, Writing-review & editing, Supervision.

Acknowledgements

This study was presented as an oral presentation at the 4th International Conference on Advanced Engineering Technologies (ICADET' 22) symposium held in Bayburt on September 28-30, 2022.

References

[1] O'connell, J. E., Fox, P. F., (2001). Significance and applications of phenolic compounds in the production and quality of milk and dairy products: A review. *International Dairy Journal*, 11(3), 103-120.

[2] Polidori, P., Vincenzetti, S., (2013). Use of donkey milk in children with cow's milk protein allergy. *Foods*, 2(2), 151-159.

[3] Gahruie, H. H., Eskandari, M. H., Mesbahi, G., Hanifpour, M. A., (2015). Scientific and technical aspects of yogurt fortification: A review. *Food Science and Human Wellness*, 4(1), 1-8.

[4] Erkaya, T., Şengül, M., (2012). A Comparative study on some quality properties and mineral contents of yoghurts produced from different type of milks. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 18(2), 323-329.

[5] Balthazar, C. F., Pimentel, T. C., Ferrão, L. L., Almada, C. N., Santillo, A., Albenzio, M., Mollakhalili, N., Mortazavian, A. M., Nascimento, J. S., Silva, M. C., Freitas, M. Q., Sant' Ana, A. S., Granato, D., Cruz, A. G., (2017). Sheep milk: Physicochemical characteristics and relevance for functional food development. C*omprehensive Reviews in Food Science and Food Safety*, 16(2), 247-262.

[6] Watkins, P. J., Jaborek, J. R., Teng, F., Day, L., Castada, H. Z., Baringer, S., Wick, M., (2021). Branched chain fatty acids in the flavour of sheep and goat milk and meat: A review. *Small Ruminant Research*, 200, 106398.

[7] Recio, I., De La Fuente, M. A., Juárez, M., Ramos, M., (2009). Bioactive components in sheep milk. *Bioactive Components in Milk and Dairy Products*, 83-104.

[8] De Barros, R. F., Torres, F. R., Silva, P. H. F. D., Stringheta, P. C., Pereira, J. P. F., Paula, J. C. J. D., Cutrim, C. S., Cortez, M. A. S., (2020). Lutein as a functional ingredient in sheep milk yogurt: development, characterization and extraction recovery. *Food Science and Technology*, 40(2), 683-690.

[9] Çakmakçı, S., Türkoğlu, H., Çağlar, A., (1997). Meyve çeşidi ve muhafaza süresinin meyveli yoğurtların bazı kalite kriterleri üzerine etkisi. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, 28(3), 390-404.

[10] Zhou, X., Chai, L., Wu, Q., Wang, Y., Li, S., Chen, J., (2021). Anti-diabetic properties of bioactive components from fish and milk. *Journal of Functional Foods*, 85, 104669.

[11] Ihemeje, A., Nwachukwu, C. N., Ekwe, C. C., (2015). Production and quality evaluation of flavoured yoghurts using carrot, pineapple, and spiced yoghurts using ginger and pepper fruit. *African Journal of Food Science*, 9(3), 163-169.

[12] Cho, Y. H., Hong, S. M., Kim, C. H., (2013). Isolation and characterization of lactic acid bacteria from Kimchi, Korean traditional fermented food to apply into fermented dairy products. *Korean Journal for Food Science of Animal Resources*, 33(1), 75-82.

[13] Ünver, İ. H., (2014). Developing probiotic yogurt using *Saccharomyces boulardii* and various prebiotics investigation of their effects on yogurts. Master Thesis, İstanbul Technical University, İstanbul.

[14] Nestle, M., (2013). Food politics: In Food Politics. How the Food Industry Influences Nutrition and Health, Berkeley, University of California Press.

[15] Meybodi, N. M., Mortazavian, A. M., Arab, M., Nematollahi, A., (2020). Probiotic viability in yoghurt: A review of influential factors. *International Dairy Journal*, 109, 104793.

[16] Perna, A., Simonetti, A., Grassi, G., Gambacorta, E., (2018). Effect of αS1-casein genotype on phenolic compounds and antioxidant activity in goat milk yogurt fortified with *Rhus coriaria* leaf powder. *Journal of Dairy Science*, 101(9), 7691-7701.

[17] Khan, I. T., Nadeem, M., Imran, M., Ullah, R., Ajmal, M., Jaspal, M. H., (2019). Antioxidant properties of milk and dairy products: A comprehensive review of the current knowledge. *Lipids in Health and Disease*, 18(1), 1-13.

[18] van den Oever, S. P., Mayer, H. K., (2021). Analytical assessment of the intensity of heat treatment of milk and dairy products. *International Dairy Journal*, 121, 105097.

[19] Li, M., Shen, M., Lu, J., Yang, J., Huang, Y., Liu, L., Huang, Y., Liu, L., Fan, H., Xie, J. Xie, M., (2022). Maillard reaction harmful products in dairy products: Formation, occurrence, analysis, and mitigation strategies. *Food Research International*, 151, 110839.

[20] Kavaz, A., (2012). Determination of organic acid contents, aroma profile and other quality characteristics of probiotic yoghurts produced with the combinations of different prebiotics. PhD thesis, Atatürk University, Erzurum, Turkey.

[21] Arslaner, A., Salık, M. A., Bakırcı, İ., (2021). The effects of adding *Hibiscus sabdariffa* L. flowers marmalade on some quality properties, mineral content and antioxidant activities of yogurt. *Journal of Food Science and Technology*, 58(1), 223-233.

[22] Urgu, M., Saatli, T. E., Türk, A., Koca N., (2017). Determination of hydroxymethylfurfural content of heat-treated milk (pasteurized, UHT and lactose-hydrolised UHT milk). *Akademik Gıda*, 15(3), 249-255.

[23] Anonim, (2006). Yoğurt Standardı, TS-1330. Türk Standartları Enstitüsü, 11 s., Ankara.

[24] Tripaldi, C., Palocci, G., Di Giovanni, S., Marri, N., Boselli, C., Giangolini, G., Amatiste, S., (2018). Microbiological and chemical characteristics of pasta filata type cheese from raw ewe milk, using thermophilic and mesophilic starters. *Journal of Food Safety and Food Quality*, 69:105-130.

[25] Tami, S. H., Aly, E., Darwish, A. A., Mohamed, E. S., (2022). Buffalo stirred yoghurt fortified with grape seed extract: new insights into its functional properties. *Food Bioscience*, 47, 101752.

[26] Diep, T. T., Yoo, M. J. Y., Rush, E., (2022). Effect of tamarillo fortification and fermentation process on physicochemical properties and nutrient and volatiles content of yoghurt. *Foods*, 11(1), 79.

[27] Ahmed, I. A. M., Alqah, H. A. S., Saleh, A., Al-Juhaimi, F. Y., Babiker, E. E., Ghafoor, K., Hassan, A. B., Osman, M. A., Fickak, A., (2021). Physicochemical quality attributes and antioxidant properties of set-type yogurt fortified with argel (*Solenostemma argel* Hayne) leaf extract. *LWT*, 137, 110389.

[28] Abdel-Hamid, M., Romeih, E., Huang, Z., Enomoto, T., Huang, L., Li, L., (2020). Bioactive properties of probiotic set-yogurt supplemented with *Siraitia grosvenorii* fruit extract. *Food chemistry*, 303, 125400.

[29] Ferreira, E. A., Siqueira, H. E., Boas, E. V. V., Hermes, V. S., Rios, A. D. O., (2016). Bioactive compounds and antioxidant activity of pineapple fruit of different cultivars. *Revista Brasileira de Fruticultura*, 38(3), 1-7.

[30] Shalabi, O. M., (2022). Antioxidant, antibacterial, and antitumor activities of goat's stirred yoghurt fortified with carob molasses. *Annals of Agricultural Sciences*, 67(1), 119-126.

[31] Chávez-Servín, J. L., Andrade-Montemayor, H. M., Vázquez, C. V., Barreyro, A. A., García-Gasca, T., Martínez, R. A. F., Ramírez, A. M. O., de la Torre-Carbot, K., (2018). Effects of feeding system, heat treatment and season on phenolic compounds and antioxidant capacity in goat milk, whey and cheese. *Small Ruminant Research*, 160, 54-58.

[32] Cui, Y., Shi, X., Tang, Y., Xie, Y., Du, Z., (2020). The effects of heat treatment and fermentation processes on the formation of furfurals in milk-based dairy products using a QuEChERS technique followed by gas chromatography coupled with triple quadrupole mass spectrometry. *Food Chemistry*, 313, 125930.

[33] Hou, Y., Zhang, X., Liu, X., Wu, Q., Hou, J., Su, P., Guo, Q., (2022). Comparison of the effects of 5-Hydroxymethylfurfural in milk powder matrix and standard water on oxidative stress system of Zebrafish. *Foods*, 11(12), 1814.

[34] Bakırcı, I., Kavaz, A., (2008). An investigation of some properties of banana yogurts made with commercial ABT-2 starter culture during storage. *International Journal of Dairy Technology*, 61(3), 270-276.

[35] Akubor, P. I., (2016). Quality evaluation and storage properties of yoghurt supplemented with pineapple juice. *International Journal Science and Knowledge*, 5(1), 23-31.

[36] Gangwar, R., Hai, H., Sharma, N., Kumar, P., (2016). Development and quality evaluation of yoghurt fortified with pineapple, apple and sweet lemon juice (fruit yoghurt). *International Journal of Engineering Research*, 5(3), 621-629.

[37] Al-Dhabi, N. A., Valan Arasu, M., Vijayaraghavan, P., Esmail, G. A., Duraipandiyan, V., Kim, Y. O., Kim, H., Kim, H. J., (2020). Probiotic and antioxidant potential of *Lactobacillus reuteri* LR12 and *Lactobacillus lactis* LL10 isolated from pineapple puree and quality analysis of pineapple-flavored goat milk yoghurt during storage. *Microorganisms*, 8(10), 1461.

[38] Khatoon, N., Ali, S., Liu, N., Muzammil, H. S., (2021). Preparation and quality assessment of fruit yoghurt with persimmon (*Diospyros kaki*). *Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental Sciences*, 58(1), 111-128.