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Research Paper / Araştırma Makalesi

Physicochemical and Microbiological Properties of Ayran Produced from Milk Treated with Different Microbial Transglutaminase

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ABSTRACT

In this study, ayran was produced from milk treated with microbial transglutaminase (MTG) under different conditions. The aim of this work was to determine the effect of treating milk with two different MTGs (MTG-MP and MTG-YG), either before or after heat treatment at different conditions (at 4°C for 12 h, at 38°C for 3 h, and at 50°C for 1 h), on the physicochemical and microbiological properties of ayran. The highest number of grains and mean perimeter of grains were determined as 140.8 per 3 mL of the sample and 3.8 mm, respectively, in ayran samples produced from milk treated with MTG-MP at 50ºC for 1 h before heat treatment. During storage, the apparent viscosity and consistency coefficient values of ayran drinks ranged from 0.10 to 0.26 Pa.s and from 1.05 to 4.13 Pa.s^n, respectively. In comparison to ayran drinks produced from milk treated with MTG before heat treatment and after heat treatment, the latter had higher apparent viscosity and consistency coefficient values, as well as lower number of grains, mean perimeter of grains, visual roughness, syneresis, and flow behaviour index values. Considering the physicochemical properties of ayran, treating milk with MTG-MP at 38°C for 3 h prior to heat treatment might be preferable in comparison to treating at 4°C for 12 h or at 50°C for 1 h. Similarly, treating milk with MTG-YG at 50°C for 1 h prior to heat treatment might be preferable compared to treating it at 4°C for 12 h or at 38°C for 3 h. This study demonstrated that changing the type of MTG used and the treatment condition of MTG to milk used in ayran production resulted in insignificant differences in microbiological properties.

Keywords: Salty drinkable yoghurt, Microbial transglutaminase, Treatment conditions, Visual properties

Farklı Mikrobiyal Transglutaminaz Uygulanan Sütlerden Üretilen Ayranın Fizikokimyasal ve Mikrobiyolojik Özellikleri

ÖZ

Çalışmada, mikrobiyal transglutaminaz (MTG) kullanılarak farklı koşullar altında işlenen sütlerden ayran üretilmiştir. Çalışmanın amacı, ısıl işlemden önce (4°C'de 12 saat, 38°C'de 3 saat ve 50°C'de 1 saat) veya ısıl işlemden sonra iki farklı MTG'ye (MTG-MP ve MTG-YG) maruz bırakılan sütlerden üretilen ayranların fizikokimyasal ve mikrobiyolojik özellikleri belirlemektir. Isıl işlemden önce 50ºC'de 1 saat MTG-MP ile muamele edilen sütten üretilen ayran örneklerinde en yüksek tane sayısı ve ortalama tane çevresi değerleri sırasıyla 140.8/3 mL ve 3.8 mm olarak belirlenmiştir. Depolama süresince ayran örneklerinin görünür viskozite ve kıvam katsayısı değerleri sırasıyla 0.10-0.26 Pa.s ve 1.05-4.13 Pa.s^n arasında değişmiştir. Isıl işlemden önce MTG ile işlenen sütten üretilen ayran örnekleriyle ısıl işlemden sonra MTG eklenen sütlerden üretilen ayran örnekleri karşılaştırıldığında, ikincisinin görünür viskozite ve kıvam katsayısı değerleri daha yüksek, ayrıca granül sayısı, ortalama granül çevresi, görsel pürüzlülük, serum ayrılması ve akış davranış indeksi değerleri daha düşük olmuştur. Ayranın fizikokimyasal özellikleri bakımından, sütün ısıl işlemden önce 38°C'de 3 saat boyunca MTG-MP ile muamele edilmesi, 12 saat boyunca 4°C'de veya 1 saat boyunca 50°C'de muamele edilmesinden daha iyi olabilir. Benzer şekilde, sütün ısıl işlemden önce 1 saat boyunca 50°C'de MTG-YG'ye maruz bırakılması 12 saat boyunca 4°C'de veya 3 saat boyunca 38°C'de muamele edilmesinden daha iyi olabilir. Bu çalışma, ayran üretiminde kullanılan süte uygulanan MTG tipinin ve MTG'nin muamele koşulunun değiştirilmesinin ayranın mikrobiyolojik özelliklerinde istatistiksel olarak anlamlı bir fark yaratmadığını göstermiştir.

Anahtar Kelimeler: Tuzlu içilebilir yoğurt, Mikrobiyal transglutaminaz, Uygulama koşulları, Görsel özellikler

INTRODUCTION

Ayran, which is a yoghurt-based, fermented, and salty dairy product, is produced both industrially and traditionally [1]. In the industrial method, milk, whose total solids content is adjusted to about 8% by adding drinkable water, is fermented using yoghurt bacteria named *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, then salt is added and mixed. Traditionally, ayran is manufactured by the addition of water and salt to yoghurt [2]. Some physical quality characteristics of ayran, such as syneresis, viscosity, graininess (non-dispersible particles), and visual roughness (irregularities in protein gel), play a key role in its quality and consumer acceptability [3]. Casein micelles possess a negative charge in the natural pH of milk, but they exhibit a net positive charge in an acidic medium, leading to a tendency to clump together and agglomerate. Due to the low pH in fermented dairy products, including ayran, milk protein sedimentation is a usual problem [4]. Fermentation leads to the aggregation of casein micelles to form gel networks, which are disintegrated into weak particle clusters through stirring [5]. Controlling the aggregation formation during processing is necessary to prevent extensive particle aggregation, which is linked to common structural defects, including graininess [6]. Graininess and visual roughness are common structural defects in fermented dairy products, such as ayran [3, 7].

Dairy products with more desired qualities can be produced by altering the physicochemical properties of milk proteins through enzymatic cross-linking [8]. The application of microbial transglutaminase (MTG, EC 2.3.2.13, protein-glutamine γ-glutamyl-transferase) in fermented dairy products to improve physicochemical properties of milk proteins, including viscosity, gel strength, and water holding capacity [9]. MTG catalyses the isopeptide bonds between amino acids (glutamine and lysine) and peptides. It has the ability to form new intra- and inter-molecular cross-links, leading to the formation of covalently cross-linked protein polymers [10, 11]. In most fermented dairy products, the treatment with MTG occurs either before fermentation or simultaneously with fermentation [12]. MTG treatment of milk before fermentation offers the benefit of maintaining a constant pH throughout the cross-linking reaction, which opens up a greater range of potential treatment conditions, but it also necessitates extra processing time and a thermal inactivation step to inactivate the enzyme. When milk is treated with MTG simultaneously with the fermentation, MTG is gradually inactivated by acidification [13]. Although MTG activity decreases significantly below pH 5, it has been reported that residual MTG activity increased gel strength and viscosity during the storage of fermented dairy products. Exceeding tolerable levels of structural strengthening may lead to undesirable sensory qualities, particularly in stirred fermented dairy products such as ayran [8]. Although it is stated that fermented dairy products produced with MTG have a smooth texture [14-16], it has also been reported that they show a grainier structure than those produced without MTG, depending on the MTG concentration, degree of protein polymerisation, and protein and fat contents [12, 13, 17, 18].

In the production of fermented dairy drink, milk can be treated with MTG prior to or after heat treatment [19]. Whey proteins, unless denatured by heat, have less tendency to form cross-link reactions by MTG, but caseins, the main milk proteins, are a suitable substrate for MTG [14]. Additionally, in heat-treated milk, casein polymerisation by MTG was observed, whereas this reaction was nearly absent in unheated milk due to the presence of indigenous MTG inhibitors [20]. However, the addition of glutathione, a food-grade reducing agent that prevents the inhibition of the MTG, can improve the low reactivity of MTG in unheated milk [13]. In this study, two different MTGs were used: one containing glutathione and one without glutathione. Moreover, it was hypothesised that the application conditions of the MTG to milk (before or after heat treatment, temperature and time) affect the MTG activity and change the properties of ayran. In the limited number of studies on the use of MTG in ayran production, studies have reported that MTG treatment conditions influence the physicochemical and microbiological properties of ayran [21, 22]. However, to our knowledge, no study has been conducted to determine the effect of using MTG on the graininess and visual roughness of ayran. In the present study, the aim is to determine the effect of treatment of milk with two different MTGs, either before heat treatment at different treatment conditions or after heat treatment concurrently with the inoculation of yoghurt bacteria, on the physicochemical properties, including the graininess and visual roughness, and microbiological properties of ayran.

MATERIALS and METHODS

Raw cow milk (total solids content of 11.6%, protein content of 3.2%, fat content of 3.1%, ash content of 0.7%, and pH value of 6.6) was obtained from the dairy plant of the Faculty of Agriculture, Akdeniz University. MTG Activa® MP (MTG-MP, standard type) and Activa® YG (MTG-YG, high functional type) with a declared specific activity of 1000 U g^{-1} protein were donated by Ajinomoto Co., Inc. (Tokyo, Japan). In addition to MTG, Activa® MP contains maltodextrin and lactose. On the other hand, Activa® YG consists of yeast extract, maltodextrin, lactose, vegetable oil, and MTG. The difference between the two MTGs used in this study is that MTG-MP does not contain glutathione, while MTG-YG contains glutathione, whose exact amount is not known but is enough to inactivate MTG inhibitors [23]. The yoghurt starter culture (Danisco YO-MIX 511) was purchased from Türker Industry Technic Machine Inc. (İstanbul, Türkiye).

Treatment of Milk with MTG and Ayran Production

In the production of ayran, raw milk was heated at 55°C and skimmed to about 0.1% fat content using a cream separator (G140 model, SMS Ltd. Co., Kayseri, Türkiye). Then, the total solids content of skimmed milk was adjusted to about 6% by adding deionised water and named ayran milk. Ayran milk was thermalised at 67°C for 20 s to inactivate MTG inhibitors in it and subsequently cooled to 4°C. Dannenberg and Kessler [24] reported that these thermalisation conditions do not lead to any measurable denaturation of whey proteins. Ayran milk was treated with MTG-MP or MTG-YG at a concentration of 0.5 U g^{-1} protein. The MTGs were added to ayran milk either before or after heat treatment at 85°C for 15 min. Treatment with MTG of milk prior to heat treatment was performed at three temperature-time combinations. Treatment with MTG of milk after heat treatment was carried out simultaneously with the inoculation of the starter culture at 42°C. The treatment conditions for the MTGs were decided using the information on the product specification sheet by Ajinomoto Co., Inc. The ayran milk was divided into nine parts prior to MTG treatments. The first and second parts were cooled to 4°C and treated with MTG-MP and MTG-YG, respectively, for 12 h. The third and fourth parts were heated to 38°C and treated with MTG-MP and MTG-YG, respectively, for 3 h. The fifth and sixth parts were heated to 50°C and treated with MTG-MP and MTG-YG, respectively, for 1 h. Following the MTG treatments, the first six parts were heat treated at 85°C for 15 min and then cooled to 42°C. The seventh and eighth parts were heat treated at 85°C for 15 min, then cooled to 42°C and treated with MTG-MP and MTG-YG, respectively. Following the treatment with MTG, no heat treatment was conducted on the seventh and eighth parts for enzyme inactivation. The ninth part was heat treated at 85°C for 15 min, then cooled to 42°C, which was used in the production of the control sample without MTG treatment. The milks at 42°C were without delay inoculated with 0.1 g L^{-1} of the yoghurt starter culture and then incubated at 42°C until pH reached about 4.6. After the incubation period, all the samples were cooled to 20°C, 0.8% salt (Billur Tuz Co. Ltd., İzmir, Türkiye) was added, and mixed for 2 min using a mechanical mixer (Bosch, Mixxo Quattro MSM 7700, Jesenice, Slovenia). Subsequently, the ayran samples were packaged in 170-mL polystyrene cups with lids and kept at 4°C for 15 days. The process flow diagram for the production of ayran from milk, with and without MTG treatment, is presented in Figure 1. Ayran samples were coded as follows: MPBH-4/12 and YGBH-4/12, which were ayran, produced from milks treated with MTG-MP and MTG-YG at 4°C for 12 h before heat treatment, respectively; MPBH-38/3 and YGBH-38/3, which were ayran, produced from milks treated with MTG-MP and MTG-YG at 38°C for 3 h before heat treatment, respectively; MPBH-50/1 and YGBH-50/1, which were ayran, produced from milks treated with MTG-MP and MTG-YG at 50°C for 1 h, respectively. Meanwhile, the ayran samples produced from milks treated with MTG-MP and MTG-YG after heat treatment were coded as MPAH and YGAH, respectively, while the control ayran sample produced from milk without MTG treatment was coded as C.

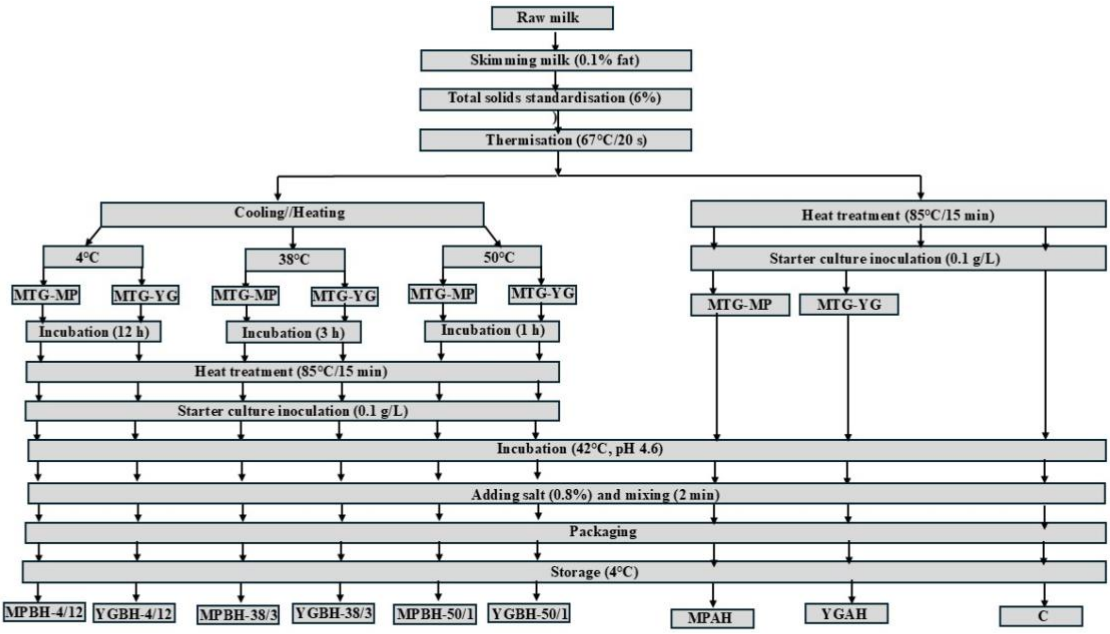

Figure 1. The process flow diagram for the production of ayran from milk with and without MTG treatment

Physicochemical and Microbiological Analyses

The pH values of raw cow milk and ayran samples were determined with a digital pH meter (Thermo Scientific Orion 2-Star, Bremen, Germany). The total solids, protein, fat, and ash contents and titratable acidity of the raw cow milk and ayran samples were determined by the gravimetric [25], Kjeldahl [26], Gerber [27], gravimetric

[28], and titration methods [29]. The number of grains and the mean perimeter of grains as a measure of graininess were determined by image analysis according to the method described by Küçükçetin, Weidendorfer, and Hinrichs [30]. A digital camera (EOS 450D, Canon Inc., Tokyo, Japan) was used to capture real-scale images of the ayran samples under the constant illumination of a digital imaging box (B430, Sanoto, Guangdong, China). Using an image processing software (Bs200, BAB Image Analysing Systems Inc., Ankara, Türkiye), the number of grains indicating a perimeter of grains greater than 1.0 mm per 3 mL ayran was determined. The visual roughness (Rvis) as an additional descriptive parameter for the graininess of the ayran samples was calculated using a method by Küçükçetin et al. [30]. The mean absolute intensity deviation of each pixel from a median smoothed intensity line through the ayran image was defined as the Rvis of the ayran sample. The measurement of the rheological parameters of the ayran samples was carried out using a Brookfield R/S plus stress-controlled rheometer (Brookfield Engineering, Middleboro, MA, USA) with double gap concentric cylinder geometry (DG3) and a Brookfield temperature control (TC-502) set to a constant 4°C. The viscosity measurements at a shear rate of 50 s^{-1} were recorded as the apparent viscosity values of the ayran samples [1]. The Rheo3000 software (Rheotec Messtechnik GmbH, Berlin, Germany) was used to obtain rheological parameters (the apparent viscosity, consistency coefficient and flow behaviour index) of the yoghurt samples and to find a suitable rheological model fitting the experimental shear stress-shear rate. The syneresis value was determined by measuring the volume of separated serum at the top after the ayran samples were transferred to 50 mL graduated cylinders and stored at 4°C for 15 days [31]. The viable counts of *Lactobacillus* spp. were determined using MRS agar (pH 6.5; Merck KGaA, Darmstadt, Germany). MRS agar plates were incubated at 37°C anaerobically for 72 h. M17 agar (Merck KGaA) containing 1% (w/w) lactose was used to determine the viable counts of *Streptococcus* spp., and the plates were incubated aerobically at 37°C for 48 h [32]. The rheological and microbiological properties, pH, and titratable acidity of the ayran samples were determined on days 1 and 15 of storage, while the syneresis, number of grains, perimeter of grains, and Rvis were conducted on day 15 of storage.

Statistical Analysis

Each experiment was conducted in duplicate, and the data were presented as the means ± standard deviations. SAS statistical software (release for Windows, SAS Institute Inc., Cary, NC, USA) and IBM SPSS statistical software version 23 (SPSS Inc., Chicago, IL, USA) were used to analyse the data. The Kolmogorov-Smirnov test was used to analyse the normal distribution of data. The treatments' means were compared using Duncan's multiple range test.

RESULTS and DISCUSSION

Total solids, protein, and ash contents of the ayran samples ranged from 6.67 to 7.01%, from 2.09 to 2.38%, and from 0.45 to 0.54%, respectively. In the present study, the drop in pH and rise in titratable acidity values occurred in the ayran samples during the storage period (Table 1).

Table 1. Some physicochemical characteristics of ayran samples produced from milk with and without MTG treatment

Samples*	Storage period (days)	pH	Titratable acidity (%)	Apparent viscosity (Pa.s)	Consistency coefficient ($Pa.s^n$)	Flow behaviour index (n)
MPBH-4/12	1	4.47±0.02Aa	0.56±0.01Ba	0.11±0.00Ah	1.15±0.10Aef	0.47±0.01Ba
	15	4.24±0.03Ba	0.62±0.02Aa	0.10±0.00Bg	0.83±0.03Bg	0.54±0.02Aa
YGBH-4/12	1	4.46±0.02Aa	0.55±0.01Ba	0.16±0.01Ae	1.58±0.04Ad	0.34±0.01Bd
	15	4.24±0.02Ba	0.63±0.03Aa	0.14±0.00Bed	1.28±0.08Be	0.38±0.01Ac
MPBH-38/3	1	4.45±0.01Aa	0.56±0.01Ba	0.12±0.00Ag	1.20±0.04Aef	0.42±0.02Bb
	15	4.26±0.03Ba	0.60±0.01Aa	0.11±0.00Bf	0.89±0.03Bg	0.47±0.01Ab
YGBH-38/3	1	4.47±0.03Aa	0.56±0.01Ba	0.17±0.01Ad	2.01±0.13Ac	0.29±0.01Be
	15	4.24±0.01Ba	0.62±0.01Aa	0.15±0.01Bd	1.56±0.08Bd	0.33±0.03Ad
MPBH-50/1	1	4.46±0.01Aa	0.55±0.02Ba	0.15±0.01Af	1.34±0.05Ae	0.37±0.01Bc
	15	4.27±0.01Ba	0.61±0.01Aa	0.14±0.01Be	1.05±0.02Bf	0.41±0.02Ac
YGBH-50/1	1	4.47±0.02Aa	0.54±0.01Ba	0.19±0.01Ac	2.50±0.11Ab	0.25±0.02Bf
	15	4.26±0.02Ba	0.61±0.01Aa	0.18±0.01Bc	2.03±0.03Bc	0.30±0.02Ae
MPAH	1	4.44±0.03Aa	0.54±0.01Ba	0.20±0.00Ab	2.84±0.09Aa	0.19±0.01Bg
	15	4.24±0.03Ba	0.61±0.01Aa	0.19±0.00Bb	2.33±0.08Bb	0.25±0.01Af
YGAH	1	4.44±0.01Aa	0.56±0.01Ba	0.22±0.01Aa	2.92±0.22Aa	0.17±0.02Bg
	15	4.24±0.01Ba	0.62±0.02Aa	0.21±0.01Ba	2.47±0.14Ba	0.22±0.01Af
C	1	4.47±0.03Aa	0.54±0.03Ba	0.11±0.01Ag	1.06±0.08Af	0.48±0.03Ba
	15	4.26±0.03Ba	0.61±0.02Aa	0.10±0.01Bg	0.78±0.05Bg	0.55±0.02Aa

*MPBH-4/12: The ayran produced from milk treated with MTG-MP at 4ºC for 12 h prior to heat treatment, YGBH-4/12: The ayran produced from milk treated with MTG-YG at 4ºC for 12 h prior to heat treatment, MPBH-38/3: The ayran produced from milk treated with MTG-MP at 38ºC for 3 h prior to heat treatment, YGBH-38/3: The ayran produced from milk treated with MTG-YG at 38ºC for 3 h prior to heat treatment, MPBH-50/1: The ayran produced from milk treated with MTG-MP at 50ºC for 1 h prior to heat treatment, YGBH-50/1: The ayran produced from milk treated with MTG-YG at 50ºC for 1 h prior to heat treatment, MPAH: The ayran produced from milk treated with MTG-MP after heat treatment, YGAH: The ayran produced from milk treated with MTG-YG after heat treatment, C: The control ayran sample produced from milk without MTG treatment. Values are the means ± standard deviations; means with different capital letters in each column for the same sample are significantly different (P < 0.05); means with different small letters in each column on the same storage day are significantly different (P < 0.05).

The metabolism of lactose by *Streptococcus* spp. and *Lactobacillus* spp. resulted in an increase in the concentrations of organic acids, leading to a fall in pH values and an increase in titratable acidity values of stirred yoghurts during storage [33]. This phenomenon, known as post-acidification, is typically caused by the acidification activity of yoghurt bacteria [34]. No statistically significant difference in both the pH and titratable acidity values was found among the ayran samples stored for one day and those stored for 15 days. These results are consistent with Sanlı et al. [22], who found no significant differences among ayran samples with and without MTG in respect to pH and titratable acidity values through the storage period of 20 days. On the other hand, Ozer et al. [35] found that MTG treatment significantly affected the pH and titratable values of yoghurt and decreased the growth rate of yoghurt bacteria, with the concentration of MTG playing a key role in this. They also reported that increasing MTG concentration slowed down the growth of yoghurt bacteria, which was presumably the reason for the delayed acidity progression in yoghurt.

Real-scale images of the ayran samples stored for 15 days are shown in Figure 2. The number of grains and mean perimeter of grains of the 15-day-stored ayran samples produced from milk treated with/without MTG varied from 84.5 to 140.8 per 3 mL of the sample and from 2.2 to 3.8 mm, respectively (Figure 3). The visual roughness values of the ayran samples stored for 15 days, as presented in Figure 4, ranged between 2.1 and 7.3 units of AID_m (mean absolute intensity deviation). Regardless of the use of different MTGs, the number of grains, mean perimeter of grains, and visual roughness values of the ayran samples made from milk treated with MTG after heat treatment were significantly ($p<0.05$) lower than those of other ayran samples. The interactions between denatured whey proteins and micellar casein surfaces are often enhanced by heat treatment of whey protein in milk through κ-casein–whey-protein sulphydryl interchange. In cross-linked casein micelles, the denatured whey protein layer may function as a protective colloid, facilitating integration into the three-dimensional gel network. This inhibits or reduces the formation of grainy structures as surface hydrophobicity decreases. The reduced hydrophobic interactions resulted in the formation of stable and firm yoghurt gel structures [12, 13].

The ayran samples made from milk treated with MTG-MP or MTG-YG at 50ºC for 1 h before the heat treatment had the highest number of grains, the highest mean perimeter of grains, and the highest visual roughness values. A mild preheat treatment of milk at 50°C for 30 min resulted in an approximate 11% decrease in the content of ionised calcium [36]. Ramasubramanian et al. [37] reported that a decrease in calcium of more than 10% in milk before heat treatment increased the graininess of stirred yoghurt. One possible explanation for this detection, made by the same authors, is as follows: The reduction in calcium content in milk at this level is anticipated to lead to the dissolution of colloidal calcium phosphate, resulting in the release of loosely bound casein aggregates from the casein micelle while preserving its overall integrity. Because of this, the milk has free casein aggregate particles that are part of the serum and not the casein micelles. Upon heat treatment of this milk, the whey proteins denature and attach to the surfaces of the casein micelles; however, some of these denatured whey proteins may also attach to the free casein aggregate particles in the serum. The whey protein-casein aggregates thus formed do not become part of the micelle and do not participate in the gel network, while the micelles integrate into the gel during acid coagulation. Instead, they may remain as dense protein particles within the continuous yoghurt coagulum.

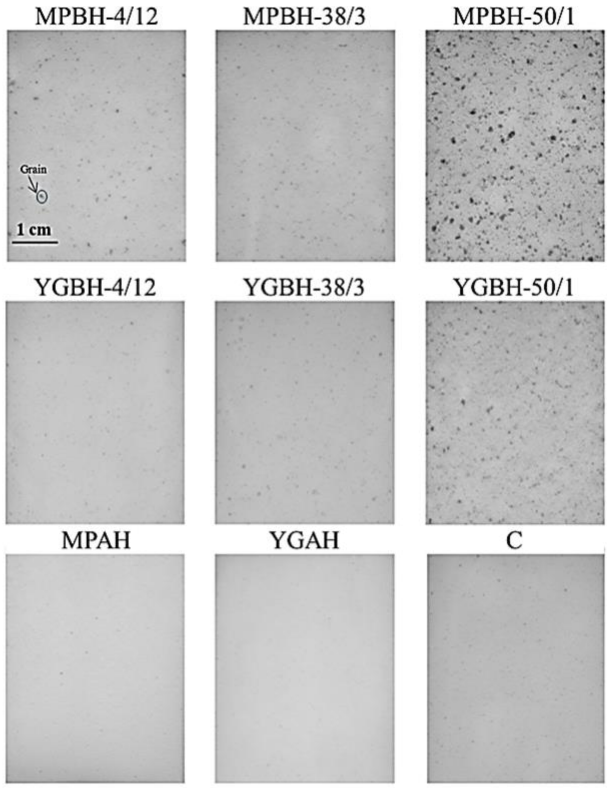

Figure 2. Real-scale images of the ayran samples (MPBH-4/12: The ayran produced from milk treated with MTG-MP at 4ºC for 12 h prior to heat treatment, YGBH-4/12: The ayran produced from milk treated with MTG-YG at 4ºC for 12 h prior to heat treatment, MPBH-38/3: The ayran produced from milk treated with MTG-MP at 38ºC for 3 h prior to heat treatment, YGBH-38/3: The ayran produced from milk treated with MTG-YG at 38ºC for 3 h prior to heat treatment, MPBH-50/1: The ayran produced from milk treated with MTG-MP at 50ºC for 1 h prior to heat treatment, YGBH-50/1: The ayran produced from milk treated with MTG-YG at 50ºC for 1 h prior to heat treatment, MPAH: The ayran produced from milk treated with MTG-MP after heat treatment, YGAH: The ayran produced from milk treated with MTG-YG after heat treatment, C: The control ayran sample produced from milk without MTG treatment).

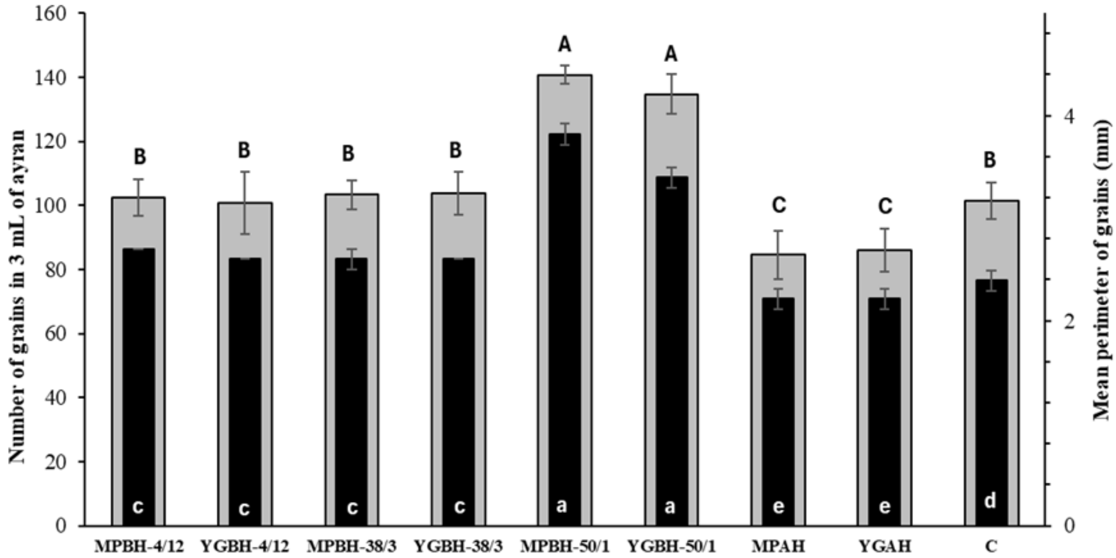

Figure 3. Number ( ) and mean perimeter ( ) of grains in the ayran samples obtained from milk with and without MTG treatment after 15 days of storage (The bars represent mean values, and the error bars represent the standard deviation of the mean. Different capital letters for the light shaded bars and small letters for the dark shaded bars indicated that the means are significantly different ($p<0.05$). MPBH-4/12: The ayran produced from milk treated with MTG-MP at 4ºC for 12 h prior to heat treatment, YGBH-4/12: The ayran produced from milk treated with MTG-YG at 4ºC for 12 h prior to heat treatment, MPBH-38/3: The ayran produced from milk treated with MTG-MP at 38ºC for 3 h prior to heat treatment, YGBH-38/3: The ayran produced from milk treated with MTG-YG at 38ºC for 3 h prior to heat treatment, MPBH-50/1: The ayran produced from milk treated with MTG-MP at 50ºC for 1 h prior to heat treatment, YGBH-50/1: The ayran produced from milk treated with MTG-YG at 50ºC for 1 h prior to heat treatment, MPAH: The ayran produced from milk treated with MTG-MP after heat treatment, YGAH: The ayran produced from milk treated with MTG-YG after heat treatment, C: The control ayran sample produced from milk without MTG treatment)

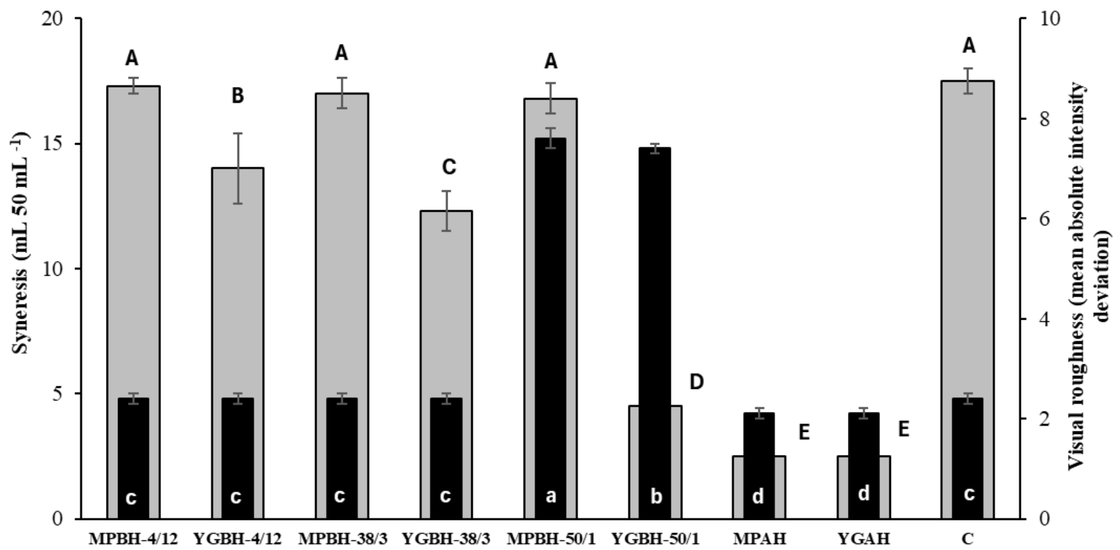

Figure 4. Syneresis ( ) and visual roughness ( ) of the ayran samples obtained from milk with and without MTG treatment after 15 days of storage (The bars represent mean values, and the error bars represent the standard deviation of the mean. Different capital letters for the light shaded bars and small letters for the dark shaded bars indicated that the means are significantly different ($p<0.05$). MPBH-4/12: The ayran produced from milk treated with MTG-MP at 4ºC for 12 h prior to heat treatment, YGBH-4/12: The ayran produced from milk treated with MTG-YG at 4ºC for 12 h prior to heat treatment, MPBH-38/3: The ayran produced from milk treated with MTG-MP at 38ºC for 3 h prior to heat treatment, YGBH-38/3: The ayran produced from milk treated with MTG-YG at 38ºC for 3 h prior to heat treatment, MPBH-50/1: The ayran produced from milk treated with MTG-MP at 50ºC for 1 h prior to heat treatment, YGBH-50/1: The ayran produced from milk treated with MTG-YG at 50ºC for 1 h prior to heat treatment, MPAH: The ayran produced from milk treated with MTG-MP after heat treatment, YGAH: The ayran produced from milk treated with MTG-YG after heat treatment, C: The control ayran sample produced from milk without MTG treatment)

These small particles, which have greater mobility compared to micelles in milk, can undergo excessive rearrangement during the acid coagulation process and place the casein micelle network under extreme local stress. Given that this kind of local stress can result in fractures when the gel network is formed by acid coagulation and graininess forms after stirring, these small protein aggregates might cause graininess to be promoted. Another explanation for the highest number of grains, the highest mean perimeter of grains, and the highest visual roughness values in the ayran samples made from milk treated with MTG-MP or MTG-YG at 50ºC for 1 h before the heat treatment was the change in fermentation time due to the decrease in the amount of calcium in the milk. In the current study, fermentation time ranged between 270 and 315 min for the ayran samples. Fermentation times for the ayran samples made from milk treated with MTG-MP or MTG-YG at 50ºC for 1 h before the heat treatment were lower than those of other ayran samples (data not shown). The reduction of calcium ions in milk results in the dissolution of micellar calcium phosphate, which might indirectly influence the rate of acidification and thereby impact acid coagulation [38]. As graininess increases with an increased rate of acidification and slight changes in the acid coagulation process, the reduction of calcium may enhance the formation of grains by changing the acidification process [37].

Table 1 displays the apparent viscosity values of the ayran samples. The apparent viscosity values of the ayran samples produced from milk treated with MTG simultaneously with the inoculation of the starter culture following the heat treatment were higher than those produced from milk treated with MTG before the heat treatment. When comparing the ayran samples produced from milk treated with MTG before heat treatment, the apparent viscosity values of the ayran samples produced from milk treated with both MTGs at 50°C for 1 h were higher. Motoki and Seguro [39] reported that the optimum temperature for MTG activity was 50°C. Additionally, this may be due to the decrease in ionised calcium content by a mild preheat treatment of milk at 50°C for 30 min. However, the level of decrease in calcium probably did not result in massive micelle disruption [37]. When all samples were compared, it was determined that the apparent viscosity values of the ayran samples produced from milk treated with MTG-YG were higher than those produced from milk treated with MTG-MP. The apparent viscosity values of the ayran samples decreased with the storage period. Degradation of gel structure because of post-acidification and proteolysis by the yoghurt bacteria may be the cause of the reduction in apparent viscosity during storage at 4ºC [40].

For all ayran samples, the correlation coefficients (R^2) exceeded 0.92 for the Ostwald de Waele model (Equation 1) that was used in the description of the rheological properties of the samples.

$$\mu = K\,(\gamma)^{n-1} \qquad (1)$$

where μ, K, γ, and n refer to the apparent viscosity (Pa.s), the consistency index (Pa.s^n), shear rate (s^{-1}), and the flow behaviour index (dimensionless), respectively [41]. In this study, all ayran samples displayed a decrease in apparent viscosity due to the increase in shear rate, as shown in Figure 5, which shows how the apparent viscosity changes with the shear rate in the ayran samples stored for 15 days. Additionally, after fitting the Ostwald de Waele model, the flow behaviour index values of the ayran samples were below 1, ranging from 0.19 to 0.55 during a 15-day storage period, as seen in Table 1. These results indicated that the ayran samples exhibited non-Newtonian pseudoplastic fluid behaviour. The non-Newtonian pseudoplastic behaviour was found for all ayran samples, as the texture of ayran was influenced by weak physical bonding, electrostatic interactions, and hydrophobic interactions [42]. The consistency coefficient values for the ayran samples are presented in Table 1. The ayran samples produced from milk treated with MTG after heat treatment had higher consistency coefficient values and lower flow behaviour index values than those of the ayran samples produced from milk treated with MTG prior to the heat treatment. The incorporation of MTG-YG before or after heat treatment of milk resulted in considerable changes in the rheological properties, with an increase in the consistency coefficient and a decrease in the flow behaviour index of the ayran samples. In the comparison of the ayran samples produced from milk treated with MTG before heat treatment, the samples produced from milk treated with both MTGs at 50°C for 1 h had higher consistency coefficient values and lower flow behaviour index values. The consistency coefficient of the samples tended to decrease, whereas the flow behaviour index tended to increase during storage. Moreover, the reduction in values for the consistency coefficient and the increase in values for the flow behaviour during the storage period may be attributed to the same reasons previously discussed, which lead to a reduction in the apparent viscosity values of the ayran samples.

Figure 4 shows the syneresis values of the ayran samples stored for 15 days. The ayran samples made from milk treated with both MTG-MP and MTG-YG concurrently with the inoculation of the starter culture after the heat treatment had the lowest syneresis values. Özrenk [43] reported that while MTG may cross-link milk proteins in unheated skim milk, heat treatment of milk prior to MTG addition increased the susceptibility of milk proteins to cross-linking. The syneresis values of the ayran samples produced from milk treated with MTG-YG before heat treatment were lower than those produced from milk treated with MTG-MP before heat treatment. No statistically significant difference in the syneresis values was found between the control sample and the ayran samples made from milk treated with MTG-MP before heat treatment. Upon comparison of the syneresis values of the ayran samples produced from milk treated with MTG-YG before the heat treatment, it was determined that the ayran samples produced from milks treated with MTG-YG at 4ºC for 12 h exhibited higher syneresis values. This was followed by the samples from milk treated with MTG-YG at 38ºC for 3 h, and lastly, those from milk treated with MTG-YG at 50ºC for 1 h. The data indicate that the incorporation of MTG-YG before or after heat treatment of milk, along with the incorporation of

MTG-MP only after heat treatment of milk, significantly diminished syneresis in ayran. This is because cross-links between the milk proteins by MTG cause the reduction in gel permeability. Furthermore, the water-holding capacity of gel is improved by MTG [22, 44].

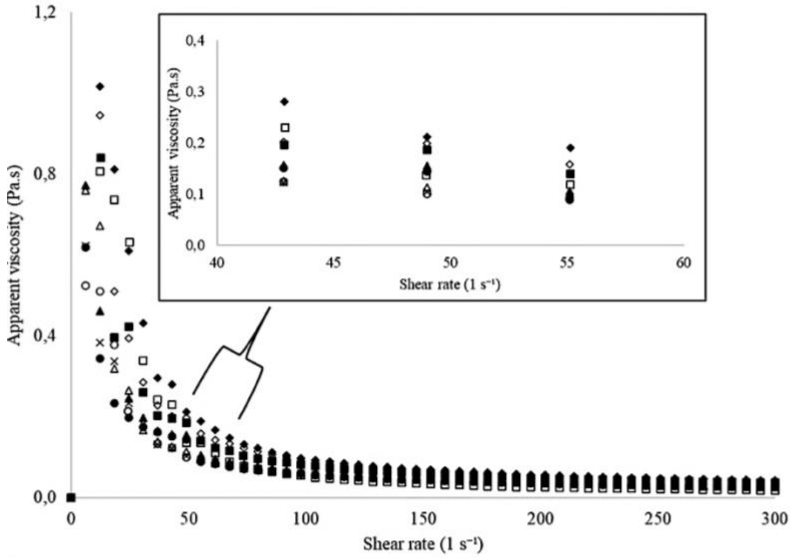

Figure 5. The changes in the apparent viscosity with the shear rate in the ayran samples stored for 15 days
(○:The ayran produced from milk treated with MTG-MP at 4ºC for 12 h prior to heat treatment, ●:The ayran produced from milk treated with MTG-YG at 4ºC for 12 h prior to heat treatment, Δ:The ayran produced from milk treated with MTG-MP at 38ºC for 3 h prior to heat treatment, ▲:The ayran produced from milk treated with MTG-YG at 38ºC for 3 h prior to heat treatment, □:The ayran produced from milk treated with MTG-MP at 50ºC for 1 h prior to heat treatment, ■:The ayran produced from milk treated with MTG-YG at 50ºC for 1 h prior to heat treatment, ◊:The ayran produced from milk treated with MT◊G-MP after heat treatment, ♦:The ayran produced from milk treated with MTG-YG after heat treatment, X: The control ayran sample produced from milk without MTG treatment.)

Table 2 displays the counts of *Streptococcus* spp. and *Lactobacillus* spp. in the ayran samples stored for 15 days. The counts of *Streptococcus* spp. and *Lactobacillus* spp. in the ayran samples produced from milk treated with/without MTG decreased during 15 days of storage. In accordance with the Fermented Dairy Products Communique of the Turkish Food Codex, which set a minimum content of yoghurt bacteria of 10^6 cfu g^{-1} for ayran, the viable numbers of *Streptococcus* spp. and *Lactobacillus* spp. in the ayran samples exceeded 10^6 cfu g^{-1} throughout storage [45]. However, although statistically significant, the differences in counts between samples due to changes in the type of MTG used or MTG treatment condition were less than 0.5 log cfu g^{-1}, which could be attributed to errors in sampling or minor differences in plate counts. The differences at this level were considered not microbiologically meaningful [46].

Table 2. Microbiological properties of the ayran samples produced from milk with and without MTG treatment

Samples*	Storage period (days)	Count of *Streptococcus* spp. (log cfu mL^{-1})	Count of *Lactobacillus* spp. (log cfu mL^{-1})
MPBH-4/12	1	9.00±0.01Aa	7.99±0.01Ab
	15	8.56±0.01Bab	7.64±0.02Bb
YGBH-4/12	1	9.03±0.08Aa	7.87±0.02Acd
	15	8.55±0.02Bab	7.62±0.04Bb
MPBH-38/3	1	8.74±0.01Ad	7.80±0.05Ade
	15	8.53±0.01Bab	7.52±0.03Bc
YGBH-38/3	1	8.98±0.02Aab	7.69±0.03Af
	15	8.58±0.02Ba	7.45±0.02Bc
MPBH-50/1	1	8.84±0.04Ac	7.77±0.03Aef
	15	8.59±0.01Ba	7.49±0.05Bc
YGBH-50/1	1	8.96±0.02Aab	7.84±0.01Acde
	15	8.51±0.01Bbc	7.72±0.02Bb
MPAH	1	8.89±0.01Abc	8.10±0.01Aa
	15	8.47±0.02Bc	7.91±0.02Ba
YGAH	1	8.68±0.02Ad	7.70±0.04Af
	15	8.53±0.01Babc	7.42±0.01Bc
C	1	8.84±0.01Ac	7.91±0.01Abc
	15	8.37±0.03Bd	7.67±0.04Bb

*MPBH-4/12: The ayran produced from milk treated with MTG-MP at 4ºC for 12 h prior to heat treatment, YGBH-4/12: The ayran produced from milk treated with MTG-YG at 4ºC for 12 h prior to heat treatment, MPBH-38/3: The ayran produced from milk treated with MTG-MP at 38ºC for 3 h prior to heat treatment, YGBH-38/3: The ayran produced from milk treated with MTG-YG at 38ºC for 3 h prior to heat treatment, MPBH-50/1: The ayran produced from milk treated with MTG-MP at

50ºC for 1 h prior to heat treatment, YGBH-50/1: The ayran produced from milk treated with MTG-YG at 50ºC for 1 h prior to heat treatment, MPAH: The ayran produced from milk treated with MTG-MP after heat treatment, YGAH: The ayran produced from milk treated with MTG-YG after heat treatment, C: The control ayran sample produced from milk without MTG treatment. Values are the means ± standard deviations; means with different capital letters in each column for the same sample are significantly different (P < 0.05); means with different small letters in each column on the same storage day are significantly different (P < 0.05).

CONCLUSION

The results of this study indicated that changes in the type of MTG used and the treatment condition of MTG to milk used in ayran production led to substantial differences in its physicochemical properties, but no significant differences in microbiological properties were found. Regardless of the use of different MTGs, the ayran samples made from milk treated with MTG after heat treatment had a lower number of grains, mean perimeter of grains, visual roughness, syneresis, and flow behaviour index values, as well as higher apparent viscosity and consistency coefficient values compared to those made from milk treated with MTG prior to heat treatment. When considering the physicochemical properties of ayran, treating milk with MTG-YG at 38ºC it at 4ºC for 12 h or at 50ºC for 1 h, while treating milk with MTG-MP before heat treatment did not lead to an effective result. In future studies, sensory evaluations should be done to better understand the results, along with determining the physicochemical and microbiological properties of ayran made from milk treated with MTG.

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