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RESEARCH ARTICLE

The effect of ultrasound process on lactic acid bacteria, physicochemical and sensory properties of yoghurt, before and after inoculation of starter cultures into milk

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The effect of ultrasound (US) applied before (US + i) and after (i + US) inoculation of yoghurt starter into cows' milk on some parameters of yoghurt was investigated. Lightness (L) values, amounts of acetaldehyde, diacetyl, acetoin, ethanol, lactic, pyruvic, and acetic acid were higher in the US-treated groups compared with control (*$P < 0.05$*). In the US + i group, the total amount of ketones increased.* Lactobacillus *and* Streptococcus *spp. numbers reached log ≥7.0 cfu/g in all groups (*$P > 0.05$*). It can be concluded that low-intensity ultrasound applied to cows' milk before or after inoculation of yoghurt starters increases the aroma components and organic acid content of yoghurt.*

Keywords Ultrasonication, Yoghurt cultures, Aroma profile, Organic acids.

INTRODUCTION

In addition to being a dairy product consumed all over the world with its unique nutritional elements, yoghurt is a functional food in terms of human health with the biochemical compounds it contains (Yuan et al. 2022). In many studies conducted in recent years, it has been reported that regular intake of yoghurt and yoghurt bacteria (LAB) and their metabolites reduces obesity, diabetes, inflammation and blood cholesterol, and that yoghurt has anti-carcinogenic, antioxidative and immunomodulatory effects (Mousavi et al. 2020). These functional effects are mostly due to aroma compounds (acetaldehyde, diacetyl, acetoin, acetone, ethanol, and 2-butanon), organic acids and bioactive peptides formed as a result of fermentation. These metabolic products are also very important in the formation of the rheological texture and quality of yoghurt (Tian et al. 2017). Due to these properties, many studies, such as fermentation with high-protein milk, enrichment with plant extras, enrichment with prebiotics, probiotic microorganisms and antioxidants have been carried out to increase the level, quality and functional attributes of metabolites formed in yoghurt (Cavalheiro et al. 2020; Gu et al. 2020; Kumar et al. 2022; Li et al. 2022). There are also studies such as microfiltration and ultrasound application to increase the rheological properties of yoghurt (Sfakianakis and Tzia 2017; Kenari and Razavi 2021).

In recent years, there has been a growing demand for minimally processed, healthy, safe and high-quality foods. New technologies such as non-thermal processing methods are gaining more and more attention as they cause less damage to the nutritional value of the product. Ultrasonication is a non-thermal technology that uses sound waves with frequencies between 16 and 100 kHz, and it has been used for many years in the food industry in many processes, such as homogenisation, emulsification, extraction, degassing, crystallisation, cutting and microbial inactivation (Yu et al. 2020; Scudino et al. 2022; Manyatsi et al. 2023). Ultrasound, which is considered as one of the alternative methods to pasteurisation especially in the dairy sector, attracts attention with its cheap, simple and fast, non-toxic, environmentally friendly and energy saving aspects. Compared with heat treatment applications, ultrasound has significant advantages in yoghurt, such

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as increasing the homogenisation efficiency, shortening the fermentation time, improving the water holding capacity and the structure (Abesinghe et al. 2019; Akdeniz and Akalın 2019; Kenari and Razavi 2021).

Many studies have been conducted on the rheological and physical effects of ultrasound applications on yoghurt fermentation. It has been revealed that ultrasound application has an effect on some components in milk. As a result of this effect, ultrasound improves the structure of yoghurt by enhancing gel strength and firmness. In addition, ultrasound provides a better homogenisation and improves the water holding capacity and viscosity of yoghurt by reducing syneresis (Akdeniz and Akalın 2019; Kenari and Razavi 2021). In some other studies, data have been presented that ultrasound application accelerates the metabolism in bacteria, causes an increase in the number of bacteria, and reduces the fermentation time (Abesinghe et al. 2019; Guimarães et al. 2019; Hashemi and Gholamhosseinpour 2020). However, in the literature review, it was observed that there are few studies on the sensory analysis of ultrasound-treated yoghurt samples. In addition, no study was found comparing the effect of ultrasound treatment applied to milk before and after starter culture inoculation on the sensory properties of yoghurt.

Based on the above information, yoghurt was produced in two different ways in the present study; (i) making yoghurt by adding yoghurt cultures to ultrasound-treated milk, (ii) making yoghurt by applying ultrasound to milk with starter culture added. The aim of the study is to determine whether there is a difference between these two applications on the viability of yoghurt starter cultures and sensory properties, physicochemical structure, organic acid type/amount and volatile aroma components of yoghurt.

MATERIALS AND METHODS

The antibiotic-free cows' milk used in the study was obtained from a farm owned by a local dairy that was controlled by a veterinarian and had a HACCP plan. Raw cows' milk was brought to the laboratory in a cold (4 °C) chain. The milk brought to the laboratory was divided into three groups of 200 mL in 250 mL beaker glasses. All milk samples were heat-treated at 90 °C for 10 min. All analyses were performed in three independent replicates.

Experimental groups

An ultrasonic processor with a 13 mm diameter probe (Bandelin Hd 2200.2, probe TT 13, 200 W max. power, Amplichron, Berlin, Germany) was used and test parameters were 15% amplitude, 20 kHz frequency, 15 min processing time.

For the control group; one of the 200 mL milk samples was cooled to 43 °C and 6 g (3%) of commercial yoghurt (Sütaş, Istanbul, Turkey) was added as a starter culture. For the pre-ultrasound starter culture inoculation (i + US) group, one of the milk samples was cooled to 4 °C, then starter culture was added as described above. Immediately afterwards, the milk was subjected to ultrasound (US) treatment until its temperature reached 43 °C. For the post-ultrasound starter culture inoculation (US + i) group; milk sample was cooled to 4 °C, then the milk was subjected to US treatment until its temperature reached 43 °C. Immediately after US treatment, the starter culture was added to the milk as described above.

All groups were incubated at 43 °C until the pH dropped to 4.6–4.7, and then analysed after cooling at 4 °C for 24 h.

Sensory analysis

Yoghurt samples removed from the refrigerator (4 °C) were kept at room temperature (21–24 °C) for 10 min before sensory analysis. The sensory evaluation was carried out by 15 untrained panellists consisting of five department staff and 10 faculty students. Appearance, colour, consistency, mouthfeel, odour and taste were assessed using a 5-point hedonic scale (1 = dislike extremely, 2 = dislike, 3 = neither like nor dislike, 4 = like and 5 = like very much). The yoghurt samples with the highest total score (30 points) was evaluated as the most liked.

Colour measurement

The colour values were evaluated using Lovibond® SV 100 colorimeter (Amesbury, UK) [L^*: (lightness) white/black; a^*: redness/greenness; b^*: yellowness/blueness].

a_w measurement

a_w values were detected with Water Activity Meter (Novasina®, Pfäffikon, Switzerland).

Determination of organic acids

For the determination of organic acid types and levels of yoghurt samples, Kordiš-Krapež et al. (2001) methods were modified and used for the preparation of the samples and their reading to high-performance liquid chromatography (HPLC). A Supelco C18 solid phase cartridge was conditioned in 3 mL of methanol and then washed with 10 mL of distilled water. A 5 g yoghurt was homogenised with 5 mL of 2% H_3PO_4 and centrifuged at 3000 rpm for 2 min. Then, 1 mL of the supernatant was diluted with 1 mL extraction solution (pH 8.0, 0.01 M KH_2PO_4). A known quantity of 1 mL of this solution was passed through the cartridge and the eluate was collected in a tube. The cartridge was washed with 1 mL extraction solution. The eluate was collected, then a 20 µL was injected to the column using a SPD-10Avp UV–VIS detector (210 nm). A Teknokroma Tracer Extrasil ODS (2) (250 × 4.6 mm) 5-µm particle column (Türkiye) was used.

SPME flavour profile

Yoghurt sample (3.0 g) was taken into a 15 mL vial and kept at 40°C for 30 min. Solvent-free technique was used for the extraction of volatile substances. Extraction was carried out

with 75 µm carboxen-polydimethylsiloxane (CAR-PDMS; Merck, Darmstadt, Germany) fibre-vial injection. The sample was placed at 40°C and then PDMS fibre was exposed in the headspace for 30 min, allowing the aroma substances to pass into the fibre structure. Desorption of the volatile compounds to be extracted was performed in a gas chromatography–mass spectrometry (GC/MS) system. During desorption phase, the fibre was immersed in the injection block and kept at 250 °C for 2 min. Helium was used as the carrier gas at a flow rate of 1 mL/min. A DB-Wax column (60 m, 0.25 mm, 0.25 µm film thickness, JandW Scientific, Folsom, CA, USA) was used to separate the compounds. Gas chromatography (GC) oven temperature was initially kept at 40°C for 2 min (desorption period) and the temperature was increased to 70 °C at a rate of 5°C/min, and then kept at this temperature for 1 min. Then, the temperature was increased to 240°C at a rate of 10°C/min and held there for 30 min. The mass spectrometer (MS) mass range was set at 33–450 atomic mass unit (amu) (threshold value 1000) with a sampling rate of 1.11 scan/min. GC–MS analysis was performed with a Shimadzu QP-2010 mass spectrometer (Shimadzu Corporation, Kyoto, Japan) equipped with a Shimadzu GC-2010 gas chromatography system to determine the volatile aroma compounds in the product. The results are given as the % ratio of each peak to the total peak area.

Microbiological analysis

A 10 g sample of yoghurt was mixed with 90 mL 0.1% sterile peptone water in a sterile sample bag and homogenised for 2 min in a stomacher to prepare a 10^{-1} dilution. Serial dilutions were prepared from 10^{-1} dilution. Microbiological analyses were carried out by inoculating serial dilutions into suitable media using pour plate method. de Man Rogosa and Sharpe (MRS) agar (Merck, Darmstadt, Germany) containing 10% lactose was used for total lactic acid bacteria count, and M17 agar (Merck, Damstadt, Germany) was used for *Streptococcus* spp. count. Plates were incubated at 30°C for 72 h (ISO 15214 1998).

Statistical analysis

The study was completed by performing three independent repetitions. The data obtained from the study was analysed using SPSS 26 (SPSS Inc., Chicago, IL, USA) statistical program. Analysis of variance (ANOVA) was applied to determine the difference between groups showing homogeneous distribution as a result of homogeneity test. A Duncan multiple comparison test was used to detect the level of difference of the data showing significance ($P < 0.05$) by analysis of variance.

RESULTS AND DISCUSSION

Food preference is highly dependent on sensory characteristics, such as appearance, taste, colour, and mouthfeel. Sensory responses to the taste and physical properties of foods are important in choosing foods (Scudino et al. 2023). Compared with the control group, the panellists gave higher overall scores to the ultrasound-treated yoghurt samples ($P < 0.05$). The scores of the control, US + i and i + US groups were determined as 18.11, 22.85 and 25.45, respectively (Table 1). A difference was detected between the control and the ultrasound groups in terms of appearance, colour, consistency and mouthfeel ($P < 0.05$). As for odour and taste, no difference was found between the samples ($P < 0.05$). There was no sensory difference between US + i and i + US groups ($P > 0.05$). The higher appreciation of the samples subjected to ultrasound treatment can be explained by the fact that the ultrasound treatment applied to milk reduces serum release, increases viscosity (Akdeniz and Akalın 2023), provides fat homogenisation (Ektik and Tavşanlı 2021; Glover et al. 2022) and partially provides protein denaturation (Sfakianakis and Tzia 2017) in yoghurts. In a study on consumer perception of the use of new technologies, such as ultrasonication in food production, it was reported that the presence of a label on the product explaining the advantages of the new technology applied was positively received by the consumer and increased the acceptability of the product (Scudino et al. 2023). Therefore, yoghurts produced with ultrasound application may be perceived positively by the consumer and may increase the market share of the producer company.

According to the colour results of the yoghurt samples, a statistical difference ($P < 0.05$) was found between the control and ultrasound groups in terms of L^* value (lightness) (Table 2). The L^* values of US + i and i + US groups were 85.76 and 84.91, respectively. The results of both ultrasound groups were closer to the white colour L^*:100. No difference was observed between the samples in terms of a^* value ($P > 0.05$). There was a difference between the control group and i + US group in terms of b^* value

Table 1 Sensory scores of yoghurt samples obtained from milk that was subjected to ultrasound at 15% amplitude and 20 kHz frequency for 15 min before and after the starter culture was added. Control: Classical yoghurt. US + i: Ultrasound application before inoculation of the starter culture into milk. i + US: Ultrasound application after inoculation of the starter culture into milk.

Appearance	Control	US + i	i ± US
Colour	3.40 ± 0.18[a]	4.43 ± 0.11[b]	4.63 ± 0.8[b]
Consistency	2.36 ± 0.16[a]	4.16 ± 0.15[b]	4.63 ± 0.12[b]
Mouthfeel	2.56 ± 0.17[a]	3.8 ± 0.16[b]	4.36 ± 0.13[b]
Odour	3.66 ± 0.16	3.23 ± 0.22	3.80 ± 0.23
Taste	3.33 ± 0.18	2.90 ± 0.23	3.50 ± 0.21
Total	18.11[a]	22.85[b]	25.45[b]

[ab]The mean values with different letters in the same line are significantly different ($P < 0.05$).

($P < 0.05$). Lightness (L^*) value of the ultrasound applied samples was higher than the control group ($P < 0.05$), and they were closer to the white colour L^*:100. The yellowness (b^*) value was lower in the i + US group compared with the control group ($P < 0.05$). The white colour of milk is due to the presence of fat globules and casein micelles that scatter light in the visible spectrum. The energy produced by ultrasound can be absorbed or reflected by colloidal milk proteins and fat globules (Scudino et al. 2022; Kenari and Razavi 2021). Gürsoy et al. (2022) reported that there was no difference between the ultrasound-treated yoghurt samples and the control group in their colorimetry measurements. However, since ultrasound application has an effect on fat homogenisation, protein denaturation and viscosity of yoghurt, it is likely to have an effect less or much on the colour values as well. Scudino et al. (2022) reported that the L^* value in the milk they applied ultrasound (800 W, 19 KHz) was higher than the milk that was not applied ultrasound. They attributed this to the increase in luminocity due to the decrease in fat globule sizes. Similarly, in the present study, L^* value was found to be significantly higher in the ultrasound-treated groups (US + i and i + US) compared with the control group ($P < 0.05$). Considering that consumers prefer white yoghurt, it is expected that the average scores of white yoghurts will be higher (Table 1).

The amounts (mg/g) of seven organic acids, mainly lactic, acetic, citric, pyruvic, oxalic, succinic and ascorbic acid, were analysed in yoghurt samples. There was a significant difference in lactic, acetic, pyruvic and succinic acid levels between the control group and the ultrasound-treated groups ($P < 0.05$). The amounts of lactic acid, which is the most important organic acid in yoghurt, were 8.19, 11.97 and 12.0 in the control, US + i and i + US groups, respectively. The amounts of acetic, pyruvic and succinic acid were 0.26, 0.03 and 0.09 for the control group, 0.42, 0.04 and 0.01 for the US + i group, and 0.38, 0.05 and 0.1 for the i + US group, respectively (Table 3). Lactic acid and pyruvic acid, which are non-volatile flavour compounds of yoghurt, and acetic acid, one of the volatile organic acids, were detected at higher levels in the ultrasound groups compared with the control group, especially in the i + US group ($P < 0.05$). In previous studies (Nöbel et al. 2016), it was reported that ultrasound application caused the release of intracellular β-galactosidase from lactic acid bacterial cells and released β-galactosidase showed a higher lactose hydrolysis activity than in cells. They reported that this situation may cause maximum hydrolysis of lactose and high level of lactic acid production. This phenomenon may explain why high lactic acid levels were detected in the ultrasound-treated groups in the present study. On the other hand, Delgado et al. (2020) reported that the lactic acid value in yoghurts produced after ultrasound application to milk was similar to the control group, while Yuan et al. (2022) reported that acetic acid was higher in control group samples. Nguyen et al. (2012) reported that the ratios of lactic acid, acetic acid and pyruvic acid in fermented milk products containing different lactic acid bacteria vary according to lactic acid bacteria species and ultrasound application times. The discrepancy between the organic acid values in this study and the values found by other researchers is probably due to the difference in the lactic acid bacteria used and the ultrasound application times.

In SPME flavour profiles of yoghurt samples (Figure 2; Table 4), a significant difference was detected between the control and ultrasound-treated groups in terms of acetaldehyde, diacetyl, propane/acetone, acetoin, methyl heptyl

Table 2 Colour measurements and a_w values of yoghurt samples obtained from milk that was subjected to ultrasound at 15% amplitude and 20 kHz frequency for 15 min before and after the starter culture was added. Control: Classical yoghurt. US + i: Ultrasound application before inoculation of the starter culture into milk. i + US: Ultrasound application after inoculation of the starter culture into milk.

	Control	US + i	i ± US
L^* (black 0/white 100)	82.88 ± 0.61^b	85.76 ± 0.43^a	84.91 ± 0.43^a
a^* (redness+/greenness−)	−0.25 ± 0.9	−0.3 ± 0.6	−0.21 ± 0.23
b^* (yellowness+/blueness−)	4.53 ± 0.24^a	4.10 ± 0.18^{ab}	3.83 ± 0.18^b
a_w	0.97 ± 0.002	0.97 ± 0.002	0.97 ± 0.001

^{ab}The mean values with different letters in the same line are significantly different ($P < 0.05$).

Table 3 Organic acid compounds and amounts in yoghurt samples obtained from milk that was subjected to ultrasound at 15% amplitude and 20 kHz frequency for 15 min before and after the starter culture was added (mg/g). Control: Classical yoghurt. US + i: Ultrasound application before inoculation of the starter culture into milk. i + US: Ultrasound application after inoculation of the starter culture into milk.

	Control	US + i	i ± US
Lactic acid	8.19 ± 0.47^a	11.97 ± 1.79^b	12.19 ± 1.33^b
Acetic acid	0.26 ± 0.01^a	0.42 ± 0.02^b	0.38 ± 0.03^b
Citric acid	1.07 ± 0.18	1.32 ± 0.15	1.30 ± 0.2
Pyruvic acid	0.03 ± 0.0^a	0.04 ± 0.0^{ab}	0.05 ± 0.0^b
Oxalic acid	0.14 ± 0.02	0.17 ± 0.01	0.36 ± 0.27
Succinic acid	0.09 ± 0.01^b	0.01 ± 0.0^a	0.1 ± 0.0^b
Ascorbic acid	0.21 ± 0.07	0.17 ± 0.09	0.36 ± 0.12

^{ab}The mean values with different letters in the same line are significantly different ($P < 0.05$).

Table 4 SPME flavour profile of yoghurt samples obtained from milk that was subjected to ultrasound at 15% amplitude and 20 kHz frequency for 15 min before and after the starter culture was added (peak area %). Control: Classical yoghurt. US + i: Ultrasound application before inoculation of the starter culture into milk. i + US: Ultrasound application after inoculation of the starter culture into milk.

	Control	US + i	i ± US
	Ratios of volatile compounds		
Acetaldehyde	7.55 ± 0.12[a]	10.94 ± 0.48[c]	8.12 ± 0.17[b]
Diacetyl*	8.17 ± 0.33[a]	10.83 ± 0.67[b]	10.97 ± 1.15[b]
2 Propanone/acetone*	3.97 ± 0.66[a]	4.85 ± 0.37[b]	3.98 ± 0.87[a]
Acetoin*	6.51 ± 0.32[a]	8.93 ± 0.23[b]	9.57 ± 0.56[b]
Methyl heptyl ketone*	5.88 ± 0.03[a]	5.90 ± 0.07[a]	2.97 ± 0.07[b]
2–3 Pentanedione*	11.19 ± 1.85	13.75 ± 0.53	10.27 ± 1.22
2-Heptanone*	19.06 ± 2.48[b]	21.2 ± 1.63[b]	12.48 ± 0.06[a]
Ethyl acetate	3.19 ± 0.6b[a]	4.0 ± 0.1[a]	6.0 ± 0.1[b]
Ethanol	1.83 ± 0.01[a]	2.54 ± 0.25[b]	2.5 ± 0.01[b]
L-limonene + Other**	10.02 ± 2.41[b]	5.04 ± 0.28[a]	5.93 ± 1.87[a]

[abc]The mean values with different letters in the same line are significantly different ($P < 0.05$).

*Ketone.

**Other: Volatile aroma compounds that could not be detected in each group in the SPME aroma profile analysis.

ketone, ethanol, ethyl acetate and L-limonene values ($P < 0.05$). The levels (%) of acetaldehyde and diacetyl were 7.55, 8.17 for the control, 10.94, 10.83 for the US + i and 8.12, 10.97 for the i + US groups, respectively (Table 4). Ketone compounds, one of the volatile aroma compounds, were detected at the highest rate in all groups. Ketone compounds were followed by aldehydes and then ester compounds. Volatile aroma compounds that could not be detected in each group in the SPME aroma profile analysis and compounds that were not main aroma compounds of yoghurt, such as L-limonene, were named as "other" in Table 4.

Acetaldehyde, which is an effective aroma component in the formation of the characteristics taste and aroma of yoghurt, is an important quality factor in determining the quality of yoghurt. *Lactobacillus delbrueckii* subsp. *bulgaricus* is the most effective lactic acid bacteria in the formation of acetaldehyde in yoghurt fermentation (Farag et al. 2021). However, it produces higher levels of acetaldehyde when used with *Streptococcus thermophilus* (Sieuwerts et al. 2010). It is stated that the ideal acetaldehyde concentration for the characteristic taste and aroma formation in yoghurt varies between 8 and 25 ppm, and classical taste and aroma do not occur at concentrations below 4 ppm (Routray and Mishra 2011). Although higher level of acetaldehyde was detected in the US + i group compared with the control and i + US groups in this study ($P < 0.05$), acetaldehyde levels were found at ideal concentration values in all three groups.

Ketones (diacetyl, 2 propanone/acetone, 2–3 Pentanedione, 2 heptanone, acetoin), which are in the second place after aldehydes in the formation of the characteristic flavour and odour of yoghurt, are produced by the oxidation of unsaturated fatty acids, deterioration by heat and fermentation of yoghurt bacteria (Mu et al. 2021). In this study, ketone compounds were the most intensely detected volatile aromatic compounds. The higher scores of the ultrasound-treated yoghurt samples compared with the control samples by the panellists may be due to the high concentration of ketone compounds together with the acetaldehyde level. Similar to our results, Yuan et al. (2022) determined the highest rate of ketone compounds among volatile aromatic compounds in yoghurt samples in their study.

Another volatile aromatic compound found in yoghurt is ethanol, which is the end product in the breakdown of glucose and catabolism of amino acids. Ethanol is also a source of acetaldehyde, the main volatile compound in yoghurt (Cheng 2010). Similar to our study, Yuan et al. (2022) reported that ethanol levels were higher in ultrasound-treated yoghurt samples compared with the control group. In our study, the high rate of acetaldehyde in ultrasound groups with high ethanol content may be due to the fact that ultrasound application increases amino acid catabolism and glucose breakdown. There are studies proving that ultrasound accelerates bacterial lactose fermentation (Nöbel et al. 2016; Abesinghe et al. 2019).

Microbiological analysis of yoghurt samples at inoculation time (immediately after) and at 1st, 2nd, 3rd and 24th hours after inoculation showed that the numbers of total *Lactobacillus* spp. and *Streptococcus* spp. were similar ($P > 0.05$). The average number of total lactic acid bacteria was log 4.6 cfu/mL at inoculation time and log 7.2 cfu/mL at the end of 24 h. The average number of *Streptococcus* spp. was 4.7 $\log_{10}$ cfu/mL at inoculation and log 7.1 cfu/mL at the end of 24 h (Figure 1).

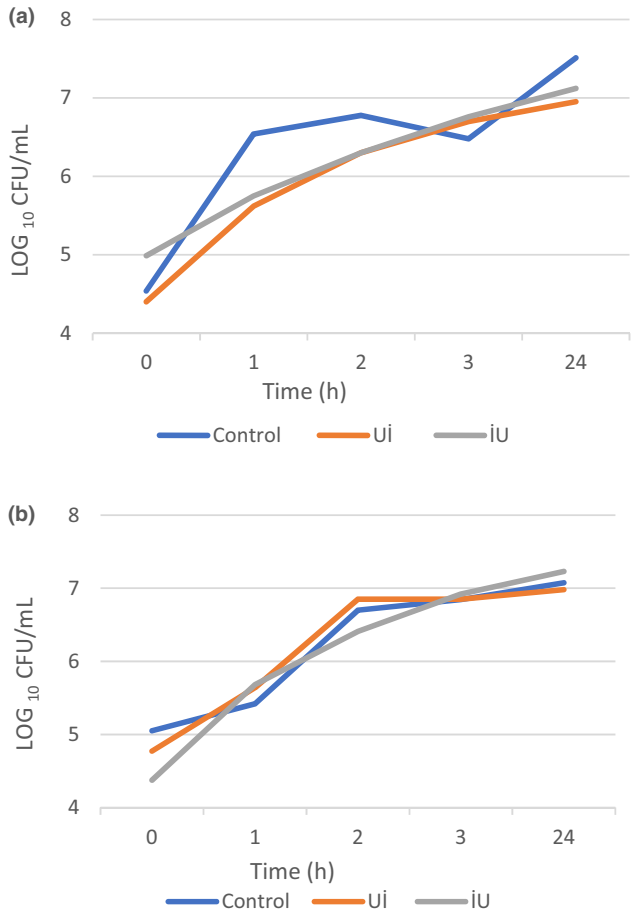

Figure 1 *Lactobacillus* spp. (a) and *Streptococcus* spp. (b) numbers in yoghurt samples applied ultrasound at 15% amplitude and 20 kHz frequency for 15 min before and after adding starter culture into milk. US + i: Ultrasound application before inoculation of the starter culture into milk. i + US: Ultrasound application after inoculation of the starter culture into milk. Control: Classical yogurt.

Depending on the intensity and duration of application, ultrasound treatment can have both acceleration and inhibition effects on microbial cell proliferation and viability (Abesinghe et al. 2019; Manyatsi et al. 2023). It has been reported that the inhibition effect is due to irreparable injuries to the cell wall caused by high intensities of ultrasound (Guimarães et al. 2019; Abesinghe et al. 2022). Balthazar et al. (2019) reported that application of ultrasound (20 Khz 78 W) to 40 mL of fresh sheep milk for 18 min increased the temperature to 63°C and caused a significant reduction in total mesophilic aerobic bacteria, lactobacilli and lactic streptococci. Tavsanli et al. (2022) reported that when they applied ultrasound with a frequency of 20 KHz and at 100% amplitude (200 W) to 100 mL of goat milk, the temperature rose above >70°C in the 5th minute and a significant decrease in *Lactobacillus* and *Streptococcus* numbers (>5.0 log cfu/mL) was achieved. There is controversy about the sensitivity of microorganisms to ultrasound. While some researchers have reported that Gram-positive bacteria are more resistant to ultrasound than Gram-negative bacteria (Scudino et al. 2022; Manyatsi et al. 2023), some have stated that cell size and shape are more important in sensitivity and resistance, while others have stated that the "softness" and "thickness" of the cell capsule are more important (Marchesini et al. 2015). Abesinghe et al. (2022) applied ultrasound (20 kHz 234 W) for 6 and 10 min immediately after the addition of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* to 50 mL buffalo milk and reported that the application provided a slightly increase in starter culture numbers and metabolic activities. In our study, no significant difference was observed between the control group and the ultrasound groups in terms of *Lactobacillus* spp. and *Streptococcus* spp. numbers during the fermentation at 43°C for 3 h and storage at 4°C for 24 h ($P > 0.05$). *Lactobacillus* spp. and *Streptococcus* spp. numbers reached log ≥7.0 cfu/g in all groups in this study. The low intensity of ultrasound applied in the present study may be the reason why there was no increase in LAB numbers compared with the study reporting that ultrasound application promoted an increase in LAB numbers. However, it should be noted that M17 agar was used for *Streptococcus* spp. counts in the present study. Mullan (2015) reported that some strains of *L. delbrueckii* subsp. *bulgaricus* can grow on M17 agar, and 23% of the *L. delbrueckii* subsp. *bulgaricus* strains tested in his study grew well on this agar. In addition, Süle et al. (2014) and Saccaro et al. (2011) showed that *L. delbrueckii* subsp. *bulgaricus* grew on M17 agar, although it was less in number than *S. thermophilus*. In the present study, bacterial counts were given as logarithms with base 10. In the study, even if some of the total colonies counted on M17 agar belong to *L. delbrueckii* subsp. *bulgaricus*, this will not cause serious deviations in logarithm with base 10. However, it would be appropriate to refer to the number of streptococci in M17 agar as the 'estimated streptococcal number' in the present study.

There was no difference between the control and ultrasound groups in terms of water activity (a_w) values ($P > 0.05$) (Table 2). It has been reported that the water activity of yoghurt is approximately 0.98 (Tapia et al. 2020). The a_w is an important factor in inhibiting or limiting the growth of microorganisms. There is no study evaluating the effect of ultrasound application on the water activity of yoghurt. According to the findings of the current study, it can be said that ultrasound treatment applied does not cause any change in the a_w value. This is important in terms of not adversely affecting the growth of lactic acid bacteria in yoghurt.

CONCLUSION

As a result, it was determined that the ultrasound treatment applied before and after the starter culture was added to the milk did not make a difference in terms of physicochemical and sensory properties of the yoghurt. It was observed that

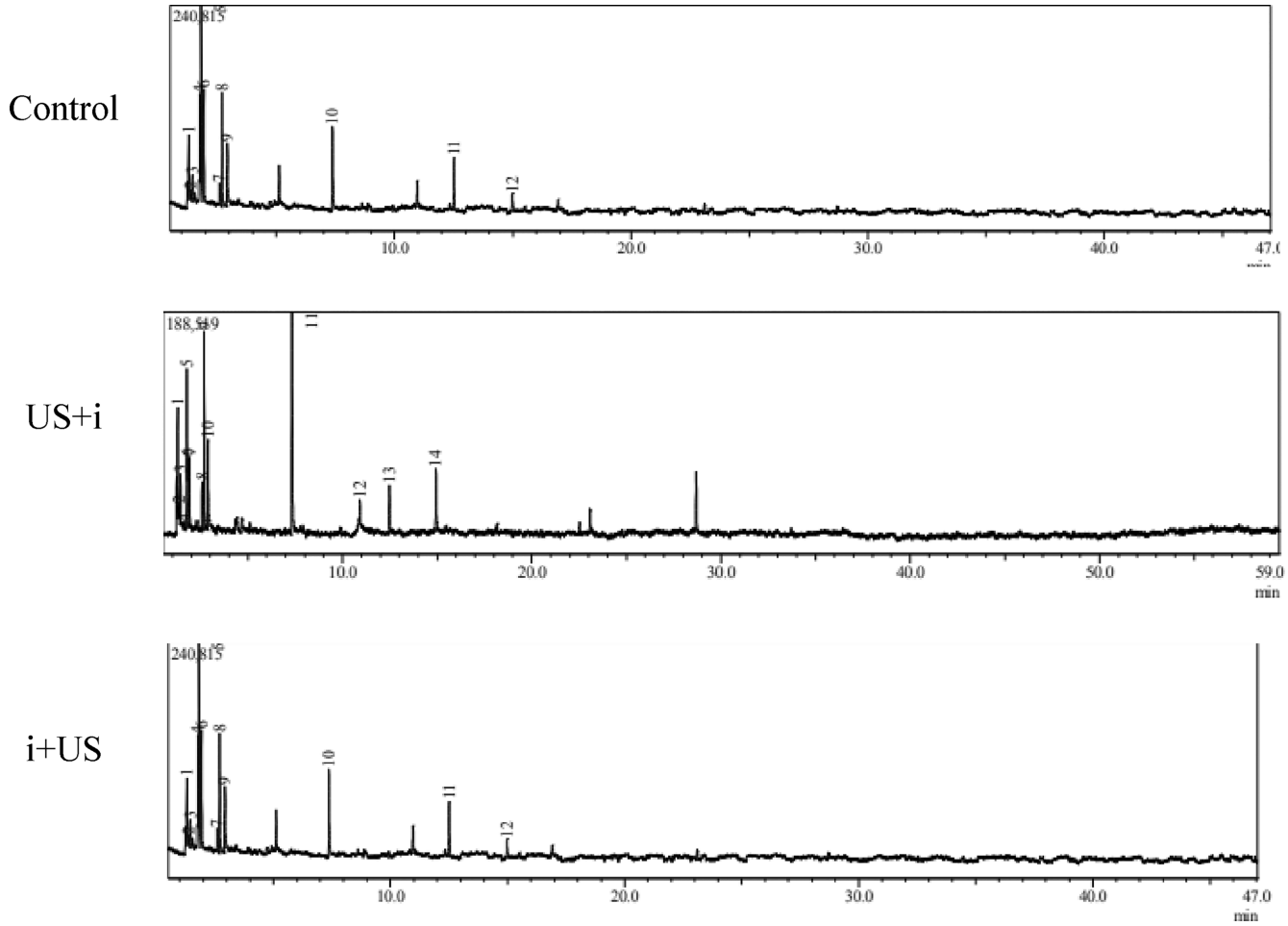

Figure 2 Chromatogram of yoghurt samples applied ultrasound at 15% amplitude and 20 kHz frequency for 15 min before and after adding starter culture into milk. Control: Classical yoghurt. US + i: Ultrasound application before inoculation of the starter culture into milk. i + US: Ultrasound application after inoculation of the starter culture into milk.

the application of low-intensity ultrasound to the milk improves the sensory properties of yoghurt and offers the chance to produce yoghurt with a whiter colour and more flavour components, which can be preferred more by consumers, compared with yoghurt produced by the traditional method. It would be appropriate to conduct research on the determination of ultrasonic parameters (frequency, amplitude, application time and temperature) to be applied to milk for the production of yoghurt with the ideal sensory and physicochemical properties.

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AUTHOR CONTRIBUTIONS

Hakan Tavşanlı: Conceptualization; investigation; methodology; project administration; writing – original draft. **Tevhide Elif Güner:** Investigation. **Berfin Altundal:** Investigation. **Nisanur Ektik:** Investigation. **Osman İrfan İlhak:** Conceptualization; methodology; writing – original draft; writing – review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

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

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