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**Research Paper / Araştırma Makalesi**

# Effect of Microfiltration, Storage Time and Temperature on Properties of Shalgam Juices

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## ABSTRACT

This study aimed to investigate the effect of microfiltration, storage temperature and time on some quality characteristics of shalgam juices. Shalgam juice samples produced by fermentation with *Lactiplantibacillus plantarum* were filtered through a 0.45µm diameter filter and stored at 4°C and 20°C temperatures for six months. Significant decreases in the microbial population of beverages were found by microfiltration, and logaritmic reductions were between 3.60 and 4.96 log cuf/mL in shalgam samples. It was found that microfiltration could be used in the production of shalgam juice to reduce the population of microorganisms before storage, and microbial growth could be inhibited by storing micro-filtered juices at 4°C during storage. During storage at 4°C, micro-filtered shalgam juices were preferred over other shalgam samples. Sensory properties of shalgam juices stored at 4°C were preserved better than those of shalgam samples stored at 20°C. Results indicated that microfiltration treatment and/or cold-storage at 4°C could increase the shelf-life and improve sensory properties of shalgam juices, and this combination of treatments could be recommended as an alternative to thermal pasteurization, which might be unfavoured by many consumers.

**Keywords:** Consumer preference, *Lactiplantibacillus plantarum*, Logarithmic reduction value, Microfiltration, Shalgam (salgam) juice, Storage temperature

## Şalgam Sularının Özellikleri Üzerine Mikrofiltrasyon ile Depolama Sıcaklık ve Süresinin Etkisi

## ÖZ

Bu çalışmada mikrofiltrasyon, depolama sıcaklık ve süresinin şalgam suyu kalitesi üzerine etkilerinin araştırılması amaçlamıştır. *Lactiplantibacillus plantarum* fermantasyonu ile üretilen şalgam suyu, 0.45µm gözenek çaplı filtreden süzülmüş ve hem 4°C hem de 20°C'de altı ay süreyle depolanmıştır. Mikrofiltrasyon işlemi ile mikroorganizma sayılarında önemli azalmalar meydana gelmiş ve logaritmik azalma değerleri 3.60-4.96 kob/mL arasında belirlenmiştir. Bu nedenle, şalgam suyu üretiminde mikrofiltrasyon, depolamadan önce ve depolama sırasında mikroorganizma popülasyonunu azaltmak için kullanılabilir ve ürünler 4°C'de tutularak mikrobiyal büyüme yavaşlatılabilir. Uzayan depolama süresine bağlı olarak, panelistler mikrofiltrasyon işlemi uygulanmış ve 4°C'de depolanmış şalgam sularını diğer örneklere tercih etmişlerdir. Ayrıca, 4°C'de saklanan numunelerin duyusal özellikleri, 20°C'de saklanan numunelere kıyasla korunmuştur. Mevcut çalışmaya göre şalgamların raf ömrünü uzatmak ve duyusal özelliklerini daha iyi hale getirmek için mikrofiltrasyon işlemi ve/veya 4°C'de depolama, birçok tüketici tarafından tercih edilmeyen termal pastörizasyona alternatif bir işlem olarak önerilebilir.

**Anahtar Kelimeler:** Tüketici tercihi, *Lactiplantibacillus plantarum*, Logaritmik azalma değeri, Mikrofiltrasyon, Şalgam suyu, Depolama sıcaklığı

## INTRODUCTION

Shalgam juice is an important commercial product produced by lactic acid fermentation on the raw materials consisting of bulgur flour which is also called setik, sourdough/baker's yeast, unrefined rock salt, the root of turnip (*Brassica rapa* L.), drinkable water and mainly black carrot [1]. There is no industrial-level starter use. However, producers can also resort to using starter cultures, especially autochthonous *Lactiplantibacillus (Lpb.) plantarum* (Formerly *Lactobacillus plantarum*) [2, 3].

The quality of shalgam juice is not stable during storage and it undergoes microbiological deterioration [1, 4]. The most important factors affecting microbial growth, especially during lacto-fermentation or storage, are mainly oxygen, temperature, fermentable sugar level, buffer capacity, pH, acidity, natural inhibitory compounds and the amount of lactic acid produced [5]. Even during cold storage, it has a shelf life of up to three months depending on storage conditions. Wild yeasts easily can develop on the surface and can deteriorate the physicochemical properties of the shalgam juice, they can reduce acidity and produce off-flavour. For this reason, it is important to inactivate and/or reduce microorganisms to preserve the shalgam juice for a longer period. Although the shelf life can be increased by heat treatment, this process can lead to a cooked carrot flavour in the product. This situation adversely affects the sensory properties of the product and cannot be accepted by consumers. Using chemical additives can increase the shelf life too but the used amount is too high and restricted legally. However, only benzoic acid is allowed and the maximum amount to be added to the shalgam juice is 200 mg/L [1, 4]. The industrial producers have been searching to satisfy the preferences of the customer on this product (essentially, good quality, healthy and safe) by creating new techniques able to overcome the limitations of the presented preservation techniques. In this regard, microfiltration (MF), as a non-thermal process seems to be a very suitable preservation method [6].

MF is used for different purposes (clarification, stabilization and cold sterilization) in a wide variety of industries such as the food and beverage industry (apple, pear, grape, orange, lemon juice, beer, wine and dairy industry), biotechnology, and biochemistry and biomedicine industries. Particulate matters including microorganism cells in many fermented liquids or beverages could be removed by MF using filters of with a diameter of 0.1-10 µm [7, 8].

As far as it is known, there is no research regarding the effect of the filtration processes of shalgam juice and their storage at different temperatures. In addition, only a few studies investigated the use of starter culture in the production of shalgam juice. However, no study has been found on shalgam juice that has been microfiltered, or even related to its storage for a long time. Regarding quality, temperature is an important parameter during the storage of food and beverages. Shalgam juice is generally stored at ambient temperatures in plants or packaged in supermarkets and sometimes at 4°C with a lesser extent. For this reason, both temperatures (ambient and 4°C) were chosen for storage in the present study. Therefore present study aimed to investigate the effect of (i) microfiltration, (ii) storage temperatures (4 and 20°C) and (iii) storage time (2, 4 and 6 months) on the microbiological, chemical and sensory quality of shalgam juice.

## MATERIALS and METHODS

Autochthonous *Lpb. plantarum* used as a starter culture was isolated from the extract in shalgam juice production in the Cukurova University, Laboratory of Biotechnology.

### Preparation of Pasteurized Black Carrot Juice and Proliferation of Culture

Autochthonous *Lpb. plantarum* was proliferated in pasteurized carrot juice. To obtain pasteurized carrot juice, freshly obtained carrots were selected, sorted and washed, then it was cut into small pieces and the juice was extracted with a juice extractor (F172 Felix Juicy, Kocaeli, Türkiye). The resulting juice was pasteurized at 85°C for five minutes to inactivate microorganisms and the pasteurized juice was cooled to 25°C. For inoculum preparation, two colonies of *Lpb. plantarum* was inoculated into sterile conical flasks containing carrot juice and 1.2% sterilized salt and it was incubated for 48 h at 25°C in an orbital shaker with a speed of 160 rev/min.

### Production of Shalgam Juice

The mixture of bulgur flour (30 g/L), rock salt (2 g/L), sourdough (2 g/L) and water was kneaded and fermented. Fermentation was carried out at 25°C for 3 days. Then, the dough was diluted 3-4 times with water and a diluted sample was used for carrot fermentation. Carrot fermentation was done by using sorted and chopped black carrot (150 g/L), rock salt (10 g/L) and sliced turnip (10 g/L) in a 10 L closed tank. The tank was filled with water. In addition, autochthonous starter culture prepared as described above, *Lpb. plantarum* (5%), was added to the tank [1] and all fermentations were performed in duplicate at 25°C.

### Microfiltration

After fermentation, all shalgam juices were clarified, then combined in a 50 liter drum and divided into two parts. One part was filtered; the other part was not filtered and used as a control. The unfiltered samples were transferred to 200 mL of closed polyethylene terephthalate plastic bottles. Filters are autoclave sterilized in aluminum foil. Samples were first passed through a coarse filter. Later on, they were passed through a 1.2 µm filter (Whatman grade, GF/C) and a 0.7 µm filter (Whatman grade, GF/F). At last, MF was performed with a 0.45 µm diameter filter (Millipore Hydrophilic HVLP) with the aid of the vacuum pump. Filtration processes were done at a room temperature of about 22±2°C.

### Storage Conditions

At the end of the filtration process, filtered and unfiltered samples were transferred to 200 mL of closed plastic bottles. Before storage, filtered SJ samples were divided into two parts. One part was stored at 4±2°C and the other one at 20±2°C, and coded respectively as F-4 and F-20. In addition, unfiltered samples were also divided into two parts. Each sample was stored at 4°C (Un-4) and 20°C (Un-20). Chemical, microbiological and sensory evaluations were carried out during storage (0, 2, 4 and 6 months).

### Microbiological Analysis

Samples were taken from the middle of the sealed bottles. For the counts of LAB, MRS Agar supplemented with 50 mg/L cycloheximide was used. Each plate was incubated at 30°C for 3 days in jars anaerobically using Gas Packs (Anaerocult A, Merck AG, Darmstadt, Germany). Total aerobic mesophilic bacteria (TAMB) number was determined on PCA agar (Merck, 1.05463, Darmstadt, Germany) and yeast counts were determined on plate including PDA (Merck, 1.10130, Darmstadt, Germany) supplemented with 0.1 g/L oxytetracycline. Violet Red Bile Agar was used for counting coliform bacteria [9, 10].

### Measurement of Logarithmic Reduction Value

LAB, TAMB and yeast counts before and after the filtration were detected. The logarithmic reduction value (LRV) was determined according to the following equation [11].

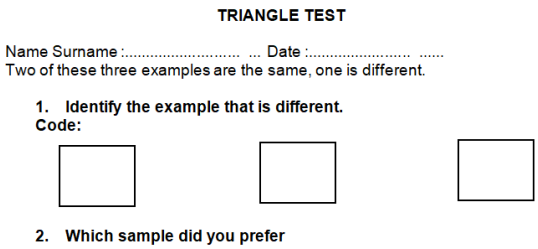
$$LRV = \log_{10} \times \left( \frac{\text{Feed sample concentration}}{\text{Filtered sample concentration}} \right)$$

### Chemical Analysis

Proximate compositions of samples, including pH, total solids, ash, and protein contents were measured according to the AOAC [12] methods. Titratable acidity was analysed by the method given by TS 11149 Shalgam Standard [13]. Total anthocyanin determination was perforrmed according to the method reported by Toktas et al. [2]. Results presented in terms of cyanidin-3-glycoside.

### Sensory Analysis

The samples were assessed by 18 panellists aged 20 to 64 years. A Triangle test (Figure 1) was carried out to find if the filtered and unfiltered shalgam juices during storage time and temperature were significantly different and the significance of the test was given in the statistical table [14]. The panellists were selected from people who are experienced in sensory testing and were informed before the evaluation. The three samples were given to the panellists in each test. Two questions were asked of the panellists. First question; which of the three examples, two of which are the same, is different? Second question; which of these two different samples did you prefer? Sensory tests, evaluators and environmental conditions were set to be the same and, were carried out in daylight, in a bright environment. For these reasons, since the evaluations of the same people were taken into account in the sensory tests performed at different storage times, the data of the panellists who could not perform some tests were not taken into account. The data of 13 panellists who performed all tests were taken into account. Panellists were given bran bread and water for taste neutralization after tasting each shalgam juice.


TRIANGLE TEST

Name Surname :..... ... Date :..... .....
Two of these three examples are the same, one is different.

1. Identify the example that is different.
Code:

2. Which sample did you prefer

Figure 1. Sensory evaluation form (triangle test) [14]

### Statistical Analysis

Shalgam juice production and analysis were carried out in parallel. The data obtained in the analyses were evaluated according to a one-way analysis of variance and the difference between the significant groups was subjected to Duncan's multiple comparison test. The SPSS 10.0 package program was used for this purpose.

## RESULTS and DISCUSSION

Due to limited knowledge of the effect of shalgam juice storage conditions (temperature and time) on microbial growth and composition, present research contributes significantly to the understanding of these processes.

### Logarithmic Reduction Value in Filtered Shalgam Juice

In this research, most of the microorganisms were physically removed by passing the samples through a filter and LRVs were determined. The cell concentration of each microbial group analysed was determined based on the colony-forming unit (cfu) on MRS, PCA and PDA agar. TAMB showed the highest value before filtration followed by yeast and LAB, whilst coliforms were not found in any sample. Because they are more sensitive to high acidity and low pH than LAB and TAMB. Interestingly, Kirlangic et al. [15] stated that the number of coliform counts in the samples at the beginning of storage was between 1.93-2.69 log cfu/mL and they could be isolated from the environment even at 30°C after 90 days of storage.

To create the desired microbial safety in fruit juices or fermented beverages, membrane filters with a 0.45 µm diameter pore size are used [11]. LRV of LAB, TAMB and yeasts were determined as 3.60, 4.13 and 4.96, respectively. As a result, the LRVs of the

microorganisms exhibited a decrease of more than 1000 times with the filtration process. The highest LRV was found with yeast because yeast cell diameter is bigger than that of bacteria. Not only yeast but also LAB and TAMB counts were also reduced by MF. It is determined that the MF process was an important parameter for decreasing microbial load before storage. The findings in the present work follow a previous study by Asano et al. [11] who found between 2.39 and 6.17 in LRV for LAB using a 0.65 µm pore-size membrane filter in beer. In addition, Purkayastha et al. [6], Hahn [16] and Renouf et al. [17] stated that MF through between 0.2-0.65 µm pore-size membrane filters are generally used for the removal of various microbial groups from heat-susceptible juice but they were not enough to remove out the microorganisms from the products. The results of this study are in consistent with the results found in freshwater samples [16], in wine during storage [17] and in coconut water [6].

**Changes in Counts of Microorganisms During Storage of Shalgam Juice**

While the LAB count was 5.68 log cfu/mL in unfiltered samples at the beginning of storage, it was determined as 2.08 log cfu/mL in filtered shalgam juices (Figure 1). Interestingly, besides LAB, TAMB counts were determined to be >2 log cfu/mL in filtered juices. This may be due to the presence of very small cells of bacteria. Asano et al. [11] stated that in brewery products the sterile filtration process has gained importance instead of pasteurization, especially due to consumer preferences. They reported that even if the pore size of the filter used for this purpose is at the recommended levels, there is a possible risk of penetration of some small LAB.

A higher storage temperature favoured microbial growth. LAB counts increased significantly at 4°C and 20°C in unfiltered samples, they reached 7.04 log cfu/mL (Un-4) at the beginning of the storage and they reached 7.60 (Un-20) log cfu/mL on month 6 (end-storage). Similarly, their counts in filtered samples increased to 3.0 (F-4) and 3.83 (F-20) log cfu/mL in month 4 and then decreased slightly to 2.91–3.42 log cfu/mL in month 6, respectively.

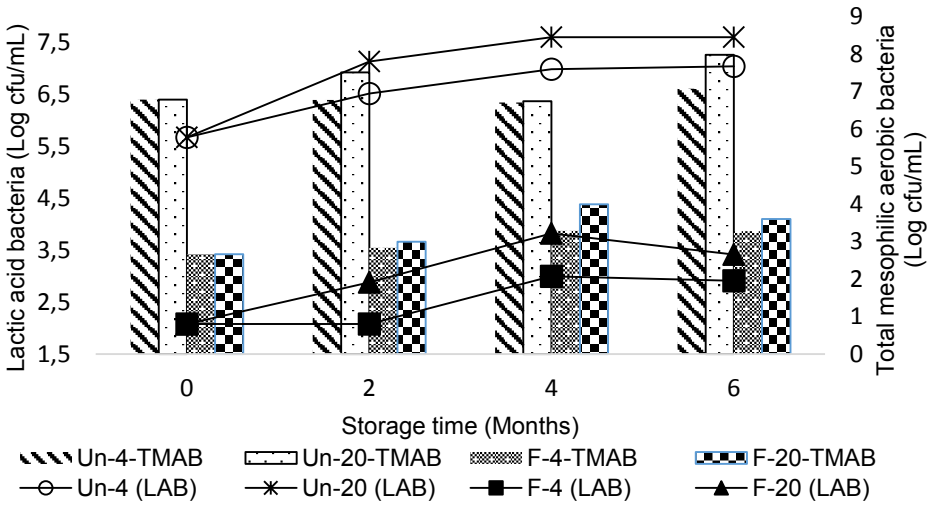



Figure 1. Population dynamics of LAB and TAMB in shalgam juices treated with MF and throughout storage at 4°C and 20°C

TAMB counts were found as 6.79 log cfu/mL in unfiltered samples. According to the Turkish Shalgam Standard, the number of TAMB in shalgam juice should be between 4.0 log cfu/mL and 5.0 log cfu/mL (TS 11149 Shalgam Standard 2003). Therefore, unfiltered samples do not comply with the standards. After MF, TAMB counts were significantly reduced to 2.66 log cfu/mL. During 6 months of storage, their counts increased, except for some minor exceptions. In unfiltered samples stored at 20°C, TAMB counts increased faster than those stored at 4°C, and their numbers increased from 6.79 to 7.98 log cfu/mL (Un-20) at the end of the observation period. It was determined as 7.08 log cfu/mL (Un-4) when stored at 4°C. Similarly, TAMB numbers in filtered samples increased more rapidly when stored at 20°C and decreased after maximum numbers were reached in the fourth month of storage. It was determined as 3.27 (4°C) and 3.60 log cfu/ml (20°C) at the end of storage. As a result, the filtration process led to a decrease in the number of microorganisms. However, as the storage temperature increased, the microorganisms increased more rapidly. Moreover, generally, an increase in TAMB numbers was determined with six months of storage. The reason for the increase in LAB and TAMB numbers in filtered and unfiltered samples during storage can be expressed as the presence of fermentable sugars in carrots remaining at the end of fermentation [15]. At the end of the storage period, while the data obtained in unfiltered samples were higher than the values reported by Standard [13], they were below the permissible limits obtained in filtered samples.

While the total yeast count in unfiltered samples was 6.26 log cfu/mL, it was determined below 30 cfu/mL after filtration. The filtration process had a very important effect on the decrease in the number of total yeast. Yeast is the main spoilage factor in shalgam juice it is very important in terms of shortening the shelf life.

In the present study, we found that not only the MF process is an important parameter for the shelf life of shalgam juice but also storage temperature is important too. The effect of temperature on the growth of microorganisms was clear (Figure 1). This suggests that after MF and throughout storage, low-temperature storage at 4°C does not inhibit the growth of these microorganisms, but slows down their growth compared to storage at 20°C. Voon et al. [18] stated that rising temperatures resulted in an enhancement of the microbial growth rates but low-temperature storage (4°C) lowered the rate of growth of microorganisms in durian pulp during storage. Peñas et al. [19] stated that storage at refrigerated temperature resulted in an important increment of TAMB and LAB counts in raw cabbage and sauerkraut.

**Chemical Composition of Shalgam Juices During Storage**

In this study, shalgam juices were sampled on months 0 (before storage), 2, 4, and 6 for analyses. The amounts of titratable acidity and total solid decreased from 8.92 g/L to 8.58 g/L ($P<0.05$) and from 26 g/L to 23.9 g/L ($P<0.05$), respectively. However, an important effect of MF treatment on pH and ash values could not be determined (Table 1). There is no previous research on the effect of MF on the composition of shalgam juice.

The effects of 2, 4 and 6 months of storage on the chemical composition of samples and the interaction between the MF process, storage temperature and time in this composition are given in Table 2 and Table 3, respectively. In particular, Table 3 indicates according to the analysis of variance whether the MF process, storage temperature and time have an effect together and whether they create synergy. As seen in these Tables, one of the most significant variables affecting the chemical composition is storage time. The total acidity of shalgam juice is one of the main quality factors that affect sensory characteristics, especially the sour taste of the product [3]. Titratable acidity decreased during storage in filtered and unfiltered samples ($P<0.05$). Similarly, Kirlangic et al. [15] reported a decrease in acidity in shalgam juice (both 4°C and 30°C) after 90 days of storage, and Voon et al. [18] reported a decrease in durian paste after 5 weeks of storage at 4°C. In contrast, Iyicinar [20], and Avci [21] stated that acidity increased from the beginning of storage to the end in unpasteurized shalgam juice, and storage time was statistically important on titratable acidity. Moreover, acidity decreased sharply in unfiltered shalgam juice, especially samples stored at 20°C, whereas in filtered shalgam juice it decreased more slowly ($P<0.01$). It is thought that during storage the most important reason for this may be due to the utilization of acids by microorganisms such as spoilage yeasts found in unfiltered juices.

Table 1. Composition of filtered and unfiltered shalgam juices before storage

| Parameter | Unfiltered shalgam juice | Filtered shalgam juice | S |
|---|---|---|---|
| pH | 3.43±0.014 | 3.46±0.007 | ns |
| Titratable acidity [1] (g/L) | 8.92±0.028 | 8.58±0.057 | * |
| Total solid (g/L) | 26.06±0.021 | 23.89±0.134 | ** |
| Ash (g/L) | 13.65±0.368 | 12.90±0.120 | ns |
| Protein (g/L) | 2.85±0.014 | 2.93±0.007 | * |
| Total anthocyanin[2] (mg/L) | 140.1±1.53 | 138.3±1.57 | ns |

[1]: as lactic acid, [2]: as cyanidin-3-glycoside, S: Significance, ** and * display the significance at 1% and 5% by LSD, respectively. ns: not significant.

According to the standard in Turkey [13], the amount of titratable acidity must be at least 6.0 g/L as lactic acid and pH 3.8 (revised standard in 2016). The findings in the present research for titratable acidity and pH follow the standard. However, in this research titratable acidity amounts of stored samples were higher than that stated by Iyicinar [20]. While MF and storage temperature had no significant effect on pH and titratable acidity (Table 3), storage time was found statistically important ($P<0.05$). Moreover, MF and storage time were found statistically important in total solid, ash and protein contents of shalgam juices, but storage temperature has no significant effect on ash and protein contents (Table 3).

The anthocyanins found in the shalgam juice derive from the black carrot used in production Therefore; the main anthocyanin is cyanidin-3-glycoside [2]. Ates et al. [22] and Ulu [23] reported that anthocyanin compounds decreased in all samples with storage time, regardless of storage temperature and high hydrostatic pressure or UV application. Urkiaga et al. [24] reported that as the pore diameter decreases, the total anthocyanin content in wines decreases partially and the use of a filter with a pore diameter of 0.45 µm instead of a 0.8 µm filter reduces the amount of anthocyanin from 220 mg/L to 217 mg/L. In addition, there was a decrease in anthocyanin content after storage at both temperatures (except for Un-4). Similarly, Wang et al. [25] stated that the anthocyanin amount of black soybean koji decreased with storage. According to the analysis of variance, the effect of the filtration process ($P>0.05$) and storage time ($P>0.05$) on total anthocyanin was

statistically insignificant, while the effect of temperature was significant (P<0.05).

Table 2. Chemical composition of shalgam juices after storage for two, four and six months

| | Two months | | | | | Four months | | | | | Six months | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unfiltered shalgam juice | | Filtered shalgam juice | | S | Unfiltered shalgam juice | | Filtered shalgam juice | | S | Unfiltered shalgam juice | | Filtered shalgam juice | | S |
| | Un-4 | Un-20 | F-4 | F-20 | | Un-4 | Un-20 | F-4 | F-20 | | Un-4 | Un-20 | F-4 | F-20 | |
| pH | 3.45±0.01 | 3.44±0.01 | 3.46±0.01 | 3.45±0.01 | ns | 3.445±0.01 | 3.455±0.01 | 3.465±0.01 | 3.46±0.0 | ns | 3.46b±0.01 | 3.54a±0.04 | 3.47b±0.01 | 3.47b±0.0 | * |
| Titratable acidity[1] (g/L) | 8.86b±0.03 | 8.97a±0.03 | 8.57c±0.1 | 8.6c±0.03 | ** | 8.87a±0.02 | 8.59b±0.1 | 8.525b±0.07 | 8.54b±0.02 | ** | 8.69a±0.04 | 7.59b±0.2 | 8.515a±0.1 | 8.5a±0.0 | ** |
| Total solid (g/L) | 24.96a±0.04 | 23.74b±0.16 | 23.28b±0.37 | 22.57c±0.24 | ** | 24.63a±0.04 | 23.05b±0.13 | 23.12b±0.37 | 22.04c±0.2 | ** | 24.82a±0.06 | 22c±0.2 | 22.87b±0.4 | 21.53c±0.44 | ** |
| Ash (g/L) | 13.97a±0.01 | 13.85a±0.26 | 12.91b±0.18 | 12.97b±0.07 | ** | 13.77a±0.04 | 13.68a±0.09 | 12.73b±0.16 | 12.81b±0.13 | ** | 13.81a±0.01 | 13.62a±0.11 | 12.59b±0.2 | 12.72b±0.09 | ** |
| Protein (g/L) | 3.01a±0.02 | 2.99a±0.02 | 2.94b±0.003 | 2.94b±0.003 | * | 3.16a±0.06 | 3.11a±0.04 | 2.95b±0.02 | 2.94b±0.01 | * | 3.19a±0.02 | 3.16a±0.02 | 2.96b±0.02 | 2.95b±0.04 | ** |
| Total anthocyanin[2] (mg/L) | 149a±0.69 | 120.1d±1.12 | 137.1b±0.8 | 128.3c±0.93 | ** | 151.3a±1.13 | 106.2d±0.5 | 140.3b±5.6 | 124.7c±2.83 | ** | 151.3a±0.14 | 104.2d±0.5 | 136.7b±4.86 | 122.1c±3.2 | ** |

[1]: as lactic acid, [2]: as cyanidin-3-glycoside, Un-4: Unfiltered shalgam juice stored at 4ºC, Un-20: Unfiltered shalgam juice stored at 20ºC; F-4: Filtered shalgam juice stored at 4ºC, F-20: Filtered shalgam juice stored at 20ºC. S: Significance, *** and * display the significance at * 1% and 5% by LSD, respectively. ns: not significant.

Table 3. The interaction among the MF process, storage temperature and time on the composition of shalgam juice according to the analysis of variance

| | Microfiltration | | S | Temperature | | S | Storage time (month) | | | S |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unfiltered shalgam juice | Filtered shalgam juice | | +4ºC | +20ºC | | 2 | 4 | 6 | |
| pH | 3.46±0.037 | 3.46±0.008 | ns | 3.46±0.01 | 3.47±0.04 | ns | 3.45b±0.01 | 3.46b±0.009 | 3.48a±0.036 | * |
| Titratable acidity[1] (g/L) | 8.59±0.49 | 8.54±0.05 | ns | 8.67±0.16 | 8.47±0.44 | ns | 8.75a±0.18 | 8.63a±0.15 | 8.32b±0.47 | * |
| Total solid (g/L) | 23.87±1.12 | 22.57±0.69 | *** | 23.95±0.93 | 22.49±0.79 | *** | 23.64a±1.0 | 23.21ab±1.0 | 22.81b±1.36 | ** |
| Ash (g/L) | 13.78±0.15 | 12.79±0.16 | *** | 13.3±0.6 | 13.28±0.48 | ns | 13.42a±0.5 | 13.25b±0.52 | 13.19b±0.6 | ** |
| Protein (g/L) | 3.10±0.08 | 2.94±0.016 | *** | 3.03±0.11 | 3.01±0.09 | ns | 2.97b±0.035 | 3.04a±0.11 | 3.06a±0.12 | ** |
| Total anthocyanin[2] (mg/L) | 130.4±21.8 | 131.6±7.6 | ns | 144±7.07 | 117.6±9.6 | *** | 133.6±11.5 | 130.6±18 | 129±18.8 | ns |

[1]: as lactic acid. [2]: as cyanidin-3-glycoside. S: Significance. ***. ** and * display the significance at 0.1%. 1% and 5% by LSD. respectively. ns: not significant.

The effect of 6 months of storage at different temperatures on the overall composition of the samples was evaluated by PCA score and loading charts (Figures 2a and 2b). According to PCA, the first principle component (PC1) explains 54.2% of the total variance, while the second component (PC2) explains 42.4%. The unexplained part is very low (3.4%). The variables that positively correlate the most with the PC1 are total solid, total anthocyanin, and titratable acidity (Figure 2b). That result is in agreement with Table 2, and the indicated parameters decreased more in samples stored at 20°C (F-20 and Un-20) than in samples stored at 4ºC. When the score plot graph (Figure 2a) was examined, it was seen that the samples formed clustering according to the microfiltration process and storage temperature. The Un-20 sample is grouped separately on the left side of the graph, and the others on the right side. According to the Loading plot (Figure 2b), protein and ash characterize the Un-20 sample, while dry matter characterizes the Un-4 sample, and total anthocyanin and titratable acidity characterize the other samples (F4 and F20). As a result, it can be said that 6 months of storage according to PCA has a significant effect on the components of MF-treated and untreated samples.

**Sensory Evaluation of Shalgam Juice During Storage**

Sensory properties greatly affect the quality of beverages and therefore sensory analyses were also carried out on the samples, as it is one of the important properties of microbiological safety for the consumer. During the storage period, a sensory analysis was carried out by 13 panellists in comparison of the samples that were microfiltered with those that were not, and the samples stored at low temperature with the samples stored at 20°C. For this purpose, two of the three samples presented to the panellists were given the same, one was different (For example Un-4, Un-4 and F-4), and the panellists were asked to find the different samples. Then, the panellists were asked which sample they preferred.

In triangle tests, 12 out of 13 panellists correctly determined that the filtered shalgam juices were different from the unfiltered samples (P<0.001). Two of those who knew it right preferred filtered shalgam juice. As can be seen from Table 4, after two months of storage, 9 out of 13 panellists correctly determined that the filtered sample was different from the control (unfiltered) sample in shalgam juices stored at both 4°C and 20°C. Three of the panellists who found the different samples in shalgam juices stored at 4°C and five of the panellists who found the different samples in shalgam juices stored at 20°C preferred the applied method (filtered sample). On the other hand, the MF process had significant effects (P<0.01) at both temperatures. At the end of 6 months of storage, 10 out of 13 panellists correctly determined that the filtered sample was different from the control sample in shalgam juices stored at 4°C and 11 out of 13 panellists in shalgam juices stored at 20°C. Six of the panellists who found the different samples in shalgam juices stored at 4°C and nine of the panellists who found the different samples in shalgam juices stored at 20°C preferred the applied method. MF process was found statistically significant at 4°C (P<0.01) and 20°C (P<0.001).

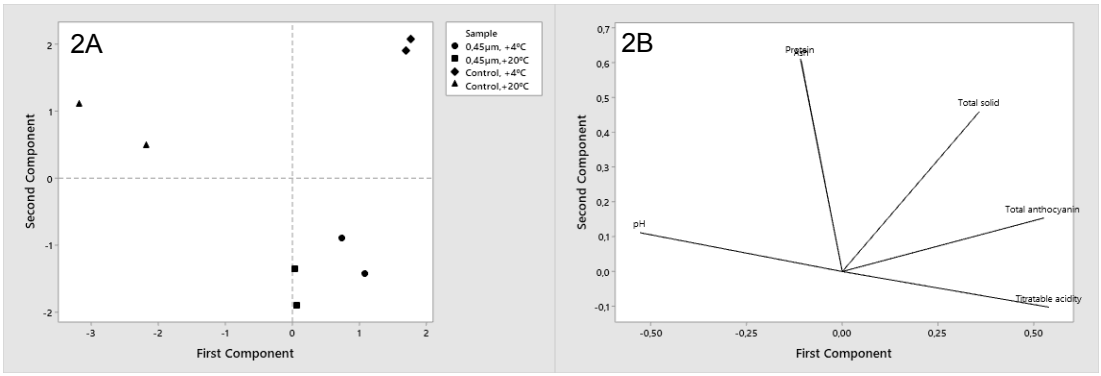



Figure 2. The PCA plots express the distribution of the general composition of shalgam juices after 6 months of storage, a: Score plot, b: Loading plot.

Table 4. The comparison of sensory evaluation according to the triangle test method during storage of shalgam juices (Panellist preferences according to the effect of microfiltration or temperature treatment during the storage period)

| Samples | | The effect of microfiltration | | | The effect of temperature | | |
|---|---|---|---|---|---|---|---|
| | | Number of panellists who found the different sample | Number of panellists who preferred the microfiltration | S | Number of panellists who found the different sample | Number of panellists who preferred the cold storage | S |
| After microfiltration – Before storage | | | | | | | |
| Unfiltered shalgam juice | Filtered shalgam juice | 12 | 2 | *** | - | - | - |
| After 2 months of storage | | | | | | | |
| Un-4 | F-4 | 9 | 3 | ** | - | - | - |
| Un-20 | F-20 | 9 | 5 | ** | - | - | - |
| Un-20 | Un-4 | - | - | - | 7 | 3 | ns |
| F-20 | F-4 | - | - | - | 10 | 3 | ** |
| After 4 months of storage | | | | | | | |
| Un-4 | F-4 | 9 | 4 | ** | - | - | - |
| Un-20 | F-20 | 10 | 7 | ** | - | - | - |
| Un-20 | Un-4 | - | - | - | 10 | 7 | ** |
| F-20 | F-4 | - | - | - | 9 | 5 | ** |
| After 6 months of storage | | | | | | | |
| Un-4 | F-4 | 10 | 6 | ** | - | - | - |
| Un-20 | F-20 | 11 | 9 | *** | - | - | - |
| Un-20 | Un-4 | - | - | - | 11 | 9 | *** |
| F-20 | F-4 | - | - | - | 9 | 7 | ** |

Un-4: Unfiltered shalgam juice stored at 4ºC. Un-20: Unfiltered shalgam juice stored at 20ºC; F-4: Filtered shalgam juice stored at 4ºC. F-20: Filtered shalgam juice stored at 20ºC.

Shalgam juice stored at 20°C was used as a control in the sensory test performed to see the effect of temperature applied in the second, fourth and sixth months of storage (Table 4). In the triangle test method performed after two months of storage, the number of panellists who found different samples in filtered shalgam juices was 10, and 3 of these 10 panellists preferred shalgam juice stored at 4°C. On the other hand, while seven panellists found the different samples in unfiltered shalgam juices, three of them preferred the applied temperature. While the effect of temperature was found statistically important (P<0.01) in filtered shalgam juice, its effect was not important in unfiltered shalgam juices (P>0.05). At the end of 6 months of storage, the number of panellists who found a different sample in filtered shalgam juices was nine, and seven of them preferred shalgam juice stored at 4°C. On the other hand, while 11 panellists found a different sample in unfiltered shalgam juices (P<0.01), nine of them preferred shalgam juice stored at 4°C (P<0.001).

According to the results of sensory analysis during the entire storage period, the number of panellists who preferred the filtered shalgam juice increased in both temperatures and the number of panellists who preferred the shalgam juice stored at 4°C with the increasing storage time. This shows that the sensory properties of the filtered samples were stable during storage at both temperatures compared to unfiltered shalgam juice. On the other hand, the sensory properties of filtered and unfiltered samples stored at 4°C were also stable compared to samples stored at 20°C. Ulu [23] reported that the control samples (22°C) of shalgam juices deteriorated in 49 days, however, among the samples kept for the same time, those that underwent UV treatment were preferred. Kirlangic et al. [15], in their study, looked at the effect of pasteurization on shalgam juice stored at different periods and reported that the most preferred samples were obtained in the first 7th days of storage, according to sensory analysis. They also reported that the preference for sensory properties decreased with storage. Iyicinar [20] stated that the sensory properties of unpasteurized samples deteriorated at the end of storage. In a study conducted by Tanguler et al. [26], it was reported that the most popular products in terms of smell and aroma were shalgam juices produced by rapid processing at 25°C and stored for 4 months. In addition, researchers reported that shalgam juices produced under suitable conditions can be stored for up to 4 months at room conditions without using any heat treatment or preservatives [26].

## CONCLUSION

The results obtained in this research provide significant information about the effect of MF, storage time and temperature on the shelf life of shalgam juice. In recent years, the tendency of consumers on safe and additivies free beverages with original characteristics have increased. Based on our knowledge, there is no information about the MF process of shalgam juice, and there are few studies on its storage. Therefore, microbiological, chemical and sensory qualities of manufactured shalgam juice were assessed after filtering and throughout 6 months of storage at 4°C and 20°C. The microfiltration process led to lower TAMB, LAB and yeast counts and titratable acidity and total solid levels. Considering that yeasts are the main spoilage factor in shalgam juice, it is important to reduce the amount and growth of TAMB, especially yeasts to prolong the shelf life. On the other hand, the effect of storage temperature and the effect of microfiltration on shalgam juice samples during six months of storage were significantly recognized by the panellists.

According to the results,

- It could be said that before the storage of shalgam juice, microfiltration could be used to reduce the population of microorganisms and during its storage; refrigerated temperature could be used to retard the growth of microorganisms.
- It can be said that microfiltration is a suitable method that can be used instead of thermal pasteurization for the preservation of shalgam juice.
- It can be said that one of the most important variables affecting the chemical composition is the storage time
- Shalgam juice could be stored at 4°C without filtration, and with filtration at 4°C and 20°C for 6 months without any important change.

Therefore, this study is a pioneering study that provides important information about shalgam juice. In addition to a more detailed investigation of the effects of different filtration technologies on the characteristics of shalgam juice produced and stored under different conditions on an industrial scale, studies on different preservation techniques should be conducted.

## AUTHOR STATEMENTS

Hasan TANGULER: Conceptualization; investigation, performing experiments, verifying the overall replication/reproducibility of results/experiments, writing - original draft. Huseyin ERTEN: Conceptualization; Data curation; funding acquisition, designing experiments, leading the relevant project, writing - review and editing.

## DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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