JUNE 2020 M.Sc. in Food Engineering ŞERİFE MERVE UZAY

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AYRAN-SHALGAM MIXTURE: A STUDY ON RHEOLOGICAL PROPERTIES, SHELF LIFE, AND SENSORY EVALUATION

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ŞERİFE MERVE UZAY

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June 2020

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Şerife Merve UZAY

ABSTRACT

AYRAN-SHALGAM MIXTURE: A STUDY ON RHEOLOGICAL PROPERTIES, SHELF LIFE, AND SENSORY EVALUATION

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The objectives of this study were to evaluate the rheological properties, shelf life and sensory properties of different ayran-shalgam mixtures. Mixtures of ayran and shalgam in different ratios [25% ayran + 75% shalgam (v/v), 50% ayran + 50% shalgam (v/v) and 75% ayran + 25% shalgam (v/v)] were subjected to rheological measurements at different temperature levels (4, 8 and 12 °C). Power law model was used to define the rheological data. It was apparent that temperature had no effect on the flow behavior index of the mixtures. On the other hand, as the temperature increased consistency index of the mixtures decreased. Ayran-shalgam mixtures had similar flow behavior index with that of shalgam no matter whatever the amount of ayran or shalgam in the mixtures was. However, consistency index increased as the amount of ayran increased in the mixtures at a constant temperature. Shelf life analyses up to 4 weeks at 4 °C indicated that serum separation increased up to second week and then stabilized for all the mixtures. Slight increase (0.05 to 0.1 unit) in the pH values was observed during the first week, then, pH values were stabilized just like the serum separation. Microbiological analyses revealed that mixtures had a similar shelf life with that of ayran. Mixture with the highest ayran content [75% ayran + 25% shalgam (v/v)] had the highest scores in sensory evaluation. It was concluded that ayran-shalgam mixture had a huge potential for commercial production.

Key Words: Ayran, Shalgam, Fermented Beverages, Rheology, Shelf life, Microbiology

ÖZET

AYRAN-ŞALGAM KARIŞIMI: REOLOJİK ÖZELLİKLER, RAF ÖMRÜ VE DUYUSAL DEĞERLENDİRME ÜZERİNE BİR ÇALIŞMA

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Yüksek Lisans Tezi, Gıda Mühendisliği
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Bu çalışmanın amacı, farklı ayran şalgam karışımlarının reolojik özelliklerini, raf ömrünü ve duyusal özelliklerini belirlemektir. Ayran ve şalgamın farklı oranlarda karışımları [%25 ayran + %75 şalgam (v/v), %50 ayran + %50 şalgam (v/v) ve %75 ayran + %25 şalgam (v/v)] farklı sıcaklık düzeylerinde (4, 8 ve 12 °C) reolojik ölçümlere tabi tutulmuştur. Reolojik verileri tanımlamak için Power Law modeli kullanılmıştır. Sıcaklığın karışımların akış davranış indeksi üzerinde hiçbir etkisi olmadığı açıktır. Öte yandan, sıcaklık arttıkça karışımların kıvam indeksinin azaldığı gözlemlenmiştir. Ayran-şalgam karışımlarının, karışımlardaki ayran veya şalgam miktarı ne olursa olsun, şalgam ile benzer akış davranış indeksine sahip olduğu anlaşılmıştır. Ancak karışımlarda sabit bir sıcaklıkta ayran miktarı arttıkça kıvamlılık endeksi artmıştır. 4 °C' deki depolama işleminde 4 haftaya kadar raf ömrü analizleri, serum ayrılmasının ikinci haftaya kadar arttığını ve daha sonra tüm karışımlar için stabilize olduğunu göstermiştir. İlk hafta pH değerlerinde hafif artış (0,05-0,1 birim) gözlemlenmiş daha sonra pH değerleri tıpkı serum ayrımı gibi stabilize olmuştur. Mikrobiyolojik analizler, karışımların ayran ile benzer raf ömrüne sahip olduğunu ortaya koymuştur. Ayran miktarı daha yüksek olan karışım [%75 ayran + %25 şalgam (v/v)] duyusal değerlendirmede en yüksek puanı almıştır. Ayran-şalgam karışımının ticari üretim için büyük bir potansiyele sahip olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Ayran, Şalgam, Fermente İçecekler, Reoloji, Raf Ömrü, Mikrobiyoloji

Dedicated to my family …

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LIST OF SYMBOLS

T	Shear Stress
Γ	Shear Rate
$\boldsymbol{a}$	Standard Error

LIST OF ABBREVIATIONS

A	Ayran
ANOVA	Analysis of Variance
CFU	Colony-Forming Units
°C	Celsius Degree
DRBC	Dichloran Rose-Bengal Chloramphenicol
Eq.	Equation
G	Gram
K	Consistency Index
L	Liter
LAB	Lactic Acid Bacteria
mg	Milligram
min	Minute
mL	Milliliter
MPN	Most Probable Number
MRS	Man Rogosa Sharpe Agar
N	Flow Behavior Index
PCA	Plate Count Agar
R^2_{adj}	Adjusted Coefficient of Determination
RMSE	Root Mean Square Error
S	Shalgam
TFC	Turkish Food Codex
TMAB	Total Mesophilic Aerobic Bacteria
TS	Turkish Standard
TSE	Turkish Standards Institute
TÜİK	Turkish Statistical Institute
v/v	Volume per Volume

CHAPTER I

INTRODUCTION

1.1 General

The word fermentation was obtained from a Latin word fervere and was later defined by French scientist Louis Pasteur (1822-1895) as "life without air." It is a metabolic process that derives energy from the organic compounds without the involvement of any oxigenous agents. Historically, fermentation was used by humans for the preservation of food. The production of fermented foods and beverages is one of the oldest manufacturing and preservation methods of human consumption, dating back to ancient times (Caplice & Fitzgerald, 1999). Since ancient times, foods and beverages have been produced by fermentation techniques in different countries. Turkey has a wide range of fermented beverages such as ayran (yogurt drink), shalgam (turnip juice), kefir and boza. Among them, ayran and shalgam are the most popular ones.

Probiotics can be defined as a food or supplement containing concentrates of defined strains of living microorganisms (Chandan, 2007). Cultures associated with health benefits are yogurt bacteria such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*, other lactobacilli, and bifidobacteria (Chandan and Nauth, 2012; Chandan and Shah, 2013). Yogurt starter bacteria, namely *S. thermophilus* and *L. delbrueckii ssp. bulgaricus*, act as probiotics because of their health-promoting effects, which are also found in ayran. Yogurt bacteria have been indicated to assist in lactose digestion, reduce or prevent diarrhea, and strengthen immune defenses of the host. They are also reported to persist and remain viable throughout the human gastrointestinal tract. Additionally, Ma et al. (2013) reported that probiotic therapy can reduce liver aminotransferase, total cholesterol and improve insulin resistance.

A daily dose of probiotics may help manage allergic responses during the pollen season (Singh et al., 2013). Beserra et al. (2015) reported that probiotics help to partially reduce cholesterol levels circulating in blood. King et al. (2014). showed that yogurt products with probiotics decreases the duration of common cold and upper respiratory tract infections.

Shalgam is a highly nutritious and microbiologically safe drink. Lactic acid gives shalgam its sour taste and helps to regulate the pH of the digestive system. Moreover, it keeps shalgam from spoiling and helps the body make better use of some minerals. The calcium, potassium, and iron in shalgam strengthen the bones and teeth. Human also uses it as a medicine because its antiseptic agents clean the digestive organs and soothe the bowels (Yardımcı et al., 2015). Shalgam provides appetizing properties and its positive effect on the digestive system also important (Canbas and Fenercioglu, 1984). The predominant phenolic compound in shalgam is anthocyanins. Black carrot anthocyanins are responsible for purplish red color of shalgam (Turker et al., 2007; Kristbergsson and Ötles, 2016). Generally, the amount of anthocyanin contained in homemade shalgam (83.19 mg/L) is higher than commercial products (41.89 – 48.40 mg/L) (Ercelebi and Özkanli, 2010; Altay et al., 2013). Anthocyanins and lactic acid bacteria from black carrots used in shalgam production cause a reduction in the risk of cancer and cardiovascular diseases (Gobbetti et al., 2010; Ekinci et al., 2016).

Shalgam microbiology is complex and not known in detail. Natural fermentation involves mainly lactic acid bacteria and yeasts. Shalgam fermentation depends on microorganisms that are present on the surfaces of raw materials, surfaces of vessels where product is produced and stored, and in the sourdough extract (Canbas and Fenercioglu, 1984; Canbas and Deryaoglu, 1993*).* *L. plantarum*, *L. arabinosus*, *L. brevis*, and *L. paracasei spp. paracasei* were the dominant Lactic acid bacteria during the fermentation of shalgam produced by traditional method (Arici, 2004; Erginkaya and Hammes, 1992).

In Turkey, some people consume ayran and shalgam together by mixing them. Although studies on ayran and shalgam are abundant, no scientific knowledge about the mixture of ayran shalgam could be found in the literature.

Nevertheless, newspapers and TV programs have mentioned about this interesting mixture beverage (see for example Arda'nın mutfağı, 2020). It is worth to investigate this beverage since it consists of two different traditional fermented products, one contains antioxidants and the other has beneficial bacteria.

1.2 Objectives of the Study

Although ayran-shalgam mixture is consumed in different parts of Turkey, to the best of our knowledge there are no studies on this mixture beverage. Therefore, the objectives of this study were to evaluate the (i) rheological properties, (ii) shelf life and (iii) sensory properties of different ayran-shalgam mixtures.

CHAPTER II

LITERATURE REVIEW

2.1 Fermentation

Fermentation is one of the most economical and oldest techniques used in food preservation (Naidu et al., 1999). The first records as long as 2000 years ago indicate that human were intaking ‘soured milks’. Lactic acid bacteria (LAB) have been found in various fermented foods. These are preservative and also beneficial for health (Hutkins, 2006; Klaenhammer, 2007; Naidu et al., 1999).

McFeeters (2004) stated that the combination of this ancient method of bio-preservation with the current biotechnology tools should result in controlled fermentation processes and the selection of starter cultures to increase the consumption of fresh-like vegetables and fruits and milk and milk-related components.

2.2 Ayran

Fermentation has been used by people in order to preserve milk since ancient times. The word yogurt is believed to initially been used by Turks in the 8th century. It is thus assumed that the first Asian Turkish nomads made yoghurt (Altay, 2017). Nowadays, the largest producers and consumers of fermented milk products are Turkey, Syria, Iran, Iraq, Balkans, India and Pakistan (Altay, 2017). Although fermented milk products and yogurt are now being commercially manufactured in most countries, in some places traditional yogurt production still takes place (Altay, 2017). Table 2.1 summarizes the different names of yogurt and yogurt-like products in the world.

Table 2.1 Yogurt and yogurt-like products around the world*

Traditional Name	Country	Traditional Name	Country
Busa	Turkestan	Mezzoradu	Sicily
Cieddu	Italy	Roba	Iraq
Dahi/Dadhi/Dahee	India, Bangladesh, Nepal	Skyr	Iceland
Filmjolk/Filbunke/Filbunk/ Surmelk	Scandinavia	Taettem-jolk/Tettemelk Tarho	Hungary
Gioddu	Sardinia	Tiaourti	Greece
Yoğurt/Ayran	Turkey	Urgotnic	Balkan Mountains
Katyk	Transcaucasia	Villi	Finland
Kissel Mleka	Balkans	Yogurt/Yogurt/Yaort	Rest of the world
Leben/Leban	Lebanon and some Arab countries	Yourt/Yaourti/Yahourth/ Yogur/Yaghourt	("Y" is replaced by "J" in some instances)
Mast/Dough	Iran and Afghanistan	Zabady	Egypt and Sudan
Mazun/Matzoon	Armenia		

*Adapted from Tamime and Deeth (1980) and Kosikowski and Mistry (1997).

The optimum temperature for the growth of bacterial cultures in milk which leads to coagulation of milk, formation of yogurt and yogurt drinks is around 40 °C. Yogurt and ayran may vary in their taste, composition, texture, characteristics and health properties based on the type of milk used. During fermentation of milk to yogurt, milk sugar is partially converted into lactic acid by the action of various enzymes, originating from the growth of thermophilic lactic acid bacteria. This causes a sufficient decrease in the pH, resulting in a dissociation of the colloidal calcium phosphate, a destabilization of the casein micelles, further liberation of individual casein molecules (Jaros and Rohm, 2003; Routray et al., 2011).

Ayran is one of the traditional fermented milk beverage which has a characteristic salty taste in Turkey. Ayran is a fermented milk product produced by the addition of water to yogurt (homemade) or by the addition of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* to standardized milk (industrially produced) (TFC, 2009). In Turkey annual production of dairy products can be broken down into the following components: fresh milk (251,632 tons), yogurt (1,1 billion tons), cheese (681,085 tons), butter (45,000 tons), ayran (51,844 tons) and cream (1,383 tons) (Table 2.2) (TÜİK, 2015).

Table 2.2 Turkish Dairy Industry Data (Kırdar, 2017).

Dairy Production (× 1000 ton)	**2010**	**2011**	**2012**	**2013**	**2014**
Liquid Milk	1,091	1,165	1,250	1,298	1,311
Cheese from cow's milk	473	519	539	574	604
Cheese from others milk	—	—	25	26	29
Whole milk powder	—	—	35	39	51
Skimmed milk powder	—	—	47	40	60
Yoghurt	908	1,007	1,053	1,081	1,101
Ayran	—	459	508	560	599
Cheese	27	31	35	40	43
Whey powder	9.6	15	19	33	41
Butter	12	11	16	20	22
Cheese	5.2	5.9	6.9	8	9.6

Several researchers reported the composition of ayran as the total dry matter (1.07–11%), protein (1.44–3.48%), salt (0.17–1.75%) and fat (0.1–3%). The titratable acidity is 0.4–1.73% and the pH varies between 3.44 and 4.44 (Gülmez et al., 2003; Kocak et al., 2006; Sanli et al., 2011; Sen and Küplülü, 2004; Patir et al., 2006; Tamucay-Özünlü and Kocak, 2010).

2.2.1 Manufacturing Technology of Ayran

Traditionally ayran may be prepared by blending yoghurt with water (30–50%) and salt (0.5–1%) (Köksoy and Kilic, 2003). On the other hand, industrial production of ayran can be carried out by two different methods; which can be produced either by the addition of water to yoghurt or the addition of water to milk first and then fermentation of diluted milk (Kocak et al., 2006; Kocak and Avsar, 2009). These two methods are shown in Figure 2.1.

Industrial production starts with the standardization of raw milk in terms of fat ratio (1.5% for full fat, 0.8% for half-fat, 0.15% for fat free). In the first method, standardized milk is diluted with water until 8% of total solid content is obtained. In the second, water is added after the fermentation step. Milk is homogenized and pasteurized in both techniques (Kocak and Avsar, 2009) (Figure 2.1).

Good microbiological quality of ayran could be obtained by heat pasteurization before fermentation (Kocak and Avsar, 2009; Sen and Küplülü, 2004). Pasteurized milk is then inoculated with yoghurt starter cultures (*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) and incubated until a pH 4.2–4.4 is obtained. After the achievement of the required acidity fermented samples are cooled (Köksoy and Kilic, 2003; Kocak and Avsar, 2009). In the 2nd technique, adequate water is added to the fermented milk until the total solid content of the sample reaches to 8% followed by salt addition (0.5%). Industrially produced ayran should be packaged (Sen and Küplülü, 2004). Acetaldehyde content may be affected as a result of two manufacturing techniques, but the difference is not detectable in sensory analysis.

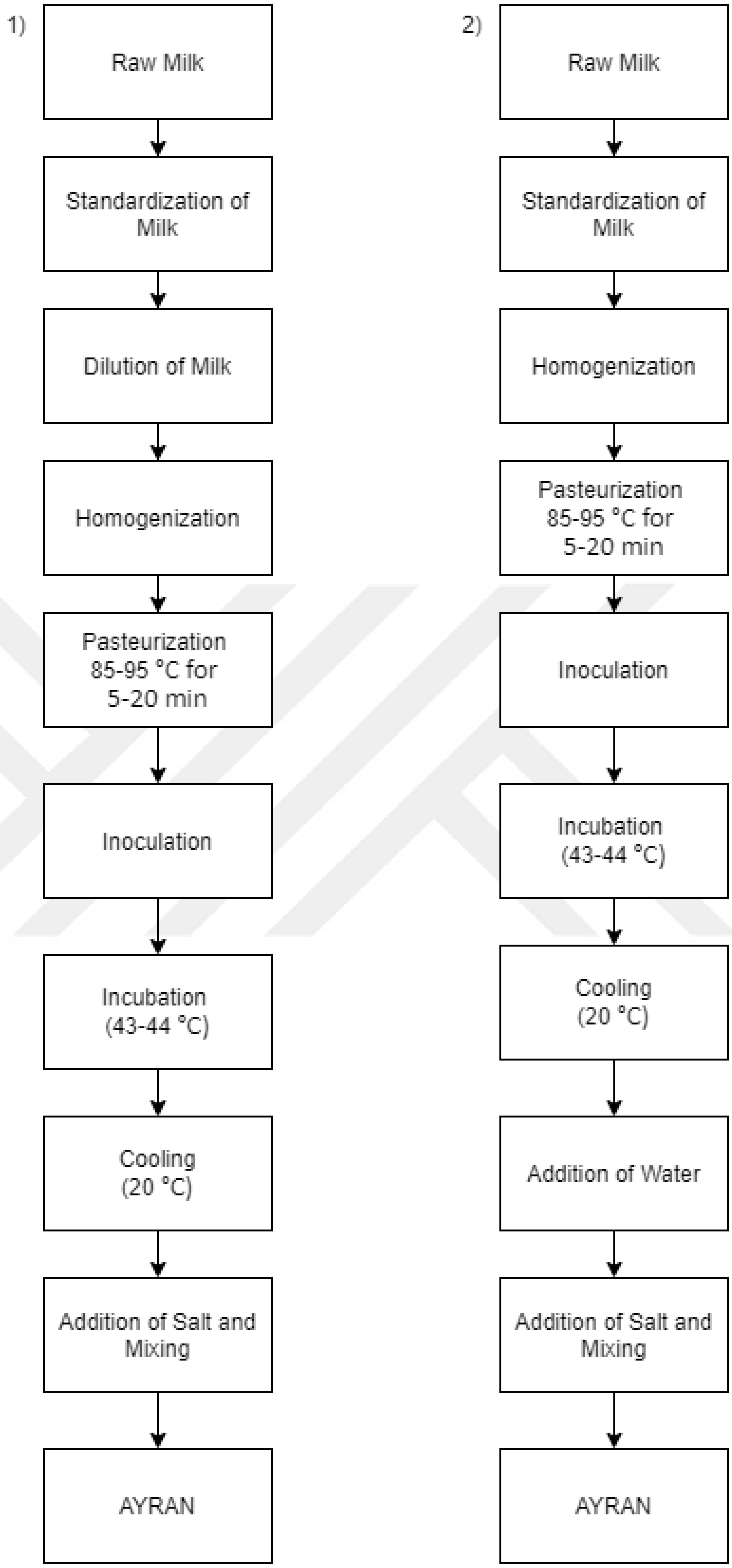

Figure 2.1 The manufacturing method of industrial production of ayran with two different techniques (Redrawn from Kabak and Dobson, 2011 and Kocak and Avsar, 2009).

2.2.2 Rheological Properties of Ayran

The rheological behavior of a food matrix is an important physical property which directly affects overall product quality and processing characteristics as well as the consumer acceptability (Yegin and Fernandez-Lahore, 2012). Moreover, rheological data could be used to assess shelf life of the food and to evaluate the food texture by correlating them to sensory properties (Steffe, 1996).

There are only a few studies on the rheological properties of ayran. Köksoy and Kilic (2003) and Lokumcu et al. (2002) observed that ayran exhibited pseudoplastic (shear thinning) or thixotropic behavior depending on its fat content. Bayraktaroglu and Obuz (2008) also found that whole ayran had pseudoplastic behavior. However, light ayran that does not contain fat exhibited showed nearly Newtonian behavior. Lokumcu et al. (2002) reported that consistency index (K) and flow behavior index (n) values of ayran samples at 9.5 °C varied between 0.07 and 0.70 $Pa \cdot s^n$ and 0.38–0.66, respectively based on the fat content of ayran samples. They concluded that the rheological properties of ayran samples varied because of different compositions of products and/or manufacturing technologies.

Köksoy and Kilic (2003) produced ayran with traditional method (by mixing the yogurt with water and salt) in the laboratory conditions. They found that the addition of salt up to 1% and water up to 50% at 10 °C decreased the K values of ayran samples from 1.214 to 0.018 $Pa \cdot s^n$. These results indicated how the ingredients could affect the rheological properties of ayran. Studies related to the rheology of ayran are given in Table 2.3.

2.2.3 Serum Separation of Ayran

The main textural problems are low viscosity and serum separation (up to 30%). Serum separation occurs in fermented milk products due to the aggregation of casein micelles to particles and sedimentation of casein particles during storage. The presence of salt in ayran may also increase the serum separation. The addition of increasing levels of salt and water was found to increase the serum separation in ayran (Köksoy and Kilic, 2003).

Table 2.3 Some rheological studies carried out on ayran

Sample	Type	Additives	Viscosity	Rheological Parameters*	Reference
Ayran	Made from yogurt	Pectin	7.33 s/10 cm for control	—	Atamer et al. (1999)
Ayran	Commercial	None	—	K = 0.07–0.70 Pa·s^n n = 0.66–0.38	Lokumcu et al. (2002)
Ayran	Made from yogurt	None	—	K = 0.508 70 Pa·s^n n = 0.466 (0.5% salt and 30% water)	Köksoy & Kilic (2003)
Ayran	Made from yogurt	Low methoxyl pectin K-carrageenan Na-alginate Agar agar Guar gum Locust bean gum High methoxyl pectin Carboxymethylcellulose Gelatine	Apparent viscosity is 15 mPa·s at 55 s^{-1} with thixotropy of 50 Pa·s	K = 37 mPa·s^n n = 0.77 for the control sample Gum additions increased K	Köksoy & Kilic (2004)

* K is consistency index; n is flow behavior index

2.2.4 Shelf-life of Ayran

Köksoy and Kilic (2003) stated that shelf life of ayran is 10-15 days at refrigeration temperature. It is not possible to apply any heat treatments or other processes for extending its shelf life because such processes would also decrease the number of yoghurt bacteria, which must be higher than 10^7 CFU/mL (TFC, 2009) according to the Turkish Food Codex (Kocak and Avsar, 2009).

2.2.5 Microbiology of Ayran

Kocak and Avsar (2009) detected that industrially produced ayran microbiota was more stable compared to the microbiota of homemade ayran. The risk of contamination was very high for homemade ayran and particularly *Kluyveromyces* spp. and *Saccharomyces* spp., were generally present. Some studies concerning microbiological properties of ayran is given in Table 2.4.

Table 2.4 Studies on microbiological quality of ayran.

Microorganisms	Type	Mean	Min.	Max.	Reference
Total aerobic bacteria (CFU/mL)	Homemade	8.02 ± 0.08	6.60	9.02	Patir et al. (2006)
	Commercial	7.03 ± 0.16	4.65	8.95	
Coliforms (MPN/mL)	Homemade	$5.08\times10^2 \pm 9.68\times10^1$	< 3	> 1100	Patir et al. (2006)
	Commercial	$5.01\times10^1 \pm 2.35\times10^1$	< 3	>1100	
Staphyloccus/ Micrococcus spp. (CFU/mL)	Homemade	2.01 ± 0.19	0.95	5.39	Patir et al. (2006)
	Commercial	2.50 ± 0.16	0.95	5.56	
Yeasts and molds (CFU/mL)	Homemade	5.52 ± 0.14	3.23	7.23	Patir et al. (2006)
	Commercial	3.76 ± 0.30	0.95	7.20	

2.3 Shalgam

Shalgam is defined as a red colored, sour, and cloudy soft beverage by Tangüler and Erten (2012). Shalgam is produced by lactic acid fermentation with mixture of turnips, black carrot bulgur (broken wheat) flour, water, and salt. It is widely consumed in the Mediterranean region of Turkey (cities such as Adana, Hatay, and Mersin). Nowadays, it also becomes popular in metropolises.

Several researchers reported the composition of shalgam as the total dry matter (2.0–4.0%), protein (0.09–0.018%), salt (1.1–2.2%), lactic acid (0.578–8.05 g/L), ash (1.46–2.06%), and ethanol (0.79–6.41). The titratable acidity is 1.06–9.1 g/L, and the pH varies between 3.15 and 4.25 (Arici, 2004; Canbas and Deryaoglu, 1993; Canbas and Fenercioglu, 1984; Özdestan and Üren, 2009; Özhan-Özer, 2009; Tangüler and Erten, 2012). According to Erten et al. (2008) and Incedayi et al. (2008), shalgam is one of the nutritious beverages due to its high mineral, vitamin, amino acid, and polyphenol contents.

Shalgam has also been reported to contain anthocyanin as cyanidin-3-glycoside (88.3–114.1 mg/L) (Canbas, 1991). The anthocyanin content of homemade shalgam (83.19 mg/L) was found to be higher than the commercial products (41.89–48.40 mg/L) (Ercelebi and Özkanli, 2010).

2.3.1 Manufacturing Technology of Shalgam

Homemade shalgam is generally produced especially in southern parts of Turkey. However, commercial production is on the rise in recent times (Canbas and Fenercioglu, 1984; Erten et al. 2008). There are two main processing methods for commercial production which are the direct and the traditional methods. The traditional method has two distinct fermentation stages: sourdough or first fermentation and carrot or second fermentation. However, the direct method has only second fermentation (Erginkaya and Hammes, 1992; Erten et al., 2008). The two production methods are shown in Figures 2.2. and 2.3.

In the traditional method, sourdough fermentation is carried out by adding sourdough, salt, and water to bulgur flour. (Erten et al., 2008). The mixture is allowed to ferment at ambient temperature for 3–5 days. The purpose of the sourdough fermentation is to increase the numbers of LAB and yeasts (Tangüler and Erten, 2012a). Then, the mixture is extracted with water.

Second fermentation starts with the addition of salt, sliced black carrot and sliced turnip are added to the extract in a tank for carrot fermentation. Traditionally, wooden vessels are used for the fermentation. While the use of fiberglass, plastic or stainless-steel tanks could be seen. Fermentation accomplishes naturally for 3 to 10 days at ambient temperature, which can vary from 10 to 35 °C. At the end of the second fermentation, shalgam is obtained (Canbas and Fenercioglu, 1984; Erten et al., 2008).

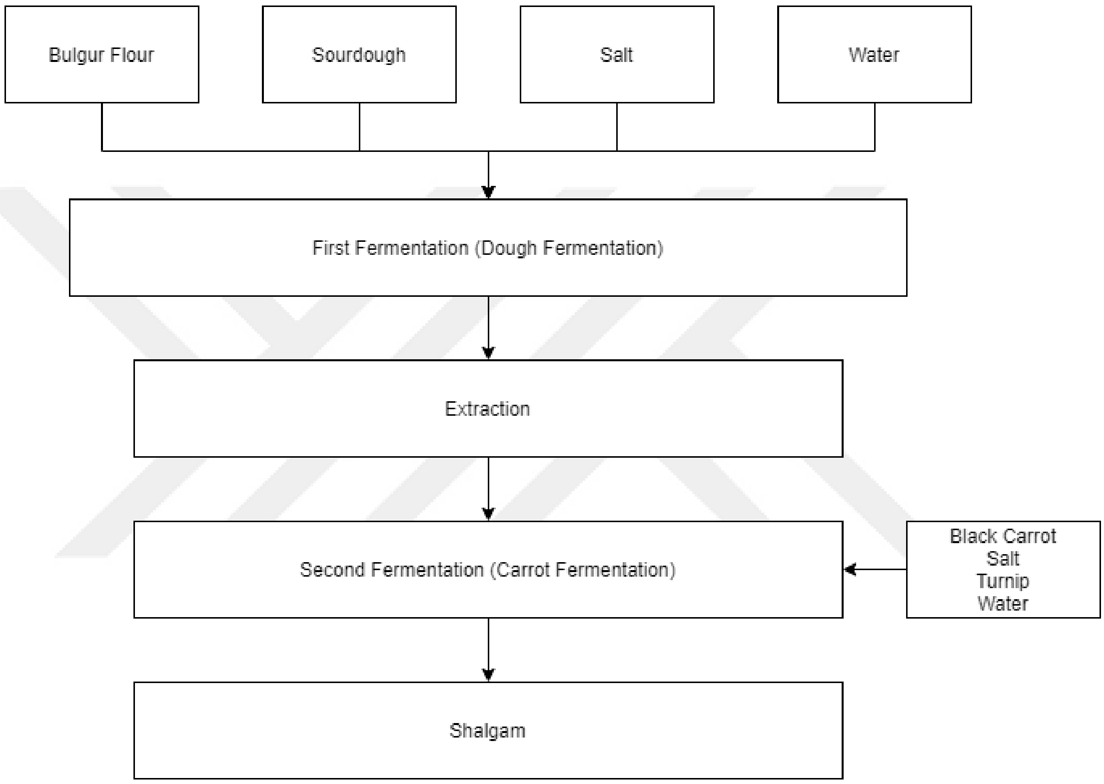

Figure 2.2 The production of shalgam by traditional method. (Erten et al., 2008).

In direct method, chopped black carrots (10–20%), salt (1–2%), if available sliced turnip (1–2%), bakers' yeast (*Saccharomyces cerevisiae*) or sourdough (0.2%) and adequate water are taken into the fermentation tank. Fermentation occurs at ambient temperature (10 to 35 °C) for 3–10 days. (Erten et al., 2008).

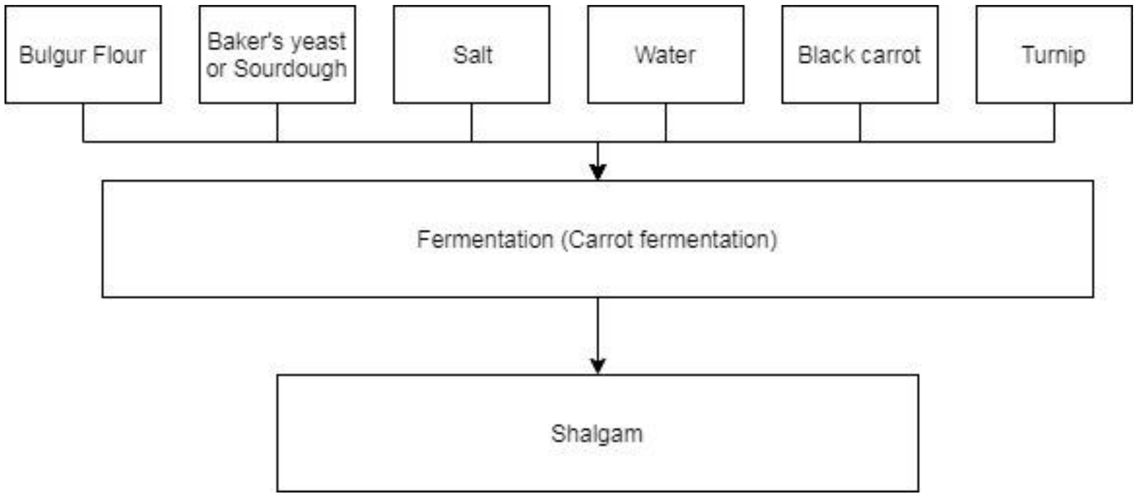

Figure 2.3 The production of shalgam by direct method. (Erten et al., 2008).

2.3.2 Shelf Life of Shalgam

Canbas and Fenercioglu (1984) and Erten et al. (2008) observed that in sealed container the shelf life of unpasteurized shalgam was about 3 months at 4 °C. However, the shelf life could be increased up to 1-2 years by heat treatment and addition of the preservatives. Özler and Kılıç (1996) pasteurized the shalgam at 65 °C for 30 min and stored at 4 °C for 6 months. Tangüler and Erten (2012) carried out a study on the addition of benzoic acid and its salts in shalgam and applied heat pasteurization. They observed that shelf life of shalgam could be extended up to 1 year. According to Turkish Food Codex Food Additives, benzoic acid or its salts (sodium, potassium, and calcium benzoate) may be used as the chemical additive up to 200 mg/L.

The pH of shalgam should be between 3.3–3.8 (TS, 2003) that is why the risk of growth of undesirable bacteria in shalgam is low. Yeasts, on the other hand, may grow in the acid environment, and may spoil shalgam by film formation, off-flavor and color changes. *Candida krusei*, *Candida pelliculosa* and *Candida lipolytica* were reported to cause spoilage in shalgam (Özhan and Özer, 2009).

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

One brand commercial ayran and shalgam were obtained from a local market in Konya, Turkey. Three different samples were used, (with different expiry date) for both ayran and shalgam. All analysis were performed in triplicate. In order to prepare the mixture beverages, ayran and shalgam were mixed in different amounts (v/v): (i) 25% ayran – 75% shalgam, (ii) 50% ayran – 50% shalgam, (iii) 75% ayran – 25% shalgam. Therefore three mixtures were obtained. All mixtures were stored at refrigerator (Arçelik, Turkey), at 4 °C until their use.

3.2 Methods of Analysis

3.2.1 Rheological Measurements

Ayran-shalgam mixture (25%, 50%, and 75%) samples were subjected to the measurement of shear stress (Pa) versus shear rate (s^{-1}) with an increasing range of 1–100 s^{-1} using an Anton Paar rheometer (RheolobQC, Austria) controlled by a computer. The rheological measurements were carried out at three different sample temperatures of 4, 8 and 12 °C. The samples were mixed about 2 min before loading the vessel of the rheometer (100 mL). Three replicates were performed for each mixture.

Power law model [Eq.(1)] was used to determine the rheological properties of the mixtures:

$$\tau = K\gamma^n \quad (1)$$

where τ is the shear stress (Pa), γ is the shear rate (s^{-1}), K is the consistency index ($Pa \cdot s^n$) and n is the dimensionless flow behavior index. If $n = 1$ the fluid is Newtonian, if $n < 1$ the fluid is pseudoplastic (shear thinning) and if $n > 1$ the fluid is dilatant (shear thickening).

Non-linear regression analysis was performed by using SigmaPlot software (Version 12, Chicago, IL, USA) in order to estimate the parameters of the model and goodness-of-fit of the model was assessed by adjusted coefficient of determination (R^2_{adj}) and root mean square error (RMSE) values.

3.2.2 Shelf Life Analyses

All mixtures were stored at refrigerator at 4 °C for 28 days. The analyses (pH measurements, serum separation, microbiological study) were performed at 0^{th}, 7^{th}, 14^{th}, 21^{st}, and 28^{th} days of storage.

3.2.2.1 pH Measurement

The pH values of the samples were measured at 4 °C using a pH meter (Seven Compact model, Mettler Toledo, Switzerland). The samples were shaken by hands about 2 min in order to have homogenous beverages before the pH measurement. Results were obtained from the 1^{st}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of storage at 4 °C.

3.2.2.2 Serum Separation

Serum separation was monitored by using the method of Köksoy, Kılıç (2003). All the mixtures individually were placed in 50 mL of graduated cylinders for 28 days and the volume of separated serum was recorded at 1^{st}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of storage at 4 °C.

3.2.2.3 Microbiological Study

For microbiological studies, each medium was accurately weighed with an analytical balance (TX4202L model, Shimadzu, Japan) and dissolved in appropriate quantity of distilled water according to the manufacturer's specification; solved with magnetic stirrer (Mr. Hei-Standard model, Heidolph, Germany); till the agar powder melted, and the medium was sterilized in an autoclave (CLG model, Alp, Japan) if it was necessary. If not, medium was boiled by using bunsen burner (Eisco, USA) and placed in a water bath at 47 °C.

Maximum recovery diluent was prepared with distilled water according to the manufacturer's specification. Nine mL of diluent was put into the tubes and they were sterilized in the autoclave.

One mL of ayran samples was transferred to 9.0 mL of sterile maximum recovery diluent and mixed carefully using a vortex mixer for 5 - 10 seconds. Then, 1 mL from the previous dilution was added to tubes containing 9.0 mL of sterile maximum recovery diluent and mixed together with the vortex tube mixer (creating a 10^{-2} dilution). That procedure was repeated for as many dilutions in the sequence of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}.

Same procedure also applied on the shalgam, 25% ayran + 75% shalgam, 50% ayran + 50% shalgam and 75% ayran + 25% shalgam (v/v) samples.

All microbiological analyses were performed at the 1st, 7th, 14th, 21st and 28th day of storage at 4 °C.

<u>Total aerobic count</u>

To determine the number of total aerobic count 0.1 mL of each dilution was seeded on Plate Count Agar (PCA) (Merck, Darmstadt, Germany) plates using spread plate method and the plates were then incubated at 30 °C for 48 h in an incubator (Nuve, Turkey). Total colonies on the surface of the plates were counted and expressed as $\log_{10}$ colony forming unit per milliliter ($\log_{10}$CFU/mL) of the samples.

<u>Yeast & molds</u>

The method of Harrigan (1998) was used for determination of number of yeast and mold. Each sample dilution (0.1 mL) was seeded on Dichloran Rose-Bengal Chloramphenicol (DRBC) Agar (Merck, Darmstadt, Germany).

Using spread plate method and the plates were incubated at 25 °C for 48 h. The colonies were counted and expressed as log_{10} colony forming unit per milliliter (log_{10}CFU/mL) of the sample.

Streptococcus

Diluted samples with the volume of 0.1 mL were spread onto plates of M17 agar (Merck, Germany). It was used to enumerate *streptococci* in samples (Vinderola & Reinheimer, 1999). Using spread plate method and the plates were incubated at 37 °C for 48 h. The colonies were counted and expressed as log10 colony forming unit per milliliter (log_{10}CFU/mL) of the sample.

Lactobacillus

Each diluted sample with 1 mL of volume was seeded on Man, Rogosa and Sharpe agar (MRS) (Merck, Darmstadt, Germany) plates using pour plate method and the plates were incubated anaerobically at 37 °C for 48 h. The colonies were counted under surface and expressed as log10 colony forming unit per milliliter (log_{10}CFU/mL) of the sample.

3.2.3 Sensory Analysis

The consumer acceptability properties were conducted by sensory evaluation of shalgam and ayran mixtures with different ratio. Sensory analysis was performed using the scoring and ranking test according to the method described by Barillère, Bénard (1986). Sensory analysis was performed by 8 trained (expert) and 6 semi-trained panelists. These panelists were the members of PANAGRO A.Ş. at Konya.

The samples were presented in random order and then the panelists were informed about the evaluation criteria. They recorded their scores on a sensory evaluation form (Table 3.1) Scoring test was performed on hedonic rating, indicating color, odor, taste, structure, and appearance. Panelist graded the taste, structure in closed cup. Subsequently, they opened the lid of the cup and ranking the color, odor, and appearance. Water was provided to the panel members for palate cleansing between samples.

Table 3.1 Sensory evaluation form of ayran-shalgam mixtures.

Name of Panelist:	
Date:	

Product#	Taste	Odor	Color	Structure	Appearance	General Evaluation

Type the hedonic rating that best describes your feelings about the product.
5: I really like **4:** Little like **3:** Both like and unlike **2:** Little unlike **1:** I really unlike
COMMENTS:

3.2.4 Statistical Analysis

All experiments were performed in triplicate and the results were presented as mean ± standard deviation. A one-way analysis of variance (ANOVA) was conducted to determine the effect of the three temperatures on the viscosity of samples using Minitab version 17 (State College, PA, USA). To determine which means are significantly different from each other, the Tukey's multiple range test method was used. Trends were considered significant when the means of compared parameters differed at $P<0.05$ significance level.

CHAPTER IV

RESULTS AND DISCUSSIONS

The effect of independent variables on viscosity, self-life and sensory properties of mixtures were investigated. All the analysis for ayran, shalgam, and their mixtures were triplicated and their averages were used. The results obtained and then analyzed by ANOVA.

4.1 Rheological Properties of Ayran-Shalgam Mixtures

A lot of rheological models have been employed to fit the viscosity data on fermented dairy drinks. (Köksoy and Kilic 2003, 2004). The power law model [Eq.(1)] has been used in the flow behaviour modeling of fermented milk beverages. (Kristensen et al. 1997; Penna et al. 2001). The power law model was also found suitable in this study to model the shear stress data of the ayran with shalgam in model system at increasing shear rate.

Shear rate versus shear stress graph of ayran, shalgam and their mixtures i.e., 25% ayran + 75% shalgam, 50% ayran + 50% shalgam and 75% ayran + 25% shalgam (v/v) at 4 °C is shown in Figure 4.1. The highest shear stresses were obtained for ayran whereas the lowest ones were obtained for shalgam. The mixtures were in between but were close to the values of shalgam. Shear rate versus apparent viscosity at 4 °C is shown in Figure 4.2. Similar to shear stress, apparent viscosity values were the highest for ayran and the lowest for shalgam, and the values for mixtures were close to that of shalgam.

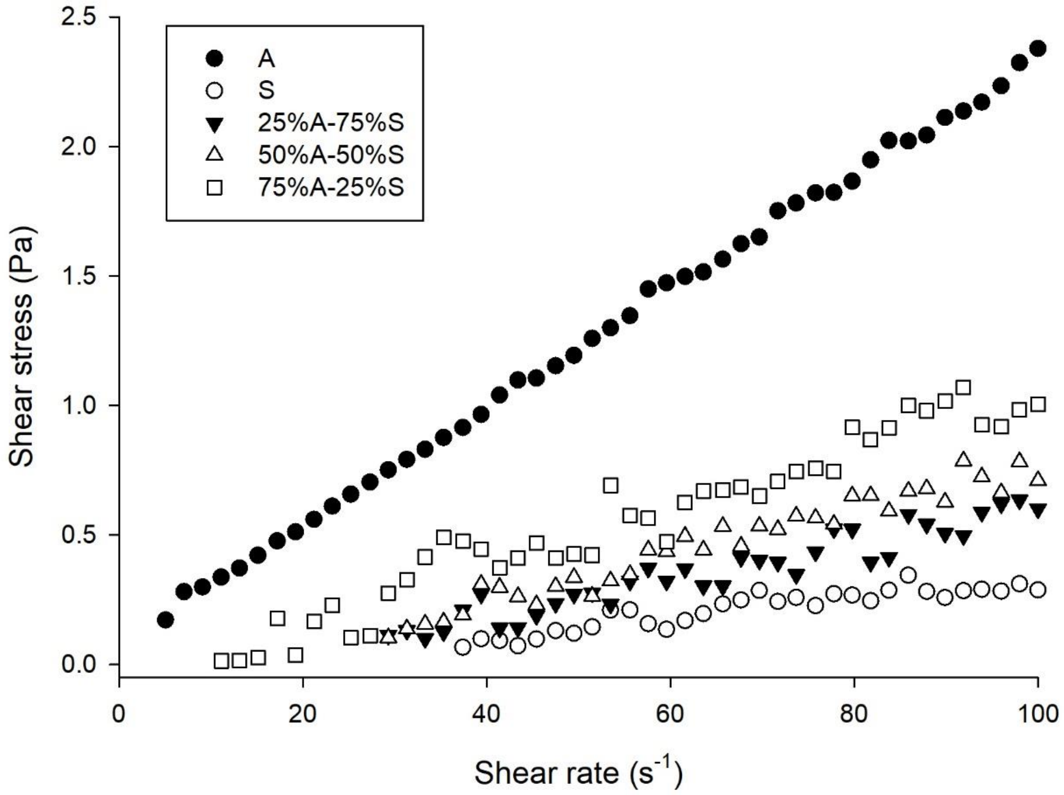

Figure 4.1 Shear stress of ayran (A), shalgam (S) and their mixtures as a function of shear rate at 4 °C.

Shear rate versus shear stress graphs for the mixtures at 8 and 12 °C are given in Figures 4.3 and 4.4, respectively. Similar trends were also observed at 8 and 12 °C.

Torre et al. (2003) found that viscosity values of set-type fermented milks made with different commercial probiotics were between 15.8 and 23.3 mPa s. Milk beverages which fermented were found to be non-Newtonian fluids with shear thinning. (Butlert and McNulty 1995; Kristensen et al. 1997; Lucey et al. 1999; Penna et al. 2001; Köksoy and Kilic 2003).

It was observed that K decreased with increasing shalgam concentrations in model ayran. This result can be based on the acidity ambiance in samples ayran with shalgam.

In consequence of acidification of milk with fermentation, the casein micelles and the fat globules covered by adsorbed casein, aggregate and form a three-dimensional network of chains and clusters (Dogan, 2011). In this research, with addition of shalgam in ayran acidification ratio increases. As a result of this condition, it may cause more aggregate formation. Well shaked samples before analysis, but due to aggregation, may have caused different results during reading, and this may cause fluctuation in the chart. Additionally, in a work studied by Schkoda, Hechler, and Kessler (1999) found that the addition of NaCl in the ayran, caused the apparent viscosity of acidified casein gels decreased. The researchers found that the decrease in viscosity to reduced tendency of casein micelles to aggregate is due to the increase in repulsive forces created by salt ions on the surface of micelles. Shalgam had saltier taste and addition of shalgam increased NaCl concentration of ayran.

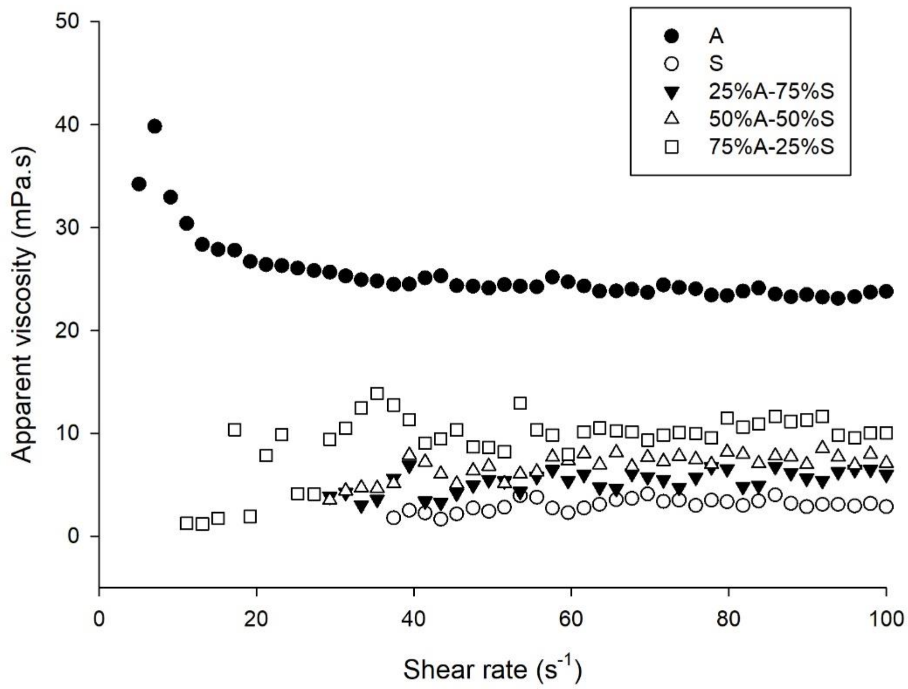

Figure 4.2 Apparent viscosity of ayran (A), shalgam (S) and their mixtures as a function of shear rate at 4 °C.

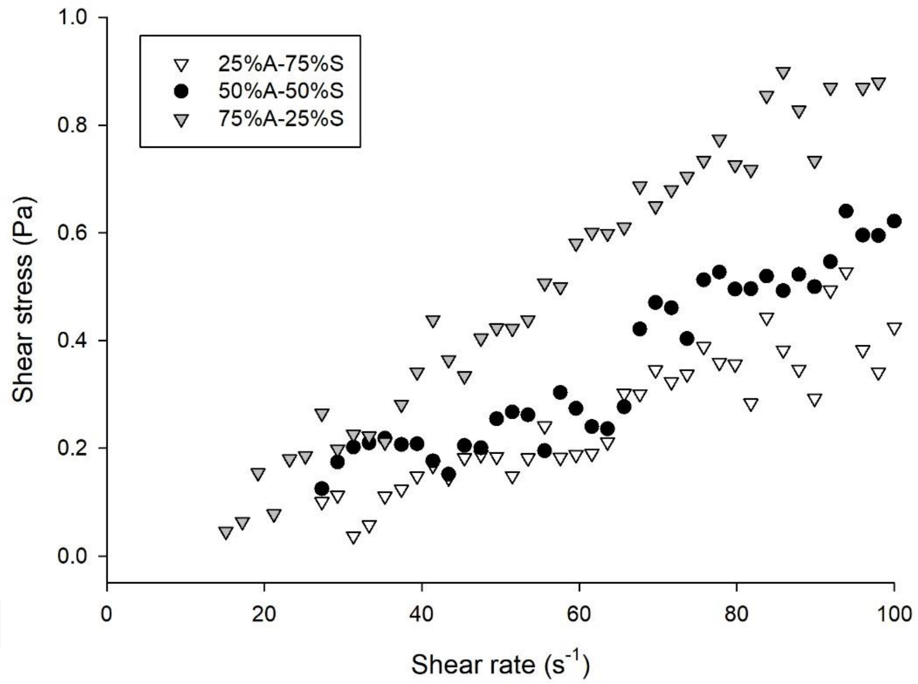

Figure 4.3 Shear stress of ayran (A), shalgam (S) and their mixtures as a function of shear rate at 8 °C.

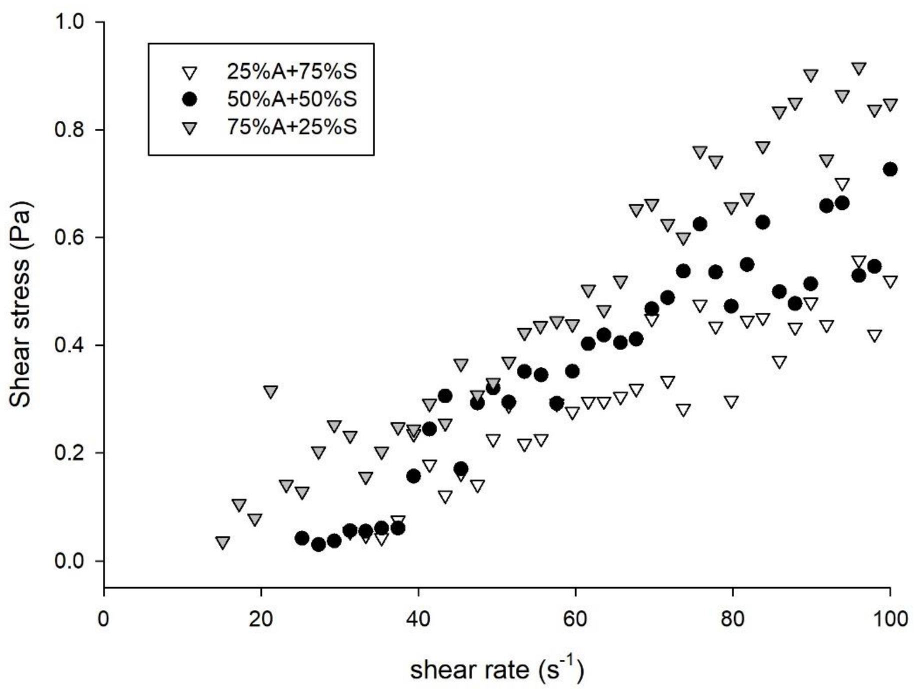

Figure 4.4 Shear stress of ayran (A), shalgam (S) and their mixtures as a function of shear rate at 12 °C.

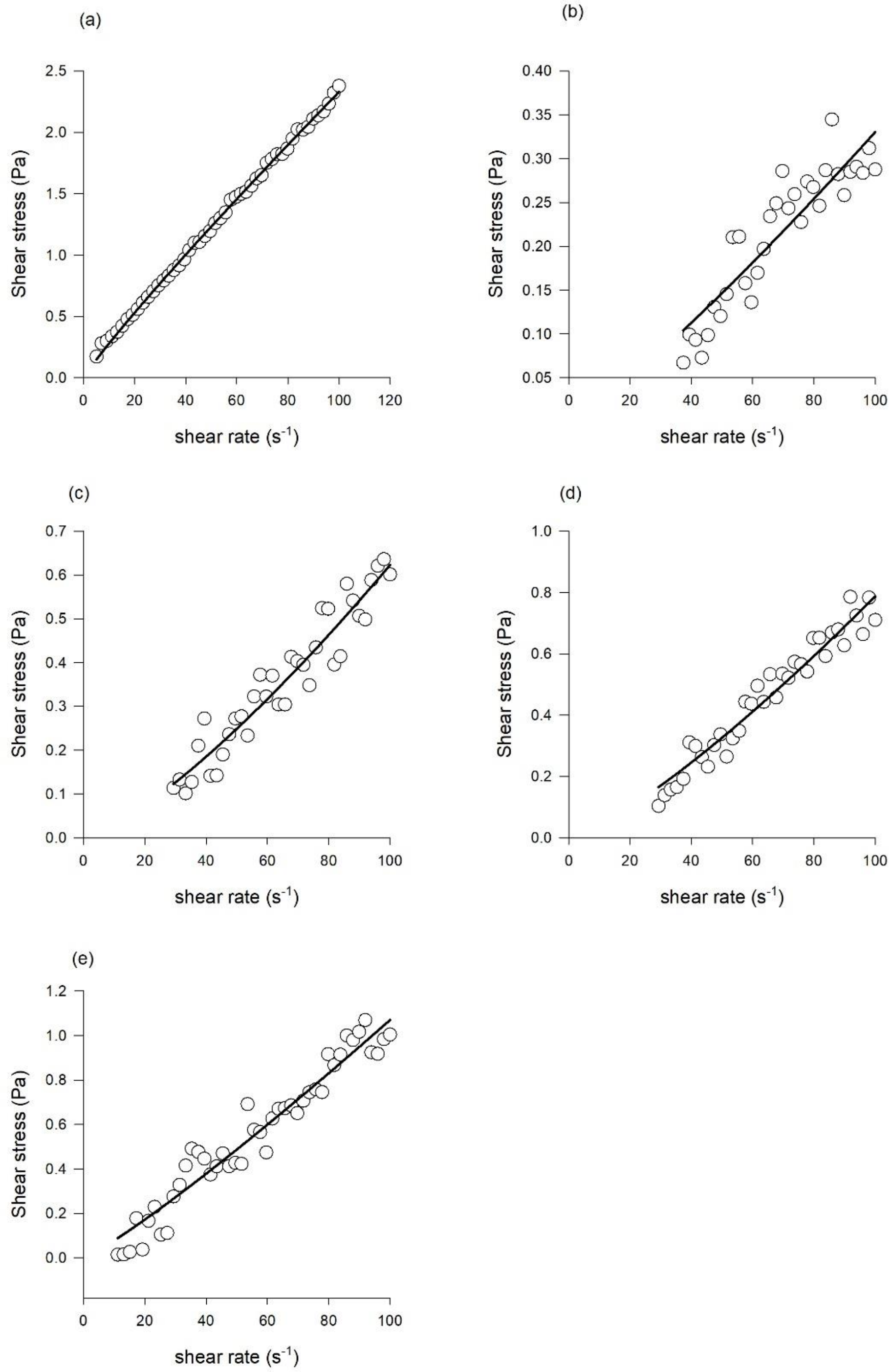

Figure 4.5 Fits of Power law model [Eq.(1)] for Ayran (a), Shalgam (b), 25% ayran + 75% shalgam (c), 50% ayran + 50% shalgam (d) and 75% ayran + 25% shalgam at 4 °C (—— : Predicted, O : Experimental).

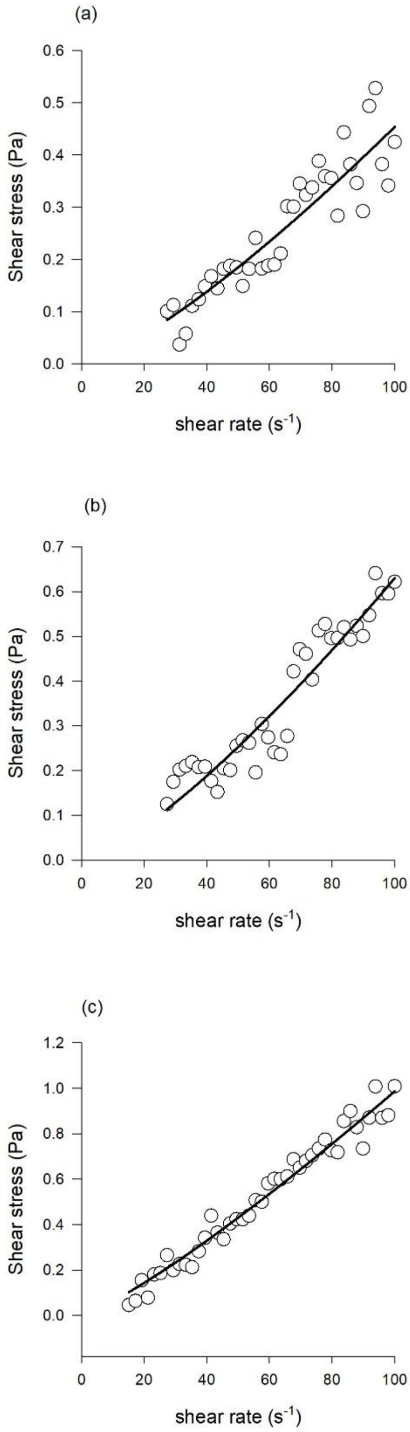

Figure 4.6 Fits of Power law model [Eq.(1)] for 25% ayran + 75% shalgam (a), 50% ayran + 50% shalgam (b) and 75% ayran + 25% shalgam at 8 °C (──: Predicted, O : Experimental).

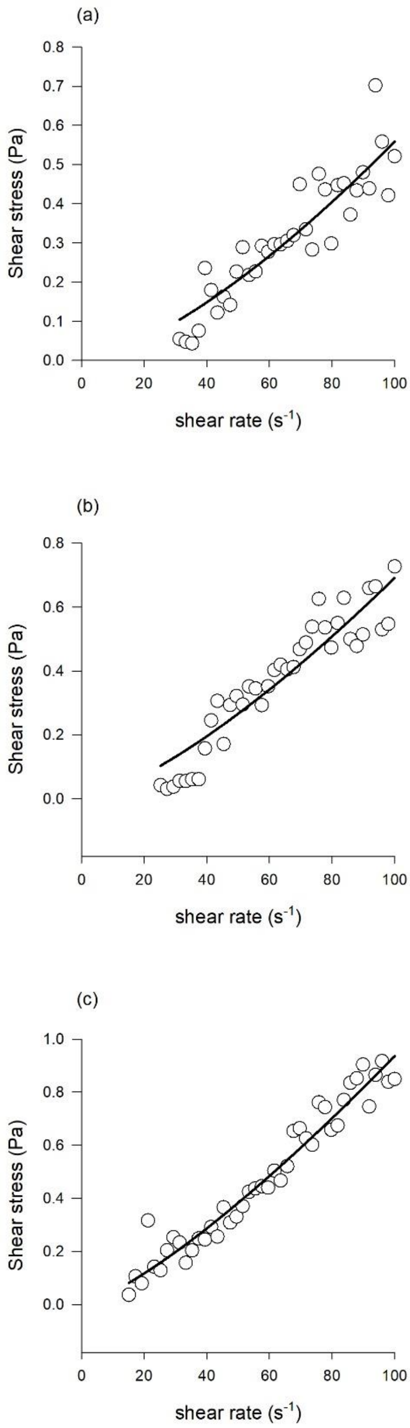

Figure 4.7 Fits of Power law model [Eq.(1)] for 25% ayran + 75% shalgam (a), 50% ayran + 50% shalgam (b) and 75% ayran + 25% shalgam at 12 °C (── : Predicted, o : Experimental).

Fits of the Power law model [Eq.(1)] is given in Figures 4.5, 4.6 and 4.7 at 4, 8 and 12 °C, respectively. The results of fits of power law model to the rheological data are given in Tables 4.1, 4.2 and 4.3. These results indicated that the power law model produced reasonable fits to the data since $R^2_{adj} \geq 0.82$ and RMSE ≤ 0.078. Ayran was slightly pseudoplastic at 4 °C because flow behavior index (n) was obtained as 0.9. On the other hand, shalgam was slightly dilatant at 4 °C (n=1.2) as well as the mixtures (n changing from 1.1 to 1.3). It was apparent that temperature had no effect on the flow behavior index (Tables 4.1, 4.2 and 4.3).

Table 4.1 Rheological parameters of ayran (A), shalgam (S) and their mixtures at 4 °C obtained by fitting the power law model [Eq.(1)].

	K ($Pa.s^n$)	**n (—)**	**R^2_{adj}**	**RMSE**
A	0.0340 ± 0.001[a]	0.9 ± 0.007	0.9985	0.025
S	0.0015 ± 0.007	1.2 ± 0.100	0.8215	0.033
75%A+25%S	0.0058 ± 0.0015	1.1 ± 0.060	0.9379	0.078
50%A+50%S	0.0022 ± 0.0006	1.3 ± 0.060	0.9431	0.047
25%A+75%S	0.0014 ± 0.0005	1.3 ± 0.080	0.9104	0.048

[a] standard error

It was observed that consistency index (K) increased as the amount of ayran increased in the mixture of ayran-shalgam at a constant temperature. Moreover, as the temperature increased K value decreased (Tables 4.1, 4.2 and 4.3).

Ertugay et al. (2012) reported that Ayran exhibited a pseudoplastic fluid with a non-Newtonian character since the values of the flow behavior index (n), a measure of the departure from Newtonian flow, ranged between 0 and 1. This research reported similar results with our findings.

No studies were found on the rheological properties of shalgam or ayran added shalgam in the literature.

Table 4.2 Rheological parameters of ayran (A) and shalgam (S) mixtures at 8 °C obtained by fitting the power law model [Eq.(1)].

	K (Pa.s^n)	n (—)	R^2_{adj}	RMSE
75%A+25%S	0.0040 ± 0.0008[a]	1.2 ± 0.05	0.9664	0.051
50%A+50%S	0.0015 ± 0.0006	1.3 ± 0.09	0.8983	0.051
25%A+75%S	0.0012 ± 0.0006	1.3 ± 0.10	0.8382	0.051

[a] standard error

Table 4.3 Rheological parameters of ayran (A) and shalgam (S) mixtures at 12 °C obtained by fitting the power law model [Eq.(1)].

	K (Pa.s^n)	n (—)	R^2_{adj}	RMSE
75%A+25%S	0.0025 ± 0.0006[a]	1.3 ± 0.06	0.9503	0.059
50%A+50%S	0.0012 ± 0.0006	1.4 ± 0.10	0.8682	0.073
25%A+75%S	0.0007 ± 0.0004	1.4 ± 0.20	0.8176	0.067

[a] standard error

4.2 Shelf Life

4.2.1 Serum Separation

Serum separation is a common phenomenon occurring in acidified dairy drinks and results in the sedimentation of large particles at the bottom or the formation of a serum layer at the top due to aggregation and sedimentation of casing particles during storage (Kiani et al., 2008). In our case serum and shalgam remained at the top and sedimentation at the bottom (Figure 4.8). Therefore, the volume measured at the top was not only the volume of the serum separated from ayran but also the volume of shalgam that existed in the mixture. Nevertheless, for simplicity the term serum separation was used throughout this study.

Figure 4.8 Serum separation picture of mixtures at the 28th day of storage [The tube on the left has 25% ayran and 75% shalgam (v/v); the tube at the center has 50% ayran and 50% shalgam (v/v); the tube on the right has 75% ayran and 25% shalgam (v/v)].

Serum separation data during storage for 28 days is shown in Figure 4.9. As it can be seen that serum separation increased up to second week and then stabilized for all mixtures. Ayran is a diluted yoghurt beverage that has the physical properties of yoghurt. During yoghurt manufacturing, high pasteurization temperature (93 °C) is applied to milk to denature whey proteins and thereby to improve the water holding capacity of resultant yoghurt (Tamime and Robinson, 1999). This gel network structure of yoghurt composed of casein and heat denatured whey proteins is soft and sensitive to mechanical damage. In the manufacture of ayran, it is mixed with water and salt, gel network is disrupted. (Köksoy and Kılıç, 2003) Moreover, as Schkoda et al. (1999) suggested addition of salt could provide charged groups that create repulsive forces and prevent aggregation of the casein micelles. In this research, with the addition of shalgam to ayran, salt content increased and gel network was more disrupted. Köksoy and Kılıç (2003) also reported that at pH values of 4.2 in ayran which is lower than the isoelectronic point of caseins, electrostatic repulsions between micelles could be reestablished. A common denominator of fermented milk drinks like ayran is their low pH and low viscosity which results in sedimentation problems due to aggregation of milk protein. (Amice and Quemeneur et al., 1995).

Addition of shalgam to ayran reduced the pH value of the mixtures. For the same reason, electrostatic repulsions reestablished. Nevertheless, protein-protein interactions might increase during storage promoting aggregation and release of serum from the network as described by Köksoy and Kılıç (2003).

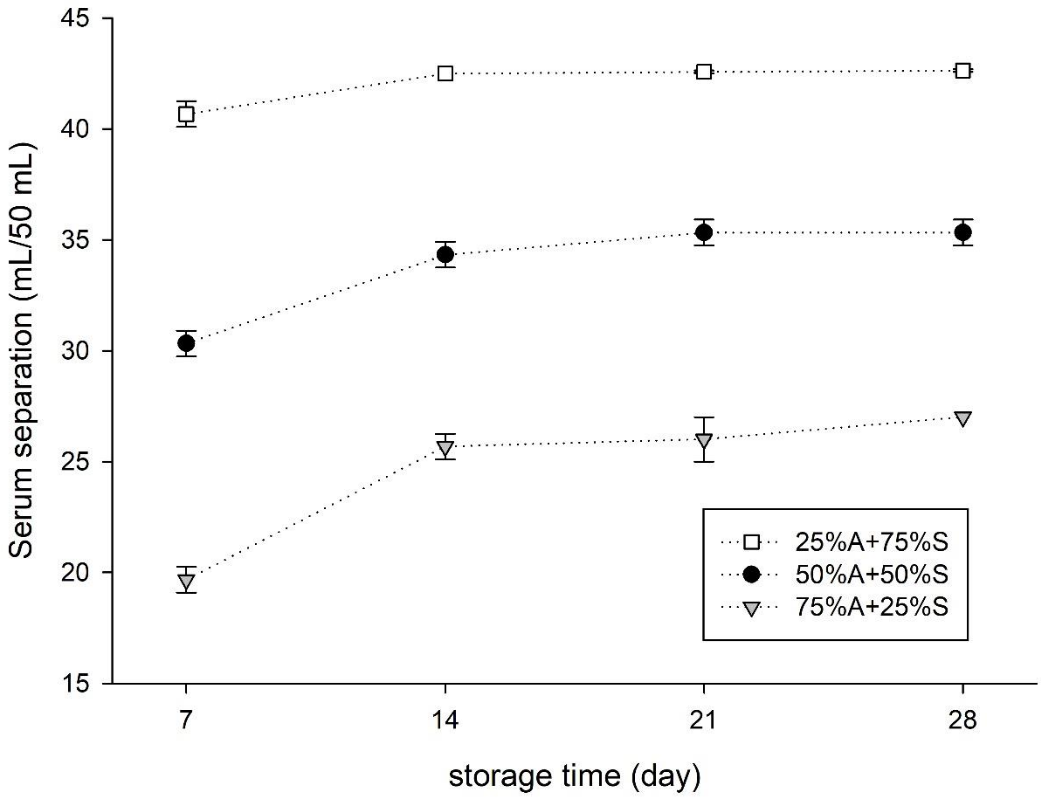

Figure 4.9 Serum separation i.e., serum + shalgam levels in ayran (A) – shalgam (S) mixtures during storage at 4 °C (Error bars represent standard deviation of three means).

4.2.2 pH

The initial pH values of the shalgam samples were 3.33±0.04 (± shows standard error). Canbas and Deryaoglu (1993) reported that the pH values of commercial shalgam samples obtained from four well-known producers at three different production periods in Adana ranged from 3.33 to 3.67. The results in this study were in agreement with the findings of Canbas and Deryaoglu (1993).

The initial pH values of ayran samples, in the current study, ranged between 4.10 and 4.14 (with an average of 4.12±0.04). The pH values of ayran samples were reported as 3.96 to 4.52 in literature (Tamucay-Özünlü and Kocak, 2010; Sanli et al., 2011) which are in agreement with our results.

The change of pH values of ayran-shalgam mixtures is displayed in Figure 4.10. Slight increase (0.05 to 0.1 unit) in pH values was observed during the first week then pH values were stabilized just like what was observed in the serum separation.

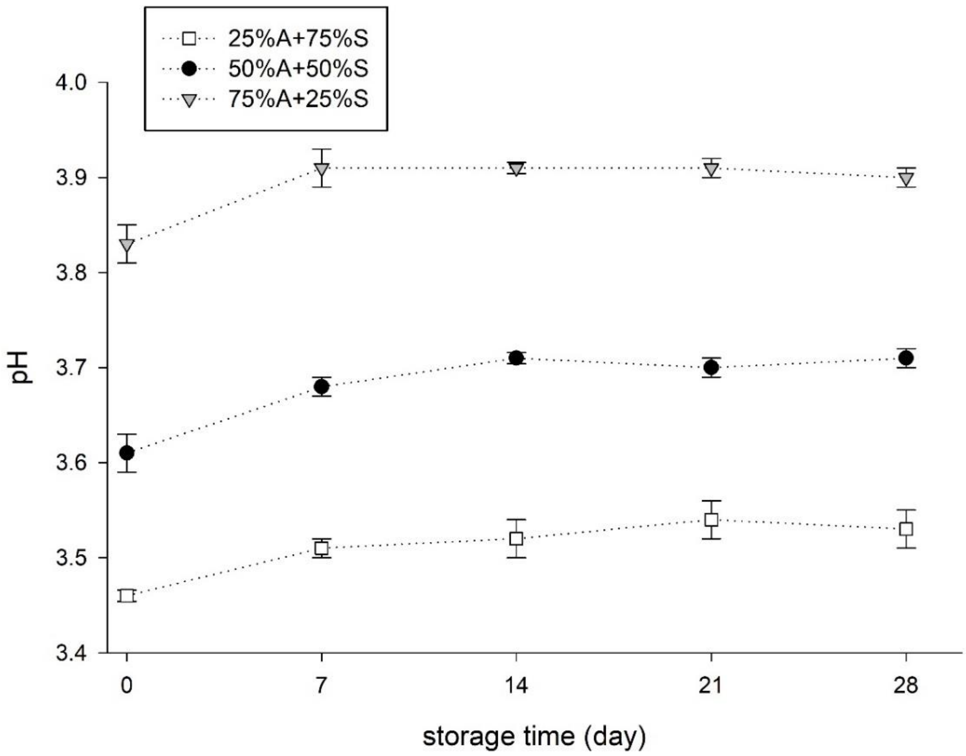

Figure 4.10 pH values of ayran (A) – shalgam (S) mixtures during storage at 4 °C (Error bars represent standard deviation of three means).

4.2.3 Microbiological Analyses

4.2.3.1 Total Aerobic Count

Beal et. al. (1999) found that bacterial concentrations of ayran were influenced by the storage time, final fermentation pH, strain association and incubation temperature. In another study conducted by Şahan et al. (1998), it was reported that total aerobic mesophilic bacteria count of yoghurt varied from 5.74 $\log_{10}$CFU/g to 7.38 $\log_{10}$CFU/g.

Aydar (2003) reported that total mesophilic aerobic bacteria in shalgam were in the range of 6.43–7.79 log_{10}CFU/mL at the end of the fermentation. According to the Turkish Standards (TS 11149), total mesophilic aerobic bacteria (TMAB) must be at a maximum level of 1.0×10^5 CFU/mL in shalgam.

Baruzzi et al. (2016) found that bacterial concentrations of ayran were influenced by the storage time, final fermentation pH, strain association, and incubation temperature. Tanguler and Erten (2012) reported the main reason for relatively high levels of total mesophilic aerobic bacteria could be related to microbiological load of raw materials and inadequate sanitary conditions during processing.

Total aerobic mesophilic bacteria count of the ayran-shalgam mixtures were determined at 0, 7, 14, 21, 28th days of storage and the results are shown in Figure 4.11. In general number of bacteria increased with respect to time and the highest count (6.2 log_{10}CFU/mL) was observed in the 75% ayran + 25% shalgam mixture at the end of the storage period.

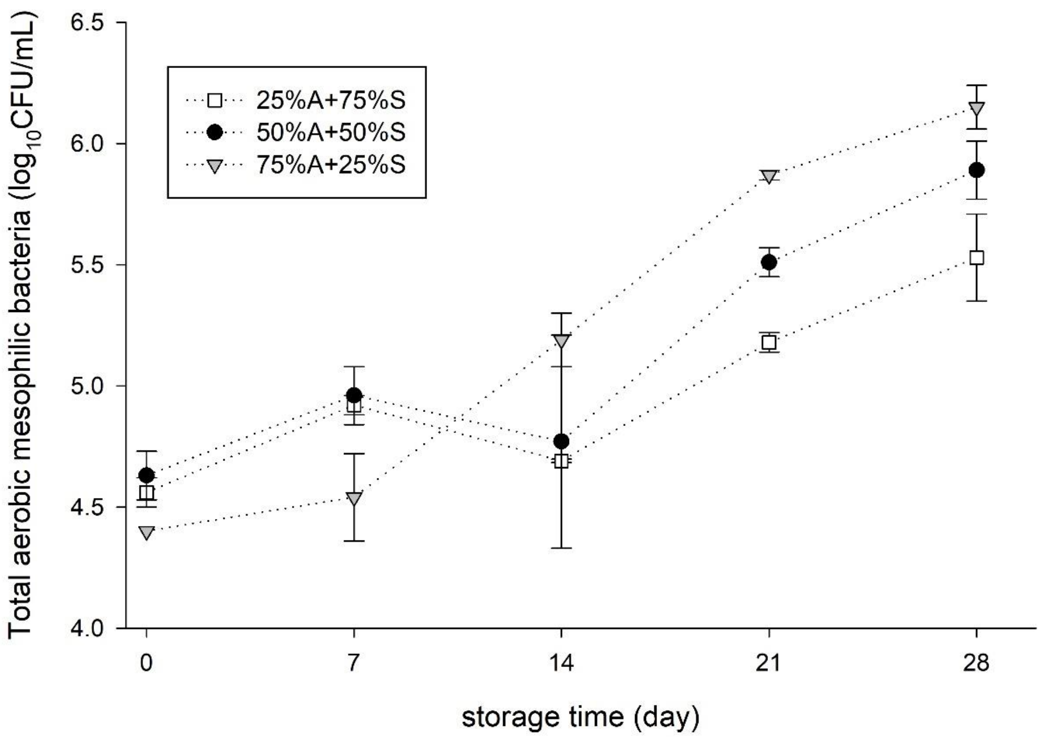

Figure 4.11 Total aerobic mesophilic bacteria count of ayran (A) – shalgam (S) mixtures during storage at 4 °C (Error bars represent standard deviation of three means).

On the 0th day, total aerobic mesophilic bacteria count of the 75% ayran + 25% shalgam mixture was lower than that of 25% ayran + 75% shalgam mixture. Nevertheless, the difference was within the experimental error range i.e., 0.5 log_{10}CFU/mL. Total aerobic mesophilic bacteria count of the 75% ayran + 25% shalgam mixture showed dramatic increase after 7th day of storage. However, counts of total aerobic mesophilic bacteria decreased for 25% ayran + 75% shalgam mixture after 7th day of storage.

At the end of the storage period, the total aerobic count of 25% ayran + 75% shalgam mixture was lower in comparison with 75% ayran + 25% shalgam mixture. Preservatives added during the production of shalgam may affect the count of total aerobic mesophilic bacteria in the mixtures which had contained more shalgam.

4.2.3.2 Yeast and Molds

Yeast and molds count are shown in Figure 4.12. Number of yeast and molds increased with respect to time and similar counts (5.5 to 6.0 log_{10}CFU/mL) were observed for all mixtures at the end of the storage period.

In a study, Jalal (2018) couldn't find any yeast and molds in ayran samples initially, but he observed yeast and molds growth on the 10th days of storage. Şahan et al. (1998) reported that yeast and molds count of yogurt samples were 4.18 log_{10}CFU /g and 6.56 log_{10}CFU/g. According to Institute of Turkish Standards (TS 1330), yeast and molds count of yoghurt should not exceed the level of 2.00 log_{10}CFU/g.

Figure 4.12 shows that yeast and molds count increased progressively throughout the storage period. Jalal (2018) also reported that yeast and mold counts increased dramatically throughout the 20 days of storage which is very similar to our findings.

Utus (2008) found that the total yeast counts was in the range of 7.18–7.61 log_{10}CFU/mL at the end of fermentation in shalgam. Erten et al. (2008) reported, the occurrence of yeasts in shalgam samples may originate from the initial microorganisms available on the surfaces of raw materials (including the bakers' yeast or sourdough) and surfaces of vessels where product is produced.

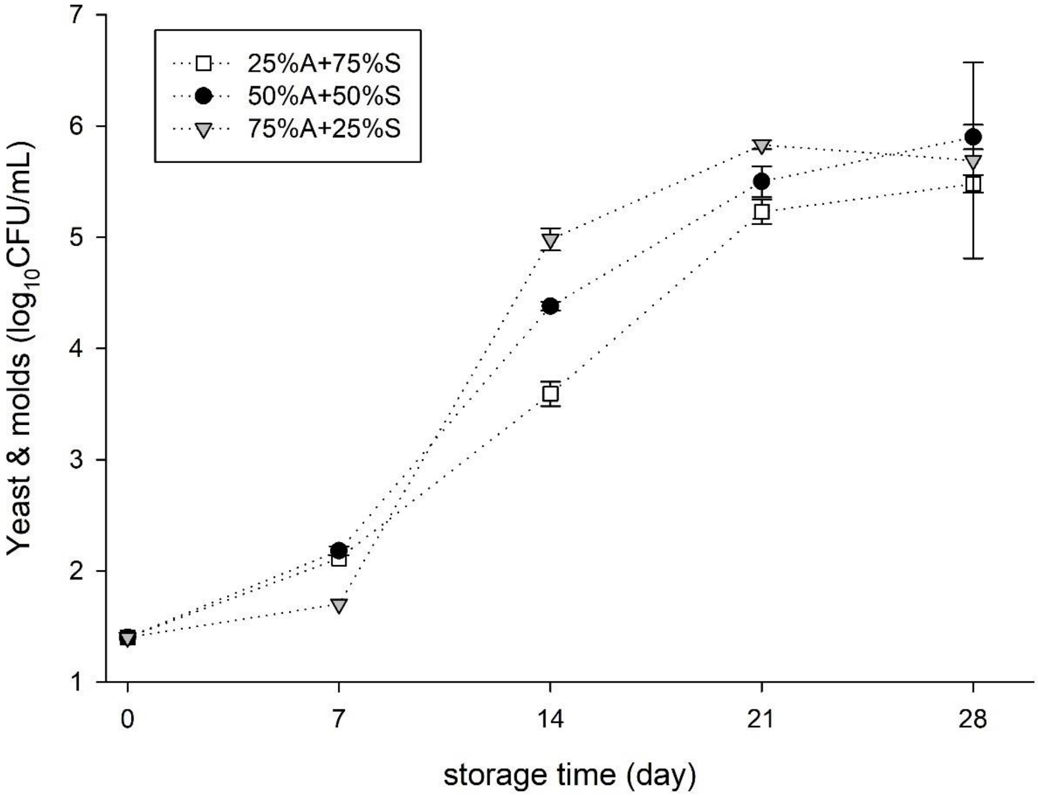

Figure 4.12 Yeast and molds count of ayran (A) – shalgam (S) mixtures during storage at 4 °C. Error bars represent standard deviation of three means.

4.2.3.3 Lactobacilli

Lactobacilli count is shown in Figure 4.13. Number of lactobacilli decreased first two weeks for 25% ayran + 75% shalgam and 50% ayran + 50% shalgam mixtures thereafter it increased; however, this increase was at most 0.4 log_{10}. It was observed that the number of lactobacilli increased while the streptococci count decreased. Similarly, such an opposite situation was also determined by Demirci et al. (2019). As for 75% ayran + 25% shalgam mixture, the decrease in lactobacilli count was up to three weeks and then about 0.7 log_{10} increase was observed.

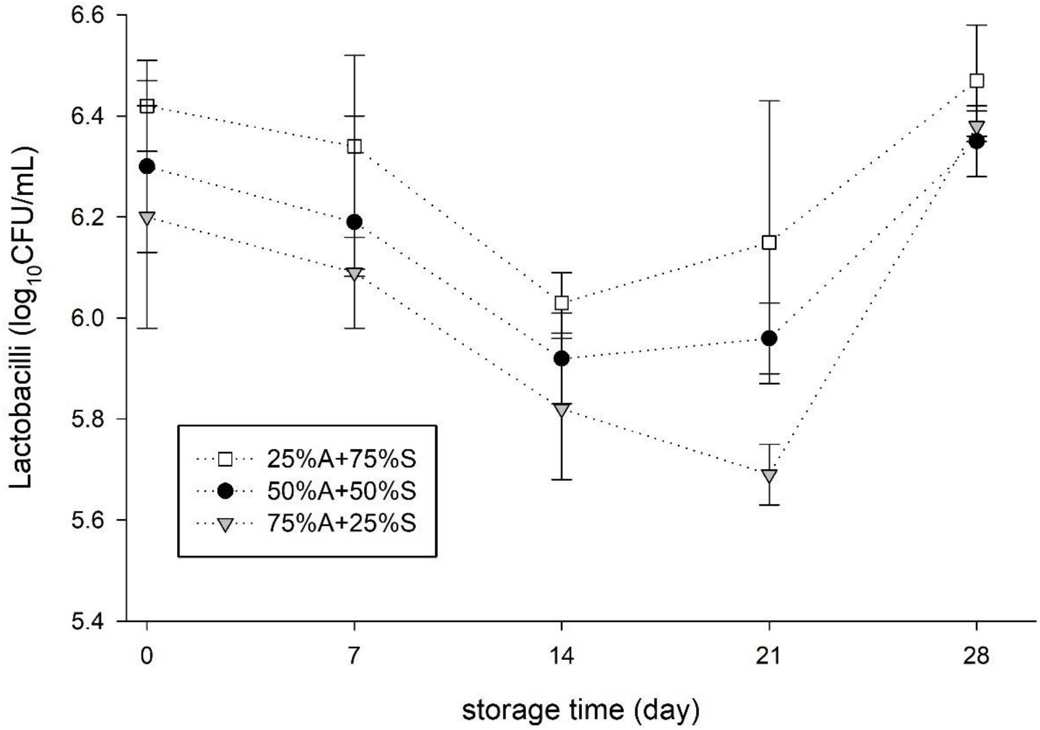

Figure 4.13 Lactobacilli count of ayran (A) – shalgam (S) mixtures during storage at 4 °C. Error bars represent standard deviation of three means.

4.2.3.4 Streptococci

Streptococci count is shown in Figure 4.14. Number of streptococci decreased exactly one $\log_{10}$ for 25% ayran + 75% shalgam mixture. However, for the other two mixtures no drastic increase or decrease was observed. This indicates that shalgam may have an adverse effect on the survival of streptococci. Tanguler and Erten (2012) defined *Lactobacillus*, *Pediococcus* and *Leuconostoc* species as lactic acid bacteria from shalgam, however, they did not identify any *Streptococcus* species.

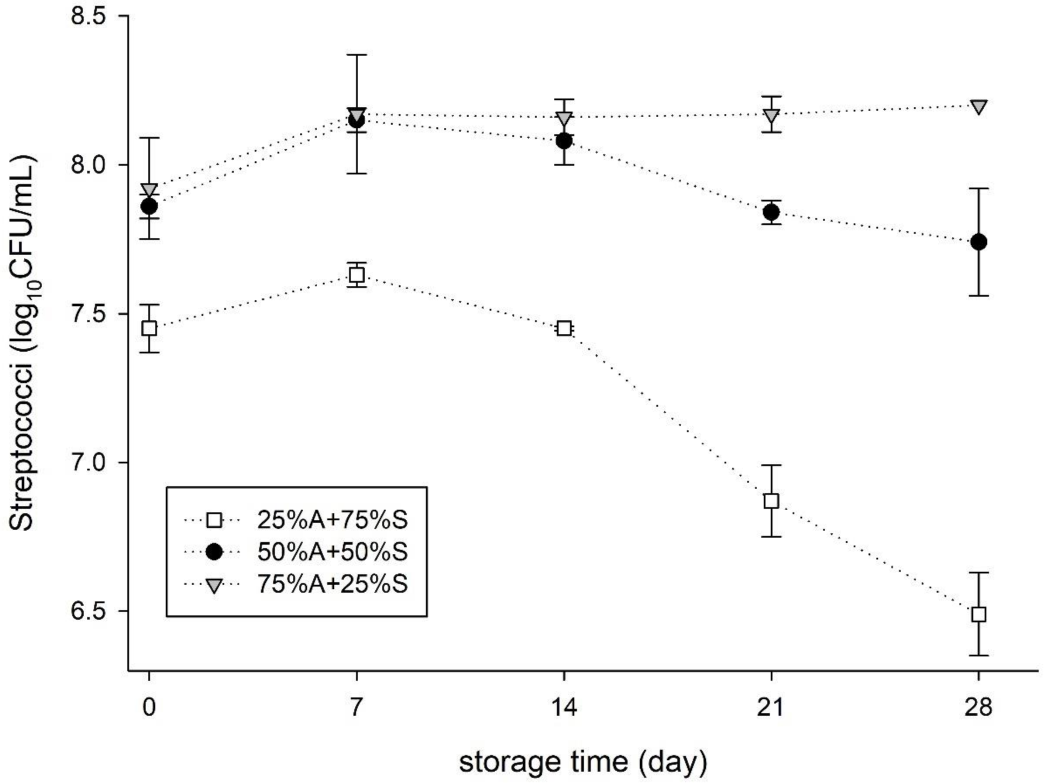

Figure 4.14 Streptococci count of ayran (A) – shalgam (S) mixtures during storage at 4 °C (Error bars represent standard deviation of three means).

Streptococcus count of bacteria was higher than that of *Lactobacillus* bacteria in all treatments during the storage period. This observation is in line with the observation of Birollo et al. (2000) and Vinderola et al. (2000). They found that the count of *Streptococcus* was significantly higher than that of *Lactobacillus* in yoghurt. This could be attributed to plastic containers used for packaging of ayran during storage periods. The use of plastic containers has been reported to improve the growth and viability of *Streptococcus* compared to glass bottles due to the high amount of oxygen permeation (Ranadheera et al., 2012). Moreover, additional salt coming from shalgam might affect the *Lactobacillus* count.

Significant decrease ($p < 0.05$) of *Streptococcus* count was observed in 25% ayran + 75% shalgam mixture (Figure 4.14) samples. It is considered that the death of *Streptococcus* during the storage is because of their relative sensitivity to acidic conditions. This result is in agreement with that of Beal et al. (1999) who reported that *Streptoccocus* bacteria are inhibited by an acidic environment.

4.3 Sensory Analysis

Sensory analysis is an important criterion to determine consumers' expectations. Sensory scores for the trained panelists (n=8) are given in Figure 4.15. It seemed that the mixture with higher ayran content had the highest scores except color. The color scores of three mixtures were all the same. The statistical analysis revealed that although different scores were obtained for the mixtures the results were not significant ($p>0.05$). Moreover, generally higher scores were obtained. The lowest score was 3.13±0.99 out of 5 for the taste of 25% ayran + 75% shalgam mixture.

Sensory scores for the semi-trained panelists (n=6) is shown in Figure 4.16. It was noticed that the scores were lower than (as low as 2.67 out of 5) that of trained panelists. Nevertheless, odor, structure and taste scores were higher for the mixture with the highest ayran content i.e., 75% ayran + 25% shalgam. Figure 4.17 shows the overall scores of all panelists (trained + semi-trained).

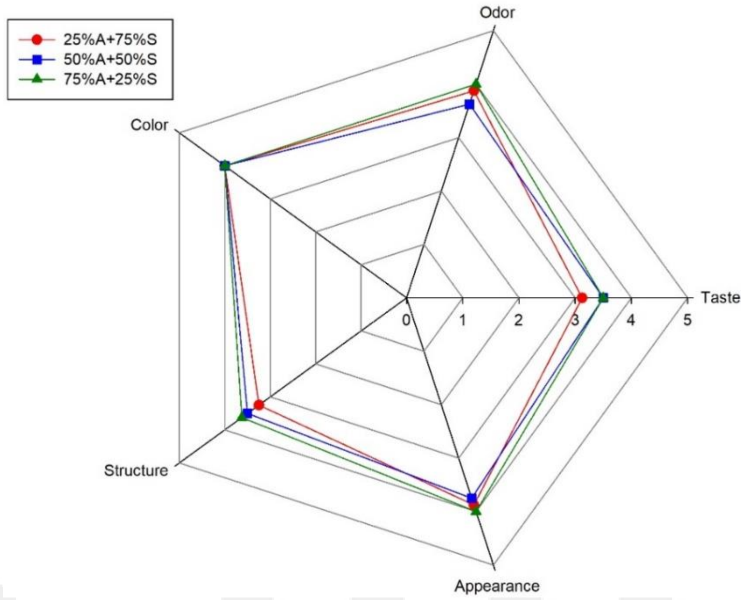

Figure 4.15 Sensory scores of ayran (A) – shalgam (S) mixtures performed by 8 trained panelists.

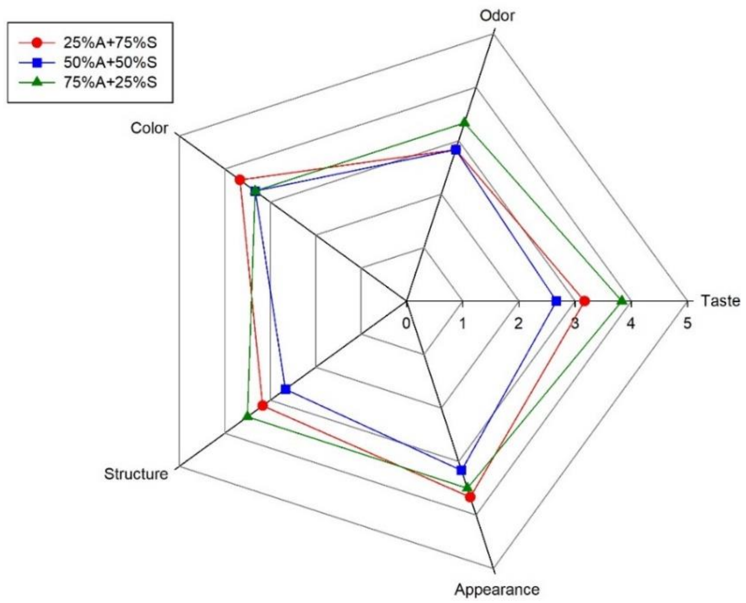

Figure 4.16 Sensory scores of ayran (A) – shalgam (S) mixtures performed by 6 semi-trained panelists.

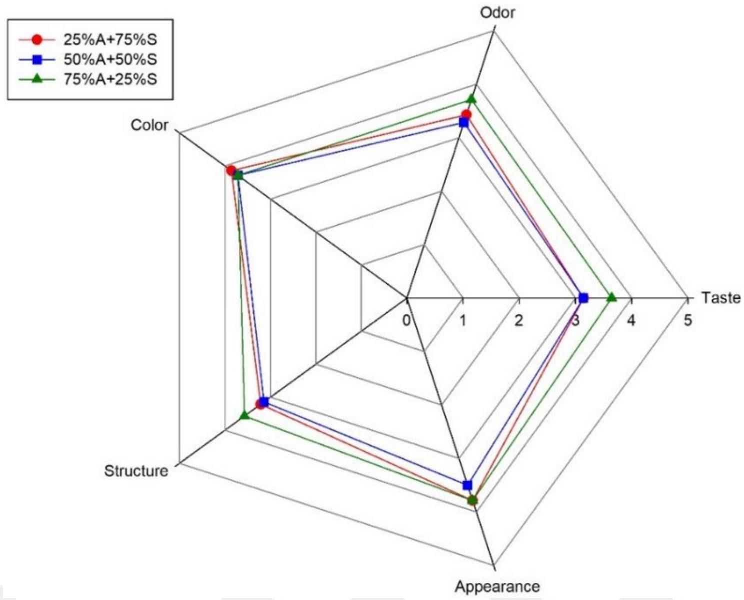

Figure 4.17 Sensory scores of ayran (A) – shalgam (S) mixtures performed by 8 trained and 6 semi-trained panelists.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The mixtures of ayran and shalgam with different ratios [25% ayran + 75% shalgam, 50% ayran + 50% shalgam and 75% ayran + 25% shalgam (v/v)] were subjected to rheological measurements at different temperature levels (4, 8 and 12 °C). Power law model was used to determine the rheological data. Results indicated that the power law model produced reasonable fits to the rheological data since high adjusted coefficient of determination ($R^2adj \geq 0.82$) and low root mean square error values were obtained ($RMSE \leq 0.078$). Ayran was slightly pseudoplastic at 4 °C because flow behavior index (n) was obtained as 0.9. On the other hand, shalgam was slightly dilatant at 4 °C ($n = 1.2$) as well as the mixtures (n changing from 1.1 to 1.3). It was apparent that temperature had no impact on the flow behavior index of the mixtures. On the other hand, as the temperature increased K values of the mixtures decreased. It was concluded ayran-shalgam mixtures had similar flow behavior index with that of shalgam no matter the amount of ayran or shalgam in the mixtures whereas consistency index increased as the amount of ayran increased in the mixtures at a constant temperature. Shelf life analyses up to 4 weeks at 4 °C revealed that serum separation increased up to second week and then stabilized for all mixtures. Slight increase (0.05 to 0.1 unit) of pH was observed during the first week then pH values were stabilized just like the serum separation. Microbiological analyses showed that mixtures had similar shelf life with that of ayran. Mixture with the highest ayran content [75% ayran + 25% shalgam (v/v)] had the highest scores in sensory evaluation.

5.2 Recommendations

Future studies could be focused on avoiding the serum separation since it may be a problem for the prolonged storage. It is also known that shalgam has high anthocyanin content therefore anthocyanin stability of ayran-shalgam mixtures during storage could also be studied.

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APPENDIX

APPENDIX A

Table A.1 Shear rate (s-1) vs. apparent viscosity (mPa.s) data of ayran (A), shalgam (S) and their mixtures at 4 °C.

AYRAN		SHALGAM		25% A + 75% S		50% A + 50% S		75% A + 25% S	
Shear Rate	**Apparent viscosity**	**Shear Rate**	**Apparent viscosity**	**Shear Rate**	**Apparent viscosity**	**Shear Rate**	**Apparent viscosity**	**Shear Rate**	**Apparent viscosity**
5.04	0.1724	37.4	0.0672	29.3	0.1135	29.3	0.1033	11.1	0.0141
7.06	0.2812	39.4	0.0992	31.3	0.1328	31.3	0.1382	13.1	0.0157
9.08	0.2991	41.4	0.0932	33.3	0.1011	33.3	0.1569	15.1	0.0267
11.10	0.3374	43.4	0.0727	35.3	0.1270	35.3	0.1651	17.2	0.1779
13.10	0.3721	45.4	0.0985	37.4	0.2100	37.4	0.1916	19.2	0.0372
15.10	0.4221	47.5	0.1307	39.4	0.2720	39.4	0.3105	21.2	0.1665
17.20	0.4771	49.5	0.1203	41.4	0.1413	41.4	0.2988	23.2	0.2294
19.20	0.5119	51.5	0.1453	43.4	0.1422	43.4	0.2625	25.2	0.1044
21.20	0.5598	53.5	0.2102	45.4	0.1898	45.4	0.2313	27.3	0.1120
23.20	0.6108	55.6	0.211	47.5	0.2361	47.5	0.3021	29.3	0.2760
25.20	0.6578	57.6	0.1578	49.5	0.2720	49.5	0.3368	31.3	0.3282
27.30	0.7039	59.6	0.1361	51.5	0.2770	51.5	0.2644	33.3	0.4152
29.30	0.7515	61.6	0.1697	53.5	0.2331	53.5	0.3235	35.3	0.4911
31.30	0.7918	63.6	0.1969	55.6	0.3224	55.6	0.3479	37.4	0.4764
33.30	0.8306	65.7	0.2341	57.6	0.3722	57.6	0.4429	39.4	0.4461
35.30	0.8768	67.7	0.249	59.6	0.3221	59.6	0.4368	41.4	0.3745
37.40	0.9147	69.7	0.2859	61.6	0.3701	61.6	0.4950	43.4	0.4118
39.40	0.9652	71.7	0.2433	63.6	0.3038	63.6	0.4426	45.4	0.4699
41.40	1.0399	73.7	0.2593	65.7	0.3038	65.7	0.5331	47.5	0.4124
43.40	1.0992	75.8	0.2275	67.7	0.4130	67.7	0.4572	49.5	0.4276
45.40	1.1063	77.8	0.2738	69.7	0.4031	69.7	0.5347	51.5	0.4224
47.50	1.1538	79.8	0.2675	71.7	0.3948	71.7	0.5215	53.5	0.6915
49.50	1.194	81.8	0.2462	73.7	0.3479	73.7	0.5737	55.6	0.5759
51.50	1.2596	83.8	0.2867	75.8	0.4347	75.8	0.5660	57.6	0.5660
53.50	1.3007	85.9	0.3447	77.8	0.5243	77.8	0.5422	59.6	0.4743
55.60	1.3468	87.9	0.2822	79.8	0.5227	79.8	0.6516	61.6	0.6264
57.60	1.4502	89.9	0.2582	81.8	0.3954	81.8	0.6522	63.6	0.6703
59.60	1.4731	91.9	0.2849	83.8	0.4141	83.8	0.5923	65.7	0.6735
61.60	1.4983	93.9	0.2905	85.9	0.5798	85.9	0.6694	67.7	0.6855
63.60	1.5155	96.0	0.2837	87.9	0.5416	87.9	0.6793	69.7	0.6505
65.70	1.5644	98.0	0.3119	89.9	0.5067	89.9	0.6278	71.7	0.7067
67.70	1.6244	100.0	0.2876	91.9	0.4983	91.9	0.7856	73.7	0.7447
69.70	1.6510			93.9	0.5881	93.9	0.7245	75.8	0.7577
71.70	1.7516			96.0	0.6207	96.0	0.6638	77.8	0.7454
73.70	1.7823			98.0	0.6359	98.0	0.7831	79.8	0.9156
75.80	1.8212			100.0	0.6011	100.0	0.7102	81.8	0.8675
77.80	1.8227							83.8	0.9139
79.80	1.8666							85.9	1.0000
81.80	1.9486							87.9	0.9796
83.80	2.0233							89.9	1.0171
85.90	2.0210							91.9	1.0696
87.90	2.0444							93.9	0.9246
89.90	2.1116							96.0	0.9175
91.90	2.1370							98.0	0.9838
93.90	2.1706							100.0	1.0046
96.00	2.2341								
98.00	2.3232								
100.00	2.3783								

Table A.2 Shear rate (s^{-1}) vs. shear stress (Pa) data of ayran (A), shalgam (S) and their mixtures at 4 °C.

AYRAN		SHALGAM		25% A + 75% S		50% A + 50% S		75% A + 25% S	
Shear Rate	**Shear Stress**	**Shear Rate**	**Shear Stress**	**Shear Rate**	**Shear Stress**	**Shear Rate**	**Shear Stress**	**Shear Rate**	**Shear Stress**
5.04	34.2115	37.4	1.7975	29.3	3.8742	29.3	3.5281	11.1	1.2723
7.06	39.8245	39.4	2.5181	31.3	4.2407	31.3	4.4154	13.1	1.1978
9.08	32.9305	41.4	2.2502	33.3	3.0333	33.3	4.7093	15.1	1.7619
11.1	30.3920	43.4	1.6751	35.3	3.5932	35.3	4.6696	17.2	10.365
13.1	28.3540	45.4	2.1670	37.4	5.6188	37.4	5.1267	19.2	1.9379
15.1	27.8735	47.5	2.7530	39.4	6.9057	39.4	7.8838	21.2	7.8505
17.2	27.7975	49.5	2.4312	41.4	3.4116	41.4	7.2155	23.2	9.8761
19.2	26.6840	51.5	2.8209	43.4	3.2746	43.4	6.0452	25.2	4.1347
21.2	26.400	53.5	3.9265	45.4	4.1756	45.4	5.0899	27.3	4.1090
23.2	26.3015	55.6	3.7989	47.5	4.9727	47.5	6.3641	29.3	9.4252
25.2	26.0555	57.6	2.7413	49.5	5.4966	49.5	6.8057	31.3	10.484
27.3	25.8180	59.6	2.2837	51.5	5.3772	51.5	5.1330	33.3	12.457
29.3	25.6605	61.6	2.7540	53.5	4.3540	53.5	6.0429	35.3	13.892
31.3	25.2910	63.6	3.0936	55.6	5.8037	55.6	6.2635	37.4	12.750
33.3	24.9220	65.7	3.5655	57.6	6.4651	57.6	7.6933	39.4	11.326
35.3	24.8050	67.7	3.6791	59.6	5.4053	59.6	7.3294	41.4	9.0436
37.4	24.4795	69.7	4.1022	61.6	6.0067	61.6	8.0348	43.4	9.4820
39.4	24.5055	71.7	3.3925	63.6	4.7743	63.6	6.9556	45.4	10.3390
41.4	25.1135	73.7	3.5165	65.7	4.6280	65.7	8.1197	47.5	8.6872
43.4	25.3110	75.8	3.0036	67.7	6.1024	67.7	6.7553	49.5	8.6395
45.4	24.3405	77.8	3.5199	69.7	5.7835	69.7	7.6727	51.5	8.1999
47.5	24.3070	79.8	3.3523	71.7	5.5053	71.7	7.2716	53.5	12.918
49.5	24.1255	81.8	3.0088	73.7	4.7181	73.7	7.7804	55.6	10.367
51.5	24.4525	83.8	3.4200	75.8	5.7384	75.8	7.4718	57.6	9.8313
53.5	24.2985	85.9	4.0145	77.8	6.7405	77.8	6.9707	59.6	7.9587
55.6	24.2435	87.9	3.2114	79.8	6.5510	79.8	8.1653	61.6	10.166
57.6	25.1895	89.9	2.8722	81.8	4.8325	81.8	7.9720	63.6	10.534
59.6	24.7200	91.9	3.0994	83.8	4.9392	83.8	7.0655	65.7	10.258
61.6	24.3170	93.9	3.0925	85.9	6.7526	85.9	7.7970	67.7	10.129
63.6	23.8150	96.0	2.9560	87.9	6.1633	87.9	7.7296	69.7	9.3336
65.7	23.8285	98.0	3.1836	89.9	5.6361	89.9	6.9836	71.7	9.8544
67.7	24.0035	100.0	2.8763	91.9	5.4216	91.9	8.5470	73.7	10.100
69.7	23.6890			93.9	6.2607	93.9	7.7124	75.8	10.002
71.7	24.4245			96.0	6.4684	96.	6.9172	77.8	9.5837
73.7	24.1715			98.0	6.4902	98.0	7.9921	79.8	11.4750
75.8	24.0410			100.0	6.0113	100.0	7.1024	81.8	10.6030
77.8	23.4345							83.8	10.9010
79.8	23.3915							85.9	11.6470
81.8	23.8165							87.9	11.1480
83.8	24.1335							89.9	11.3130
85.9	23.5395							91.9	11.6370
87.9	23.2635							93.9	9.84210
89.9	23.4890							96.0	9.56170
91.9	23.2490							98.0	10.0400
93.9	23.1065							100.0	10.0460
96.0	23.2820								
98.0	23.7105								
100.0	23.7830								

Table A.3 Shear rate (s^{-1}) vs. shear stress (Pa) data of ayran (A), shalgam (S) and their mixtures at 4 °C.

25% A + 75% S		50% A + 50% S		75% A + 25% S	
Shear Rate	**Shear Stress**	**Shear Rate**	**Shear Stress**	**Shear Rate**	**Shear Stress**
27.3	0.1008	27.3	0.1249	15.1	0.0455
29.3	0.1128	29.3	0.1746	17.2	0.0636
31.3	0.0373	31.3	0.2019	19.2	0.1544
33.3	0.0579	33.3	0.2100	21.2	0.0779
35.3	0.1111	35.3	0.2187	23.2	0.1798
37.4	0.1238	37.4	0.2072	25.2	0.1858
39.4	0.1484	39.4	0.2084	27.3	0.2649
41.4	0.1681	41.4	0.1765	29.3	0.1981
43.4	0.1447	43.4	0.1520	31.3	0.2260
45.4	0.1821	45.4	0.2049	33.3	0.2224
47.5	0.1880	47.5	0.2008	35.3	0.2116
49.5	0.1847	49.5	0.2549	37.4	0.2816
51.5	0.1492	51.5	0.2672	39.4	0.3413
53.5	0.1820	53.5	0.2618	41.4	0.4389
55.6	0.2411	55.6	0.1955	43.4	0.3643
57.6	0.1827	57.6	0.3035	45.4	0.3342
59.6	0.1881	59.6	0.2740	47.5	0.4043
61.6	0.1901	61.6	0.2404	49.5	0.4235
63.6	0.2115	63.6	0.2362	51.5	0.4229
65.7	0.3019	65.7	0.2770	53.5	0.4387
67.7	0.3010	67.7	0.4216	55.6	0.5071
69.7	0.3453	69.7	0.4709	57.6	0.5000
71.7	0.3234	71.7	0.4609	59.6	0.5809
73.7	0.3376	73.7	0.4035	61.6	0.6010
75.8	0.3886	75.8	0.5130	63.6	0.5992
77.8	0.3592	77.8	0.5272	65.7	0.6106
79.8	0.3558	79.8	0.4957	67.7	0.6866
81.8	0.2835	81.8	0.4964	69.7	0.6498
83.8	0.4434	83.8	0.5199	71.7	0.6800
85.9	0.3821	85.9	0.4927	73.7	0.7046
87.9	0.3463	87.9	0.5232	75.8	0.7347
89.9	0.2925	89.9	0.5003	77.8	0.7740
91.9	0.4938	91.9	0.5468	79.8	0.7264
93.9	0.5280	93.9	0.6404	81.8	0.7176
96.0	0.3824	96.0	0.5960	83.8	0.8548
98.0	0.3414	98.0	0.5949	85.9	0.8997
100.0	0.4250	100.0	0.6216	87.9	0.8281
				89.9	0.7342
				91.9	0.8702
				93.9	1.0073
				96.0	0.8694
				98.0	0.8799
				100.0	1.0088

Table A.4 Rheological parameters of ayran (A) and shalgam (S) mixtures at 12 °C

25% A + 75% S		50% A + 50% S		75% A + 25% S	
Shear Rate	**Shear Stress**	**Shear Rate**	**Shear Stress**	**Shear Rate**	**Shear Stress**
31.3	0.0543	25.2	0.0420	15.1	0.0362
33.3	0.0473	27.3	0.0304	17.2	0.1062
35.3	0.0433	29.3	0.0371	19.2	0.0794
37.4	0.0754	31.3	0.0560	21.2	0.3163
39.4	0.2356	33.3	0.0552	23.2	0.1419
41.4	0.1792	35.3	0.0608	25.2	0.1287
43.4	0.1217	37.4	0.0610	27.3	0.2039
45.4	0.1625	39.4	0.1573	29.3	0.2527
47.5	0.1415	41.4	0.2448	31.3	0.2328
49.5	0.2264	43.4	0.3057	33.3	0.1568
51.5	0.2889	45.4	0.1710	35.3	0.2033
53.5	0.2178	47.5	0.2929	37.4	0.2483
55.6	0.2268	49.5	0.3209	39.4	0.2445
57.6	0.2920	51.5	0.2944	41.4	0.2918
59.6	0.2770	53.5	0.3513	43.4	0.2556
61.6	0.2963	55.6	0.3450	45.4	0.3660
63.6	0.2962	57.6	0.2922	47.5	0.3083
65.7	0.3049	59.6	0.3516	49.5	0.3303
67.7	0.3194	61.6	0.4027	51.5	0.3705
69.7	0.4496	63.6	0.4191	53.5	0.4241
71.7	0.3346	65.7	0.4048	55.5	0.4365
73.7	0.2827	67.7	0.4117	57.6	0.4452
75.8	0.4761	69.7	0.4680	59.6	0.4395
77.8	0.4360	71.7	0.4887	61.6	0.5035
79.8	0.2982	73.7	0.5379	63.6	0.4661
81.8	0.4469	75.8	0.6250	65.7	0.5205
83.8	0.4519	77.8	0.5359	67.7	0.6530
85.9	0.3719	79.8	0.4727	69.7	0.6633
87.9	0.4338	81.8	0.5498	71.7	0.6258
89.9	0.4800	83.8	0.6285	73.7	0.6010
91.9	0.4389	85.9	0.4995	75.8	0.7609
93.9	0.7019	87.9	0.4777	77.8	0.7429
96.0	0.5585	89.9	0.5141	79.8	0.6576
98.0	0.4210	91.9	0.6589	81.8	0.6739
100.0	0.5210	93.9	0.6640	83.8	0.7701
		96.0	0.5297	85.9	0.8344
		98.0	0.5465	87.9	0.8512
		100.0	0.7267	89.9	0.9034
				91.9	0.7453
				93.9	0.8646
				96.0	0.9165
				98.0	0.8383
				100.0	0.8489

Table A.5 Serum separation (serum + shalgam levels) in ayran (A) – shalgam (S) mixtures during storage at 4 °C.

	25% A + 75% S		50% A + 50% S		75% A + 25% S	
Time (days)	**mL/50 mL**	**SD**	**mL/50mL**	**SD**	**mL/50mL**	**SD**
7	40.67	0.58	30.33	0.58	19.67	0.58
14	42.50	0.00	34.33	0.58	25.67	0.58
21	42.57	0.06	35.33	0.58	26.00	1.00
28	42.63	0.06	35.33	0.58	27.00	0.00

Table A.6 pH values of ayran (A) – shalgam (S) mixtures during storage at 4 °C.

	25% A + 75% S		50% A + 50% S		75% A + 25% S	
Time (days)	**pH**	**SD**	**pH**	**SD**	**pH**	**SD**
0	3.46	0.006	3.61	0.020	3.83	0.020
7	3.51	0.010	3.68	0.010	3.91	0.020
14	3.52	0.020	3.71	0.006	3.91	0.006
21	3.54	0.020	3.70	0.010	3.91	0.010
28	3.53	0.020	3.71	0.010	3.90	0.010

Table A.7 Total aerobic mesophilic bacteria count of ayran (A) – shalgam (S) mixtures during storage at 4 °C.

	25% A + 75% S		50% A + 50% S		75% A + 25% S	
Time (days)	Total Aerobic Mesophilic Bacteria	SD	Total Aerobic Mesophilic Bacteria	SD	Total Aerobic Mesophilic Bacteria	SD
0	4.56	0.06	4.63	0.10	4.4	0.00
7	4.92	0.04	4.96	0.12	4.54	0.18
14	4.69	0.01	4.77	0.44	5.19	0.11
21	5.18	0.04	5.51	0.06	5.87	0.02
28	5.53	0.18	5.89	0.12	6.15	0.09

Table A.8 Yeast and molds count of ayran (A) – shalgam (S) mixtures during storage at 4 °C.

	25% A + 75% S		50% A + 50% S		75% A + 25% S	
Time (days)	Yeast & Molds count	SD	Yeast & Molds count	SD	Yeast & Molds count	SD
0	1.40	0.00	1.40	0.00	1.40	0.00
7	2.11	0.00	2.18	0.04	1.70	0.00
14	3.59	0.11	4.38	0.04	4.98	0.10
21	5.23	0.11	5.50	0.14	5.83	0.04
28	5.48	0.08	5.90	0.11	5.69	0.88

Table A.9 Lactobacilli count of ayran (A) – shalgam (S) mixtures during storage at 4 °C.

	25% A + 75% S		50% A + 50% S		75% A + 25% S	
Time (days)	**Lactobacilli count**	**SD**	**Lactoba-cilli count**	**SD**	**Lactobacilli count**	**SD**
0	6.42	0.09	6.30	0.17	6.20	0.220
7	6.34	0.18	6.19	0.21	6.09	0.007
14	6.03	0.06	5.92	0.09	5.82	0.140
21	6.15	0.28	5.96	0.07	5.69	0.060
28	6.47	0.11	6.35	0.07	6.38	0.030

Table A.10 Streptococci count of ayran (A) – shalgam (S) mixtures during storage at 4 °C.

	25% A + 75% S		50% A + 50% S		75% A + 25% S	
Time (days)	**Streptococci count**	**SD**	**Streptococci count**	**SD**	**Streptococci count**	**SD**
0	7.45	0.08	7.86	0.04	7.92	0.17
7	7.63	0.04	8.15	0.04	8.17	0.20
14	7.45	0.007	8.08	0.08	8.16	0.06
21	6.87	0.120	7.84	0.04	8.17	0.06
28	6.49	0.140	7.74	0.18	8.2	0.00