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EVALUATION OF PHYSICOCHEMICAL AND SENSORIAL PROPERTIES IN RECONSTITUTED AYRAN DRINKS FROM YOGURT POWDERS LYOPHILIZED UNDER DIFFERENT VACUUM PRESSURES

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ABSTRACT
To extend the shelf life of yogurt and to use it in different formulations, a drying process is performed. Among the drying processes, lyophilization comes to the forefront in terms of preserving the properties of dried products at a high level. In this study, the effect of different lyophilization pressures (0.5 and 1.0 mbar) on the physicochemical properties of reconstituted ayran samples (0.5A and 1.0A) was investigated during 28 days of cold storage. Besides, sensory analyses of the reconstituted ayran samples were also performed. The outcomes were compared with the ayran produced directly (control). According to the results, serum separation increased more in reconstituted samples, however, syneresis declined with decreasing lyophilization pressure. The highest viscosity was determined in the control sample (6.97 mPa.s), followed by 0.5A (5.56 mPa.s) and 1.0A (4.66 mPa.s), respectively. Consequently, the increased lyophilization pressure negatively affected the physicochemical and sensorial properties of reconstituted ayran.
Keywords: Reconstituted ayran, syneresis, viscosity, lyophilization pressure, powdered yogurt

FARKLI VAKUM BASINÇLARINDA LİYOFİLİZE EDİLEN YOĞURT TOZLARINDAN REKONSTİTÜYE EDİLMİŞ AYRANLARDA FİZİKOKİMYASAL VE DUYUSAL ÖZELLİKLERİN DEĞERLENDİRİLMESİ

ÖZ
Yoğurdun raf ömrünü uzatmak ve farklı formülasyonlarda kullanmak amacıyla kurutma işlemi gerçekleştirilmektedir. Kurutma işlemleri arasında liyofilizasyon, kurutulan ürünlerin özelliklerinin yüksek düzeyde korunması açısından ön plana çıkmaktadır. Bu çalışmada, farklı liyofilizasyon basınçlarının (0.5 ve 1.0 mbar) rekonstitüye edilmiş ayran numunelerinin (0.5A ve 1.0A) fizikokimyasal özellikleri üzerindeki etkisi 28 günlük soğuk depolama süresince incelenmiştir. Ayrıca, rekonstitüye ayran örneklerinin duyusal analizleri de yapılmıştır. Analiz bulguları doğrudan üretilen ayran (kontrol) ile karşılaştırılmıştır. Elde edilen sonuçlara göre, rekonstitüye edilmiş örneklerde serum ayrılması daha fazla artmıştır, ancak, azalan liyofilizasyon basıncı ile sineresiz azalmıştır. En yüksek viskozite kontrol numunesinde (6.97 mPa.s) belirlenmiş olup, bunu sırasıyla 0.5A (5.56 mPa.s) ve 1.0A (4.66 mPa.s) takip etmiştir. Sonuç olarak, artan liyofilizasyon basıncı, rekonstitüye edilmiş ayranın fizikokimyasal ve duyusal özelliklerini olumsuz yönde etkilemiştir.
Anahtar kelimeler: Rekonstitüye ayran, sineresiz, viskozite, liyofilizasyon basıncı, toz yoğurt

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INTRODUCTION

Ayran is a traditional drinkable fermented milk product produced by mixing yogurt with water and salt or by adding yogurt cultures to standardized milk (Altay et al., 2013). Ayran is produced traditionally in Turkey by diluting yogurt with 30-50% water and adding about 1% salt (Koçak and Avsar, 2009). The chemical composition of ayran is directly associated with the type of milk used, the dilution rate, and the desired fat content (whole fat, semi-skimmed, or skimmed) (Kabak and Dobson, 2011). It is widely consumed in Turkey due to its nutritional value, health benefits, and desirable aroma and taste (Sarhir et al., 2019). Ayran, which is rich in calcium and vitamins, is a valuable and easily digestible beverage (Aladeboyeje and Sanli, 2021). Besides that, it has been reported to have anticarcinogenic (especially against colorectal cancer), antioxidant, and immunomodulatory properties (Grishina et al., 2011).

The primary texture defects observed in ayran and yogurt are serum separation and low viscosity (Altay et al., 2013). During the storage of these products, optimum viscosity and no serum separation are quality criteria requested by consumers for a pleasant mouthfeel (Ozdemir and Kilic, 2004). Serum separation occurs as a result of the precipitation of casein micelles by forming aggregate. In this regard, Köksoy and Kılıç (2003) have asserted that increasing water and salt levels in the composition of ayran enhance serum separation. Moreover, Gursoy et al. (2016) have reported that developing acidity throughout the storage of ayran triggers whey separation.

In the powder form of fermented milk products, it is aimed to produce a product with a long shelf life that can be used at any time and that does not need cold storage due to reduced enzymatic and microbial activities as a result of the decrease in moisture content (Routray and Mishra, 2012). Moreover, the drying process reduces product volume, thereby decreasing packaging, storage, and transportation costs (Song and Aryana, 2014). For these reasons, food manufacturers and consumers tend towards to powdered foods. Yogurt powder is used in a wide variety of food products as a nutritional supplement, texture enhancer, and flavor improver (Koç et al., 2010; Song and Aryana, 2014). Another advantage is that yogurt powder can be used by diluting it at the desired rate in the production of ayran, which has a very short shelf life (15-30 days) (Köksoy and Kılıç, 2003; Soysona Ar and Ocak, 2022).

Among the various food drying methods; the freeze-drying is the most preferred one, as it overcomes unwanted Maillard reactions during the drying process, offers a product with better rehydration capability, protects flavor components at the highest level, provides better sensorial characteristics, and preserves bacterial viability at the highest rate in fermented products (Kumar and Mishra, 2004; Santos et al., 2018a). The freeze-drying, also known as the lyophilization, involves subjecting the product frozen at very low temperatures to low pressure provided by vacuum and removing the frozen water in the product by sublimation (Santos et al., 2018b). Sublimation in the lyophilization process is accomplished as a result of lowering the ambient temperature by keeping the pressure below the triple point of water (Doran, 2013). In this process, the vacuum pump has been stated to cause the most energy consumption (Kovaci et al., 2021). Accordingly, the selection of high vacuum pressures in the lyophilization process can boost electricity consumption.

A previous study on the production of reconstituted ayran from yogurt powders produced by conventional drying and freeze-drying was conducted by Soysona Ar and Ocak (2022). According to the results of their research, the reconstituted ayran produced from lyophilized yogurt powder showed superiority in its quality compared to that produced from conventionally dried. However, no study examining the effect of different vacuum pressures on the final properties of ayran has been found in the literature. From this point of view, this study was carried out to determine the effect of lyophilization pressure on the quality characteristics of reconstituted ayran. For this purpose, the physicochemical properties of ayran samples reconstituted from yogurts dried at 0.5 and 1.0 mbar absolute pressures were examined during 28 days of cold storage. In addition, the sensory properties of the reconstituted ayran samples were also investigated. The findings were compared with ayran samples produced by diluting yogurt, which is one of the industrial methods.

MATERIALS AND METHODS

Materials

Yogurt cultures (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) were provided from the natural isolate collection of Panagro Meat and Milk Integrated Facilities (Konya, Turkey). Pasteurized and standardized yogurt milk to 14% total dry matter content was also supplied from this company.

Yogurt making

Yogurt production was carried out according to the procedure mentioned by Öztürk (2022). Yogurt milk was inoculated with 2% of bacterial cultures (1:1, *S. thermophilus*: *L. bulgaricus*) activated in a commercial TW60 medium based on whey (Danisco, Niebüll, Germany) and incubated at 43 °C. The incubation was terminated when the acidity reached pH 4.6. Following this, the yogurts were stored at 4 °C and then used in the production of lyophilized yogurt powders. The yogurts used in the control ayran samples were produced one day before the ayran production.

Lyophilization conditions and production of yogurt powders

The method defined by Öztürk (2022) was used in the production of lyophilized yogurt powders. The yogurts, which were kept at -80 °C for a day to solidify, were placed in a freeze-dryer (LyovaporTM L-200, Buchi, Italy) and dried separately for 1 day at vacuum pressures of 0.5 and 1.0 mbar. The dried samples were powdered with the help of a spatula and transferred to sterile plastic closed containers. Prepared powder samples were stored at room temperature.

Electricity consumption

The socket plug wattmeter (TT-technic,16A 3680W, China) was used to determine electrical consumption of the lyophilizer at different vacuum pressures. The results were recorded as energy consumption (kWh) and used electrical current (A).

Experimental samples

Experimental production of ayran samples is presented in Figure 1. To produce the control ayran, the dry matter ratio of the prepared yogurt samples was adjusted to approximately 8% by adding water and salt. After adding water and salt, ayran samples were mixed homogeneously for 2 min at 10000 rpm by using Ultra Turrax T25 mixer (IKA, Staufen, Germany) and transferred into 200 mL sealed sterile plastic containers. The proximate composition of the control ayran samples has been found to consist of 7.75±0.10% total dry matter, 1.85±0.07% fat, and 0.72±0.00% salt. For reconstituted ayran samples, yogurt powders were formulated to have total dry matter and salt contents similar to those of control ayran. To that end, after adding salt and water to the yogurt powders, respectively, they were homogenized for 2 min at 10000 rpm by using the Ultra Turrax T25 mixer. The prepared reconstituted ayran samples were also transferred into 200 mL sterile sealed plastic containers. All ayran samples were produced in two replicates. The prepared samples were kept in refrigerated conditions for 28 days. Ayran samples were referred to as follows: control, 0.5A, and 1.0A for produced conventionally, produced from yogurt powder dried with 0.5 mbar, and produced from yogurt powder dried with 1.0 mbar, respectively.

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Titratable acidity (%lactic acid) and pH values

Titratable acidity of ayran samples was figured out by titration with 0.1 N NaOH and expressed as percentage of lactic acid as described by Simsek et al. (2007). As for the pH value of the samples, it was directly measured with a calibrated pH-meter (Starter 3100 model, Ohaus).

Serum separation

For this analysis, ayran samples were stored in 50 mL graduated cylinders at 4 °C for 28 days. Serum separation was calculated by the volumetric proportioning of the whey separated at the top and the results were provided as a percentage (%) (Köksoy and Kılıç, 2003).

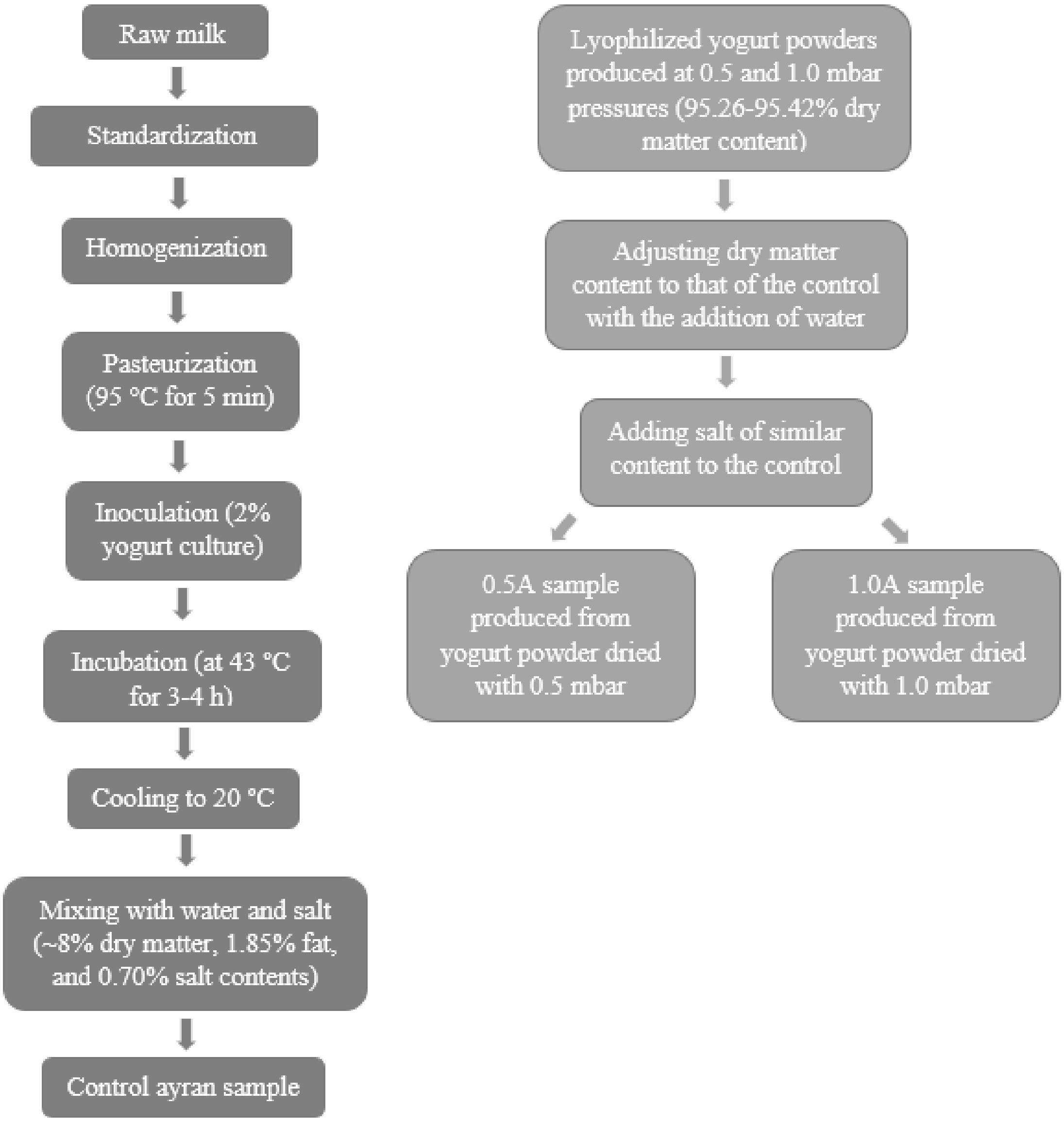

Figure 1. Experimental production schemes of ayran samples.

Viscosity measurements
Viscosity values of ayran samples were determined at 4 ± 1 °C using a viscometer (Sine-wave Vibro Viscometer SV-10/Japan), which records the electric current required to resonate two sensor plates at a fixed frequency (Celik and Temiz, 2020). The obtaining data over the storage time were expressed in millipascal second (mPa.s).

Sensorial assessment
Sensory analyzes of the samples were performed on the 14th day of the cold storage time by a panel of eleven members, who were familiar with ayran. Samples were evaluated in terms of the following parameters: appearance, color, odor, structure, and taste. Panelists scored the samples with a hedonic scale from 1 to 5 describing dislike very much to like very much, respectively (Soysona Ar and Ocak, 2022).

Statistical evaluation
The resulting data were subjected to one-way analysis of variance (ANOVA) in Minitab software version 17 (State College, USA). All data

are given as the mean ± standard deviation of two replicates. The mean of the results was compared with the Tukey test at a 95% confidence interval to identify significant differences between the ayran samples and different storage days. Additionally, the two-sample independent t-test was performed to compare the effects of different pressure treatments on electricity usage in the lyophilization process.

RESULTS AND DISCUSSION

Energy consumption in the lyophilization process is higher compared to other drying systems since it is carried out for longer times under very low pressures (Kovaci et al., 2021). Among the equipment of freeze-dryer, the vacuum pump has been reported to be the greatest individual energy consumer (Kešelj et al., 2017). The energy consumptions were 0.827 and 0.822 kWh, and the used current values were 4.450 and 4.392 A for 0.5 and 1.0 mbar pressure applications, respectively. However, the amount of consumed energy and the used current values were not statistically different for 0.5 and 1.0 mbar vacuum pressure applications ($P > 0.05$).

The mean dry matter value of the control ayran samples was determined as 7.75% whilst the dry matter values of the samples produced by rehydration were found to be between 7.70-7.85%. From a microbiological point of view, in our previous study, it was determined that the 0.5 and 1.0 mbar pressures used for drying of yogurts decreased the number of *L. bulgaricus* by 7.76% and 6.94%, respectively, and there was no statistical difference between these reductions depending on the pressure (Öztürk, 2022). Besides, in the aforementioned study, the number of *S. thermophilus* decreased by 5.17% and 6.86% for 0.5 and 1.0 mbar pressures, respectively.

Table 1. pH values of ayran samples during refrigerated storage

	Storage days				
Samples *	0	7	14	21	28
Control	4.07±0.04^{B}	4.07±0.05^{B}	4.13±0.01^{B}	4.07±0.05^{B}	4.14±0.01^{B}
0.5A	4.38±0.04^{A}	4.34±0.03^{A}	4.41±0.04^{A}	4.30±0.04^{A}	4.32±0.02^{A}
1.0A	4.40±0.01^{A}	4.35±0.01^{A}	4.42±0.04^{A}	4.34±0.01^{A}	4.36±0.02^{A}

* 0.5A and 1.0A denote reconstituted ayran samples from yogurt powders produced using 0.5 and 1.0 mbar, respectively.
Values are expressed as mean ± standard deviation (n=2).
Significant differences were determined with the Tukey test at the $P \leq 0.05$ confidence interval.
The means indicated by different capital letters in the same column present differences between the samples.

Commercially produced ayran has a shelf life of 15 days to one month at refrigerated storage (Baruzzi et al., 2016; Köksoy and Kılıç, 2003). Considering this situation and other literature studies (Baruzzi et al., 2016; Çelekli et al., 2019; Uzay et al., 2021), ayran samples examined in this study were analyzed weekly throughout the 28 days of cold storage time. During the storage time, the pH values of the samples were between 4.07 and 4.42 (Table 1). The pH change during the storage time was insignificant for all samples ($P > 0.05$). Soysona Ar and Ocak (2022) found the pH values between 4.40-4.48 in ayran samples rehydrated from lyophilized yogurt powder, which is in accord with the pH findings (4.30-4.42) detected for the reconstituted ayran samples in this study. The pH values of the control sample were lower than those of the reconstituted samples throughout the whole cold storage days ($P \leq 0.05$). It has been reported that the pH values of fermented products are related to their load of acid-producing microorganisms (Erkaya et al., 2015). Accordingly, the pH values may have been higher in the reconstituted ayran samples due to the decrease in the number of yogurt bacteria by lyophilization. However, no effect of different pressure applications on pH value was observed ($P > 0.05$). Moreover, although slight decreases were observed in pH values during the cold storage time, these changes were not found to be statistically significant ($P > 0.05$).

Titratable acidity values of ayran samples were between 0.47-0.55% lactic acid as shown in Table 2. Different pressure treatments resulted in similar %lactic acid contents of reconstituted samples during the storage time (P >0.05). Consistent with the change in pH values, the % lactic acid content of the control sample was found to be higher than the reconstituted samples (P ≤0.05) and the titratable acidity of all samples remained stable during storage (P >0.05). The obtaining data on titratable acidity revealed the superiority of reconstituted ayran production. Because acidity is among the important factors affecting the quality and stability of ayran during storage (Erkaya et al., 2015). The improved acidity has been reported to increase serum separation, which is one of the physical quality defects in ayran (Gursoy et al., 2016).

Table 2. Titratable acidity (%lactic acid) values of ayran samples during refrigerated storage

	Storage days				
Samples *	0	7	14	21	28
Control	0.52±0.02^{A}	0.51±0.03^{A}	0.54±0.05^{A}	0.52±0.02^{A}	0.55±0.04^{A}
0.5A	0.50±0.01^{AB}	0.49±0.01^{A}	0.49±0.04^{A}	0.50±0.02^{A}	0.50±0.04^{A}
1.0A	0.47±0.02^{B}	0.48±0.01^{A}	0.51±0.03^{A}	0.48±0.01^{A}	0.50±0.02^{A}

* 0.5A and 1.0A denote reconstituted ayran samples from yogurt powders produced using 0.5 and 1.0 mbar, respectively.
Values are expressed as mean ± standard deviation (n=2).
Significant differences were determined with the Tukey test at the $P \leq 0.05$ confidence interval.
The means indicated by different capital letters in the same column present differences between the samples.

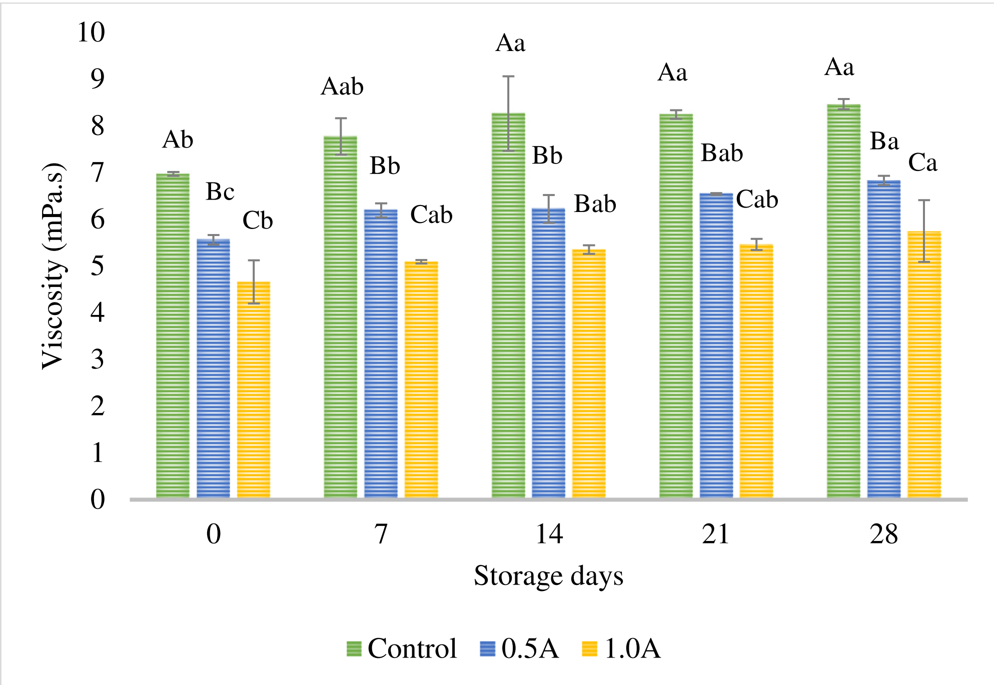

Figure 2. Viscosity of ayran samples during refrigerated storage (mPa.s). 0.5A and 1.0A denote reconstituted ayran samples from yogurt powders produced using 0.5 and 1.0 mbar, respectively. Values are expressed as mean ± standard deviation (n=2). Significant differences were determined with the Tukey test at the $P \leq 0.05$ confidence interval. The means indicated by different capital letters present differences between the samples and by different lowercase letters present differences between the storage days.

Viscosity of fermented milk products plays an important role in their shelf life (Erkaya et al., 2015). While the viscosity values of the samples were found to be between 4.66-6.97 mPa.s at the beginning of the storage time, these values were between 5.75-8.46 mPa.s at the end of the storage (Figure 2). Although these values were found to be compatible with the viscosity values determined in the ayran samples by Ozdemir and Kilic (2004) and Yilmaz et al. (2015), they were lower than the findings of Erkaya et al. (2015) and Ertugay et al. (2012), who applied ultrasonication to ayran. Throughout the storage time, the viscosity value of the control sample was higher than the reconstituted samples ($P \leq 0.05$). It has been asserted that the lyophilization process causes irreversible damage to the acid casein gel structure, especially by affecting the hydrogen bonds (Jaya, 2009; Kumar and Mishra, 2004). The mechanical energy used in mixing for rehydration has also been reported to weaken this gel structure (Santos et al., 2018b). Generally, the lyophilization pressure was observed to be effective on the viscosity during the storage time, and the viscosity decreased as the application pressure increased ($P \leq 0.05$). In our previous study, we determined that the connections between protein aggregates in yogurt powders increased with the pressure reduction (Öztürk, 2022). Therefore, this difference between pressure treatments may be due to the increased interaction of casein micelles with decreased pressure. For the control sample, the viscosity increased after the 14th day, and then similar viscosity values were observed until the 28th day ($P > 0.05$). As for the reconstituted samples, their viscosity began to increase after the 7th day and the highest viscosity values were detected at the end of the storage time. Accordingly, prolonged storage time in reconstituted ayran may increase the quality of the final product. Moreover, although the rehydration process seems to be a disadvantage for ayran, it is considered that the possibility of adjusting the product to the desired viscosity via diluting yogurt powder can be an advantage for reconstituted ayran.

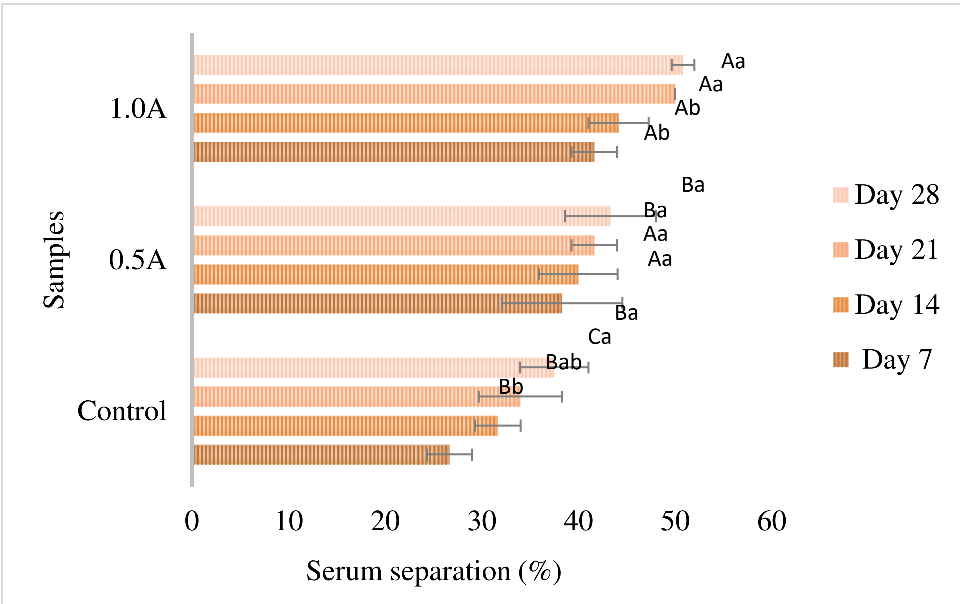

Figure 3. Serum separation of ayran samples during refrigerated storage (%). 0.5A and 1.0A denote reconstituted ayran samples from yogurt powders produced using 0.5 and 1.0 mbar, respectively. Values are expressed as mean ± standard deviation (n=2). Significant differences were determined with the Tukey test at the $P \leq 0.05$ confidence interval. The means indicated by different capital letters present differences between the samples and by different lowercase letters present differences between the storage days.

The levels of serum separation from the samples during the storage time are shown in Figure 3. While serum separation was observed between 26.67-41.67% in ayran samples at the onset of the storage, the rate of syneresis at the end of storage was between 37.50-50.83%. Serum separation was less in the control sample with the highest viscosity value ($P \leq 0.05$). As mentioned earlier, serum separation was greater in reconstituted samples because the lyophilization process caused an irreversible rearrangement of protein networks in the acid gel matrix (Kumar and Mishra, 2004). Also, serum separation observed in reconstituted ayran samples was found to be higher than that of ayran drinks produced by traditional methods in the literature (Değirmenci et al., 2021; Ozdemir and Kilic, 2004). Regarding this situation, it has been suggested that increasing the dry matter content of fermented dairy products can solve textural problems in maintaining the quality of the final product (Doğan, 2011). Therefore, it can be experienced to increase the dry matter content of reconstituted ayran for lower serum separation.

While serum separation in 0.5A sample was statistically constant during the storage time ($P > 0.05$), serum separation in control and 1.0A samples increased after the 14th day of storage ($P \leq 0.05$). Similarly, Köksoy and Kılıç (2003) observed an increase in serum separation of ayran samples over the cold storage. In addition, Soysona Ar and Ocak (2022) also detected that serum separation increased in reconstituted ayran with storage time. However, producing reconstituted ayran on demand will overcome the disadvantage of storage time on serum separation. Interestingly, on days when viscosity increased, the serum separation in the samples also increased. During the storage of ayran, serum separation occurs as a result of aggregation of casein proteins and loss of water from the protein matrix (Gursoy et al., 2016). Although it has been suggested that high viscosity alleviates serum separation by reducing particle movement in fermented milk products, gel strength which indicates water holding capacity is more important for serum separation (Priyashantha et al., 2021). The mixing process of acid coagulum in the ayran production causes changes in the protein network and affects the gel strength (Ozdemir and Kilic, 2004). Hence, in this study, the production of control ayran by breaking the coagulum of yogurt instead of being standardized at the beginning and the effect of the lyophilization process on protein network structures in reconstituted ayran may have affected the syneresis behavior of the samples during the storage time.

There was no difference in serum separation between different pressures until the 14th day of storage, however, it was determined that serum separation increased as the application pressure increased on the following storage days. The less serum separation observed in 0.5A sample compared to 1.0A may be due to the increased interaction between the casein micelles regarding the reduced vacuum pressure. Eventually, serum separation stability was found to be better in 0.5A sample during the storage time. In this regard, it is anticipated that it would be more appropriate to use lyophilized powders produced with a vacuum pressure of 0.5 mbar to produce reconstituted ayran.

Sensory analyzes of ayran samples were carried out on the 14th day of the storage time and the results are presented in Figure 4. Control ayran sample got the highest score for the sensory parameters except for taste ($P \leq 0.05$). In terms of the taste, all samples were found to be statistically similar ($P > 0.05$). In the control sample, in which dense and thicker structures were observed, the appearance score was determined as 4.67. However, there was no effect of different application pressures on the appearance scores of reconstituted ayran samples ($P > 0.05$). The structure scores of the ayran samples were found to be compatible with their viscosity values. The scores for structure were determined as 2.50 and 2.39 in 0.5A and 1.0A samples, respectively ($P > 0.05$), while it was determined as 4.56 in control sample. In one study, it was recommended to rehydrate the yogurt powder to have 30% dry matter content to obtain structural properties similar to fresh yogurt with 14% dry matter content (Sakin-Yilmazer et al., 2014). Therefore, it is considered that the production of reconstituted ayran with higher dry matter can overcome the structural score differences with the control ayran. Although there was no statistical difference in the odor, color, and taste scores of the reconstituted samples (P >0.05), 0.5A sample received slightly higher scores in terms of these features. Based on the sensorial aspect, yogurt powders produced with 0.5 mbar pressure can be recommended for reconstituted ayran production.

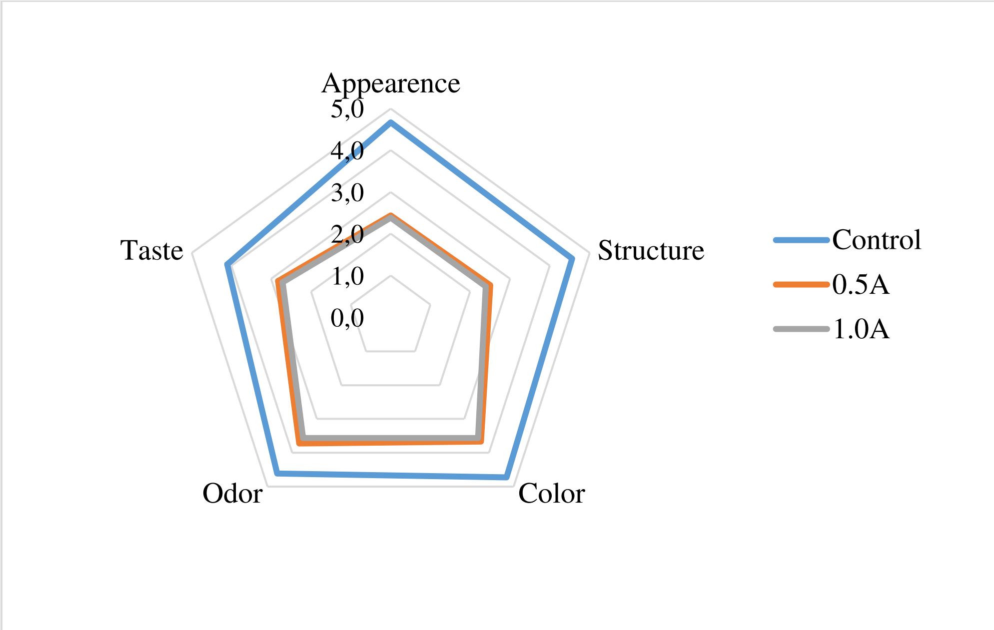

Figure 4. Comparison of sensory properties of control ayran with reconstituted ayran samples 5 points hedonic scale). 0.5A and 1.0A denote reconstituted ayran samples from yogurt powders produced using 0.5 and 1.0 mbar, respectively.

CONCLUSION

This study investigated the physicochemical characteristics of reconstituted ayran drinks made from lyophilized yogurt powders with different pressures and the findings were compared with plain ayran. Besides, ayran samples were also subjected to sensory evaluations. Throughout the 28 days of storage time, the pH values of the reconstituted ayran samples were found to be higher than the control ayran, thereby resulting in lower titratable acidity. The viscosities and serum separation levels of the samples showed a trend with respect to application pressure. Increasing applied pressure resulted in enhanced whey separation and decreased viscosity. Control samples received the highest sensory scores in terms of appearance, structure, color, and odor. In reconstituted samples, the sensory characteristics were generally found to be close to each other, however, 0.5A samples had slightly higher sensory scores. To improve the physicochemical and sensory properties, the production of reconstituted ayran with higher dry matter can be suggested. After all, if such a production methodology is to be used to extend the shelf life of ayran, it can be recommended to o use lyophilized powders dried at 0.5 mbar pressure in reconstituted ayran production for obtaining the best sensorial and physicochemical properties.

CONFLICT OF INTEREST

Author declares no potential conflict of interest.

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