

## Processing of waste yogurt serum and commercialization as a healthy food product

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**Abstract** – This study aims that the yogurt serum, which is the waste of yogurt production, to be included in daily food consumption. First, pineapple and pomegranate flavoured yogurt serums have been produced by carrying out the required process. Then, having characterized the pure and the fruit-flavoured yogurt serums, protein determination by Kjeldahl method, moisture and ascorbic acid determinations, fat analysis by Gerber test, density, colour, microbiotic analyses as well as ash determination has been carried out. These analyses were carried out on the first, fourth, seventh and the tenth days and the findings were compared by their nutritive values.

**Keywords:** Yogurt serum, pineapple, pomegranate, nutritional values, characterization.

### 1. INTRODUCTION

Yogurt is a fermented milk product which results from the lactic acid fermentation and the precipitation of the proteins in the milk. The coagulated proteins are casein and denatured serum proteins. In the structure resembling a three-dimensional network formed by these proteins, there are fat globules, dissolved proteose-peptone, and NPN, non-protein nitrogen compounds. Yogurt, containing high-quality protein, carbohydrates and lipids, having a high dry matter content, and being a milk product high in calcium, potassium, phosphorus, magnesium, zinc and B vitamins, is appreciated as a functional food, and its importance in human nutrition is widely recognized. Besides, it is a popular food since it does not spoil for a long time when refrigerated, and having a low pH value, it does not allow the pathogen microorganisms to stay alive for long. It is also known that yogurt not only keeps food allergies and intolerances under control, but it is also able to eliminate the symptoms of osteoarthritis and rheumatoid arthritis. Furthermore, yogurt is known to be effective in the symptomatic treatment of different types of diarrhea, in the reduction of serum cholesterol level and blood pressure, and in keeping diabetes under control. In addition, yogurt is known to prevent bowel cancer. The observed anticarcinogenic effect is correlated to the suppression of the bacterial enzymes, the decrease in the intestinal pH, and the activation of the immune system (Behare et al., 2015).

Although there have been many studies in the literature on the benefits of yogurt, yogurt is thrown away and wasted, and its nutritional values are not taken advantage of. Therefore, yogurt juice is not manufactured and commercialized. This study aims to support the inclusion of yogurt juice in the production by investigating its nutritive values, physical and textural properties. For this purpose, yogurt juice is produced, and the properties of the plain, pineapple flavoured and pomegranate flavoured varieties are compared to each other. A literature review on the subject has been carried out, and very few related studies have been found.

### 2. MATERIALS AND METHODS

#### 2.1 SITE DESCRIPTION

Boric acid ( $H_3BO_3$ ), hydrochloric acid (HCl), Kjeldahl tablet, methylene blue – methylene red, oxalic acid, 2,6 dichlorophenolindophenol (dye) solution, ascorbic acid, sulfuric acid ( $H_2SO_4$ ), amyl alcohol ( $C_5H_{11}OH$ ), phenolphthalein solution, sodium hydroxide (NaOH), Salmonella, Listeria monocytogenes, E. coli, Staphylococcus Aureus bacteria have been provided by Istanbul Aydin University Aziz Sancar Technology Center.

## 2.2 EXPERIMENTAL DESIGN AND SAMPLING TECHNIQUES

Yogurt serum production: After fat and dry matter standardizations, raw milk was first purified by centrifugal cleaning separators named Clarifier. It was homogenized under 200 kg/cm<sup>2</sup> (bar) pressure at 55-60 °C temperature. In order to obtain the distinctive structure of yogurt, high- temperature heat treatments are required. For this reason, 5-10-minute heat treatment norms at temperatures between 90-95 °C were used. After the heat treatment, the pasteurized milk was cooled down to the incubation temperature (42-45 °C) so that the microorganisms could activate, and the yogurt could form. The incubation process was applied by inoculating the cool milk. 1-2 % of the culture prepared according to what was indicated on the yogurt module in the incubation tank. *Lactobacillus delbrueckii* Subsp. *bulgaricus* and *Streptococcus thermophilus* mixture was used as a starter. The end of incubation is determined by checking the acidity. The acidity was observed in the interval pH 4,6–4,7, and the incubation was terminated. Since yogurt juice is obtained as the waste product of strained yogurt, it was cooled down to 10 °C. The yogurt was strained with the help of a mechanical separator, and yogurt juice, which is also called yogurt serum, was obtained.

Protein Determination by Kjeldahl Method: The samples prepared in the ratio of 3/7 for protein determination were placed in the Kjeldahl tube. 10 ml sulfuric acid and one Kjeldahl tablet were added to the Kjeldahl tube. The Kjeldahl device was switched on, and starting from 100 oC, the temperature was increased by +50 oC every half-hour. After keeping it at 400 oC for one hour, it was burned. When the solutions turned green, the burning process was terminated. The balloon was cooled down to room temperature and then placed in the distillation device. After a conical flask was filled with 50 ml boric acid, two drops of the indicator solution consisting of methylene blue and methylene red were added, and the conical was placed under the condenser so that the mouth of the adapter could touch the bottom of the conical flask. The probes of the Kjeldahl device were dipped into a 33 % NaOH and distilled water solution. At the end of the process, the solution inside the conical flask was titrated with an adjustable 0,1 N HCl acid solution until it changed to the violet color that was observed when the first indicator was added. The consumptions were recorded and necessary calculations were made (Varghese and Pare 2019; Cardines et al., 2018)

Determination of Total Dry Matter: Samples were prepared in the ratio of 3/7 for dry matter determination. First, empty drying containers and their lids (with their lids open) were dried in the oven set to 102 °C for 30 minutes. Then, their lids were closed and after they were cooled down to room temperature in the desiccator, they were weighed. 5 ml of the prepared sampled was put in the drying container whose tare was obtained and ready for constant weighing, and it was weighed again. In order to evaporate some of the yogurt juice, the dry matter containers were exposed, without its lid on, to water vapor inside a boiling water bath until the juice in the container formed a dry and cracked skin, for approximately 30 minutes. After the pre-drying that was mentioned, the external surfaces of the drying container were dried off with a dry cloth and left for drying further inside an oven set to 102 °C for three hours. Later, it was put into the oven the same way and kept there for another hour. Finally, after it was cooled in the desiccator, it was weighed for the last time and the result was recorded.

Moisture Analysis: First, the samples prepared homogeneously in the ratio of 3/7 were distributed to the Petri dishes prepared for constant weighing so that each would have 5 ml of the sample, and then each was weighed (M1). Drying containers were put into the oven whose temperature was raised gradually to 100oC. After three hours, the containers were placed into the desiccator to cool down. Once they were cool enough, their weight was recorded (M2).

Ascorbic Acid Analysis: 5 ml of ascorbic acid solution (Standard ascorbic acid solution: 10 mg ascorbic acid is dissolved in 50 ml oxalic acid solution. 0,2 mg/1 mL) (5 ml solution contains exactly 1 mg ascorbic acid) was put into a 50 ml-conical flask for adjusting, and after 5 ml of oxalic acid was added, it was titrated with a dye solution in a burette. Titration continued until a slightly pink colour was achieved and maintained for 15 seconds after the last drop of dye solution was added. The dye solution consumed (ml) corresponds to 1 mg ascorbic acid. The factor of the solution, in other words, the amount of the ascorbic acid corresponding to 1 ml of the dye solution is obtained (Lenghor et al., 2002)

Gerber Oil Analysis: Plain yogurt juice and pineapple and pomegranate flavoured juice samples prepared in the ratio of 3/7 were mixed and the mixtures were homogenized. 10 ml sulfuric acid was put in the butyrometer, and

11 ml of the sample was added in it. The butyrometer was finally added 1 ml of amyl alcohol, and its lid was closed. Amyl alcohol clarifies the medium. The acid was ensured to burn the sample by inverting the butyrometer. Shaking continued until the samples turned brown completely, and the contained fat was released. They were centrifuged for 5 minutes at 1100 rpm spin and 68 oC. Upper level of the fat was observed on the scale of the butyrometer, and the fat percentage of the sample was determined.

Density Analysis: Plain yogurt juice and pineapple and pomegranate flavoured juice samples prepared in the ratio of 3/7 were put in the conical flask. The lactodensimeter was slowly dipped into the flask, its movement was waited to stop, and the value was read.

Colour Analysis: The samples were placed in the Lovibond tindometer cell. By looking through the observation screen, the colours were equated with the help of appropriate filters, and L\*, a\*, b\* colour values were observed.

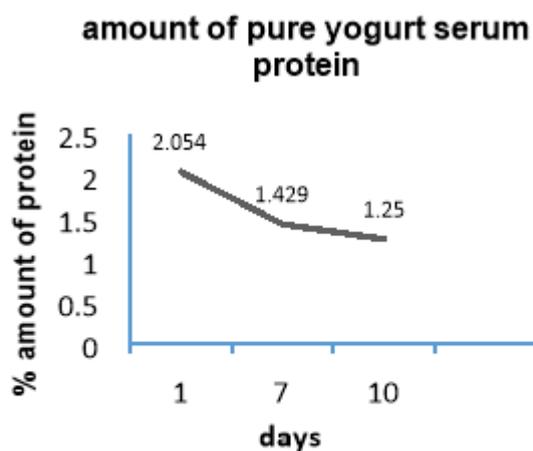
Ash Analysis: The temperature and the duration of the ash oven were set, and the plain yogurt juice and pineapple and pomegranate flavoured juice samples prepared in the ratio of 3/7 were put in the crucible. The crucibles were placed into the ash oven, and they were burnt until a white-grey colour was achieved. After the burning process, the crucibles were left to cool down in the desiccator and were weighed. This process was carried out in parallel for each of the samples, and it was made sure that the weight difference between the parallels was never more than 0,02 % (Liu, 2019).

Antimicrobial Analysis: In this study, the “paper disc agar diffusion method” was used for antimicrobial analyses. Autoclave sterilized paper discs with an 11.5 cm diameter were picked up one by one by pliers at aseptic conditions. Each disc was dipped into several solutions with different AMM dilutions given enough time to absorb. After the paper disc was taken out and dabbed gently on the mouth of the solution tube to drain the excess liquid, it was then laid on the surface of the agarized medium that had been inoculated with test microorganisms. By applying pressure with the pliers, the lower surface of the paper was ensured to touch the medium under. Another one of the sterilized paper discs was dipped into distilled water in the same manner, drained and laid on the agarized medium with the control sample (sample without AMM). The Petri dishes were incubated at 37 oC for 24 – 48 hours in the incubator. There isn’t always a direct relation between the zone diameter and the inhibition power of AMM. The diameter of the inhibition zone depends on not only the inhibition power of AMM, but also the degree of its diffusion in the medium (Nam and An, 2016).

### 3. RESULTS

#### 3.1 PROTEIN ANALYSIS BY KJELDAHL METHOD

There is a close relationship between the amount of protein and physicochemical properties of 3 different samples. Therefore, protein determination is an important analytical quality control criterion for yogurt serum. The protein values between 1-10 days are shown in Figure 1.



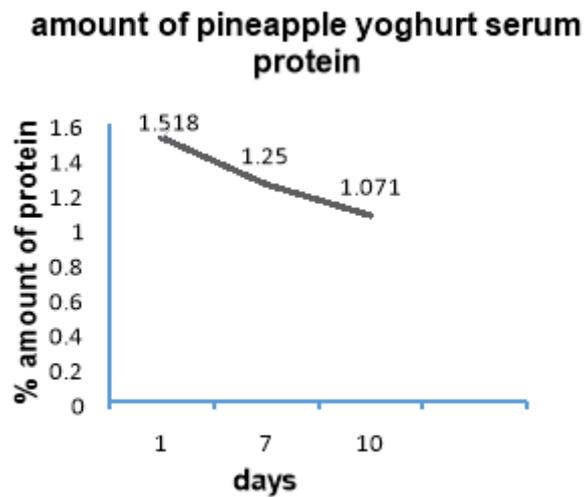
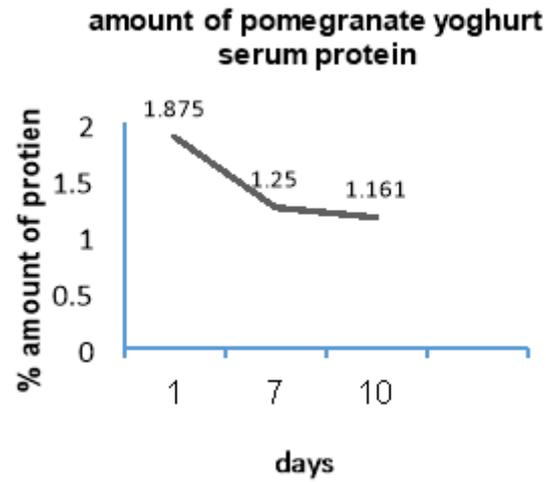


Fig. 1. Protein amounts of pure, pomegranate and pineapple yogurt serums

### 3.2. ANALYSIS OF TOTAL DRY MATTER

The dry matter determination analysis results of the samples are shown in Table 1 and Figure 2.

Table 1. % dry matter determination results of samples

Yogurt serum	Pomegranate yogurt serum	Pineapple yogurt serum
18.26	19.26	20.13

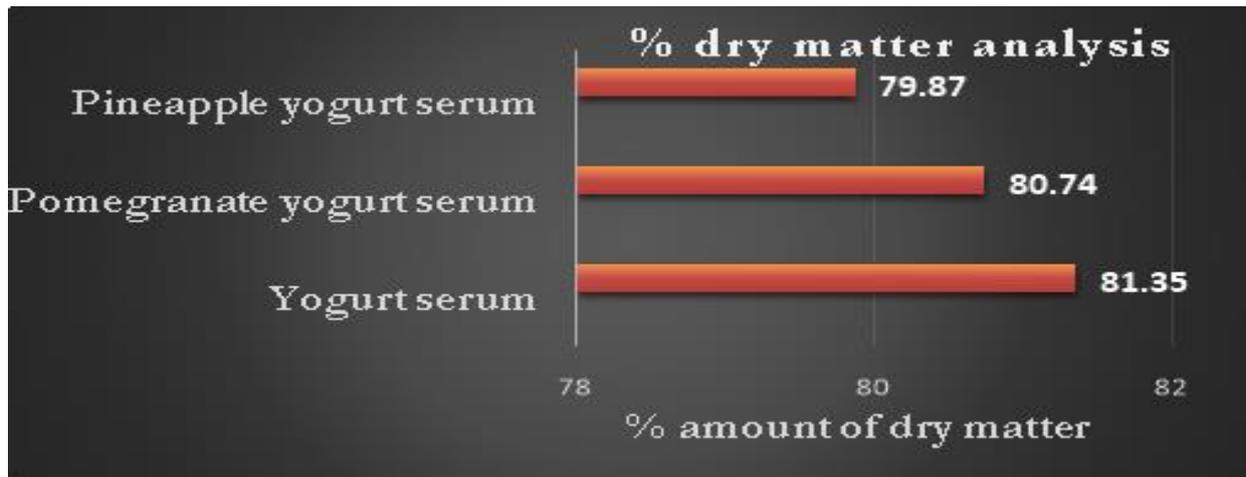


Figure 2. % dry matter determination

### 3.3. MOISTURE ANALYSIS

The moisture analysis results of the samples are shown in Table 2 and Figure 3. According to the results obtained, % moisture content in all three samples is directly proportional to total dry matter content.

Table 2. Moisture analysis results of samples

Yogurt serum	Pomegranate yogurt serum	Pineapple yogurt serum
81,35	80,74	79,87

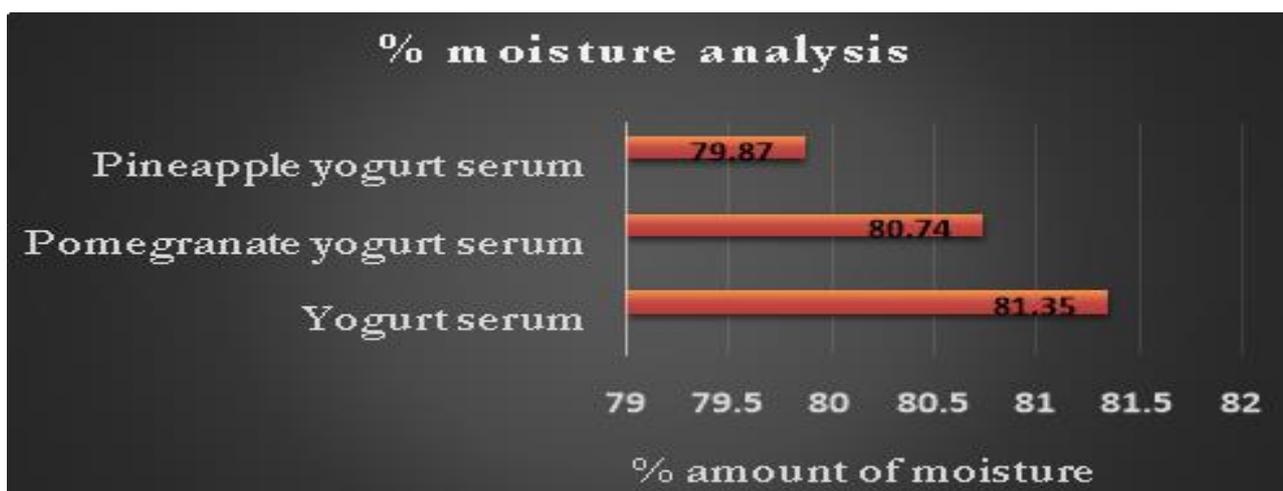


Fig. 3. % Moisture content of pure, pomegranate and pineapple yogurt serums

### 3.4. ASCORBIC ACID ANALYSIS

The amount of ascorbic acid of pure, pomegranate and pineapple yogurt serums is given in Figure 4.

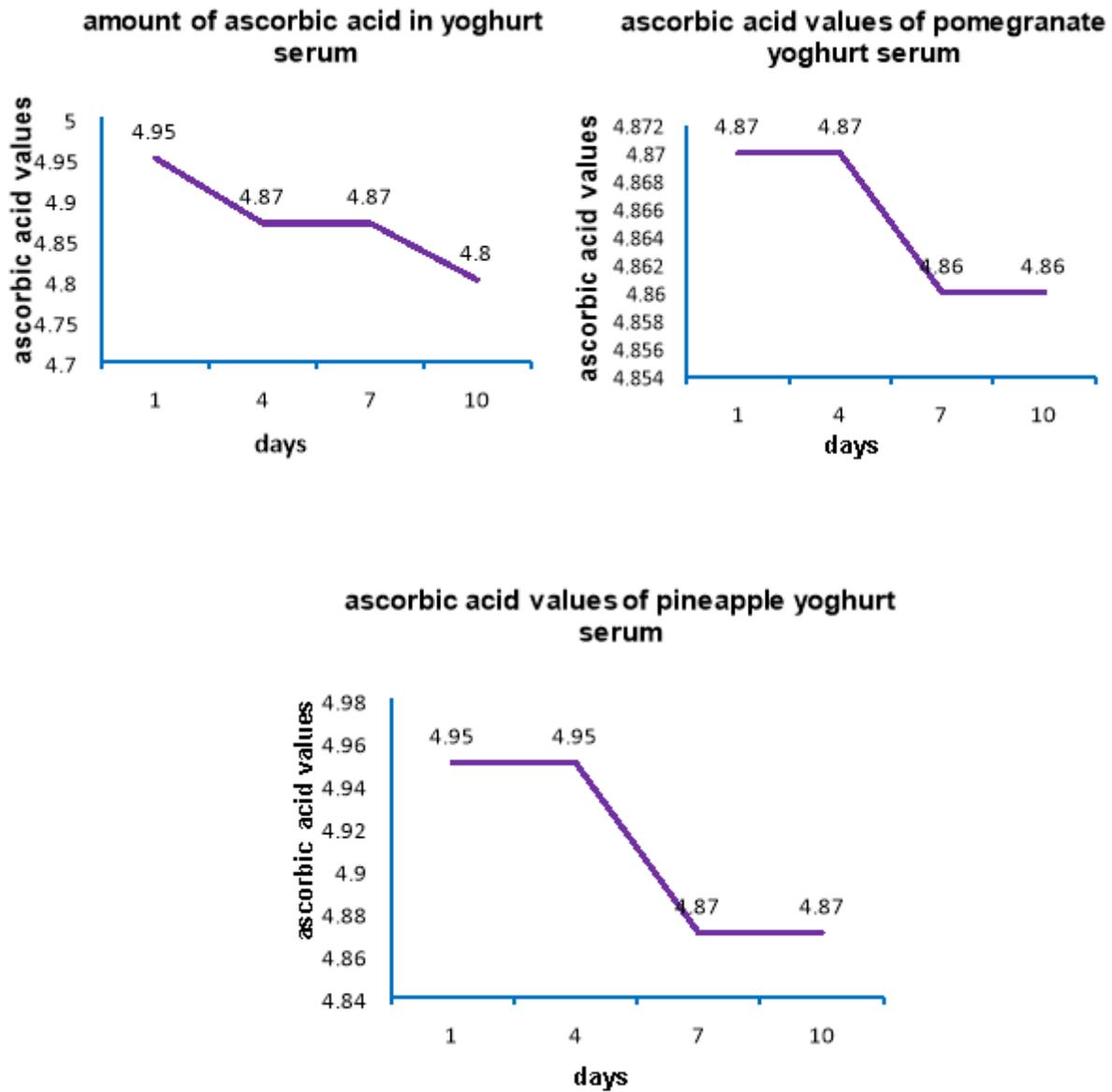


Fig. 4. Ascorbic acid content of pure, pomegranate and pineapple yogurt serums

### 3.5. GERBER OIL ANALYSIS

As a result of the Gerber oil analysis, the expected fat value did not occur on the butyrometer scale and no measurement could be made on the scale.

3.6. COLOUR ANALYSIS

Color and color difference are generally evaluated instrumentally according to the method developed by CIE.

Table 3. Color analysis values of samples

Sample	Yoghurt serum			Pomegranate yoghurt serum			Pineapple yoghurt serum		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
1.day	55,20	-2,18	13,16	4,90	2,60	1,27	17,58	2,19	13,83
7.day	40,49	0,44	14,10	-6,57	1,00	0,79	12,46	2,99	12,08
10.day	19,09	0,31	8,19	-2,20	1,46	2,23	12,8	0,31	8,19

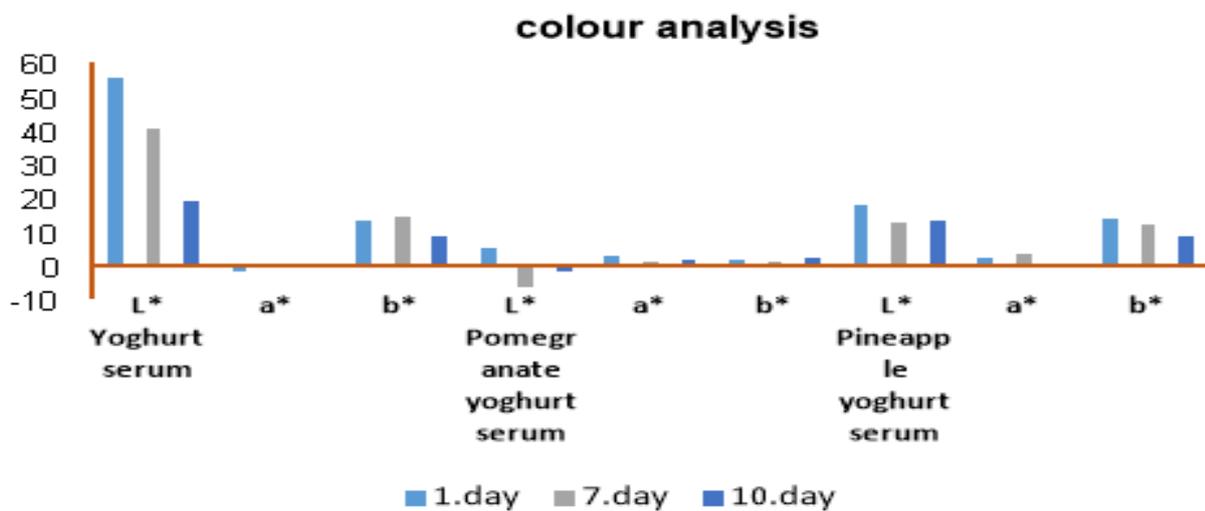


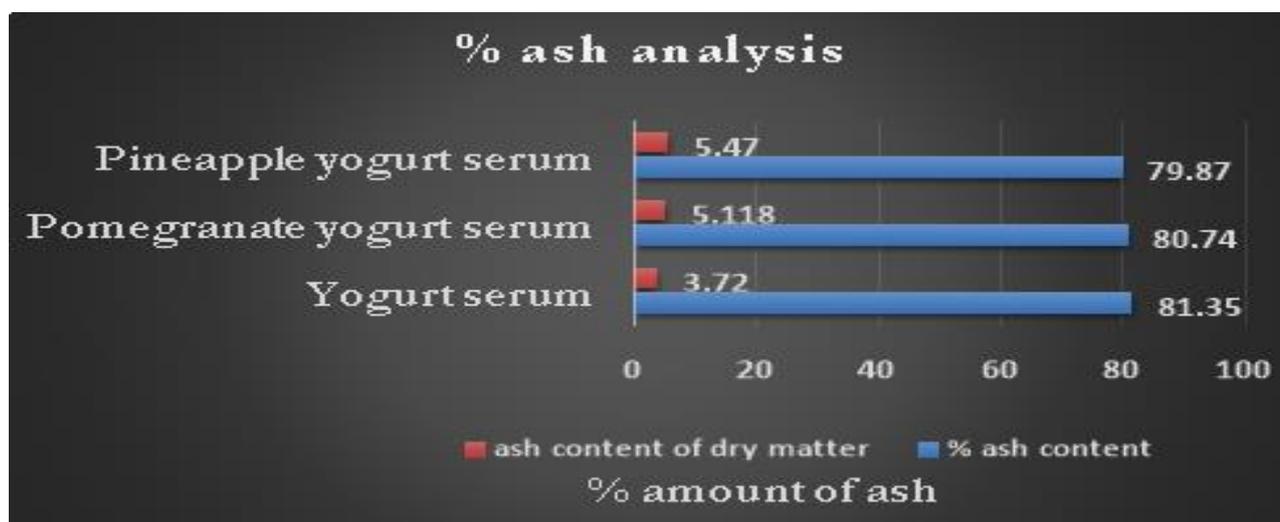
Fig. 5. Color analysis results of pure, pomegranate and pineapple yogurt serums

### 3.7. % ASH ANALYSIS

The results of ash determination analysis are given in Table 4 and Figure 6.

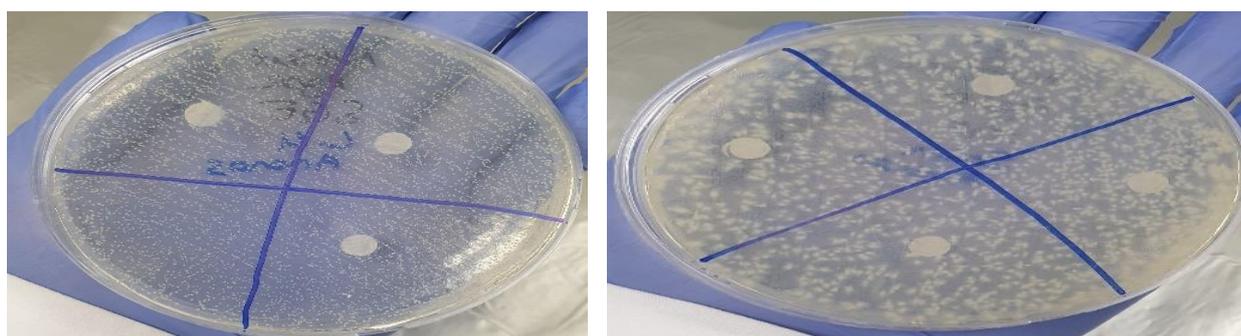
Table 4. % Ash analysis results of samples

	Yogurt serum	Pomegranate yogurt serum	Pineapple yogurt serum
% ash content	0.6799	0.9858	1.10261
ash content of dry matter	3,72	5,118	5,47



### 3.8. ANTIMICROBIAL ANALYSIS

Nutrient broth was used as liquid medium and Nutrien agar was used as solid medium in this analysis.



(a)

(b)

Fig. 7. Antibacterial resistance analysis of pineapple yogurt serum (a) and pomegranate yogurt serum (b) against Salmonella, Listeria monocytogenes, E.coli, Staphylococcus Aureus

## 4. DISCUSSION

### 4.1 PROTEIN ANALYSIS BY KJELDAHL METHOD

According to the results, while the highest protein value was observed in pure yogurt serum, protein values of pomegranate and pineapple yogurt serums decreased. Approximately 0.5% difference decrease is experienced between 1-7 days and there is not much decrease at the end of 10th day. According to the Turkish Food Codex Communiqué on Milk and Dairy Products, if yogurt is considered, it is seen that the protein value is at least 2.0% and accordingly, pure yogurt serum is in compliance with the communiqué.

### 4.2. ANALYSIS OF TOTAL DRY MATTER

The values obtained according to Table 1, % dry matter content of the products are in accordance with Turkish Food Codex Table 2. TS 3810. Although the food is liquid, high water-insoluble matter ratio shows that it maintains its mineral values.

### 4.3. MOISTURE ANALYSIS

According to the results obtained, % moisture content in all three samples is directly proportional to total dry matter content.

### 4.4. ASCORBIC ACID ANALYSIS

As shown in Figure 4, there was no or little decrease in vitamin C values. The first day values of pure yogurt serum and pineapple yogurt serum are the same and pineapple yogurt serum is seen to maintain vitamin C values even on the 4th day. This is also related to the high amount of vitamin C in pineapple content. Vitamin C values of pomegranate yogurt serum are preserved in almost 10 days. This generally means that yogurt serum is rich in ascorbic acid.

### 4.5. GERBER OIL ANALYSIS

Accordingly, the fat content of yogurt serum is between 0.01-0.09% and is quite low. Because most of the total fat is kept in the bag. Since the fat globules are located in the clot structure, they move away from the environment with the serum during the filtration stage.

### 5.6. COLOUR ANALYSIS

This method is also known as CI 1976 CIELab, CIELab three point measurement method ". In this three-point measurement method, L\*/L, light transmittance values, 0 (no transmittance) and 100 (completely transmittance), a\*/a red (- a\*/- a, green) and b\*/b yellow (-b\*/ - b, blue) indicates the colour values. As a result of the analysis, L\*, a\*, b\* values of the products are shown in Table 3 and Figure 5 (Tontul et al., 2018)

### 4.7. % ASH ANALYSIS

The obtained results showed that the amount of ash in the samples increased. This increase indicates that the products are rich in minerals.

### 4.8. ANTIMICROBIAL ANALYSIS

Antimicrobial activity analysis was performed by disc method against 4 different bacteria (Salmonella, Listeria monocytogenes, E.coli, Staphylococcus Aureus) for 3 different samples. However, as shown in Figure 7, no inhibition zone was observed in pure, pomegranate and pineapple yogurt serums since there was no resistance to bacteria.

## 5. CONCLUSION

A product obtained by filtering the yogurt obtained by the conventional method and removing the serum portion is called strained yogurt. Approximately 33% of the yogurt is kept in the bag, while 67% is excreted as serum. This serum has a nutritious value, such as churn and whey. During the production of yogurt, most of the nutritional value of yogurt is transferred to this serum. However, this serum is left to the environment as waste, wasted and mixed with sewage. This situation also creates serious economic losses. In this study, quality studies were performed on yogurt serum and durability of nutritional values between 1-10 days were measured by experimental methods. As a result of all these studies, it was concluded that according to Turkish Food Codex Communiqué on Fermented Dairy Products, it should be presented to consumption due to its suitable structural and content features. In addition, due to insufficient research on this subject, we think that this study will benefit the literature for the development and investigation of the subject.

## 6. RECOMMENDATIONS

In this study, A, D and E vitamin and mineral analysis by using HPLC is recommended.

## 7. ACKNOWLEDGEMENT

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