

Effect of Adding Different Spices and Flavours on Serum Separation, Viscosity, Acetaldehyde Content and Antioxidant Capacity in Yoghurts Produced with Adding Probiotic Culture*

Probiyotik Kültür İlave Edilerek Üretilen Yoğurtlara Farklı Baharat ve Aromaların Eklenmesinin Serum Ayrılması, Viskozite, Asetaldehit İçeriği Ve Antioksidan Kapasitesi Üzerine Etkisi

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Abstract

This research was carried out to determine the differences in quality properties of yoghurts obtained by using dried forms of various plants and their aromas with the addition of probiotic culture (*Lactobacillus casei*) in yoghurt production. In this study, mint, thyme, dill and coriander plants were obtained, cleaned and dried under appropriate conditions and conditions. Some of the plants obtained were added to the yoghurt sample in dried form at a rate of 1%. The volatile oils obtained from the remaining dried plant material were added to the yoghurt samples at a rate of 0.05%. The prepared yoghurt samples were analyzed in terms of serum separation, viscosity, acetaldehyde content and antioxidant capacity within the scope of physicochemical properties. Serum separation values in yoghurt samples varied between 6.73% and 7.84%. Viscosity values were measured in the range of 4.198–5.879 Pa. s at 20 rpm and 1.798–2.410 Pa. s at 50 rpm. The lowest serum separation value was determined in the yoghurt sample to which dill was added, and the highest value was determined in the sample to which mint was added. A statistically significant decrease was observed in the serum separation values with the increase in storage time ($p < 0.01$). A decrease was observed in the viscosity values of the yoghurt samples in parallel with the increase in storage time. Acetaldehyde contents varied between 14.188 ppm and 17.090 ppm. The lowest acetaldehyde concentration was determined in the yoghurt sample to which mint aroma was added, and the highest value was determined in the sample to which dried mint plant was added. In addition, a decrease was detected in the amount of acetaldehyde in the yoghurt samples throughout the storage periods. It was determined that the antioxidant activities of the yoghurt samples varied between 20.373% and 30.800% DPPH. It was determined that there was a significant ($p < 0.01$) increase in antioxidant activity levels of probiotic yoghurt samples to which dried aromatic plants and their essential oils were added, compared to the control group. Antioxidant activity, which was detected at lower levels on the 1st day of storage, increased significantly ($p < 0.01$) throughout the storage period (7th, 14th, 21th and 28th days). The findings obtained as a result of the study reveal that these yoghurt samples obtained by adding aromatic plants or their essential oils to probiotic yoghurts can be consumed as functional foods.

Keywords: Probiotic, Yoghurt, Serum separation, Antioxidant activity, Acetaldehyde

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Öz

Bu araştırma, yoğurt üretiminde probiyotik kültür (*Lactobacillus casei*) ilavesiyle birlikte çeşitli bitkilerin kurutulmuş formları ve bunların aromalarının kullanılmasıyla elde edilen yoğurtların kalite özelliklerindeki farklılıkların belirlenmesi amacıyla yürütülmüştür. Bu çalışmada, nane, kekik, dereotu ve kişniş bitkileri temin edilerek uygun şartlarda ve koşullarda temizlenerek kurutulmuştur. Elde edilen bitkilerin bir kısmı, yoğurt örneğine %1 oranında kurutulmuş olarak ilave edilmiştir. Geriye kalan kurutulmuş bitki materyalinden elde edilen uçucu yağlar yoğurt örneklerine % 0.05 oranında ilave edilmiştir. Hazırlanan yoğurt örnekleri, fizikokimyasal özellikler kapsamında serum ayrılması, viskozite, asetaldehit içeriği ile antioksidan kapasite açısından analiz edilmiştir. Yoğurt örneklerinde serum ayrılması değerleri % 6.73 ile %7.84 arasında değişmiştir. Viskozite değerleri ise 20 rpm’de 4.198–5.879 Pa.s, 50 rpm’de ise 1.798–2.410 Pa.s aralığında ölçülmüştür. En düşük serum ayrılması değeri, dereotu ilavesi yapılan yoğurt örneğinde, en yüksek değer ise nane ilavesi yapılan örnekte tespit edilmiştir. Muhafaza süresinin artmasıyla birlikte serum ayrılması değerlerinde istatistiksel olarak anlamlı bir azalma gözlenmiştir ($p<0.01$). Muhafaza süresinin artmasına paralel olarak yoğurt örneklerinin viskozite değerlerinde azalma gözlemlenmiştir. Asetaldehit içerikleri ise 14.188 ppm ile 17.090 ppm arasında değişmiştir. En düşük asetaldehit konsantrasyonu nane aroması ilave edilmiş yoğurt örneğinde, en yüksek değer ise kuru nane bitkisi ilavesi yapılan örnekte belirlenmiştir. Ayrıca, muhafaza süresi boyunca yoğurt örneklerindeki asetaldehit miktarında bir azalma tespit edilmiştir. Yoğurt örneklerinin antioksidan aktivitelerinin %20.373 ile %30.800 DPPH arasında değiştiği belirlenmiştir. Kurutulmuş aromatik bitkiler ve bunlara ait esansiyel yağlar ilave edilen probiyotik yoğurt örneklerinin antioksidan aktivite düzeylerinde, kontrol grubuna kıyasla önemli düzeyde ($p<0.01$) bir artış olduğu belirlenmiştir. Depolamanın 1. gününde daha düşük seviyelerde tespit edilen antioksidan aktivite, depolama süresi boyunca (7., 14., 21. ve 28. günler) önemli düzeyde ($p<0.01$) artış göstermiştir. Çalışma sonucunda elde edilen bulgular, probiyotik yoğurtlara aromatik bitkiler veya bunların esansiyel yağlarının ilavesiyle elde edilen bu yoğurt örneklerinin fonksiyonel gıda olarak tüketilebileceğini ortaya koymaktadır.

Anahtar kelimeler: Probiyotik, Yoğurt, Serum ayrılması, Antioksidan aktivite, Asetaldehit

1. Introduction

Among animal-derived food products, dairy items hold significant importance due to their nutritional composition. Milk is considered an essential dietary component globally, including in Türkiye, owing to its consumability, accessibility, and, most critically, its nutritional value derived from its various constituents. Yogurt and other fermented dairy products are notable examples of such foods; they are favored for their high digestibility, their ability to regulate intestinal health, and their role in inhibiting the proliferation of pathogenic microorganisms. Moreover, these products exhibit anticarcinogenic and cholesterol-lowering properties. Additionally, they are particularly beneficial for individuals with lactose intolerance. As a source of animal protein, dairy products also contribute to bone health through an appropriate balance of fats, carbohydrates, and proteins, as well as their elevated calcium content (Akyüz and Coşkun, 1995). Currently, yogurt is recognized as the most prevalent fermented dairy product, enhancing the nutritional value of milk while also extending its shelf life. The final product must comply with the specifications detailed in TS/1330, which governs yogurt processing standards, and it does not include variations such as fruity, flavored, sugary, diet, strained, or probiotic yogurts (Anonymous, 2021). Alternatively, yogurt can be characterized as a distinct dairy product with a unique texture achieved through the fermentation of cooled milk at the appropriate temperature by introducing specific yogurt cultures (Demirci and Şimşek, 1997). Probiotics can refer to selected strains of lactic acid bacteria, such as *Enterococcus*, *Bifidobacterium*, and *Lactobacillus*, which are introduced into the body through fermented dairy products or dietary sources and are noted for their ability to maintain viability within the intestinal environment (Gürsoy and Kımık, 2006).

The production of fermented dairy products utilizing probiotic cultures has experienced significant growth in recent years. This trend is particularly relevant in countries where traditional fermented foods, which serve various functional roles, are widely consumed. The incorporation of probiotic microorganisms is essential in this context. A substantial body of research on fermented dairy products emphasizes that the choice of microorganisms employed during production is a critical factor. Recently, there has been an increased interest in a diverse array of probiotic microorganisms aimed at enhancing the nutritional attributes, dietary applications, and therapeutic benefits of these products. Carbonyl compounds such as acetaldehyde, diacetyl, acetone, and acetoin are integral to the characteristic composition of yogurt. These components are formed as a result of fermentation (Fernandez-Garcia and McGregor, 1994). Furthermore, compounds like 2,3-pentanedione, dimethyl sulfide, and 2,3-butanedione have also been identified as components of yogurt. Research indicates that *Lactobacillus delbrueckii subsp. bulgaricus* is particularly proficient in producing acetaldehyde, which contributes to the distinctive flavor and aroma associated with yogurt (Adhikari et al., 2002). While some studies suggest that *Lactobacillus delbrueckii subsp. bulgaricus* generates more acetaldehyde than *Streptococcus thermophilus*, there is a consensus among researchers that the synergistic interaction between these two microorganisms during yogurt production significantly influences bacterial concentration, acid production, aroma development, and textural characteristics, reflecting the individual activities of each strain (Nongonierma et al., 2007). Lactic acid bacteria (LAB) play a crucial role in the fermentation of lactose present in milk, leading to the production of various fermented dairy products. A significant proportion of these LAB are classified as probiotic microorganisms. Notable species commonly utilized in food production include *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Enterococcus faecium* (Quigley, 2019). In instances where the concentration of acetaldehyde is inadequate, diacetyl emerges as a vital carbonyl compound that contributes to the balance of taste and aroma in yogurt. Both *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* possess the capability to synthesize diacetyl (Beshkova et al., 1998). There exists a divergence of opinion regarding the role of diacetyl; some researchers contend that it predominantly generates aroma when the acetaldehyde levels in yogurt are low (Kaminarides et al., 2007), while others assert its significant contribution to overall aroma development (Beshkova et al., 1998). Additionally, minor aromatic compounds such as propionic acid, propanone, butyric acid, isobutanol, n-butanol, and 2,3-butanediol interact synergistically with acetaldehyde and diacetyl to achieve a balanced aroma profile (Gallardo-Escamilla et al., 2005). Furthermore, starter bacteria produce acetone and butanone-2 during lipid metabolism; however, these compounds have a limited impact on flavor and aroma development and are not consistently generated (Rasic and Kurman, 1978).

In the context of their health benefits, plant-based ingredients are increasingly recognized as primary components in numerous food supplements. Various plants cultivated across different regions globally serve as flavor enhancers or spices (Akgül and Kıvanç, 1989). In Türkiye, various spices are predominantly utilized in culinary applications, particularly in meals and cheeses; however, their consumption rates differ significantly across regions, reflecting local taste preferences. Certain spices are not merely culinary additives but are also recognized in the literature as medicinal aromatic plants. Historically, plants rich in essential oils were employed by early humans to inhibit spoilage of meat and related products, thereby extending their shelf life and masking undesirable odors. In recent years, advancements in technology and food preservation techniques have somewhat overshadowed these traditional benefits. Currently, essential oil-rich plants are primarily utilized to enhance product diversity and flavor (Özel et al., 2015). Moreover, research has identified a range of physiological effects associated with these plants, including antimicrobial, antihypertensive, antioxidative, analgesic, carminative, and sedative properties. The incorporation of nettle extract into yogurt samples resulted in a greater reduction of pathogenic microorganisms (Gülhan and Yangılar, 2024). The composition and concentration of essential oils in these plants can vary due to factors such as species type, climatic conditions, cultivation practices, production methods, and soil fertility (Özcan and Erkmen, 2001). Essential oils are typically extracted through distillation or pressing techniques. Although these oils can crystallize easily, they generally exist in liquid form at ambient temperature. They are characterized as natural products that are predominantly colorless or light yellow and possess a strong aroma (Özdikmenli, 2011).

A review of the existing literature reveals that the primary additive to yogurt is typically fruit. While there have been investigations into the incorporation of spices in certain traditional and regional products, no research has been identified that explores the simultaneous addition of both spices and aromatic compounds to yogurt. The objective of the present study is to enhance the palatability and health benefits of yogurt a food widely recognized for its nutritional value by incorporating spices known for their medicinal and aromatic properties, as well as their antifungal, anticarcinogenic, and antimicrobial effects. This research aims to modify traditional yogurt formulations and assess the health benefits of the newly developed functional food. Additionally, it seeks to evaluate the physicochemical and sensory alterations induced by these modifications in the yogurt product.

2. Materials and Methods

2.1. Material

In the research conducted, mint, thyme, coriander (ashotu) and dill spices and their aromas were added to yogurt. Production was carried out at Atatürk University Pilot Milk Factory. Cow milk taken from Atatürk University Pilot Milk Factory was used to yogurt samples production. Lyophilized YC-350 culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) produced by Chr. Hansen (Peyma-Hansen, Türkiye) are used as starter culture. *Lactobacillus casei* strain provided by Chr. Hansen (Peyma-Hansen, Türkiye) are used as probiotic culture. All cultures were provided by a medical company in Istanbul. The spices used are grown in the provinces of Erzurum and Tokat. The plants were washed several times and the water was removed and subjected to the drying process. The plants, which were dried in a way that would prevent external factors such as insects and dust from entering, were then separated from their stems and obtained as spices with the help of a grinder. The vacuumed packages used in production were obtained from the local market.

2.2. Methods

2.2.1 Extraction of aromatic compounds from spices

Extraction of aromatic compounds from spices was performed using a distillation technique. Initially, the spice samples were dried in an oven at 35°C and subsequently ground to a fine consistency. A total of 40 grams of the ground spice was then placed into a flask, to which 1000 milliliters of water was added. The mixture was heated in a mantle heater, ensuring that the temperature did not exceed 120°C. After a heating duration of three hours, the mixture was allowed to cool for 5 to 10 minutes. The quantity of essential oil that separated and floated on the water's surface was then measured. This oil was carefully extracted using a pump-pipette and a glass syringe, and subsequently transferred into vials for storage at 4°C (Telci et al., 2004).

2.2.2. The production of yogurt samples

In the preliminary phase of the study, a total of 110 liters of raw milk were heated at 85°C for a duration of 20 minutes. Subsequently, the heat-treated milk was transferred to a double-walled cooling tank, where it was cooled to 45°C using cooling water. At this temperature, the prepared yogurt culture (DVC) was added to the milk after being previously melted. However, the number of *L. casei* added was adjusted to 10⁸ pieces/ml. The milk added yoghurt culture and probiotic were divided 9 equal parts of 12 Kg of that to 1st part spices or their aromas was'nt added and used as control. Following this, the specified amounts of 12g spices (1.0%) and 0.6g aromas (0.05%) diluted in 100ml sterile water to milk were added. The amount of herbs and aromas added to the yogurt was determined in preliminary tests in an amount that would not cover the yogurt's own flavour. Then, milks were introduced into sterile yogurt buckets and incubated at 43°C for 3 hours. The yogurt samples was removed from the incubation chamber and subsequently transferred to a cooling cabinet maintained at 4±1°C for storage. The samples were analyzed on the 1th, 7th, 14th, 21th, and 28th days of storage. Spices and aromas adding to yogurt samples were given in Table 1.

Table 1. Spices and flavour sused in the preparation of trial yogurt samples

Samples	Mint Spice (1.0%)	Mint herbal flavour (0.05%)	Oregano Spice (1.0%)	Oregano herbal flavour (0.05%)	Dill Spice (1.0%)	Dill herbal flavour (0.05%)	Coriander spice (1.0%)	Coriander herbal flavour (0.05%)
Control	-	-	-	-	-	-	-	-
Mint spice	+	-	-	-	-	-	-	-
Mint flavour	-	+	-	-	-	-	-	-
Oregano spice	-	-	+	-	-	-	-	-
Oregano flavour	-	-	-	+	-	-	-	-
Dill spice	-	-	-	-	+	-	-	-
Dill flavour	-	-	-	-	-	+	-	-
Coriander spice	-	-	-	-	-	-	+	-
Corianderflavour	-	-	-	-	-	-	-	+

2.2.3. Serum seperation analysis

Filter paper was placed on the funnel placed on the Erlenmeyer and 25 g of yoghurt sample was weighed and filtered for 2 hours at 4±1°C and the filtrate remaining at the bottom was measured in ml (Atamer and Sezgin 1986).

2.2.4. Viscosity determination

Viscosity measurements were conducted utilizing a viscometer at rotational speeds of 20 and 50 revolutions per minute (rpm), with the sample maintained at a temperature of 40°C. The viscosities of the yogurt samples were determined using a digital Brookfield viscometer (Model DV-II) (Brookfield Engineering Laboratories, USA) The viscosity values were recorded in centipoise (cP) as indicated on the device's display. Then, data were calculated as Pa.s. (Abrahamsen and Holman, 1980).

2.2.5. Determination of acetaldehyde

10 g of yogurt sample was added to 30 ml of pure water and placed in a micro kjeldahl apparatus, then 10 ml of distillate was taken. In order to bind the acetaldehyde in the distillate, 1 ml of 0.25 M sodium bisulphite solution was added to the medium, and in order for the reaction between sodium bisulphite and acetaldehyde to be irreversible, the pH value of the resulting mixture was adjusted to 9 with 0.1 N NaOH solution, and the mouth was kept in a closed environment for 15 minutes without light, and 1 ml of 1% starch solution was added on top, and titration was performed with 0.1 N iodine solution. At the end of the titration, 1 g of NaHCO₃ was added and titrated with 0.005 N iodine solution until it turned purple again, and the amount consumed was placed in the necessary places in the formula and the value was found in ppm (Jeong et al.,2015).

$$\text{Acetaldehyde (ppm)} = (44 \times \text{Spent Iodine (ml)} \times N \times 100) / (\text{Sample Amount (g)}) \times 2 \quad (\text{Eq. 1})$$

N: Normality of spent iodine solution

2.2.6. DPPH analysis

Extractions were carried out by solid phase microextraction (SPME) using 50/30µm thick Sulpeco@divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS) fibers. It is based on the elimination of DPPH (2-2-Diphenyl-2-picrylhydrazyl), a free radical, by antioxidant substances in the sample. During the analysis, 50 µl of sample was placed in the test tube and the supernatant was obtained by centrifugation, and 450 µl of tris HCl and 1 ml of DPPH (0.2mm DPPH) solution were added, and the tubes were kept in the dark at 37°C for 30 min. 517 nm absorbance was measured in the spectrophotometer and antioxidant activity was determined with the formula given below (Aqil et al., 2006).

$$\% \text{ Inhibition} = [(Abs \text{ control} - Abs \text{ sample}) / Abs \text{ control}] \times 100 \quad (\text{Eq. 2})$$

2.2.7. Statistical analyses

A completely random trial plan was established and conducted 9 treatments (as 1 control 4 (spice) + 4 (spice flavored)) with 5 different storage times and 2 replications. The results were analyzed with the SPSS 26 package program and Duncan multiple comparison test was applied for the means determined to be significant.

3. Results and Discussion

The results of serum separation, viscosity, acetaldehyde and antioxidant activity analysis were given in Table 2.

Table 2. Results of viscosity, serum separation, antioxidant and acetaldehyde analysis of probiotic yoghurt samples stored for different periods

Treatment	Storage days	Serum separation (ml)	Viscosity 20 rpm (Pa.s)	Viscosity 50 rpm (Pa.s)	Acetaldehyde (ppm)	Antioxidan activity (%)*
Control	1	7.95±0.071	6.056±0.574	2.378±0.056	20.00±1.980	19.50±0.382
	7	6.95±0.071	6.070±0.141	2.307 ±0.033	14.60±1.414	22.93±1.210
	14	6.85±0.919	6.144±0.112	2.357±0.108	17.00±6.845	23.61±0.945
	21	6.45±0.636	6.047±0.036	2.472±0.049	17.02±1.945	28.40±1.242
	28	6.65±0.636	5.610±0.204	2.432±0.043	11.78±2.171	28.78±1.338
Mint spice	1	7.90±0.000	6.177±1.237	2.473±0.045	17.85±0.785	14.05±1.975
	7	7.00±0.141	5.955±0.438	2.310±0.024	12.02±2.708	19.57±0.096
	14	7.40±0.141	5.793±0.157	2.035±0.369	14.46±7.566	20.00±0.149
	21	7.00±0.141	5.733±0.054	1.719 ±0.044	15.84±2.553	26.37±1.624
	28	7.20±0.141	5.300±0.206	1.685±0.015	10.34±1.725	26.82±1.433
Mint flavour	1	6.40±0.990	6.152±0.098	2.480 ±0.040	20.26±0.785	19.28±0.064
	7	7.40±0.707	5.958±0.065	2.373 ±0.061	15.09±2.546	26.49±6.254
	14	6.80±0.707	4.453±0.200	2.405±0.110	16.07±7.488	28.21±7.443
	21	7.15±0.212	3.868±0.141	2.372 ±0.091	16.09±2.192	33.74±8.791
	28	7.65±0.636	3.629±0.140	2.110 ±0.27	9.80±4.158	36.08±11.658
Oregano spice	1	7.45±0.071	5.677±0.376	1.808±0.066	19.77±4.066	23.15±3.249
	7	6.40±0.990	5.118±0.118	1.682±0.038	15.30±1.075	37.21±9.938
	14	7.35±0.354	5.465 ±0.716	1.964±0.496	16.31±8.556	34.30±1.450
	21	6.70±0.849	5.419±0.184	1.544±0.019	18.30±1.987	29.13±2.081
	28	6.75±0.354	4.866±0.161	1.493 ±0.013	13.10±2.729	40.41±0.382
Oregano flavour	1	6.50±0.566	6.144±0.036	2.456 ±0.048	22.23±6.244	9.14±2.940
	7	6.30±1.414	6.111±0.020	2.216±0.110	16.98±1.492	19.16±4.863
	14	6.20±0.424	4.609±0.182	2.313±0.035	16.90±1.010	23.36±2.251
	21	6.30±0.283	3.138±0.014	1.835±0.062	17.01±1.959	37.07±1.784
	28	6.70±0.566	2.821 ±0.710	1.635±0.04	9.34±1.103	37.36±1.624
Dill spice	1	7.50±0.000	5.937±0.288	2.032 ±0.111	19.70±2.913	16.08±0.255
	7	6.70±0.566	5.252±0.105	1.767 ±0.024	13.64±1.273	14.71±1.295
	14	8.00±0.566	5.556±0.487	1.948 ±0.357	14.35±1.105	16.82±1.168
	21	7.30±0.000	5.905±0.135	2.144±0.056	17.02±1.945	19.62±0.159
	28	6.10±0.566	5.582±0.026	1.924±0.056	11.78±2.171	31.06±0.032

Table 2. Continued

Dill flavour	1	6.55±0.636	6.183±0.113	2.487 ±0.021	21.54±4.448	24.98±2.070
	7	5.85±2.051	5.949±0.082	2.411 ±0.118	12.87±3.889	28.57±2.538
	14	6.85±0.495	5.446±0.400	2.374±0.127	14.58±8.514	27.27±4.618
	21	7.25±0.212	4.964±0.141	2.269±0.106	12.63±1.450	29.13±2.081
	28	7.20±0.141	5.000±0.016	2.632 ±0.047	12.23±2.249	40.41±0.382
Coriander spice	1	7.55±0.071	5.882±0.049	2.355±0.051	21.03±1.881	16.08±0.255
	7	7.95±0.071	5.731±0.059	2.179±0.030	15.96±1.315	14.71±1.295
	14	7.85±0.354	5.292±0.436	2.088±0.169	17.89±9.504	16.82±1.168
	21	6.95±0.071	5.521±0.126	2.102±0.285	20.21±2.022	19.62±0.159
	28	7.40±1.273	5.246±0.593	1.924±0.084	14.24±5.657	31.06±0.032
Coriander flavour	1	6.85±0.919	5.714±0.085	2.426±0.016	23.54±2.574	16.64±0.541
	7	6.25±1.344	5.296±0.160	2.007±0.020	16.59±2.899	21.07±2.155
	14	6.45±0.636	5.309±0.303	1.994±0.265	17.64±7.071	24.04±1.295
	21	6.70±0.849	5.635±0.546	2.283±0.290	14.48±0.587	36.01±0.287
	28	6.65±0.636	5.435±0.156	1.985±0.253	12.40±3.026	43.47±0.127

The lowest serum separation values (5.85±2.051ml) of the samples was determined in the yoghurt sample with dill spice addition and for 7 days, while the highest value (8.00±0.566ml) was determined in the yoghurt sample with dill spice addition and stored for 14 days. The viscosity values of the yogurt samples for 20 rpm shear rate were the lowest (2.821±0.710 Pa. s) on the 28st day of the yogurt with oregano flavour, while the highest value (6.114±0.113 Pa.s) was determined on the 1st day of the control yogurt sample. For 50 rpm shear rate, the lowest (1.493±0.013 Pa.s) was determined on the 28th day of the yogurt sample with thyme flavor and probiotics, while the highest value (2.632±0.047 Pa.s) was determined on the 28th day of the yogurt sample with dill flavour and probiotics addition. The lowest acetaldehyde values of the yogurt samples (6.08±1.103 ppm) were determined on the 28th day of the probiotic yogurt sample with dill and coriander addition, while the highest (23.54±2.574 ppm) were determined on the 1st day of storage of the probiotic yogurt sample with coriander addition. The lowest antioxidant activity values of yogurt samples were determined as 6.08±0.255% DPPH on the 1st day of the yogurt sample with dill spice addition, while the highest was determined as 43.47±0.255% DPPH on the 28th day of the yogurt sample with coriander flavour addition (Table 2).

3.1. Serum separation

The analysis of variance conducted on yogurt samples revealed a statistically significant difference between the samples and storage durations, From variance analysis results made on serum separation, the differences of yoghurt samples and storage times were statistically significant ($p < 0.01$). Serum separation values are presented in Table 3.

Table 3. Serum separation averages (ml) of probiotic yogurt samples

Treatments	n	Serum separation*	Storage times (days)	n	Serum separation*
Control	20	7.19 ^{bcd}	1	36	7.52 ^a
Mint spice	20	7.84 ^a	7	36	7.14 ^b
Mint flavour	20	7.26 ^{bc}	14	36	7.12 ^b
Oregano spice	20	6.88 ^{cde}	21	36	7.07 ^b
Oregano flavour	20	7.31 ^{bc}	28	36	7.09 ^b
Dill spice	20	6.73 ^c			
Dill flavour	20	7.31 ^{bc}			
Coriander spice	20	6.82 ^{de}			
Coriander flavour	20	7.39 ^b			

*Means indicated with the same letter are statistically no different.

Table 3 illustrates that the yogurt sample containing dill exhibited the lowest serum separation value, whereas the yogurt sample with mint showed the highest serum separation value. Akbal (2013) found that addition of mint

and thyme flavors to yogurt samples reduced the level of serum separation compared to the control. The found of Akbal (2013) weren't parallel with our founds. The addition of both turmeric and clove extracts significantly reduced the serum separation of the yoghurt samples (Ogunyemi et al., 2021). As different from our founds, Khalil et al (2024) note that adding aqueous mint extract at concentrations less than 4% led to a reduction in the level of serum separation in yoghurt samples. Furthermore, it was observed that serum separation in yogurt samples was significantly ($p < 0.01$) higher on the first day of storage compared to subsequent days. Khalil et al. (2024) found that after 21 days of storage, the serum separation levels decreased from the first day for all treatments as parallel to our founds. Although overall serum separation remained relatively stable from the first to the twenty-eighth day for yogurts supplemented with probiotics, certain samples did exhibit an increase in serum separation over time. In a study conducted by Burak Çınar et al. (2020), it was found that the incorporation of quince powder into probiotic yogurt resulted in a reduction in both hardness and serum separation of the yogurt samples. Demirci and Gürbüz (2023) observed that the added of xanthan gum into yogurt samples resulted in an increase in syneresis. However, they noted that, with the exception of the yogurt sample containing 0.2% xanthan gum, there were no significant differences in syneresis among the various samples at the conclusion of the storage period. The heat treatment applied to mitigate serum separation during the storage period led to a slight increase in dry matter, attributable to alterations in the structure of casein and serum proteins, while serum separation tended to decrease as the storage duration extended. It was determined (Göçer et al., 2016) that there was a decrease in serum separation values as the storage time increased in probiotic yoghurt samples.

3.2. Viscosity values of yogurt samples

The results of the Duncan test concerning viscosity values of yogurt samples at 20 rpm shear rate are presented in *Table 4*.

Table 4. Viscosity values of yogurt samples at 20 rpm shear rate

Treatments	n	Viscosity 20 rpm (Pa.s)*	Storage times (days)	n	Viscosity (Pa.s)*
Control	20	5.350 ^b	1	36	5.751 ^a
Mint spice	20	4.473 ^c	7	36	5.429 ^b
Mint flavour	20	5.879 ^a	14	36	5.234 ^b
Oregano spice	20	4.198 ^c	21	36	4.994 ^c
Oregano flavour	20	5.449 ^b	28	36	4.860 ^c
Dill spice	20	5.657 ^{ab}			
Dill flavour	20	5.554 ^b			
Coriander spice	20	5.347 ^b			
Coriander flavour	20	5.377 ^b			

* Averages indicated with the same letter are not statistically different.

Upon examining *Table 4*, it was found that the oregano-spiced probiotic yogurt sample exhibited the lowest viscosity value at a shear rate of 20 rpm (4.198 Pa. s), while the highest viscosity value (5.879 Pa.s) was found for the mint aroma added yogurt sample. Unlike our study, Khalil et al. (2024) note that from the viscosity results that high concentrations of mint extract had a negative effect. Throughout the storage period, a consistent decrease in viscosity values for the yogurt samples at 20 rpm was observed, with no significant differences noted between days 7 and 14 (as shown in *Table 4*). This finding was parallel with the research conducted by Burak Çınar et al. (2020). It was determined that the viscosity values of probiotic yogurt samples decreased with the increase in the incubation termination pH (Göçer et al., 2016). As the storage time increased, the structure of the yogurt became looser, leading to a significant reduction ($p < 0.01$).in viscosity. Khalil et al. (2024) found that after storage 21 days, there was an increase in the viscosity values for all treatments. The found of their were not parallel with our founds.

The results of the Duncan test concerning viscosity values of yogurt samples at 50 rpm shear rate are presented in *Table 5*.

Table 5. Viscosity values of yogurt samples at 50 rpm shear rate

Treatments	n	Viscosity 50 rpm (Pa.s)*	Storage times (days)	n	Viscosity (Pa.s)*
Control	20	2.215 ^{bc}	1	36	2.332 ^a
Mint spice	20	2.309 ^b	7	36	2.191 ^b
Mint flavour	20	2.169 ^{cd}	14	36	2.177 ^b
Oregano spice	20	2.145 ^{cd}	21	36	2.074 ^c
Oregano flavour	20	1.798 ^e	28	36	1.963 ^d
Dill spice	20	2.410 ^a			
Dill flavour	20	2.076 ^d			
Coriander spice	20	2.084 ^d			
Coriander flavour	20	2.124 ^{cd}			

* Averages indicated with the same letter are not statistically different.

An analysis of *Table 5* reveals that the yogurt sample flavored with oregano had the lowest viscosity value at a shear rate of 50 rpm, while the samples enriched with minth had the highest viscosity at 2.309 Pa.s. But, Akbal (2013) found that the effect of thyme extract addition on the viscosity values of yoghurts was found to be statistically insignificant ($p > 0.05$). Throughout the storage period, a consistent decline in viscosity values for the yogurt samples at 50 rpm was observed, with no significant differences noted between the measurements taken on the 7th and 14th days (*Table 5*). This decrease in viscosity can be attributed to acidity increase during storage periods and consequently weakening of interprotein bonds. These findings align with the research conducted by Burak Çınar et al. (2020). In total, as storage times extended, the structural integrity of the yogurt breaks down and this leads to a significant ($p < 0.01$) reduction in viscosity.

3.3. Acetaldehyde values of yogurt samples

Acetaldehyde, in conjunction with diacetyl, contributes significantly to the characteristic taste and aroma of yogurt. In *S. thermophilus*, serine hydroxymethyltransferase (SHMT) also possesses threonine aldolase (TA) activity, the interconversion of threonine into glycine and acetaldehyde. In this work (Chaves et al., 2002), several wild-type *S. thermophilus* strains were screened for acetaldehyde production in the presence and absence of l-threonine. Supplementation of the growth medium with l-threonine led to an increase in acetaldehyde production. According to Rasic and Kurman (1978) and Tamime and Robinson (1999), a good aroma is provided in yoghurts with acetaldehyde content ranging from 23 to 41 ppm. Görner et al. (1973) stated that a yoghurt with acetaldehyde content ranging from 10 to 20 ppm will have a good aroma. The amounts of acetaldehyde we found were within the values determined by Görner et al. (1973). The findings from the variance analysis indicated that the differences observed across the storage times were statistically significant at the $p < 0.01$ level, as evidenced by the acetaldehyde concentrations in the yogurt samples. This is further supported by the results of the Duncan test presented in *Table 6*.

The results of the Duncan test concerning acetaldehyde values of yogurt samples are presented in *Table 6*. The results of the Duncan test indicated that the yogurt sample flavored with mint exhibited the lowest acetaldehyde concentration, while the highest concentration was also found in the mint-flavored yogurt. However, statistical analysis revealed no significant ($p > 0.05$) differences between the samples. It was observed that the acetaldehyde levels in the yogurt samples varied during all storage periods, with the highest concentration recorded on the first day, followed by a decline during subsequent storage days. In a study investigating yogurt enriched with linseed oil, Mahdian (2007) reported that acetaldehyde concentrations ranged from 20.42 to 49.10 ppm, which were higher than those found in the current study. Conversely, Özer et al. (2021) assessed the acetaldehyde content in ginger-flavored yogurt and noted a maximum concentration of 65.8 ppm in the control group, while the lowest concentration of 11.6 ppm was observed in yogurt samples containing 1.5% ginger juice. These findings differ from those reported data in the present study. Additionally, Atamer and Sezgin (1986) conducted an analysis over a 14 day storage period and found a decrease in acetaldehyde levels, as parallel with of this research results. Similarly, Karagözlü (1997) produced solid and mixed fruit yogurts as well as bio-yogurt and reported a decrease

in acetaldehyde concentrations over a 28 day storage period, aligning with the observations made in this study. In yoghurt samples, the acetaldehyde amounts were found to be 25.9 to 35.5 mg/L (Tian et al., 2020). The findings of Tian et al. (2020) were higher than that of our findings.

Table 6. Acetaldehyde Values of Yoghurt Samples

Treatments	n	Acetaldehyde (ppm) *	Storage times (days)	n	Acetaldehyde (ppm)**
Control	20	15.912 ^a	1	36	19.381 ^a
Mint spice	20	17.090 ^a	7	36	13.808 ^{cd}
Mint flavour	20	14.072 ^a	14	36	15.664 ^{bc}
Oregano spice	20	14.778 ^a	21	36	16.879 ^b
Oregano flavour	20	15.016 ^a	28	36	11.823 ^d
Dill spice	20	15.273 ^a			
Dill flavour	20	14.188 ^a			
Coriander spice	20	16.905 ^a			
Coriander flavour	20	16.368 ^a			

*Averages with the same letter are statistically indistinguishable.

**Averages with different letters are statistically different.

3.4. Antioxidant activities of yoghurt samples

According to the results of variance analysis, the effects of treatment, storage time and treatment x storage time were found to be significant at the $p < 0.01$ level for antioxidant activity in yoghurt samples. The results of the Duncan test concerning of antioxidant activity of yoghurt samples are presented in Table 7.

Table 7. Antioxidant Activity of Yoghurt Samples

Treatments	n	Antioxidant activity (%) *	Storage times (days)	n	Antioxidant activity (%) *
Control	20	20.373 ^d	1	36	16.111 ^c
Mint spice	20	30.800 ^a	7	36	24.998 ^b
Mint flavour	20	26.314 ^{bc}	14	36	23.919 ^b
Oregano spice	20	24.948 ^c	21	36	26.077 ^b
Oregano flavour	20	28.458 ^{ab}	28	36	34.432 ^a
Dill spice	20	29.415 ^{ab}			
Dill flavour	20	20.015 ^d			
Coriander spice	20	24.774 ^c			
Coriander flavour	20	20.867 ^d			

*Averages with the same letter are statistically indistinguishable.

The antioxidant analysis of the yoghurt samples revealed that the lowest antioxidant activity was found in the dill and coriander -flavored yogurt, whereas the highest activity was observed in the mint-spiced added yogurt samples. Statistical comparisons indicated that the control, dill, and coriander-flavored samples did not differ significantly from one another, while thyme and coriander also showed no significant difference. However, there were statistically significant differences among the mint, dill, and coriander flavors. In a related study by Zainoldin and Baba (2009) on probiotic yogurt enriched with dragon fruit, an increase in antioxidant levels was noted in fruit-added probiotic yogurt samples compared to the control group, which was parallel with the findings of the present research. Akan (2022) found that the antioxidant activities of probiotic yoghurts made by adding oat bran and blackberry were higher than plain yoghurt samples as parallel to our findings. The antioxidant activity of the yogurt samples was found to be lower on the first day of storage, with significant differences emerging on the seventh, fourteenth, and twenty-first days. Overall, a trend of increasing antioxidant activity was observed over the storage periods (Table 7). The increase in the amount of polyphenols, phenolics and flavonoids formed during fermentation increases the antioxidant activity (Zhou et al., 2020). Fermentation increases the antioxidant

properties of red cabbage by releasing phenolic compounds bound to cell walls. This finding has shown that the release of bioactive compounds by fermentation may increase an oxidant property (Hunaefi et al., 2013). In the study (Chen et al., 2023), the total phenolic substance level of strawberry juice fermented with *L. plantarum* and *Lactobacillus delbrueckii* subsp. *acidophilus* was found to be higher than that of unfermented strawberry juice, and fermented strawberry juice showed more antioxidant properties. Probably, hydrolyzed proteins during storage times may also act as “sacrificial” antioxidants (Halliwell and Gutteridge, 2015). Proteins and their fractions are sources of bioactive peptides have a positive effect on the human organism (El-Sayed et al., 2021) Milk proteins are an important source of bioactive peptides, which are being increasingly identified in milk protein hydrolyzates and fermented dairy products (Nongonierma et al., 2015). Bioactive peptides are widely used due to their numerous health benefits (antioxidant activity). Their numerous antioxidant effects are the basis of the production of functional foods, nutraceuticals, and drugs of natural origin (Muro et al., 2011). Milk and dairy products are a source of peptides with antioxidant properties. These peptides have the ability to scavenge free radicals, chelate metal ions, and inhibit lipid peroxidation (Marcone et al., 2017). This suggests that the incorporation of spices and flavorings enhances the antioxidant activity in both probiotic and conventional yogurt. In a similar investigation conducted by Özcan et al. (2021), it was found that the addition of beetroot and stevia to probiotic yogurt not only elevated its antioxidant activity but also supported the viability of live microorganisms. Furthermore, Abou El Samh et al. (2013) reported that after a 10-day storage period, the lowest antioxidant values were found in pumpkin-flavored yogurt (42.73%), followed by black carrot (45.35%) and strawberry (46.80%). In contrast, our study indicated that the maximum antioxidant activity observed in the mint-flavored yogurt (30.08%) was lower than that of the spices and flavours evaluated in Abou El Samh et al. (2013)'s research. Mbaeyi-Nwaoha et al. (2024) added different amounts (0, 4, 6 and 8 g) of cinnamon, garlic and ginger to yoghurt samples and made herbal yoghurt. Researches (Mbaeyi-Nwaoha et al., 2024) found that the yoghurt samples added ginger powder had a high increase in DPPH, the DPPH increased but the DPPH of cinnamon adding samples were not increased as much as the ginger added samples.

3.5. Sensory Analyses Results of Probiotic yogurt Samples

Sensory analysis results of probiotic yogurt samples were given in *Table 8* and *Table 9*. According to the appearance results of the samples, the lowest value was determined in the yoghurt sample with oregano spice addition and in the control group yoghurt sample. It was determined that the control group, mint and thyme were statistically different from each other (*Table 8*). The texture score results are also supported by the instrumentally measured viscosity values of yoghurts (Akbal, 2013). The found of Akbal (2013) were parallel with our finds. Panelists gave the the highest taste values the dill flavour, coriander spice and coriander flavour samples. The samples added oregano flavour, It was rated as the yogurt with the lowest taste score by the panelists. The lowest texture values were found in oregano spice yoghurt samples, while the highest values were gave to control and mint flavour added yoghurt samples. Panelists gave the highest serum separation appreciation scores to samples with dill flavoring. However, as a result of the physical analysis (*Table 2*), the lowest serum separation level was detected in the samples containing dill spice (herb). Panelists gave the lowest general acceptability scores to the plain ice cream samples. But, Akbal (2013) found that control samples had the highest general acceptability. It was determined that when the thyme extract rate was increased to 0.3% and above the taste and aroma scores of the yoghurts decreased (Akbal, 2013).

Table 8. Sensory Analyses Results of Probiotic yogurt Samples

Treatments	n	Appearance	Taste	Texture	Serum Separation	General Acceptance
Control	12	7.192 ^a	7.069 ^{ab}	7.430 ^a	6.840 ^{abc}	7.045 ^c
Mint Spice	12	6.792 ^d	6.639 ^{bc}	7.070 ^{ab}	6.743 ^{bc}	7.216 ^a
Mint flavour	12	7.160 ^a	6.535 ^c	7.473 ^a	7.188 ^{ab}	7.372 ^a
Oregano Spice	12	6.056 ^e	6.548 ^c	6.628 ^b	7.098 ^{abc}	7.278 ^a
Oregano flavour	12	7.082 ^{ab}	6.083 ^d	7.198 ^{ab}	6.638 ^c	7.180 ^a
Dill Spice	12	7.103 ^{ab}	6.905 ^{abc}	6.764 ^{ab}	7.208 ^{ab}	7.110 ^b
Dill flavour	12	7.040 ^{abc}	7.195 ^a	6.767 ^{ab}	7.278 ^a	7.278 ^a
Coriander spice	12	6.833 ^{cd}	7.221 ^a	6.812 ^{ab}	6.888 ^{abc}	7.291 ^a
Coriander flavour	12	6.902 ^{bcd}	7.257 ^a	6.805 ^{ab}	7.062 ^{abc}	7.110 ^b

*Means indicated with the same letter are statistically no different.

Table 9. Sensory Analysis Results of Storage Periods

Storage times (Days)	n	Appearance	Taste	Texture	Serum Separation	General Acceptance
1	36	6.539 ^b	7.098 ^a	6.869 ^a	6.909 ^a	7.361 ^a
7	36	6.573 ^b	6.883 ^a	7.003 ^a	6.938 ^a	7.113 ^b
14	36	7.609 ^a	6.502 ^b	7.110 ^a	7.134 ^a	7.090 ^b
21	36	6.319 ^c	6.300 ^c	6.550 ^b	6.560 ^b	6.825 ^c
28	36	6.020 ^d	6.150 ^d	6.325 ^c	6.440 ^c	6.670 ^d

As the storage time increased, appearance, taste, serum separation and general acceptability values decreased, whereas texture value increased (Table 8). It was also determined by Bayram et al. (2024) that the sensory properties of concentrated yoghurt decreased as the storage times increased. The findings of Bayram et al. (2024) were parallel to the results of this research.

4. Conclusions

Upon examination of the analytical results pertaining to yogurt samples infused with probiotics, plant spices, and aromatic substances, it was observed that the extent of serum separation diminished over the storage period. This phenomenon may have compromised the protein stability associated with mint and its aroma, leading to the release of water previously bound by these proteins. In contrast to the control sample, the inclusion of other plant and aromatic additives did not result in an increase in serum separation within the yogurt. A review of viscosity values indicated a general decline in viscosity during the later stages of storage. Over time, the structural integrity of the yogurt deteriorated, resulting in a reduction in viscosity. When evaluating acetaldehyde formation in yogurt, the lowest concentration was found in the sample flavored with mint, while the highest concentration was observed in the yogurt containing mint spice. Notably, there was a decrease in acetaldehyde levels observed until the 28th day of storage. Additionally, an assessment of antioxidant activity revealed that the yogurt sample flavored with dill exhibited the lowest antioxidant capacity, whereas the sample flavored with mint were the highest. Antioxidant activity appeared to increase with prolonged storage. In conclusion, it was determined that the incorporation of herbs and flavors specifically mint, thyme (oregano), dill, and coriander into yogurt did not adversely affect its quality. On the contrary, these additions enhanced the yogurt's antioxidant capacity. Therefore, it can be posited that the combination of herbs and flavors with significantly contributes to the production of functional yogurt. Panelists preferred all samples containing herbs and flavours highly.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Kara, T., Özdemir, S.; Design: Kara, T., Özdemir, S.; Data Collection or Processing: Kara, T., Özdemir, S.; Statistical Analyses: Kara, T.; Literature Search: Kara, T., Özdemir, S.; Writing, Review and Editing: Kara, T., Özdemir, S.

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