

Microstructure and Rheological Properties of Ayran Made using the Most Widely Used Commercial Cultures

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ABSTRACT

Ayran, a popular fermented dairy product in Türkiye, the Balkans and the Middle East, is commonly made by incorporating salt and water into yoghurt. Viscosity and rheological properties are key in determining the quality properties of ayran drinks. This study is aimed to determine the effect of the most available culture (whether exopolysaccharide (EPS)-producing or not), which is globally marketed, on ayran rheology, viscosity, microstructure, and sensory properties. Four ayran treatments were produced as follows: T1 (ayran made using classic yoghurt culture, *Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus*); T2: ayran made using ABT-5 culture (*L. acidophilus*, *Bifidobacterium bifidum* and *S. thermophilus*); T3: ayran manufactured by EPS-producing culture (*L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*); and T4: ayran made using EPS-producing culture + *Bifidobacterium Animalis* subsp. *lactis* BB12. Ayran samples were analyzed for rheology, viscosity, microstructure, serum separation, and sensory properties. Unlike T2 and T1, the ayran sample T4 recorded the highest viscosity, the lowest flow behaviour, and the lowest serum separation. Sensory analysis indicated that all ayran samples were found acceptable by the panellists, and T3 followed by T4 gained the highest scores over the cold storage period. Therefore, using EPS-producing strains could be a successful and promising strategy to produce ayran with enhanced quality attributes.

Keywords: Ayran, Viscosity, Exopolysaccharides-producing culture, Sensory properties

En Yaygın Ticari Kültürler Kullanılarak Yapılan Ayranın Mikroyapısı ve Reolojik Özellikleri

ÖZ

Ayran, Türkiye ve Orta Doğu bölgesinde popüler olan fermente bir süt ürünü olup, genel olarak yoğurda tuz ve su ilave edilerek üretilmektedir. Viskozite ve reolojik özellikler, elde edilen ayranın kalite özelliklerinin belirlenmesinde anahtar rol oynamaktadır. Bu çalışma, küresel olarak pazarlanan en yaygın kültürün (ekzopolisakkarit (EPS) üreten ya da üretmeyen) ayran reolojisi, viskozitesi, mikroyapısı ve duyu özellikleri üzerindeki etkisini değerlendirme amacını taşımaktadır. Dört ayran örneği şu şekilde üretilmiştir: T1 (klasik yoğurt kültürü, *Streptococcus thermophilus* ve *L. delbrueckii* subsp. *bulgaricus* kullanılarak üretilen ayran); T2: ABT-5 kültürü kullanılarak üretilen ayran (*L. acidophilus*, *Bifidobacterium bifidum* ve *S. thermophilus*); T3: EPS üreten kültür ile üretilen ayran (*L. delbrueckii* subsp. *bulgaricus* ve *S. thermophilus*); T4: EPS üreten kültür + *Bifidobacterium animalis* subsp. *lactis* BB12 kullanılarak üretilen ayran. Ayran örnekleri reoloji, viskozite, mikroyapı, serum ayrılması ve duyu özellikler açısından analiz edilmiştir. T2 ve

T1'den farklı olarak, T4 ayran örneği en yüksek viskoziteyi, en düşük akış davranışını ve en düşük serum ayrılmasını göstermiştir. Duyusal analiz, tüm ayran örneklerinin panel tarafından kabul edilebilir olduğunu ve T3'ün bunu takiben T4'ün soğuk depolama döneminde en yüksek puanlar aldığını doğrulamıştır. Sonuç olarak, EPS üreten suşların kullanılması, geliştirilmiş kalite özelliklerine sahip ayran üretimi için başarılı ve umut verici bir stratejidir.

Anahtar Kelimeler: Ayran, Viskozite, Eksopolisakkarit üreten kültür, Duyusal özellikler

INTRODUCTION

Currently, there is a growing interest in fermented dairy products worldwide, driven by an increasing awareness of their nutritional value and functional properties. In Türkiye, ayran is a popular drinking yoghurt that is commonly consumed in the summer season for refreshment due to its acceptable flavour [1]. To impart the desired aroma and flavour, it is traditionally prepared by incorporating salt (0.5-1%) and water (3-5%) into yoghurt [2]. Industrially, ayran is produced using homogenized and pasteurized milk, which is subjected to fermentation using different starter cultures, including exopolysaccharides (EPS)-producing lactic acid bacteria [3]. In this context, it has been shown that fermented milk such as yoghurt and ayran has high nutritional value and therapeutic properties linked to the rich nutrients present in the milk used, and the microbial diversity [4] and the interactions between them. Beyond their impact on nutritional and therapeutic values, the microbial diversity strongly influences the fermented milk texture, microstructure, flavour, and aroma [5].

Although fermented milk has a desired flavour and thick texture, it has decreased gel firmness, lower viscosity, and higher wheying off resulting from the disruption of the protein gel matrix, especially at the end of cold storage [6]. This rheological instability is recognized by most consumers as the main drawback of fermented milk, which greatly decreases its overall acceptability [7]. To overcome this technological challenge, several approaches have been used, including adding thickening agents such as pectin, gums, and polysaccharides or in situ production of EPS. The later approach is widely employed to enhance fermented milk's texture and viscosity, which aligns with consumer preference for clean label products, serving as the best alternative to food additives [8], which reduce the acceptability of fermented dairy products like Ayran [9].

Numerous lactic acid bacteria strains can produce EPS, which may be released into the environment or remain attached to the bacterial cell wall. Although it is produced at low levels (25-600 mg/L), EPS has a significant effect on some quality attributes of the final fermented dairy product, including texture, sensory acceptability and mouthfeel [10]. Many reports pointed out its role in improving product stability, reducing serum separation [9-11], and enhancing product overall acceptability. Moreover, some health benefits have been reported for EPS, where they can alter the intestinal microflora diversity and structure [12]. Consequently, EPS is recognised as a contemporary and preferred method for producing fermented milk with

optimal consistency, enhanced functional properties, and greater overall acceptability. However, the effectiveness of these natural bio-thickeners depends on multiple factors, including their type (homo- or hetero-polysaccharide) and diversity (sugar subunits and glycosidic linkages), production level, and proximate composition of the final product. These characteristics, in turn, can be impacted by variables, such as the type of starter culture used, as well as incubation time and temperature [13].

In this context, various lactic acid bacteria strains, such as *Streptococcus thermophilus* [13], have been utilised as EPS producers to enhance the quality attributes of fermented milk during cold storage. However, research on the impact of commercially available cultures used in fermented milk processing within the dairy industry sector is quite limited. Thus, the objective of this study was to evaluate and compare the effect of four different globally marketed commercial culture blends, which are globally marketed, on selected quality attributes of ayran, with a particular focus on rheological properties and microstructure.

MATERIALS and METHODS

Milk, Reagents, Chemicals and Cultures

The fresh raw cow milk used for ayran preparation was obtained from the Animal Production Research Institute farm (Giza, Egypt). The proximate analysis of cow milk was as follows: pH 6.60, acidity 0.18%, fat 4.3%, total protein 3.68% and total solids 13.23%. The different direct vat set (DVS) commercial cultures, including traditional yoghurt starter culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, 1:1), ABT-5 (*Lactobacillus acidophilus*, *Bifidobacterium*, and *S. thermophilus*), YO-Flex Mild 1.0 (EPS-producing, *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*), and BB-12 (*Bifidobacterium animalis* subsp. *lactis*), were purchased from Chr. Hansen's Laboratories (Copenhagen, Denmark). All other reagents and chemicals used throughout the study were of analytical grade.

Ayran Preparation

Ayran samples were prepared according to the following flowchart shown in Figure 1 [14]. A 1% salt solution (previously heat-treated to 85°C/10 min and cooled to ambient temperature) was mixed with yoghurt samples in each treatment at a ratio of 60% yoghurt to 40% salt solution. After blending for 5 min, the resulting mixtures were homogenized for 3 min at 3000 rpm at 20°C using a mixer. All samples were then stored for 14 days at 4°C. Ayran samples were analysed at 7-day

intervals over the cold storage period. The Turkish Food Codex Communiqué on Fermented Dairy Products stipulates that ayran must contain at least 10^6 CFU/g of viable yoghurt culture. All ayran samples in this study met this criterion, with viable bacterial counts as follows: T1 (7.7 and 7.4 Log_{10} CFU/g), T2 (8.2, 9.5, and 7.3 Log_{10} CFU/g), T3 (7.8 and 7.3 Log_{10} CFU/g), and T4 (7.8, 7.3, and 9.4 Log_{10} CFU/g). Furthermore, the Codex requires the presence of both yoghurt starter

bacteria-*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in ayran samples. Based on this criterion, the T2 sample could not be classified as ayran. Nevertheless, its inclusion in this study provided a valuable point of comparison, as ABT culture has been commonly employed in yoghurt production within the dairy industry and would be a valuable mixed culture that enhances ayran quality.

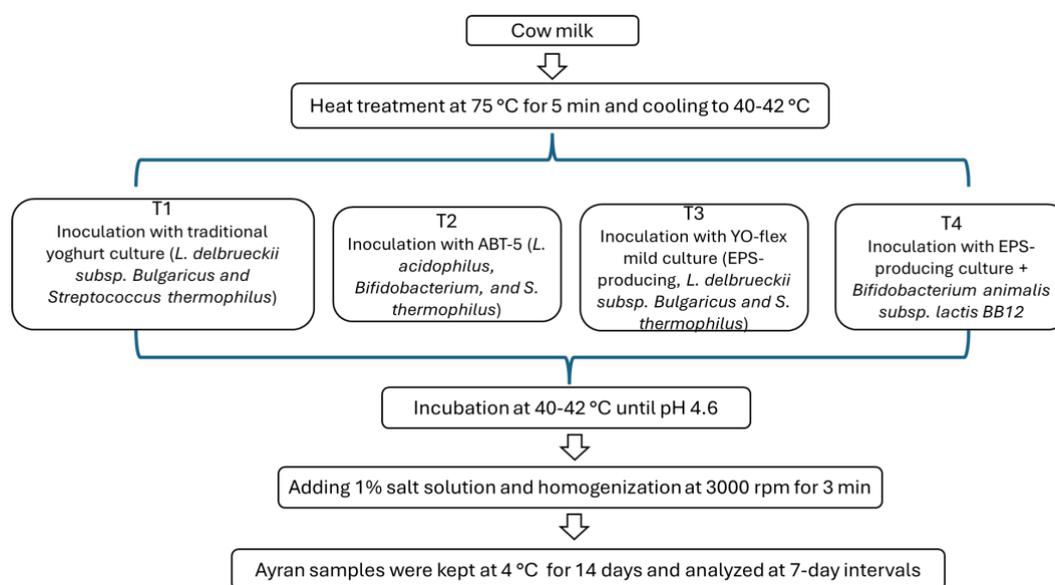


Figure 1. Processing steps of ayran samples using different cultures.

Physico-Chemical Analyses

Total solids, protein, and fat contents were estimated according to [15]. The Kjeldahl method was used for the determination of total nitrogen content, which was multiplied by a factor of 6.38 for protein content calculation. Changes in pH values were monitored by a digital pH meter (Boeco, Hamburg, Germany).

Rheological Properties of Ayran

Rheological properties were determined according to [16] by using a Brookfield Digital Rheometer model DV-III+ (Brookfield Engineering Lab., INC., Middleboro, USA) at 10°C. The Brookfield small sample adapter and Sc4-21 spindle were used. The data were analysed by using the Bingham plastic and Power Law mathematical models to provide a numerical and graphical analysis of the behaviour of data sets. These models are: $\tau = \tau_0 + \eta\dot{\gamma}$, and $\tau = K\dot{\gamma}^n$, respectively, where τ = shear stress (Pa), τ_0 = yield stress, shear stress at zero shear rate (Pa), η = plastic viscosity (mPa/s), $\dot{\gamma}$ = shear rate (1/s), K = consistency coefficient (mPa/sn), n = flow behaviour index. Apparent viscosity was measured at 10 rpm.

Serum Separation of Ayran

Serum separation was determined according to [9]. Briefly, ayran samples were poured into 50 mL graded cylinders and stored for 14 days at 4°C. On day 14, the

volume of the clear serum layer that separated at the top was measured as an indicator of instability.

Ayran Microstructure Measurement

Scanning electron microscopy (SEM) was employed to examine the particle morphology of all Ayran samples using the JXA-840A Electron Probe Microanalyzer, JEOL, Japan. Sample preparation involved drying with a Critical Point Dried apparatus, followed by gold coating using an Edwards S150A Sputter Coater (England). The prepared samples were then analysed under the scanning electron microscope, following the method described by [17].

Sensory Evaluation of Ayran

Ayran samples were organoleptically tested according to [18] for colour and appearance (15 points), body and texture (35 points), flavour (50 points), and overall acceptability (100 points). Ten expert panellists, who were frequent consumers of fermented milk and had no milk allergies, were recruited from the research staff of El-Serw Animal Production Research Station for sensory evaluation. Additionally, the panellists underwent two training sessions to familiarize themselves with the sensory descriptors and to discuss variations in colour, texture, and flavour among the yoghurt samples. Ayran samples were presented to the panellists at room temperature (25°C) in white, randomly coded polystyrene cups (50 g in 100 mL

cups), and the data were collected using a specifically designed ballot. For mouthwash, water was provided between samples.

Statistical Analysis

Data were presented as mean \pm standard deviation from three independent replicates. Statistical analysis was conducted using one-way ANOVA to evaluate significant differences among sample means and across storage periods. Differences between means were further assessed using Duncan's multiple range test at a significance level of $p < 0.05$.

RESULTS and DISCUSSION

Composition of Ayran Samples

Fermented milk products, including ayran, are known for their high acceptability, with quality attributes being a key factor. In this regard, lots of research has been conducted to enhance the quality attributes of ayran, particularly rheological and microstructure properties.

The results presented in Table 1 show the physicochemical analysis of ayran samples manufactured using different cultures over 14 days of cold storage time. The current findings revealed that all ayran samples had almost similar values of total solids, with the highest value recorded for T4 (6.40%) and the lowest value for T3 (6.31%), with insignificant changes ($p < 0.05$) between all samples on the first day of storage. Slight increases in total solids values were observed, accompanied by statistically significant changes at 14 days of cold storage. Generally, the total solids values of ayran samples in the current study were slightly lower than those reported by [13]. This change in total solids might have resulted from the evaporation that occurred during the storage period, which is reported by [19]. This difference could have partially resulted from the variation in the used strains, processing method, and milk composition, as well as the type, amount, and composition of EPS produced by these strains, or the added amount of salt and water [3]. Similar results reported that inoculation and incubation conditions affected the dry matter content of ayran samples, which ranged between 6.98 and 6.42% [13].

Table 1. Chemical composition of Ayran manufactured using different cultures over the cold storage period

Treatments ¹	Storage period (days)	Total solids (%) [*]	Fat (%)	Protein (%)	pH
T1	1	6.37 \pm 0.04bB**	2.31 \pm 0.1bA	2.07 \pm 0.03aA	4.68 \pm 0.3aA
	7	6.38 \pm 0.02aA	2.30 \pm 0.1aB	2.06 \pm 0.02aB	4.63 \pm 0.2aB
	14	6.38 \pm 0.02bA	2.29 \pm 0.1cC	2.07 \pm 0.04aA	4.60 \pm 0.1aC
T2	1	6.36 \pm 0.01cB	2.32 \pm 0.2aA	2.06 \pm 0.01bB	4.64 \pm 0.3bA
	7	6.37 \pm 0.01bA	2.30 \pm 0.1aB	2.06 \pm 0.01aB	4.60 \pm 0.2bB
	14	6.37 \pm 0.02cA	2.30 \pm 0.2bB	2.07 \pm 0.02aA	4.58 \pm 0.2Bc
T3	1	6.37 \pm 0.07bB	2.32 \pm 0.3aA	2.06 \pm 0.03bA	4.61 \pm 0.1cA
	7	6.37 \pm 0.05bB	2.30 \pm 0.1aC	2.06 \pm 0.01aA	4.59 \pm 0.2cB
	14	6.38 \pm 0.03bA	2.31 \pm 0.1aB	2.05 \pm 0.01bB	4.58 \pm 0.1bBC
T4	1	6.38 \pm 0.04aA	2.29 \pm 0.0cB	2.07 \pm 0.02aA	4.59 \pm 0.1dA
	7	6.38 \pm 0.05aB	2.30 \pm 0.1aA	2.05 \pm 0.02bB	4.56 \pm 0.2dB
	14	6.39 \pm 0.02aA	2.30 \pm 0.2bA	2.05 \pm 0.03bB	4.55 \pm 0.1cBC

¹T1: Ayran made using traditional yoghurt culture, T2: Ayran made using ABT-5 culture, T3: Ayran made using YO-Flex Mild 1.0 (EPS-producing) culture, T4: Ayran made using YO-Flex Mild 1.0 (EPS-producing) culture + *Bifidobacterium animalis* subsp. *lactis* BB-12. *Values are means \pm SD of three independent replicates. **Means among different treatments with different superscripts (e.g., a, b, c) are significantly different ($p < 0.05$), and means among different storage times with different superscript letters (e.g., A, B, C) are significantly different ($p < 0.05$).

A similar trend was reported for fat content over the storage period, where no large fluctuations were noticed in the different ayran samples. All samples showed a slight decrease in fat content during the cold storage period. Fat content ranged between 2.29-2.32%, which was almost higher than the fat content recorded for ayran samples in previous studies [13] and [20].

Moreover, the protein content of ayran samples remained remarkably steady throughout the study and across all treatments and over storage time, ranging between 2.05 and 2.07%. The variations in protein content were minor, which indicates a minimal impact of treatment type and storage time on protein content. Comparable findings have been reported by [21], where

the obtained protein contents varied between 1.44 and 3.48% in ayran samples.

The pH values of all ayran samples have been monitored throughout a 14-day cold storage period in this study (Table 1). pH values gradually decreased as the cold storage period progressed, with significant differences observed between different ayran samples. This phenomenon can mainly be attributed to the fermentation of lactose into organic acids, such as acetic and lactic acids, leading to a reduction in pH values. Moreover, significant changes in pH values were recorded in different ayran samples, which were mainly caused by the different cultures used. Therefore, the changes in pH are both time-dependent and strain-dependent. Notably, the highest value of pH (4.68) was recorded for the T1 ayran sample, which was produced

using a traditional yoghurt starter culture (*L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*). Conversely, the lowest pH values (4.54) were recorded for the T4 ayran sample, which utilized an EPS-producing culture combined with *Bifidobacterium animalis* subsp. *lactis* BB-12. The lower pH values could not solely be attributed to the accelerated growth and acidification rates of the EPS strains, but also to the inclusion of the probiotic strain *Bifidobacterium animalis* subsp. *lactis* BB-12, which contributed to the increased acidity of ayran samples. In this regard, the produced EPS not only acted as a texture-modifying agent but also served as an additional energy source for lactic acid bacteria, contributing to lowering pH and increasing acidity. These results were generally consistent with those reported by [13, 22, 23]. A similar tendency of pH changes was observed by [24], who reported that the variation in pH values among ayran samples decreased over time, and the differences became largely negligible after the seventh day. This phenomenon might be attributed to a reduction in bacterial activity/growth and the steady production of acid once a specific acidity threshold is reached.

Rheological Properties of Ayran

Rheological properties represent a key factor of consumer acceptability of the ayran product. The results shown in Table 2 display that ayran samples showed non-Newtonian behaviour, and all samples were considered a pseudoplastic fluid because the values of the flow behaviour index were below 1. Plastic viscosity and apparent viscosity were selected as they describe different aspects of ayran's flow: apparent viscosity shows overall resistance to flow, while plastic viscosity (from the Bingham model, expressed in mPa·s) reflects resistance once yield stress is overcome [25]. Results indicated an inverse relationship between the viscosity value and the flow behaviour index. Unlike the T2-coded sample, the T4

sample exhibited the highest viscosity value (Figure 2) and the lowest flow behaviour index.

Regarding the yield stress, which refers to the minimum shear stress required to initiate flow. It is a measure of the structural strength of the yoghurt, indicating the amount of force required to move or deform it. A higher yield stress means the yoghurt is more resistant to flow and will feel firmer. Results indicated that T4 had the highest yield stress and thereby the highest consistency coefficient, indicating a thicker consistency of this ayran sample. Our results were generally in line with previous data obtained by [26, 27].

Viscosity is regarded as one of the key quality attributes of fermented milk. The data in Figure 2 show that the highest viscosity values were recorded for the T4 sample, while the lowest were obtained for the T2 sample. Viscosity values significantly decreased over the cold storage period. It was noted that the ayran sample made using traditional culture (non-EPS-producing) resulted in samples with lower shear stress values, compared to EPS-producing cultures. During fermentation, exopolysaccharides (EPS) produced by bacteria do not interact well with these casein-whey protein particles, allowing the formation of larger and denser protein aggregates before the gelation point. As aggregation progresses, EPS is likely to concentrate in the continuous phase rather than integrating with the protein clusters. Consequently, the final protein network in the yoghurt is expected to be more tightly packed when EPS is present. Additionally, research by [28] found that EPS production leads to gelation at higher pH levels, potentially allowing for greater structural rearrangement post-gelation, further contributing to a more compact protein matrix. This not only affects viscosity but also affects syneresis, which is considered a significant defect, lowering the acceptability of yoghurt and ayran samples during the cold storage period.

Table 2. Rheological and viscosity properties of ayran manufactured using different cultures over the cold storage period.

Treatments ¹	Storage period	Plastic viscosity* (mPa·s)	Yield stress (Pa)	Consistency coefficient (K, mPa/sn)	Flow behaviour index (n)
T1	1	74.9±0.1cA**	1.58±0.10cA	97.9±9.07cA	0.59±0.01bA
	7	66.6±1.0cB	1.46±0.6cB	88.5±10cB	0.48±0.02bB
	14	58.3±10Cc	1.25±0.05cC	80.8±0.8cD	0.41±0.01bC
T2	1	70.2±10dA	1.43±0.03dA	88.8±0.8dB	0.81±0.01aA
	7	61.7±1.0dB	1.31±0.01dB	79.3±1.0dC	0.70±0.1aB
	14	52.9±0.3dC	1.15±0.1Dc	136.0±1.0aA	0.57±0.03Ac
T3	1	89.3±1.0bA	1.63±0.03bA	114.6±4.0bA	0.45±0.05cA
	7	78.5±0.5bB	1.57±0.03bB	103.7±3.0bB	0.38±0.02cB
	14	69.4±0.4bC	1.43±0.06Bc	97.4±3.0cC	0.30±0.05cC
T4	1	105.9±5.0aA	1.95±0.05aA	129.9±1.0aA	0.38±0.02dA
	7	90.6±10aB	1.81±0.01aB	120.8±0.8aB	0.32±0.01dB
	14	74.9±4.0aC	1.70±0.1aC	109.0±9.0bC	0.25±0.05dC

¹T1: Ayran made using traditional yoghurt culture, T2: Ayran made using ABT-5 culture, T3: Ayran made using YO-Flex Mild 1.0 (EPS-producing) culture, T4: Ayran made using YO-Flex Mild 1.0 (EPS-producing) culture + *Bifidobacterium animalis* subsp. *lactis* BB-12. *Values are means ± SD of three independent replicates. **Means among different treatments with different superscripts (e.g., a, b, c) are significantly different ($p < 0.05$), and means among different storage times with different superscript letters (e.g., A, B, C) are significantly different ($p < 0.05$).

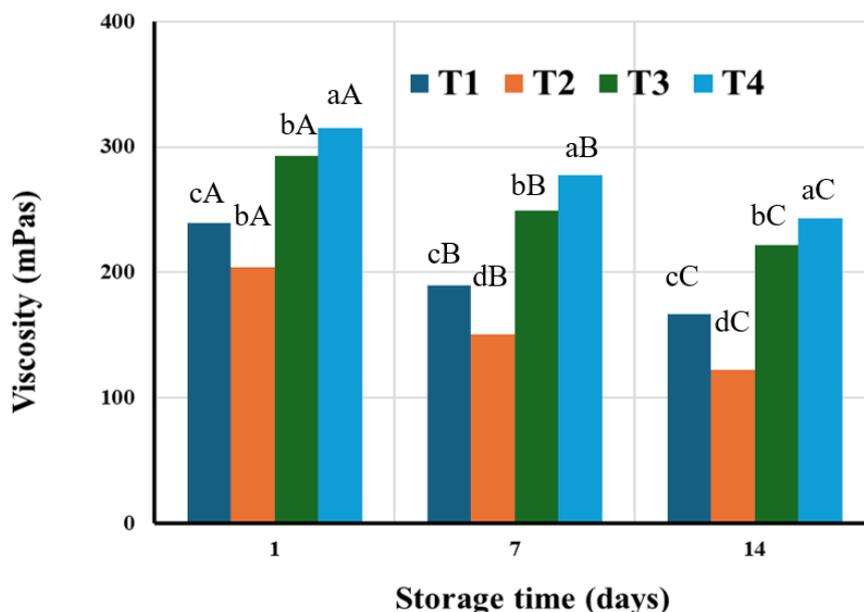


Figure 2. Viscosity of ayran samples made using different cultures during the cold storage period. Values are means \pm SD of three independent replicates. T1: Ayran made using traditional yoghurt culture, T2: Ayran made using ABT-5 culture, T3: Ayran made using YO-Flex Mild 1.0 (EPS-producing) culture, T4: Ayran made using YO-Flex Mild 1.0 (EPS-producing) culture + *Bifidobacterium animalis* subsp. *lactis* BB-12. Means among different treatments with different superscripts (e.g., a, b, c) are significantly different ($p < 0.05$). Means among different storage times with different superscript letters (e.g., A, B, C) are significantly different ($p < 0.05$).

Serum Separation

Serum separation is a common phenomenon in fermented dairy milks, occurring during storage as a result of protein particle aggregation and sedimentation. This leads to either the settling of large particles at the bottom or the formation of a clear serum layer on the surface. It is considered a determining factor for the quality and overall acceptability of ayran. The data shown in Figure 3 indicated that the highest serum separation (13 mL/50mL) was recorded for the T1 sample, and the lowest value (7 mL/50mL) was recorded for the T4 sample. It was evident that using the EPS-producing culture led to lower serum separation values.

EPS plays a vital role in the dairy industry, acting as a stabilizer, emulsifier and thickening agent, improving the product's viscosity, rheology, and reducing syneresis [29]. Therefore, the dairy industry sector tends to add various types of hydrocolloids, EPS or use the in-situ EPS production by some selected bacterial strains. In this context, EPS is gaining increasing attention as a safe and functional ingredient in the food industry [30]. In the dairy industry, EPS plays a crucial role in enhancing product viscosity, rheology, and texture and decreasing the serum separation [32, 32]. Therefore, identifying new EPS-producing LAB strains presents a promising approach for developing safe and bio-thickening agents [33], offering an attractive alternative and allowing for a clean-label product and eliminating costly ingredients [34].

Arab et al. [35] reported that the bacterial cultures employed in the preparation of fermented milks significantly influence their quality, particularly the rate of syneresis. The traditional starter cultures, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*, are widely used in yoghurt manufacturing. The strains employed in the present study are non-exopolysaccharide (EPS)-producing bacteria, which likely explains why the T1 sample exhibited the lowest viscosity and syneresis values compared to the other samples.

Consequently, several studies indicated that the use of exopolysaccharide (EPS)-producing strains may represent a successful strategy for improving texture and water-holding capacity of fermented milk products [36]. EPS functions as a safe, natural bio-thickening agent, with its effectiveness largely determined by its monosaccharide composition, charge, molecular structure (including chain length and degree of branching), and interactions with milk constituents, particularly proteins [35]. In line with this, the current findings demonstrated that EPS-producing bacterial strains (T2, T3, and T4) played a significant role in reducing serum separation. Our results were consistent with the observations of [37], who pointed out that in-situ EPS production in yoghurt enhances product firmness through its thickening effect, which is closely associated with reduced serum separation.

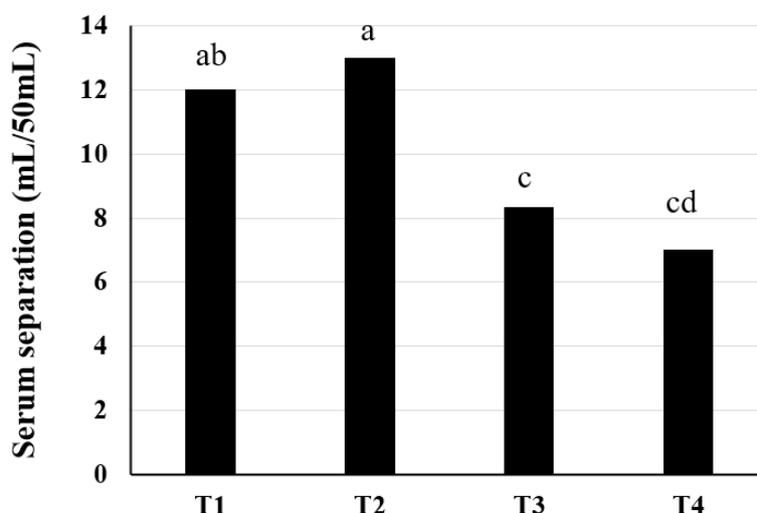


Figure 3. Serum separation of ayran samples made using different cultures. Values are means \pm SD of three independent replicates. T1: Ayran made using traditional yoghurt culture, T2: Ayran made using ABT-5 culture, T3: Ayran made using YO-Flex Mild 1.0 (EPS-producing) culture, T4: Ayran made using YO-Flex Mild 1.0 (EPS-producing) culture + *Bifidobacterium animalis* subsp. *lactis* BB-12. Means among different treatments with different superscripts (e.g., a, b, c) are significantly different ($p < 0.05$).

Similar results were obtained by [38], where the use of EPS-producing strains led to reduced whey separation. This was mainly attributed to changes in the yogurt's microstructure and the enhanced water-binding ability of EPS. One possible explanation for this effect lies in the way proteins aggregate and form gels. Since EPS molecules are not fully compatible with milk proteins, they tend to separate and occupy the spaces within the gel network. This helps to fill the pores, resulting in a softer texture and less syneresis, as also noted by [27].

Microstructure of Ayran

SEM analysis was performed to investigate structural variations in ayran samples produced using different cultures. As observed in Figure 4, the T1 sample, made with a traditional yoghurt culture, exhibits a fine, uniform matrix characterized by small, evenly dispersed protein particles and thin, interconnected strands that form the protein network. Although the particles in sample T1 were nearly spherical, uniform, and had a rough surface, some particles were nearly large and not spherical.

In contrast, the samples T2, T3, and T4 show a marked shift in microstructure, with larger protein clusters and a thicker, denser strand network. This alteration was likely to be attributed to the existence of EPS in the continuous phase, which influenced the protein aggregation process, resulting in a more compact and structured matrix. The differences observed in the T2, T3, and T4 samples might be attributed to variations in the metabolic byproducts produced by the respective bacterial cultures, of which EPS was considered the main element, especially their content, composition and structural properties, which ultimately shaped the final product properties. The ultimate protein network

present in the yogurt was anticipated to exhibit a denser aggregation in yogurt produced with EPS+ [27]. Additionally, [28] reported that EPS production led to gelation at higher pH levels, which might allow for greater structural rearrangement after gel formation. This, in turn, could promote the development of more densely aggregated gel networks [39]. The formation of the denser aggregates and highly compacted protein network had a substantial effect on viscosity, syneresis and WHC of the resulting stirred fermented milk. In this regard, unlike the ayran sample made using non-producing EPS culture, ayran samples made using EPS+ LAB tended to be homogenous.

When subjected to shear, samples made with EPS-producing strains (EPS+) are expected to have a continuous phase enriched with EPS surrounding the protein aggregates. This structure helps reduce syneresis by increasing the overall viscosity. However, due to the incompatibility between EPS and milk proteins, the EPS in the continuous phase may reduce the interactions between protein aggregates, further influencing the texture and stability of the product. This can probably be explained by the higher susceptibility of EPS+ samples to breakdown compared to EPS-samples, which is the reduced number of protein-protein interactions present at the critical sites within the network that need to be surpassed [27].

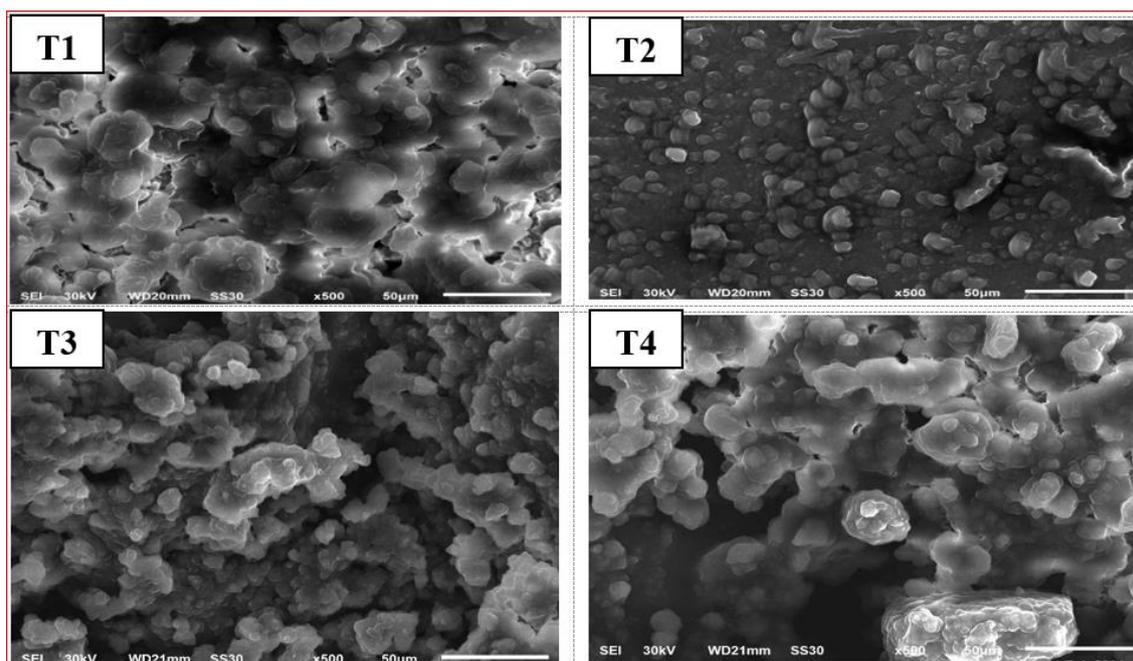


Figure 4. Scanning electron microscopy (SEM) images at 500x magnification of fresh ayran samples made using different cultures. T1: Ayran made using traditional yoghurt culture, T2: Ayran made using ABT-5 culture, T3: Ayran made using YO-Flex Mild 1.0 (EPS-producing) culture, T4: Ayran made using YO-Flex Mild 1.0 (EPS-producing) culture + *Bifidobacterium animalis* subsp. *lactis* BB-12.

In line with this, ayran samples made using EPS-producing cultures (T3 and T4) gained the highest overall acceptability (Table 3). This would be associated with the type, structure, and amount of the produced EPS. Similar results were obtained by [9], who reported that in situ-EPS production had no direct adverse impact on the overall acceptability of ayran samples. This was mainly explained by the fact that EPS is a safe bio-thickening agent, thereby having no

impact on sensory analysis descriptors such as odour and flavour. This observation was in general agreement with [29], indicating that in-situ EPS production could have a positive impact on various yoghurt parameters, including stability, texture, and sensory acceptability. This was largely due to the water-binding capacity of EPS and its ability to interact with casein aggregates, which contributed to enhanced viscosity and a creamier consistency in the final products.

Table 3. Sensory properties of ayran manufactured using different cultures over the cold storage period.

Treatments ¹	Storage times (days)	Storage period (days)			
		Colour & Appearance* (15)	Body & Texture (35)	Flavour (50)	Overall Acceptability (100)
T1	1	14±1.0aA**	33±2.0bA	46±3.0bA	93±2.0bA
	7	14±1.0aA	33±1.0bA	45±2.0cB	92±2.0cB
	14	14±0.0aA	33±2.0bA	45±4.0bB	92±3.0bC
T2	1	14±1.0aA	31±3.0cA	44±5.0cA	89±4.0cA
	7	13±2.0bBA	30±1.0cB	42±3.0dB	85±3.0dB
	14	14±0.0aA	29±2.0cC	41±3.0cC	84±3.0cD
T3	1	14±0.0aA	34±0.0aA	49±1.0aA	97±1.0aB
	7	14±0.0aA	34±0.0aA	49±1.0aA	97±0.5aB
	14	14±0.0aA	34±1.0aA	48±1.0bA	98±1.5aA
T4	1	14±1.0aA	34±1.0aA	49±0.0aA	97±1.0aA
	7	14±0.0aA	33±1.0bB	48±0.0bB	95±0.5bB
	14	14±0.0aA	33±1.0bB	48±1.0aB	95±1.0bB

¹T1: Ayran made using traditional yoghurt culture, T2: Ayran made using ABT-5 culture, T3: Ayran made using YO-Flex Mild 1.0 (EPS-producing) culture, T4: Ayran made using YO-Flex Mild 1.0 (EPS-producing) culture + *Bifidobacterium animalis* subsp. *lactis* BB-12. *Values are means ± SD of three independent replicates. **Means among different treatments with different superscripts (e.g., a, b, c) are significantly different ($p < 0.05$), and means among different storage times with different superscript letters (e.g., A, B, C) are significantly different ($p < 0.05$).

CONCLUSION

The impact of using different cultures in the preparation of ayran samples was investigated. The findings revealed that EPS-producing cultures can be used to prepare ayran samples characterized by higher sensory acceptability, improved viscosity and rheological properties, and reduced serum separation. Using EPS-producing cultures such as YO-Flex Mild 1.0 (*L.delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, 1:1) and YO-Flex Mild 1.0 combined with *Bifidobacterium animalis* subsp. *lactis*, BB-12 (1:1:1), to develop ayran with enhanced attributes, may offer a good alternative to traditional starter cultures. Future studies should focus on determining the quantity, types, and structure of the EPS produced, as well as their impact on the bioactivity of ayran.

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