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Research Paper / Araştırma Makalesi

Ultrasound-Assisted Extraction of Okra Mucilage: Rheological Properties of its Aqueous Solutions

Tolga Akcan ✉, Şelale Öncü Glaue

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ABSTRACT

Using chemical and physicochemical techniques, we extracted the mucilaginous component of okra (*Abelmoschus esculentus* L.) by the ultrasound-assisted extraction method, then evaluated the resulting polysaccharide extract's rheological properties. Our investigation encompassed examining the flow behavior of polysaccharides extracted under different okra to distilled water ratios (1:10 and 1:30) and various polysaccharide concentrations (1, 2, 3 and 4%, w/v) over a temperature range of 10°C to 80°C. Employing the power law model, we derived parameters and found that okra polysaccharides displayed non-Newtonian pseudoplastic flow characteristics. The flow behavior index ranged from 0.234 to 0.947, with the consistency coefficient ranging from 4.37 to 244.50 mPa.s. Increasing temperature resulted in a decrease in both the consistency coefficient (K) and flow behavior index (n), while concentration elevation led to higher consistency coefficient values. However, the flow behavior index did not exhibit consistent trends with concentration variations. Three statistical parameters; correlation coefficient (R^2), root mean square error (RMSE) and chi-square (χ^2) were used to evaluate the fit of the power law model to the experimental data. Our study further explored temperature's impact on the apparent viscosities of okra polysaccharide samples and modeled the influence of temperature on the consistency index using the Arrhenius equation. Samples with solid-to-solvent ratios of 1:10 and 1:30 showed increasing activation energy with concentration rise, with the highest value recorded at 275.84 kJ/mol for the 1:10 ratio sample with a 4% concentration. In SEM images, okra polymers exhibit irregular, wavy, rough textured surface, and amorphous appearance. These findings hold promise for optimizing ultrasound extraction protocols and enhancing the industrial utilization of mucilages through their rheological properties.

Keywords: Okra mucilage, Rheological properties, Ultrasound, Extraction method

Bamya Müsilajının Ultrason Destekli Ekstraksiyonu: Sulu Çözeltilerinin Reolojik Özellikleri

ÖZ

Kimyasal ve fizikokimyasal teknikler kullanılarak, bamyanın müsilajinöz bileşeni ultrason desteği ile ekstrakte edilmiş, ardından elde edilen polisakkarit ekstraktının reolojik özellikleri değerlendirilmiştir. Araştırmamız, 10°C ile 80°C sıcaklık aralığında farklı bamya-damıtık su oranları (1:10 ve 1:30) ve çeşitli polisakkarit konsantrasyonları (%1, 2, 3 ve 4, w/v) altında ekstrakte edilen polisakkaritlerin akış davranışlarının incelenmesini kapsamaktadır. Güç yasası modelini kullanarak parametreler türetilmiş ve bamya polisakkaritlerinin Newtonyen olmayan psödoplastik akış özellikleri gösterdiğini bulunmuştur. Akış davranış indeksi 0,234 ile 0,947 arasında değişirken, kıvam katsayısı 4,37 ile 244,50 mPa.s arasında değişmiştir. Artan sıcaklık hem kıvam katsayısında (K) hem de akış davranış indeksinde (n) düşüşe neden olurken, konsantrasyon artışı daha yüksek kıvam katsayısı değerlerine yol açmıştır. Ancak, akış davranış indeksi konsantrasyon değişimleri ile tutarlı eğilimler sergilememiştir. Güç yasası modelinin deneysel verilere uyumunu değerlendirmek için üç istatistiksel parametre; korelasyon katsayısı (R^2), kök ortalama kare hatası (RMSE) ve ki-kare (χ^2) kullanılmıştır. Çalışmamızda ayrıca sıcaklığın bamya polisakkarit örneklerinin görünür viskoziteleri üzerindeki etkisi araştırılmış ve Arrhenius denklemi kullanılarak sıcaklığın kıvam indeksi üzerindeki etkisi modellenmiştir. Bamya-damıtık su oranı 1:10 ve 1:30 olan numuneler konsantrasyon artışıyla birlikte artan aktivasyon enerjisi göstermiş, en yüksek değer %4 konsantrasyona sahip 1:10 oranlı numune için 275,84 kJ/mol olarak kaydedilmiştir. SEM görüntülerinde, bamya polimerleri düzensiz, dalgalı, pürüzlü dokulu yüzey ve amorf görünüm sergilemektedir. Bu bulgular, ultrason ekstraksiyon protokollerinin optimize edilmesi ve reolojik özellikleri aracılığıyla müsilajların endüstriyel kullanımının artırılması için umut vaat etmektedir.

Anahtar Kelimeler: Bamya müsilajı, Reolojik özellikler, Ultrason, Ekstraksiyon yöntemleri

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is a widely cultivated plant globally in tropical, subtropical, and temperate regions, including coastal areas bordering the Mediterranean Sea. This widespread cultivation is primarily due to the plant's significant economic and nutritional value [1]. It is often grown extensively in the Aegean part of our country and eaten fresh, frozen, or dried [2]. The viscous and mucilaginous consistency observed in extracts derived from okra is primarily ascribed to its polysaccharide composition, garnering significant attention in both food and non-food sectors for its inherent technological properties. Extensive examination of its various applications has been previously documented in scientific literature [3-5]. Early studies characterized the latter as acidic polysaccharides composed of galactose, rhamnose, and galacturonic acid, with reports indicating partial acetylation of these polysaccharides. Lengsfeld et al. [6], along with Deters et al. [7], identified the sugar components of okra polysaccharides to include rhamnose, galacturonic acid, galactose, glucose, and glucuronic acid. Okra mucilage, derived from the pods of okra plants, is a dense fluid composed of polysaccharides with random coil structures, including the aforementioned sugars [4, 8, 9]. Numerous studies have focused on investigating the rheological behavior of okra polysaccharide extracts in solution to better understand the relationship between their structure and function [10, 11].

Historically, conventional techniques including hydrodistillation, squeezing, cold pressing, maceration, and stirring-based extraction were employed for obtaining mucilage from plant origins. Nevertheless, these approaches were largely dependent on solvents, heat, and extended extraction durations, frequently resulting in the diminishment of antioxidant properties and overall phenolic content due to oxidation, hydrolysis, and ionization processes. Consequently, the exploration of more advanced alternative methods became essential [12]. Nonetheless, in recent years, there has been significant exploration into non-thermal methods as alternatives or supplements to traditional heat-based treatments. One such technique, sonication, utilizes ultrasonic waves for food processing without applying heat. This method relies on piezoelectric materials to convert electrical energy into mechanical energy, generating ultrasonic waves. When these waves pass through a liquid, they create cavitation bubbles due to fluctuating pressure. The collapse of these bubbles along the sound wave's path leads to localized areas of increased temperature and pressure. The energy transferred to the food during ultrasonic treatment can be characterized using terms such as ultrasonic power, intensity, acoustic energy density, or cavitational intensity [4, 13, 14].

The rheological properties of these extracts hold significant importance for prospective industrial utilization. Despite this, there has been a scarcity of research concerning the rheology of such systems when in solution [15, 16].

The significance of rheological properties lies in their crucial role in elucidating heat transfer phenomena and in the design, assessment, and simulation of continuous processes. The measurement of these properties offers valuable insights into the behavior and predictive outcomes related to processing, alterations in formulation, aging effects, and overall quality across diverse product categories[11]. Previous studies on rheology have demonstrated that okra mucilage exhibits characteristics of a structured fluid, displaying properties such as viscoelasticity, shear-thinning behavior, adhesion, stringiness, ductility, and cohesion [17]. In addition, the researchers stated that the extraction protocols used had distinct impacts on the rheological properties of okra mucilage, suggesting the potential for weak gel-like behavior in okra mucilage. Additionally, the viscosity of okra mucilage highlights the intimate connection between shear and extension directions. The extensional viscosity of okra mucilage significantly surpasses its shear viscosity, often by two to three orders of magnitude [9]. The mentioned rheological characteristics attributed to okra polysaccharides underscore their significant technical value for various applications, serving as viscosity enhancers, gelling agents, and thickeners and are widely used in food and medical fields [18].

Hence, the primary objective of the current investigation was to employ ultrasound extraction technology to procure okra polysaccharides under varying solid-to-solvent ratios, aiming to elucidate the solution behavior of these biopolymers across both dilute and concentrated states. These results provide a valuable data base for the potential utilization of okra mucilage obtained by ultrasound technology in various industries.

MATERIALS and METHODS

Materials

The soft and ripe okra of about 5 - 7 cm in length and 3 cm in diameter grown in Tire (Izmir, Türkiye) were obtained from a local market and immediately dried, ground and stored under suitable storage conditions. A solar dryer provided by Tartes Tarim in Izmir, Türkiye, was employed to dry the okra. Subsequently, the dried okra was pulverized into powder with particle sizes ranging from 400 to 500 µm using a hammer mill (Brook Crompton Controls, Wakefield, UK) and stored appropriately. The ethanol (96% ultra-pure) utilized for purifying polysaccharides was sourced from Tekkim Kimya (Bursa, Türkiye).

Methods

Ultrasound-Assisted Extraction Method

For ultrasound-assisted extraction of polysaccharides from okra, the extraction and purification steps used by Öncü Glaue et al. [4], 2023 were performed using Hielscher UP400S, 24 kHz, Germany, equipped with H14 probe (14 mm diameter; 90 mm height). In the extraction process, a power of 200 watts and an extraction time of 5 min, which were determined by preliminary trials, were used. To initiate the extraction procedure, samples were prepared from powdered okra using two distinct ratios of distilled water, namely 1:10 g/mL and 1:30 g/mL.

The extract was centrifuged at 2000× g for 15 minutes to separate the supernatant. The supernatant was combined and filtered through a fine-mesh cheesecloth before being concentrated in an evaporator at 70°C and 100 rpm, reducing its volume to approximately 1/3–1/4 of the original. The concentrated solution containing polysaccharides underwent precipitation and purification by adding six times its volume of ethanol, followed by centrifugation at 5000 g for 10 minutes to remove impurities. Finally, the samples were freeze-dried and stored at -20°C for preservation.

Rheological Measurements

The rheological properties of polysaccharide solutions obtained from ultrasound-assisted extracts with 1:10 and 1:30 okra/distilled water ratios and prepared at different concentrations (1, 2, 3 and 4%, w/v) were determined at different temperatures (10, 20, 40, 60 and 80°C) using a TA DHR3 (TA Instruments Inc., New Cattle, DE, USA) rheometer with concentric cylinder probe (diameter 27.99 mm, length 41.07 mm, measuring cup diameter 30 mm). The experiments were performed in two parallel runs and each run was performed with a new sample.

Apparent viscosity is a function of shear rate and in this study shear calculations (flow curves) were used to determine the flow behavior of polysaccharide solutions. The samples were completely dissolved by means of vortex and ultrasonic bath before measurement and then the temperature of the samples was adjusted to the desired temperatures using a circulating water bath. Shear stress, shear rate and viscosity values were noted for each speed. The experimental data were tested to find the most appropriate flow model based on the shear stress-shear rate data.

Flow Behavior

Shear stress (τ) and shear rate (γ) played important roles on okra polysaccharide extracts viscosity. Shear rate was systematically increased within the range of 0-100 s^{-1}. To characterize the flow behavior of polysaccharide solutions prepared at various concentrations, the two-parameter Power-Law model, extensively utilized in both theoretical analysis and practical engineering computations, was employed for testing purposes (Equation 1.) [19, 20].

$$\tau = K\gamma^{n} \tag{1}$$

where τ is the shear stress (Pa), γ is the shear rate (1/s). The constants K and n are the coefficient of consistency (Pa.s) and the flow behavior index, respectively.

In Equation 2, the logarithm of both sides is taken,

$$log\,\tau = log\,K + n\,log\,\gamma \tag{2}$$

The values of K and n are determined by logarithmic plots of shear stress (τ) versus shear rate (γ) [21].

Apparent Activation Energy

The impact of temperature on the apparent viscosities of okra polysaccharides was determined using an Arrhenius-type equation (3);

$$K = K_0 e^{\frac{-E_a}{R\left(\frac{1}{T_0}-\frac{1}{T}\right)}} \tag{3}$$

In the equation, K_0 represents the consistency factor in units of Pa.s, R stands for the universal gas constant in kJ/mol K, E_a denotes the apparent activation energy measured in kJ/mol, and T represents the polysaccharide temperature in Kelvin (K). For this analysis, a reference temperature (T_0) of 20°C (293.15 K) was assumed.

To linearize Equation 3, we can take the natural logarithm of both sides (Equation 4).

$$ln\,K = lnK_0 - \frac{E_a}{R}.\left(\frac{1}{T_0} - \frac{1}{T}\right) \tag{4}$$

K_0 and E_a values are calculated by plotting $\left(\frac{1}{T_0} - \frac{1}{T}\right)$ against ln K.

Conversely, a correlation coefficient (R^2) was computed to assess the strength of the relationship between the consistency index and activation energy. Correlation coefficient values exceeding 0.8 are typically considered strong. R^2 is valuable as it indicates the proportion of variance in one variable that can be predicted from another variable [22].

SEM Analysis

The surface structure of the prepared beads was examined using scanning electron microscopy (SEM) (JEOL, JSM-5800, Japan). The beads were affixed to a brass stud with double-sided adhesive tape and gold-coated under vacuum using ion sputtering to produce a thin layer of gold (3-5 nm) for 75 seconds. SEM images were captured at an accelerating voltage of 15 kV and a chamber pressure of 1.0 mm Hg to study the morphology. The magnification levels for the samples were set at ×10000 and ×25000.

Statistical Analysis

The fit of the rheological model used to determine the flow behavior with the experimental data was determined using Microsoft Excel (Microsoft Office 365 ProPlus, version 1810). The coefficient of determination (R^2) close to 1 was taken into consideration as an indicator of goodness of fit, while the root mean square error of the mean square (RMSE) and chi-square (χ^2) values were considered to increase the fit. For RMSE, values less than 0.1 represent perfect fit [23]. RMSE and χ^2 values were calculated using Equation 5. and 6. It is possible to detect differences between empirical data and model predictions using the following statistical tools.

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N} \left(M_{predicted,i} - M_{actual,i}\right)^2\right]^{1/2} \quad (5)$$

$$\chi^2 = \frac{\sum_{i=1}^{N} \left(M_{actual,i} - M_{predicted,i}\right)^2}{N-n} \quad (6)$$

Here, $M_{actual,I}$ is the measured value in the i. experimental analysis, $M_{predicted,i}$ is the predicted value in the i. analysis in the model, N is the number of experimental data and n is the amount of coefficients in the model used [2].

RESULTS and DISCUSSION

Rheological Measurements

Rheology provides valuable insights into the type of fluid, solution state, and phase transformation of polysaccharide solutions. Studying the rheological properties of polysaccharides is significant for enhancing their development and utilization in food-related industries [24]. This study examined how solutions of polysaccharides at various concentrations (1, 2, 3 and 4%, w/v) [25] extracted using different ratios of okra to distilled water (1:10 and 1:30), behave under different temperatures (10, 20, 40, 60 and 80°C).

After conducting the extraction process with a solid-to-solvent ratio of 1:10, the apparent viscosity of the samples increased with higher concentrations (1, 2, 3 and 4%) (Figure 1). Higher polysaccharide concentrations can often result in higher viscosity and more pronounced flow properties. This means that the polysaccharide solution shows more resistance when more force is applied. Furthermore, the amount of polysaccharide can also influence other rheological properties such as gel formation. Higher polysaccharide concentrations can generally lead to the formation of a firmer and less fluid gel, while lower concentrations can result in the formation of a looser and more fluid gel. Therefore, the percentage of polysaccharide solution can have a significant influence on the properties studied in rheology studies. However, it was noted that the apparent viscosity decreased as the shear rate increased within each concentration, showing shear-thinning behavior characteristic of a pseudoplastic fluid. All samples exhibited pseudoplastic behavior, maintaining constant viscosity values at high shear rates. The shear behavior of okra mucilage arises due to the intricate composition of its complex mixture, comprising polysaccharides, protein, and mineral components. Additionally, when subjected to shear, each sample of okra mucilage exhibits a plateau region at elevated shear rates. These constraints emerge because escalating shear rates compel the structural constituents to align with the direction of fluid flow[9]. When the shear rate increases, the extended polymer molecules within the substance gradually align themselves in the direction of the flow, leading to a reduction in interactions between neighboring polymer chains. This alignment phenomenon ultimately causes the observed shear-thinning properties as the shear rate rises [26].

In contrast, when the extraction process was conducted with a solid-to-solvent ratio of 1:30, the apparent viscosity declined as the shear rate increased (Figure 1). However, unlike the 1:10 ratio, no significant viscosity differences were observed among the concentrations. The viscosity values were quite similar to each other at the 1:30 ratio. The decrease in viscosity as the shear rate increases can be associated with hydrodynamic forces and the structural breakdown of less stable molecules resulting from an increase in component alignment.

Comprehending the alterations in apparent viscosity with temperature holds significant importance within the food industry, given the diverse range of temperatures involved in numerous heating processes, and the way viscosity responds to temperature can differ depending on the hydrocolloid source. It can be seen from the figures that at all concentrations of 1:10, at constant shear rate, as the temperature increases, the viscosity and therefore the resistance to flow decreases. At increased temperatures, molecular movement intensifies, leading to an increase in intermolecular distance and a weakening of interactions, consequently resulting in a decrease in the apparent viscosity of water-soluble systems. Additionally, various bonding forces like hydrogen, electrostatic, and hydrophobic interactions between molecules may weaken under high temperatures, leading to a similar adverse effect on apparent viscosity [26]. In the graph depicting a 1:10 dilution with a 3% concentration, it's noted that at the outset of the measurement, particularly at low shear stress levels, the viscosity value at 20°C is observed to be lower than that at 10°C. This discrepancy may stem from the fact that the initial data was collected prior to the rheometer system achieving the necessary stabilization time to reach a consistent operational state. Alternatively, variations in environmental conditions could have influenced the precision of the data.

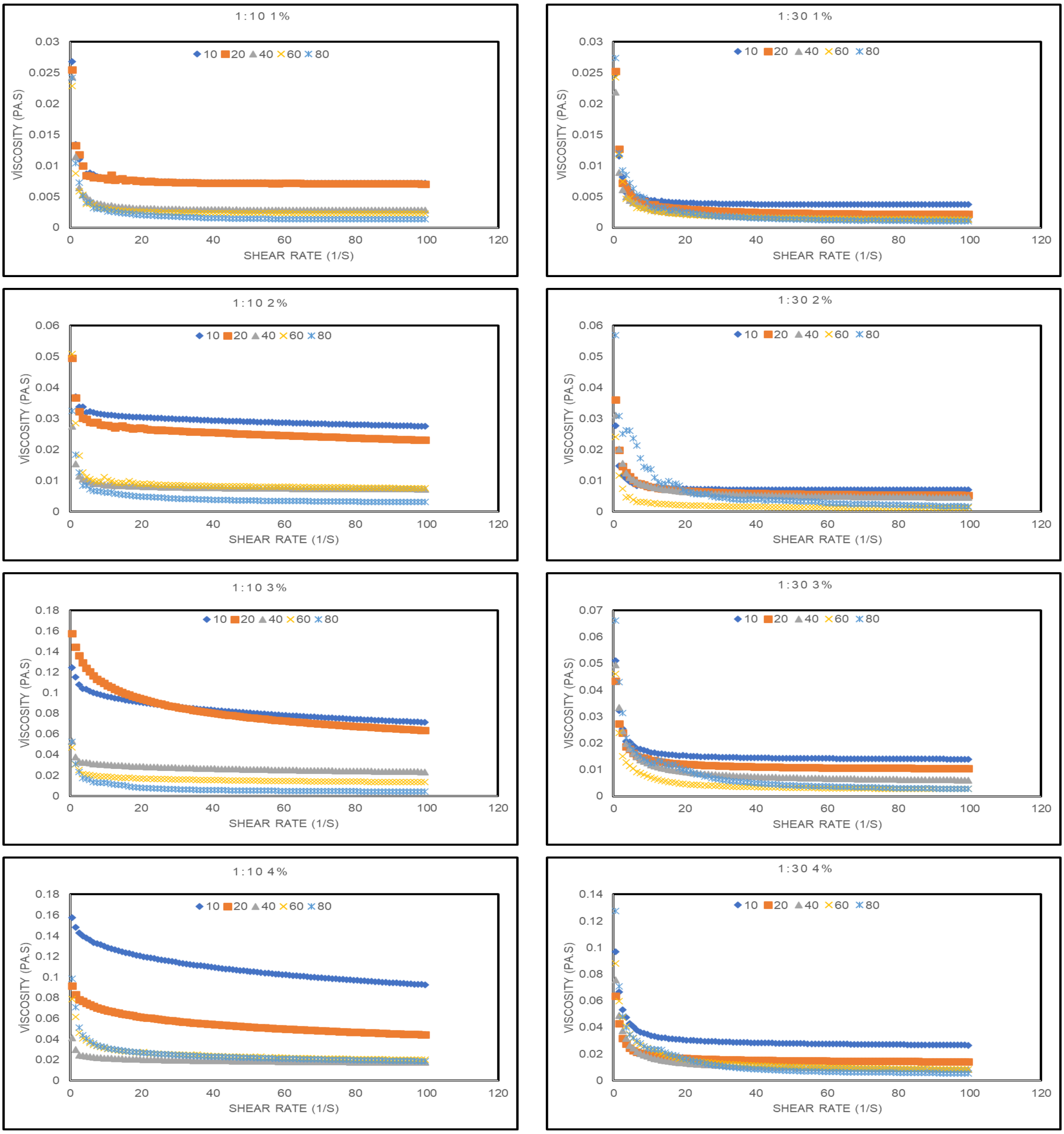

Figure 1. Viscosity - Shear Rate graphs of 1, 2, 3, 4% concentrations of polysaccharides obtained by applying ultrasound treatment at 1:10 and 1:30 okra/distilled water ratio measured at different temperatures.

As a result of the 1:10 solid/solvent ratio extraction process, when the apparent viscosity versus shear rate data of the samples at different temperatures are transferred to the graphs, it is seen that the viscosity increases with increasing concentration at all temperatures at constant shear rate (Figure 2). Our study yielded results similar to those of previous studies on the rheological properties of okra polysaccharides [11, 27-29].

At all concentrations of 1:30, at constant shear rate, viscosity decreases with increasing temperature, but the difference in viscosity between temperatures is very small (Figure 2). Consequently, the viscosity of all samples decreased as temperature rose, indicating their temperature-sensitive viscosity property. This occurrence can be attributed to the heightened thermal motion of molecules at elevated temperatures, which weakens intermolecular interaction forces, resulting in decreased viscosity [24].

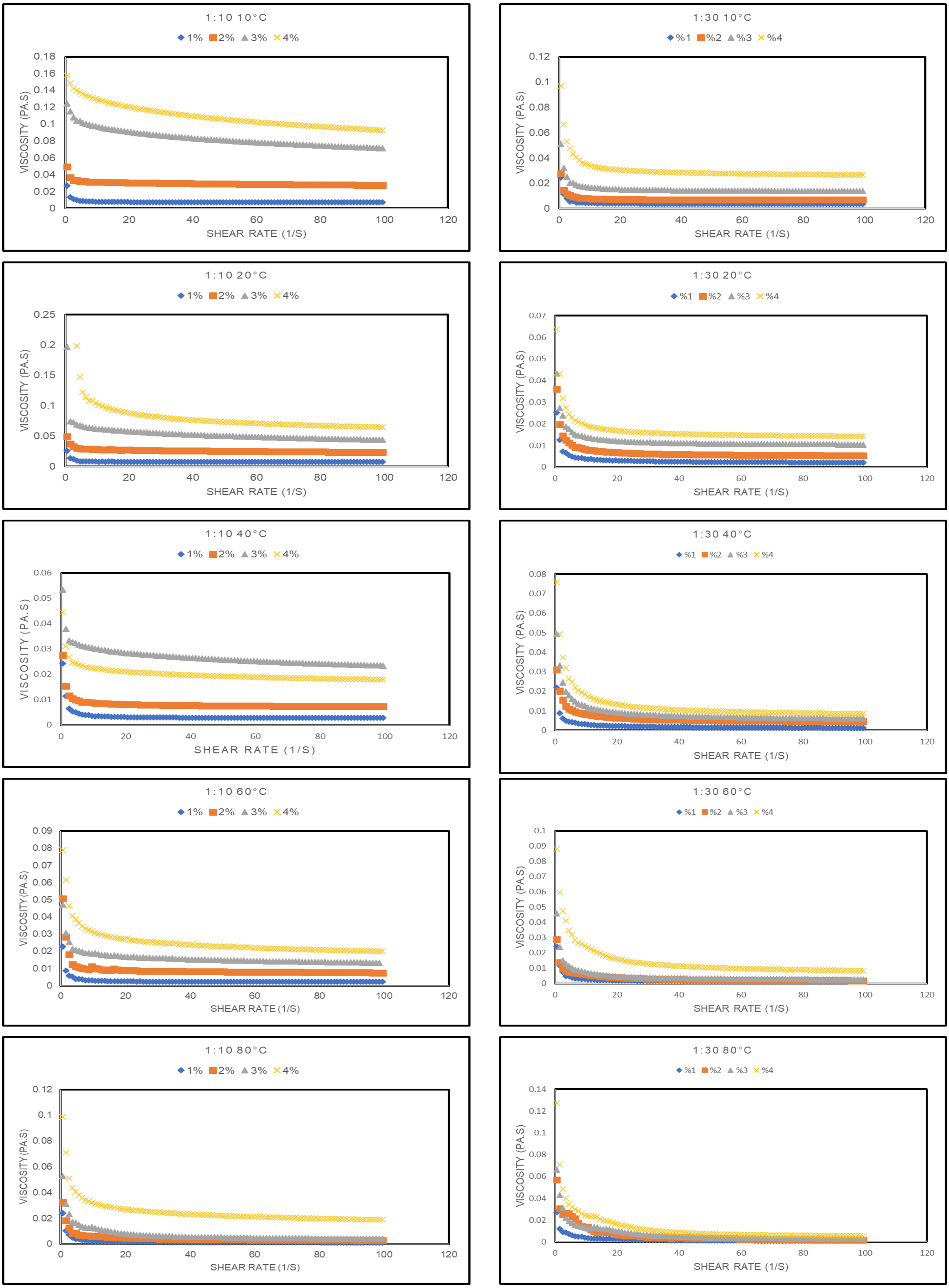

Figure 2. Viscosity - Shear Rate graphs of polysaccharides obtained by applying ultrasound treatment at 1:10 and 1:30 okra/distilled water ratio at different concentrations at 10, 20, 40, 60 and 80°C.

Viscosity - shear rate graphs of polysaccharides obtained from ultrasound assisted extractions using 1:10 and 1:30 okra/distilled water ratios were very different from each other. Polysaccharides play a pivotal role in rheology owing to the substantial volume they occupy when hydrated, compared to the non-hydrated chain's total volume. Intrinsic viscosity assessments hold significant value in biopolymer analysis, providing insights into the volume enclosed by individual polymer molecules [11].

As a result of the 1:30 solid/solvent ratio extraction process, when the apparent viscosity versus shear rate data of the samples at different temperatures were analyzed, the viscosity increased as the concentration increased at all temperatures of 1:30 solid/solvent ratio at constant shear rate (Figure 2). In general, it can be concluded that the viscosity values of all polysaccharide solutions increased as the concentration increased.

Zaharuddin et al. [29] showed in their study that the viscosity of a 1% solution of okra mucilage was greater than that of a lower concentration (0.5% solution) and this proved that viscosity increases with concentration. He said that higher viscosity means more viscosity and denser material with more important cross-linked molecules. This has been shown to facilitate more efficient retention of ingredients within tablets.

The flow behavior of the samples was explained by applying the power law (exponential) model to the shear stress and shear rate experimental data. The experimental data exhibited a strong fit ($R^2 > 0.99$) with the Power Law model. Qasem et al. [30] suggested that the pseudoplasticity observed in solutions containing macromolecules arises from the disentanglement of long-chain molecules, leading to decreased intermolecular resistance to flow during shear conditions. Graphs depicting shear stress versus shear rate curves for ratios of 1:10 and 1:30, across various concentrations and temperatures, are provided in Figure 3 and Figure 4.

As can be seen from the shear stress - shear rate graphs above, the shear stress increased with increasing shear rate and this increase is non-linear. The natural logarithms of the curves were utilized alongside nonlinear regression to ascertain the K and n values of the equation. The outcomes of the nonlinear regression for the conducted measurements are detailed as K and n values and are displayed in Table 1.

Shear stress values show lower values at high temperatures and low concentrations, which is a typical behavior of pseudoplastic (non-Newtonian) fluids [31]. The rheological properties of okra polysaccharide can be described by the coefficient of consistency and the flow behavior index. The consistency coefficient gauges the resistance of the sample to flow, while the flow behavior index delineates the rheological characteristics of a substance. Various materials exhibit distinct flow behavior indices [32]. The samples' flow behavior was analyzed concerning both concentration and temperature.

The flow behavior index, represented by "n," reflects how much the sample's flow deviates from the Newtonian flow, where n = 1. As shown in Table 1, all samples exhibit n values below 1, indicating pseudoplastic behavior. This aligns with previous research findings, suggesting that apparent viscosity decreases with increasing shear rates. Smaller n values reflect greater pseudoplasticity. As evident from the table, the n value decreases with rising temperature, indicating an increase in the product's pseudoplastic properties as temperature increases.

The viscosity coefficient (K) describes the viscosity of the sample. In other words, high K values define high viscosity [26]. It is seen that the K values decrease as the temperature increases at the same concentration and increase as the concentration increases at the same temperatures. At higher temperatures, a reduction in the consistency coefficient (K) was noted, indicating a decrease in viscosity with increasing temperature.

The fit of the power law model to the empirical data was evaluated using three statistical parameters: coefficient of determination (R^2), root mean squared error (RMSE), and chi-square (χ^2). Using these statistical tools, it is possible to detect differences between empirical data and model predictions. In addition to considering the coefficient of determination (R^2) close to 1 as an indicator of fit, it is accepted that the fit increases with lower root mean square error (RMSE) and chi-square (χ^2) values [33]. If the root mean square error is less than 0.1, it represents perfect fit [23]. The closer the chi-square (χ^2) values are to zero, the better the models fit the data. The results obtained are shown in Table 1 and according to these results, the power law model fits the flow curves of okra polysaccharides.

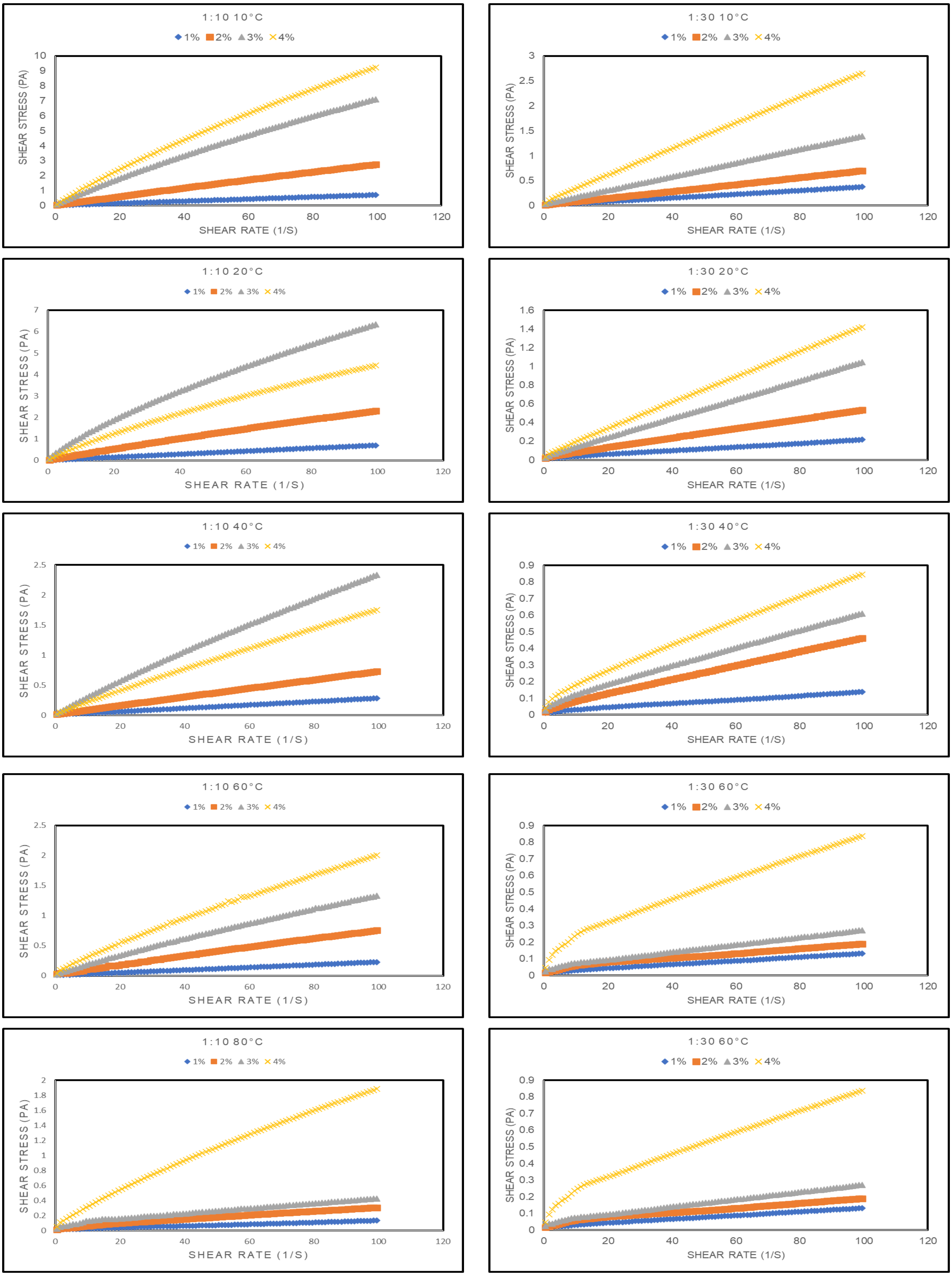

Figure 3. S Shear Stress - Shear Rate graphs of 1, 2, 3 and 4% concentrations of polysaccharides obtained by applying ultrasound treatment at 1:10 and 1:30 okra/distilled water ratio measured at different temperatures.

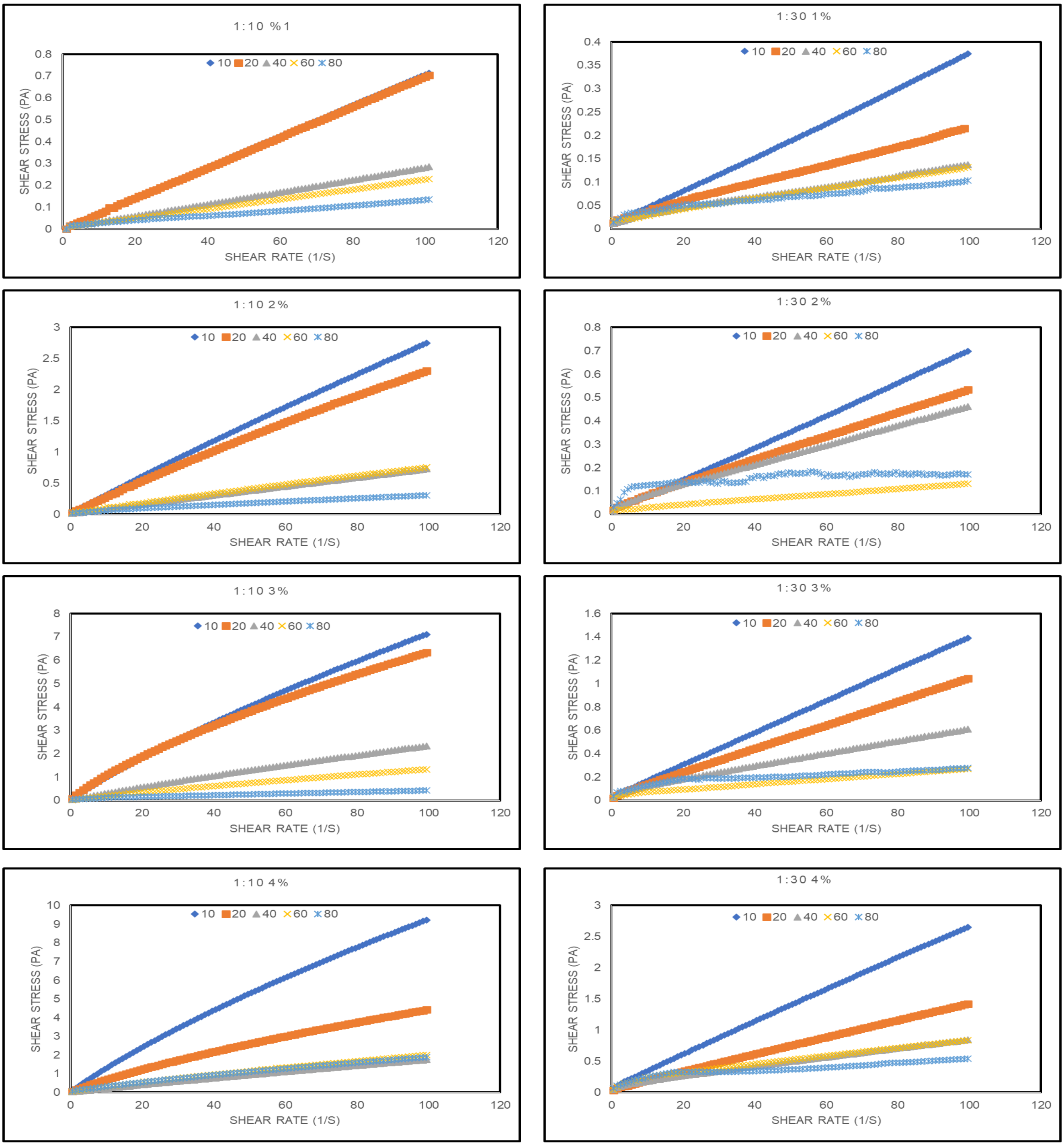

Figure 4. Shear Stress- Shear Rate graphs of polysaccharides obtained by applying ultrasound treatment at 1:10 and 1:30 okra/distilled water ratio at different concentrations 10, 20, 40, 60 and 80°C.

Temperature Dependency (Arrhenius Equation)

The Arrhenius model was employed to characterize how temperature influences the rheological properties. Assessing the temperature effect was vital due to the potential impact of different heating temperatures on food items incorporating gums and starches throughout processing. The impact of temperature on the apparent viscosity of okra polysaccharide samples was examined across a temperature range of 10 – 80°C. Following the measurements, the consistency coefficient was graphed, and the influence of temperature on the consistency index was analyzed using the Arrhenius equation. Activation energies, consistency factors and correlation coefficients are shown in Table 2.

Table 1. Rheological parameters of okra polysaccharides with different solid/solvent ratios at different temperatures and concentrations

Concentration	Temperature (°C)	Solid/Solvent Ratio (g/mL)									
		1/10					1/30				
		K	n	R^2	$\chi2$	RMSE	K	n	R^2	χ^2	RMSE
1%	10	0.009103	0.9418	0.99905	0.0000416	0.0063790	0.00745	0.87655	0.99685	0.0000301	0.005427
	20	0.008565	0.92075	0.99735	0.0000596	0.0075488	0.006951	0.73665	0.9948	0.0000225	0.004671
	40	0.005908	0.8932	0.9964	0.0000228	0.0047241	0.006361	0.63855	0.98935	0.0000180	0.004197
	60	0.005067	0.86685	0.99505	0.0000218	0.004619	0.005572	0.6613	0.9953	0.0000071	0.002617
	80	0.004704	0.67025	0.98515	0.0000251	0.004937	0.004373	0.36455	0.95845	0.0000279	0.005123
2%	10	0.03506	0.94725	0.9999	0.0000795	0.0088209	0.019602	0.92585	0.9982	0.0000967	0.009649
	20	0.030036	0.9277	0.9998	0.0000986	0.0117502	0.013993	0.8173	0.997	0.0000708	0.008323
	40	0.016457	0.9137	0.9995	0.0000200	0.0044217	0.012572	0.73735	0.99455	0.0001056	0.01016
	60	0.015249	0.8645	0.99545	0.0001374	0.010676	0.008936	0.5082	0.982	0.0000351	0.005861
	80	0.012205	0.7258	0.9972	0.0000532	0.006609	0.006512	0.23405	0.88355	0.0001084	0.009986
3%	10	0.1275	0.8815	0.9993	0.0078253	0.0872258	0.032954	0.90805	0.99835	0.0002015	0.013954
	20	0.134985	0.8143	0.9994	0.0018174	0.0421870	0.020143	0.8753	0.99805	0.0002021	0.014056
	40	0.051053	0.8892	0.99965	0.0001209	0.0107004	0.01816	0.6901	0.9938	0.0001664	0.01275
	60	0.036105	0.856	0.9999	0.0018506	0.003717	0.01205	0.54325	0.9758	0.0001005	0.009906
	80	0.03342	0.5363	0.9902	0.0004011	0.019058	0.01044	0.3388	0.9661	0.0002515	0.014775
4%	10	0.2445	0.8523	0.9994	0.0069765	0.0825000	0.095827	0.88285	0.9985	0.0007445	0.026983
	20	0.193125	0.8095	0.9995	0.0013318	0.0354762	0.068926	0.86215	0.9981	0.0003087	0.017384
	40	0.09755	0.9054	1	0.0000040	0.0018884	0.045348	0.66565	0.9938	0.0002706	0.01627
	60	0.064785	0.7387	0.99585	0.0073437	0.008656	0.037323	0.52495	0.99135	0.0006028	0.024275
	80	0.053403	0.7363	0.99855	0.0002170	0.00576	0.026303	0.3679	0.96545	0.0005094	0.022295

The rise in temperature induces alterations in the consistency index, with decreasing values indicating increased resistance to flow. The high R^2 values suggest that the consistency factors of the samples exhibit a good fit to the Arrhenius-type model equation concerning temperature. R^2 values ranging from 0.90 to 1.00 across all samples signify a robust correlation of the Arrhenius model with hydrocolloids in describing the temperature dependencies. A higher activation energy implies a greater impact of temperature on viscosity [22].

An elevation in the activation energy (E_a) of a biological system suggests its reliance on temperature. For both samples with different solid/solute ratios, the activation energy increased with increasing concentration (Table 2). The maximum activation energy value was achieved for 4% concentration (275.8434 kj/mol). A higher E_a value implies the potential for more pronounced changes in viscosity. Therefore, viscosity is more difficult to control at high concentration.

Table 2. The activation energy (E_a) and consistency factor (K_o) values of samples with different solid/solute ratios and concentrations

Solid/Solvent Ratio (g/mL)	Concentration	E_a (kJ/mol)	K_o (Pa.s)	R^2
1/10	%1	123.65	0.0080	0.96
	%2	185.84	0.0280	0.94
	%3	270.30	0.1040	0.91
	%4	275.84	0.1807	0.98
1/30	%1	85.36	0.0070	0.94
	%2	174.18	0.0157	0.96
	%3	183.67	0.0240	0.93
	%4	211.14	0.0723	0.98

Bai et al. [24] demonstrated that the activation energy values for okra polysaccharides ranged from 6.67 to 13.26 kJ/mol. It is thought that the reason for this difference may be the extraction method they applied and the pH values they tested.

Marcotte et al. [34] found that food systems containing xanthan gum exhibited lower activation energy, while systems containing starch and pectin had intermediate values. The increase in activation energy noted with the inclusion of okra extract may be associated with its notable water solubility, as indicated by the extraction procedure.

Scanning Electron Microscope

The macro and microstructures of the samples extracted for 5 minutes with 1:10 and 1:30 okra/distilled water ratios were visualized by scanning electron microscopy at two different magnifications and the irregular, wavy, rough-textured surface and amorphous appearance of okra polymers were shown (Figure 5). SEM images of polysaccharides obtained through ultrasound-assisted extraction at identical durations but varying solid-to-solvent ratios demonstrate noticeable morphological differences. Lower solid-to-solvent ratios result in denser and more compact polysaccharide structures, with fewer visible void spaces or pores. Conversely, higher solid-to-solvent ratios lead to more porous and interconnected polysaccharide structures, accompanied by an increased presence of void spaces or pores evident in the SEM images. In general, both samples consist of flake bundles, but the flakes in the sample with 1:30 okra/distilled water ratio were found to contain larger holes than the other [35].

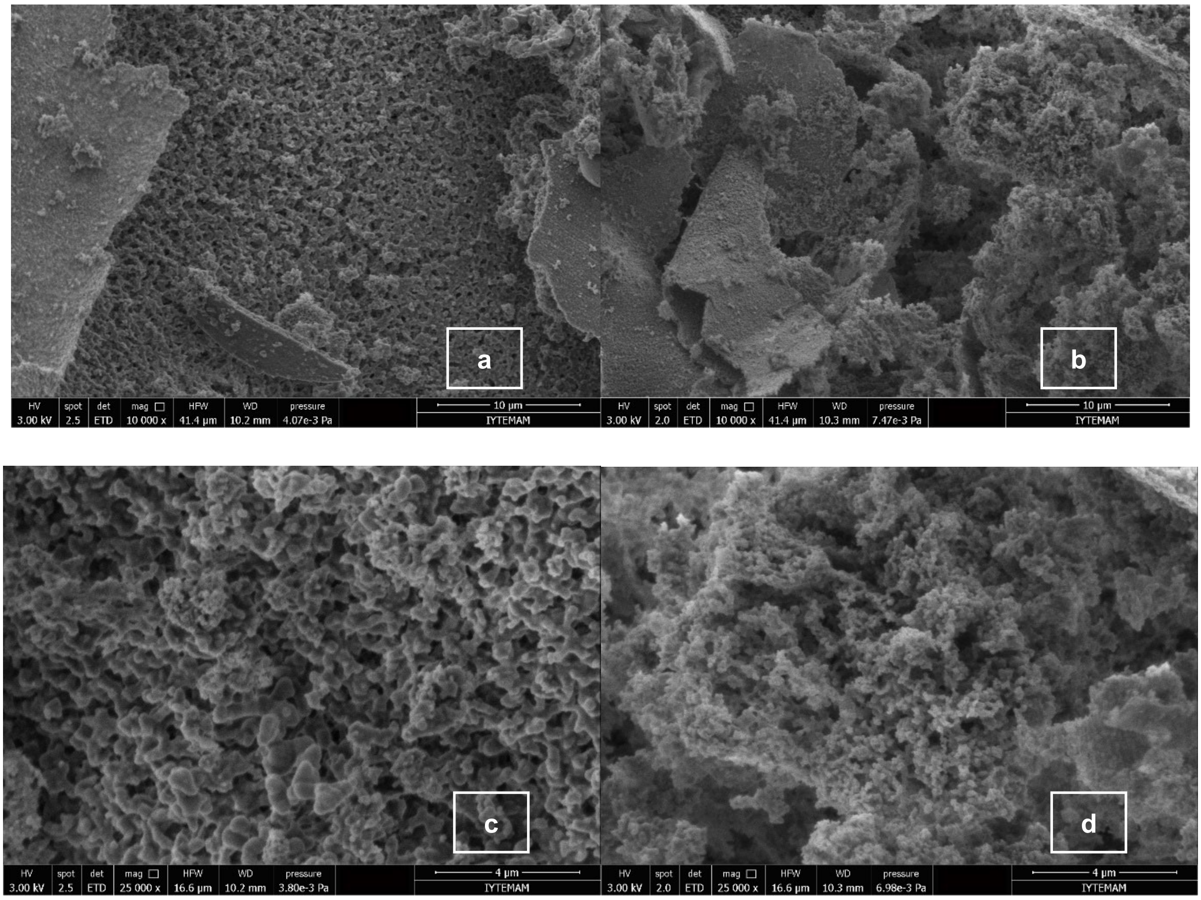

Figure 5. SEM images of polysaccharides obtained as a result of ultrasound treatment at different solid/solvent ratios. a) 1:10 / 10000x magnification, b) 1:30 / 10000x magnification, c) 1:10 / 25000x magnification, d) 1:30 / 25000x magnification

Nagpal et al. [36], characterized the structure of powdered polysaccharides in their study, focusing on the extraction of gum from Abelmoschus esculentus using an ultrasonically assisted method. Their research aimed to explore the physicochemical, functional, and antioxidant properties of the gum for potential applications in food and pharmaceuticals.

The correlation between SEM images and rheology lies in their collective capacity to offer understanding into the structure and characteristics of materials, especially concerning intricate substances such as polymers, gels, and colloidal systems. A substance showcasing a network structure that is more interconnected, as visualized in SEM images, could potentially display elevated viscosity or elasticity owing to heightened resistance against flow or deformation. Conversely, substances characterized by dispersed or irregular structures might flow more readily and showcase reduced viscosity. Large and irregular particles can lead to increased resistance in the flow and increased viscosity, while smaller and regular particles can lead to a lower viscosity flow. Furthermore, structural properties such as porosity also affect the rheological behavior of the material. Higher porosity can increase the fluidity of the material, while lower porosity can result in a higher viscosity flow. The SEM images reveal that the sample containing a 1:10 ratio of okra to distilled water exhibits a larger and irregular network structure, which is more interconnected. This structural characteristic could lead to elevated viscosity as a result of heightened resistance to flow or deformation.

As Zaharuddin et al. [29], also described, the higher the viscosity of a sample, the stickier it is. Higher viscosity leads to denser material with heavier cross-linking of molecules and the structure of okra polysaccharides appears more compact as can be seen in SEM images.

In summary, the outcomes indicate that the proportion of solid to solvent in the ultrasound-assisted extraction technique for isolating polysaccharides impacts the structure of the resulting powdered products, thereby potentially influencing their functional properties.

CONCLUSION

In conclusion, our study delved into the extraction and characterization of mucilaginous polysaccharides from okra utilizing both chemical and physicochemical techniques, with a focus on their rheological behavior. Through a systematic investigation encompassing various ratios of okra to distilled water and polysaccharide concentrations, we elucidated the non-Newtonian pseudoplastic flow characteristics of okra polysaccharides across a temperature range. The derived parameters, including flow behavior index and consistency coefficient, provided insights into the viscosity behavior of the extracted polysaccharides under different conditions.

Furthermore, our analysis revealed the significant influence of temperature and concentration on the rheological properties of okra polysaccharides. The observed trends in flow behavior index and consistency coefficient with temperature and concentration variations were systematically analyzed. Additionally, the fit of the power law model to the experimental data was rigorously evaluated using statistical parameters, further validating our findings.

Moreover, our exploration of temperature's impact on the apparent viscosities of okra polysaccharide samples, along with the modeling of temperature influence using the Arrhenius equation, provided valuable insights into the thermal behavior of these polysaccharides.

SEM imaging allowed us to visualize the structural characteristics of okra polysaccharides, highlighting differences in morphology based on solid-to-solvent ratios. These observations offer valuable guidance for optimizing extraction protocols and enhancing the industrial applications of okra mucilages based on their rheological properties.

In essence, our study contributes to the understanding of okra polysaccharides' rheological behavior and lays the groundwork for further research aimed at optimizing extraction processes and leveraging these polysaccharides in various industrial applications.

As a result, these polysaccharides obtained as a result of the analyses can be widely used in the food industry as a thickener thanks to their sticky and gamy structure, in wastewater treatment systems due to the high adsorption capacity of their wastes, and in pharmaceuticals as emulsifying and suspending agents.

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Research Paper / Araştırma Makalesi

Pumpkin Pulp Flour as a Source of Dietary Fiber: Chemical, Physicochemical and Technological Properties

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ABSTRACT

In this study, the characteristic properties of pumpkin pulp flour (PPF) obtained from the waste of the pumpkin seed production were determined. The pulp parts of the de-seeded and peeled pumpkins were used for this purpose. Proximate composition, total dietary fiber (TDF), insoluble dietary fiber (IDF), soluble dietary fiber (SDF), mineral content, amino acid content, β-carotene content, pH, color, glass transition temperature (T_g), water holding capacity (WHC), oil holding capacity (OHC), swelling capacity (SC), emulsion stability (ES) and emulsion activity (EA) values of PPF were determined. PPF had a low lipid content (0.72±0.06%) and a high ash content (8.34±0.19%). The pH, T_g, TDF, IDF, SDF, WHC, OHC and SC values are 5.61±0.01, 19.19±1.86°C, 26.13±0.17%, 19.82±0%, 6.31%±0.35, 12.91±0.40 g/g, 3.74±0.10 g/g, 12.48±0.57 mL/g, respectively. PPF were a rich source of glutamic acid, glycine, and aspartic acid, and contains high levels of potassium among major elements, and iron among minor elements.

Keywords: Pumpkin pulp flour, Dietary fiber, Glass transition temperature, Amino acid, Water-oil holding capacity

Diyet Lifi Kaynağı Olarak Bal Kabağı Posası Unu: Kimyasal, Fizikokimyasal ve Teknolojik Özellikleri

ÖZ

Araştırmada, çekirdekleri için kullanılan kabağın atıklarından elde edilen kabak posası ununun (PPF) karakteristik özellikleri belirlenmiştir. Çekirdekleri çıkarılmış kabukları soyulmuş olan kabakların posa kısımları bu amaç için kullanılmıştır. Kabak posası ununun kompozisyonu, toplam diyet lifi (TDF), çözünmez diyet lifi (IDF), çözünebilir diyet lifi (SDF), mineral madde içeriği, amino asit içeriği, β-karoten içeriği, pH, renk, camsı geçiş sıcaklığı (T_g), su tutma kapasitesi (WHC), yağ tutma kapasitesi (OHC), şişme kapasitesi (SC), emülsiyon stabilitesi (ES) ve emülsiyon aktivite (EA) değerleri tespit edilmiştir. PPF'nin düşük lipid (%0.72±0.06), yüksek kül (%8.34±0.19) içeriğine sahip olduğu belirlenmiştir. pH, T_g, TDF, IDF, SDF, WHC, OHC ve SC değerleri sırasıyla 5.61±0.01, 19.19±1.86°C, %26.13±0.17, %19.82±0.18, %6.31±0.35, 12.91±0.40 g/g, 3.74±0.10 g/g, 12.48±0.57 mL/g olarak belirlenmiştir. Kabak posası ununun glutamik asit, glisin ve aspartik asit bakımından zengin bir kaynak olduğu ve majör elementlerden potasyum, minör elementlerden ise demiri yüksek seviyede içerdiği tespit edilmiştir.

Anahtar Kelimeler: Kabak posası unu, Diyet lifi, Camsı geçiş sıcaklığı, Amino asit, Su-yağ tutma kapasitesi

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INTRODUCTION

Agricultural by-products attract attention in terms of dietary fiber, sugars, organic acids, pigments, aroma compounds and bioactive components that have antimicrobial-antioxidant- effects, and studies to evaluate these gain importance day by day. Especially as a result of the processing of fruits and vegetables, a large amount of waste consisting of shell, seed and pulp and corresponding to 30-90% of the total weight of the fruit and vegetable depending on the processed fruit and vegetable could be obtained [1]. Considering the functional, technological and nutritional properties of these products, it is important to evaluate them due to economic and environmental issues. One way of keeping agricultural by-products in a stable form and incorporating them into various food formulations as needed is to dry them into powder. Thus, a product rich in functional components such as dietary fiber could be obtained.

There is no single accepted definition for dietary fiber. According to the Codex Alimentarius Commission, dietary fiber is defined as carbohydrate polymers with three or more monomers that cannot be digested or absorbed in the human small intestine [2]. Dietary fibers are very important components for health. Maphosa and Jideani [3] stated that these components have therapeutic effects by preventing heart complications, obesity, diabetes, hemorrhoids and some types of cancer. Soluble dietary fibers have the ability to reduce serum cholesterol levels, reduce glycemic response, and improve glucose tolerance, and insoluble dietary fibers have low density and porous, have the ability to improve normal laxation, increase fecal bulk, and decrease intestinal transit [3]. In addition to these benefits in terms of health, they also improve technological properties such as water-oil binding, increasing viscosity and ion exchange capacity in the used products [4]. Therefore, there is a need to find new dietary fiber sources that could be used in the food industry.

Pumpkins belong to the family Cucurbitaceae and the genus Cucurbita [5]. Cucurbitaceae family has five major genera: *Cucurbiticae Citrullus*, *Cucurbiticae Cucumis*, *Cucurbiticae Lagenaria*, *Cucurbiticae Sechium* and *Cucurbiticae Cucurbita*. There are five species of *Cucurbiticae Cucurbita*, which are economically important, *C. turbaniformis, C. maxima*, *C. moschata*, *C. ficifolia* and *C. pepo* [6]. In Türkiye, the most common pumpkin species grown is *C. pepo* [7]. This species is mostly grown in Nevşehir. 21 526 tons of the 60 970 tons of pumpkin produced as seed pumpkin in Türkiye were grown in Nevşehir [8]. After the seeds are separated from the pumpkin, the remaining shelled flesh part is discarded without being evaluated. This part constitutes approximately 92-95% of the seed pumpkin. Considering the stated situation, it is possible to state that tons of waste will be produced. These wastes are usually left to rot in the harvested areas and cannot be used sufficiently. This situation is both damage the country's economy and causes environmental pollution, and thus researches on the use of waste is of great importance both environmentally and economically. The aim of this research, the planned considering the stated situations, is to determine the various physicochemical and technological properties of PPF obtained from pumpkin (*Cucurbita pepo* L.) pulp and to reveal whether it can be used as a potential dietary fiber source.

MATERIALS and METHODS

Materials

Fresh whole pumpkins (*Cucurbita pepo* L.) were obtained from a local vegetable farm after harvest, Nevşehir, Türkiye. After they were washed, peeled and seeded, their pulp parts were cut into 0.5-cm^3-thick cubes. The samples were frozen at -80ºC, and then freeze drying was carried out in the freeze drier (Operon FDU-8612, South Korea). Dried samples were crushed into a fine powder and powdered samples were sieved through a 100mm sieve. Powdered samples were vacuum packaged (KV-600, Novivac, Türkiye) and stored at 18ºC until usage.

Composition of Pumpkin Pulp Flour

Analysis of moisture, ash, protein, and lipid contents in PPF were determined by AOAC official methods 925.10, 920.153, 928.08, and 991.36, respectively [9]. The conversion factor of nitrogen to crude protein was 6.25.

The contents of TDF and IDF were determined by the enzymatic-gravimetric method 991.43 [9]. SDF was calculated by subtracting of the IDF from TDF. Analyzes were performed in triplicate.

Starch content (w/w) was determined using a commercially available starch quantification kit (R-Biopharm AG, Darmstadt, Germany).

Extraction and saponification of carotenoids were performed according to Koning and Roomans [10]. The resulting residue was dissolved in a methanol/ tetrahydrofuran mixture (75:25, v / v) using an ultrasonic shaker. Aliquot portions (20 µL) were injected into the Zorbax ODS column (5µm particle size, 4.6 mm id) of the reversed-phase LC system (LC20, Shimadzu, Tokyo, Japan) equipped with a UV detector. The elution mixture was methanol and tetrahydrofuran 95:5 (v/v) with a flow rate of 0.8 ml/min.

Separation and analysis of amino acids were carried out in a high-performance liquid chromatography (Series 1100, Agilent, California, USA) equipped with a fluorescence detector. The chromatographic column was a 5 µm, 4.6 x 150 mm, cartridge, Hypersil BDS-C18 (Agilent, California, USA). The mobile phase used was a mixture of 40 mM Na_2HPO_4, pH 7.8 (A) and methanol (B) with a linear gradient starting in a 50:50 (v/v) and ending, after 10 min, in a 10: 90 (v/v) mixture of A and B.

Analysis of minerals was carried out by a Scott Spray chamber (Norwalk, CT, USA) ICP-MS (PerkinElmer

ELAN DRC-e model) after the microwave digestion process.

Physicochemical Properties of Pumpkin Pulp Flour

For analysis, 0.5 g of PPF in triplicated were homogenized with 50 mL of distilled water for 30 seconds with Ultra-Turrax (MTOPS SR 30, Republic of Korea), and pH values were measured by using a pH meter (Titroline 5000, SI Analytics, Germany) calibrated in buffer solutions at pH: 4 and pH: 7.

Color values of PPF were measured using a Minolta colorimeter CR-400 (Konica Minolta Corp. Japan), with an 8 mm aperture, a 10° observer angle and a D65 illuminant. L*(lightness), a* (redness) and b* (yellowness) were analyzed according to the system of the International Commission of Illumination (CIE LAB System).

X-ray analysis of PPF was taken using an X-ray diffractometer (Rigaku, Miniflex 600, Tokyo, Japan) with Cu Kα radiation, 1.54–1.56 °A. The radiation intensities of the PPF samples were measured in the range of 3°–100° of 2θ diffraction angle, with a scan step size of 0.02° with a scanning speed of 5°/min. The determination was conducted at room temperature. The degree of crystallinity (%) was calculated as shown in equation (1) according to Stevenson et al. [11].

$$\text{Crystallinity (\%)} = \left(\frac{A_c}{A_c + A_a}\right) x100 \qquad (1)$$

where A_c is the crystalline area on the X-ray diffractogram and A_a is the amorphous area on the X-ray diffractogram.

The T_g of PPF was determined using a DSC (DSC 6000, Perkin Elmer, USA). The DSC was calibrated with indium and water for temperature and heat flow calibration (melting point: 156.6°C, ΔH= 28.47 J/g for indium, melting point: 0°C, ΔH= 333.20 J/g for water). To detect the T_g of PPF samples approximately 10 mg were weighed into the hermetic DSC pans (product number 03190029, Perkin Elmer, USA). The samples were cooled from 20°C to -60°C at a 5°C/min cooling rate, held at this temperature for 30 min, and then heated from -60°C to 200°C at 5ºC/min heating rate in a nitrogen atmosphere (flow rate 30 mL/min). An empty pan was used as a reference. The DSC measurements were done in triplicate. The onset, midpoint and end point values of the glass transition region were determined, and the midpoint value reported as the T_g.

Pasting properties of PPF were determined by using a Rapid Visco Analyser (RVA 4500, Perten, Sweden). The flour sample (3.0 g, dry basis) was weighted into the RVA vessels and dispersed in 25.5 g of distilled water. The suspension was stirred in RVA at 960 rpm for 10 seconds and stabilized for 1 minute at 50°C, and then heated to 95°C in 4 minutes and held for 2.5 minutes at 95°C. Finally, the suspension was cooled from 95°C to 50°C in 4 minutes and held for 2 minutes at 50°C. RVA analysis was carried out in triplicate under constant stirring at 160 rpm. The RVA parameters (pasting temperature, peak, trough, breakdown, final, and setback viscosity) were obtained from the RVA viscogram.

Techno-Functional Properties

The WHC and OHC of PPF were measured as described by Lopez-Marcos et al. [12]. The WHC and OHC were expressed as the weight of water and oil held by 1 g PPF sample.

The SC of PPF was determined by the method of Gomez-Ordonez et al. [13], and expressed as mL/g of corresponding PPF sample.

The EA and ES of the PPF were determined by the method of Chau and Huang [14]. The EA as a percentage was calculated from the ratio of the depth of the emulsified layer to the depth of the total volume of content inside the centrifuge tube. The ES was calculated in the same way as EA and also expressed as percentage of the unheated control.

WAI and WSI of PPF were determined by the method of Anderson et al. [15]. WAI and WSI were calculated according to equations 2 and 3.

$$\mathrm{WAI} = \frac{\text{pellet weight (g)}}{\text{dry weight of original sample (g)}} \qquad (2)$$

$$\mathrm{WSI} = \frac{\text{weight after drying (g)}}{\text{dry weight of original sample (g)}} \qquad (3)$$

Data Analysis

All the analyses were performed in triplicate. Results were presented as mean ± standard deviation (SD).

RESULTS and DISCUSSION

Composition of Pumpkin Pulp Flour

The compositions of PPF are shown in Table 1. The results showed that PPF have low contents of fat. Such low lipid content could provide and opportunity in its potential application as ingredient in foods. The PPF had a high ash content. The use of a product with such a high ash content may pose a problem for potential applications in foods due to the possibility of increasing oxidation due to the many metal ions it contains. As a result of a study conducted by Ahmed et al. [16] using pumpkin (*Cucurbita moschata*), ash, lipid and protein contents were determined as 5.6%, 1.04%, and 9.1%, respectively. In another study performed on the peeled and unpeeled pumpkins (*Cucurbita moschata*), these values were determined as 7.39%, 0.70%, 4.91% and 7.32%, 1.08%, 5.43%, respectively. Nakhon et al. [17] determined the ash, lipid and protein contents in pumpkin (*Cucurbita moschata*) flour as 5.91%, 6.74%, and 10.88%, respectively. The differences in results may be attributed to the used cultivar and the geographical location.

Table 1. Composition of pumpkin pulp flour

Composition	Value
Moisture (%)	13.58±0.21
Protein (%)	7.89±0.18
Fat (%)	0.72±0.06
Ash (%)	8.34±0.19
SDF (%)	6.31±0.35
IDF (%)	19.82±0.18
TDF (%)	26.13±0.17
Starch (%)	1.52±0.03
β-carotene (µg/100g)	903.50±14.50

±: Standard deviation of three replicate. SDF: Soluble dietary fiber, IDF: Insoluble dietary fiber, TDF: Total dietary fiber

TDF, SDF and IDF contents of PPF are shown in Table 1. As a result of the research conducted by Kalala et al. [18], the TDF, IDF and SDF contents were determined as 27.7, 19.4, 8.3 in *Cucurbita pepo*, and 23.8, 13.9, 9.9 in *Cucurbita maxima duchene* spp, respectively. The values obtained as a result of this research are similar to the values obtained from *Cucurbita pepo*. As a result of studies conducted with fruit and vegetable by-products, it has been revealed that these products are rich sources of dietary fiber. TDF contents of the olive, papaya, blueberry and pineapple byproducts powders were found to be 53.68, 32.23, 47.51 and 45.23g/100 g dry matter, respectively [1]. The amount of TDF obtained from fruit-derived by-products such as pear, grapefruit, mango and peach was determined as 36.1, 44.2, 28.05 and 35.8g/100g dry matter. The TDF content in PPF is close to that in mango. Depending on the values obtained, the ratio of IDF to SDF was 3.14. Dietary fiber has an important place in the human diet. The recommended dietary fiber intake for adults is 25 to 30g/day based on epidemiological and clinical data and the IDF/SDF ratio should be 3:1 [19]. However, the average dietary fiber intake is about half the recommended value in many countries [20]. For example, it is around 15g/day in the USA, 16g /day in Spain. IDF and SDF have different properties and therefore have different physiological effects. IDF is responsible for intestinal regulation, increasing fecal bulk, and water absorption. Water absorption is one of the most important properties, as it provides laxative effects and improves peristalsis. SDF, on the other hand, has prebiotic potential and plays an active role in the reduction of cholesterol levels and decreasing the amount of glucose absorbed in the small intestine [3].

β-carotene content of PPF is shown in Table 1. Obtained results indicated that PPF is a good source of carotene which is the precursor of vitamin A. The β-carotene content of yellow pumpkin is reported to be 1180 µg/100 g [27]. Carotenoids could be inhibited lipid peroxidation by reacting with any radical species presumably to be encountered in the biological system and reducing the cellular release of lactate dehydrogenase [21].

Amino acid contents of PPF are shown in Figure 1. Among the essential amino acids, mostly arginine, lysine and leucine amino acids were detected. From the non-essential amino acid group, the most asparagine, glycine and glutamine amino acids were determined. As a result of a research conducted on pumpkin by-products, the highest amino acid contents were determined in the peels of *C. maxima* species and in the seeds of *C. pepo* species [22]. Fernandez-Segovia et al. [23] stated that the asparagine and glutamine contents in seaweeds were high, and these amino acids were associated with the umami flavour. Therefore, the presence of these amino acids in PPF would be play an important role in the used as flavour enhancer.

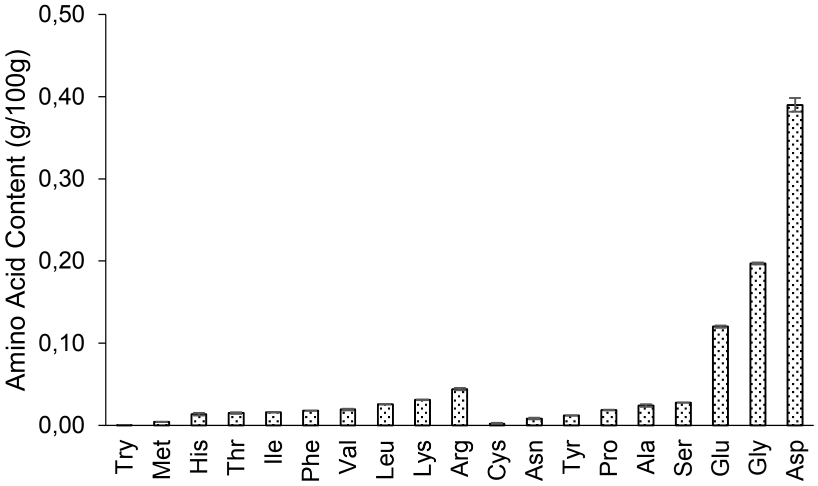

Figure 1. Amino acid content of pumpkin pulp flour

The mineral contents of PPF are shown in Figure 2a and Figure 2b. Among the main mineral substances that are essential for human beings, potassium was found to be at the highest level, followed by calcium, sulfur, phosphorus and magnesium, respectively (Figure 2a). Main and trace elements have many functional roles such as electrolyte, enzyme components, building materials in bones and teeth. Sodium, plays a role in maintaining of the osmotic pressure of the extracellular fluid. Potassium plays a functional role in the regulation of osmotic pressure within the cell, in the activation of various respiratory and glycolytic enzymes. Magnesium is present in the structure of enzymes that are particularly involved in the conversion of energy-rich phosphate compounds and plays a role in the activation of these enzymes. It also plays an important role as the stabilizer of nucleic acids, intracellular and plasma membranes. Calcium is an essential ingredient as it is involved in the structure of the muscular system and controls essential processes such as muscle contraction. Loughrill et al. [24] reported that the calcium/phosphorus ratio (Ca:P) is important for bone growth and development in infancy, and this ratio should be between 1:1 and 2:1. As a result of this research carried out on PPF, this ratio was found 2:1.

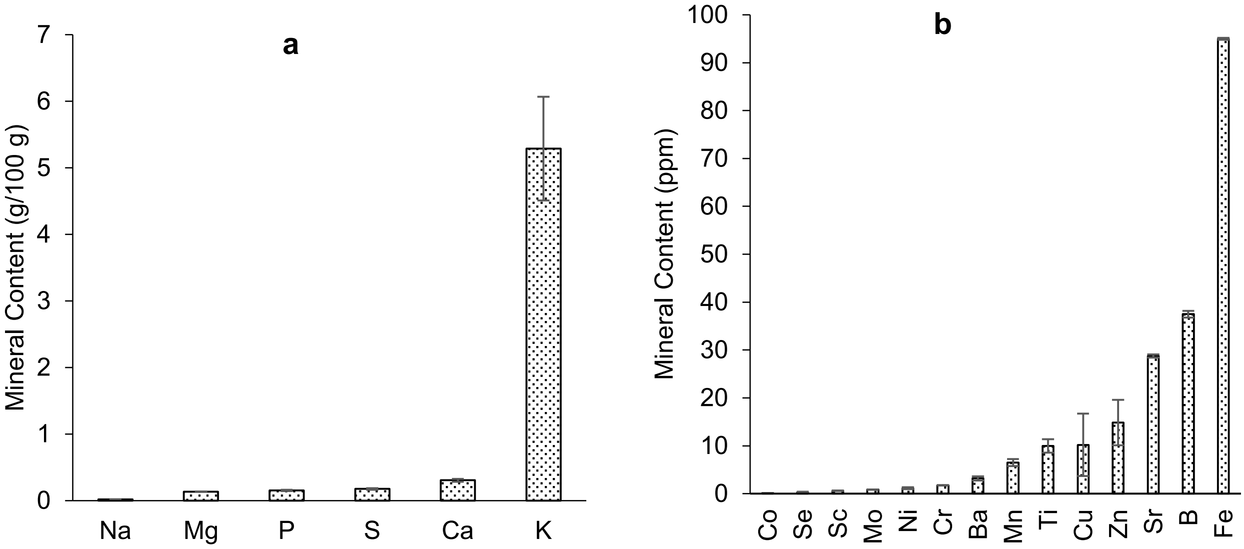

Figure 2. The mineral contents of pumpkin pulp flour. a: Main minerals, b: Trace elements.

Among the trace elements, the highest level of iron was detected in PPF, followed by B, Sr, Zn, Cu, Ti, and Mn, respectively (Figure 2b). Since high iron content causes lipid oxidation, it is not a desirable element in food processing. Copper element plays a role in the functionality of many oxido-reductase enzymes. However, since it causes many problems during the processing and storage of foods, copper is not desired to be present in the high levels such as iron. As a result of a study carried out by our research group, an increase in the thiobarbituric acid reactive substances values of bologna type sausages produced using PPF was determined, and it was concluded that the minerals contained in PPF played an active role in this increase [25]. Another important element determined in PPF is zinc. Zinc is an essential micronutrient element for human health, and present in many enzymes (glutamate dehydrogenase, lactate dehydrogenase, alcohol dehydrogenase, malate dehydrogenase, carboxypeptidase, dipeptidase, alkaline phosphatese, lecithinase and enolase) and acts as a cofactor. Wessels and Brown [26] reported that approximately 17.3% of the world's population was faced with the risk of Zn deficiency. Another important result of this study is that PPF contains selenium even if it is not very high. Similar to Zn, selenium is an important micronutrient element. Kubachka et al. [27] emphasized that Se is not only vital for the development and functions of organs but also has antioxidant properties.

Physicochemical Properties

pH value of PPF is shown in Table 2. The pH of dietary fiber is one of the most important characteristics of fiber. Because the pH value indicates which dietary fiber can be added to product without changing the technological properties of the product. For example, dietary fibers with acidic pH can be used in acidic products such as yogurt, and dietary fibers with basic or near neutral pH values can be used in emulsified meat products. As a result of a research carried out by our research group, it was determined that there was a slight decrease in both dough pH and final product pH [25].

Color is one of the most important quality criteria that affect the attractiveness of consumers in foods. Color values of PPF are shown in Table 2. As can be seen in Table 2, a high L value was determined for PPF. The L* value close to 100 indicates that the lightness of the sample is high. Obtained negative a* value represents the greenness, while the +b* value represents yellowness. This situation may be attributed to the carotene content of PPF. A similar situation was found in the result of a study carried out on peeled and unpeeled pumpkin flour [28].

Table 2. Physicochemical properties of pumpkin pulp flour

Physicochemical properties	Value
pH	5.61±0.01
L*	85.23±0.39
a*	-1.43±0.07
b*	21.57±0.43
T_g (°C)	19.19±1.86
Pasting temperature (°C)	84.00±0.01
Peak viscosity (RVU)	210.00±0.05
Trough viscosity (RVU)	109.20±1.13
Breakdown viscosity (RVU)	103.30±2.40
Final viscosity (RVU)	324.48±0.74
Setback viscosity (RVU)	217.78±3.15

±: Standard deviation of three replicate

The X-ray pattern of the PPF is shown in Figure 3. The PPF exhibited a peak at diffraction angle 2θ=21.96°. The resulting diffractogram showed the presence of a substantially amorphous structure (97.10%), and the degree of crystallization was 2.9%. This result is consistent with the low starch content (1.5%, dry basis) determined in PPF. Nakhon et al. [17] reported that the pumpkin (*Cucurbita moschata* L.) flour had a low crystallinity degree (8.63%) due to non-starch components (ash, fiber, protein and fat) in its structure and low starch concentration (25%, dry basis). Similarly, in the present study, the very low starch content (1.5%, dry basis) of PPF samples can be cited as the reason why the crystallinity was too small to calculate.

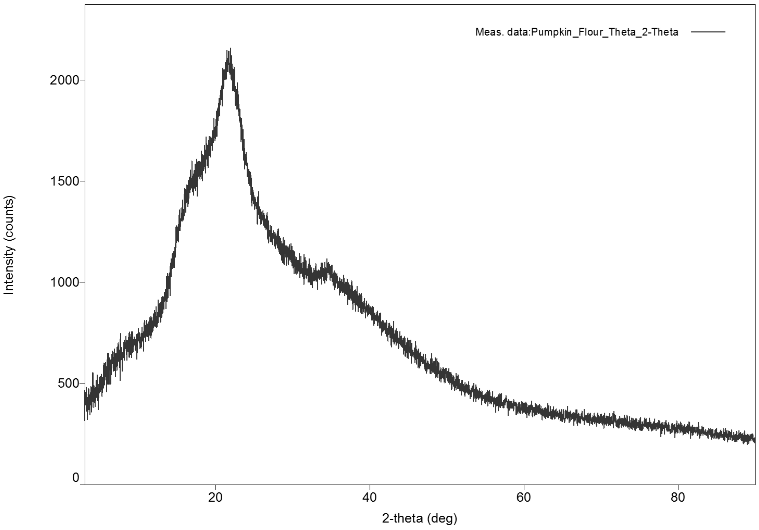

Figure 3. X-ray pattern of pumpkin pulp flour

T_g is a reference temperature that provides information about the storage temperature. It is stated that any food remains stable if stored at a temperature lower than the T_g [29]. The T_g occurs over a wide range of temperatures. Therefore, the onset, midpoint and endset temperatures of the glassy change are given, and the midpoint is generally expressed as the T_g. As can be seen in Table 2, the T_g was determined as 19.19°C. This situation shows that PPF should be stored at a temperature lower than this temperature. As a result of a study conducted on pumpkin powders (*Cucurbita moschata*) obtained by grinding in different sizes after freeze-drying, it was found that the T_g increased as the particle size decreased [16]. The researchers reported that the mid-point values for T_g ranged from 6.80°C to 12.97°C. As a result of DSC analysis performed between 20°C and 120°C in dispersions prepared by adding water to PPF, no endothermic change was detected. This might be attributed to the low level of starch and lipid content.

Pasting parameters determined by RVA of PPF are shown in Table 2. The pasting temperature, peak, trough, breakdown, final, and setback viscosity values of the PPF were higher than those of the values obtained from the flour of *Cucurbita moschata* and starch isolated from C*ucurbita maxima* [11, 17]. This situation may be related to the high protein, fiber, and fat contents and low starch concentration in PPF. Nakhon et al. [17] stated that the protein and lipid fractions in pumpkin flour interacted with amylose and it caused an increase in pasting temperature and a decrease in the viscosity values of pumpkin flour.

Techno-Functional Properties

WHC of PPF is shown in Table 3. WHC is defined as the quantity of water retained by the hydrated fiber following the subjected to an external force such as centrifugation or pressure. WHC of dietary fibers is correlated to the chemical structure, porosity, particle size, pH, the ratio of IDF/SDF, and source of vegetable. The WHC of the dietary fiber obtained from yellow passion albedo was 13 g water/g sample, papaya powder 8.93 g water/g sample, and pineapple powder 6.06 g water/g sample [1, 30]. The value obtained from the PPF is close to the value obtained from passion albedo. Such a high WHC value suggests that PPF may have potential applications in products that require viscosity development, hydration, and preservation of freshness, such as cooked food products. Cui et al. [31] stated that dietary fibers with high WHC could be prolonged mastication, and thus affecting satiation and increasing fecal volume.

Table 3. Technological properties of pumpkin pulp flour

Technological properties	Value
WHC (g/g)	12.91±0.40
OHC (g/g)	3.74±0.10
SC (mL/g)	12.48±0.57
EA (%)	70.00±0.16
ES (%)	85.71±0.19
WAI (%)	9.34±0.58
WSI (%)	0.09±0.02

±: Standard deviation of three replicate. WHC: Water holding capacity, OHC: Oil holding capacity, SC: Swelling capacity, WAI: Water adsorption index, WSI: Water solubility index.

OHC of PPF is shown in Table 3. The OHC for passion fruit albedo and passion fruit seeds and pulp mix (2.03 and 1.43 g oil/g sample), for pomegranate bagasse (5.9 g oil/g sample), for mango (1.6 g/g sample), passion fruit (0.9 g oil/g sample), guava (0.7 g oil/g sample), for insoluble dietary fibers obtained from citrus and grapefruit peels (3.6 to 8.2 g oil/g sample) were obtained [30, 32-33]. OHC is a technological property associated with the chemical structure of dietary fiber. This property varies depending on the surface properties of the fiber, total charge density, shape, size, porosity, hydrophobicity of the fiber particles [30, 34-35]. OHC is important to product yield during the cooking process, to flavor retention, to stabilize food emulsions and to decrease fat digestion and absorption in the gastrointestinal tract, to influencing in the body weight control and to the regulation of blood lipid profiles [12, 36].

SC of PPF is shown in Table 3. The SC showed a situation similar to the WHC. Thus, these properties imply that PPF could be used as an agent to improve the texture and juiciness in the food industry such as meat and fish products.

The values of ES and EA of the PPF are shown in Table 3. These results showed that PPF has a good ES and EA. High ES and EA values are preferred for emulsion formation and extending shelf life in the food industry. The ES of oat, bamboo, potato, pea, apple, and wheat dietary fibers were 51.9, 51.0, 29.9, 48.2, 35.0, 56.8, and their EA were 53.0, 56.4, 30.6, 49.0, 37.0 and 57.9, respectively [35]. ES of lemon, grapefruit, pomegranate, lemon albedo and tiger nut dietary fibers were 93.18, 89.88, 90.52, 95.80, 96.15, and EA were 53.67, 54.67, 53.0, 80.0, 78.0, respectively [12].

WAI and WSI of PPF are given in Table 3. WAI is a function of the internal voids in the sample powder [37]. The more these voids, the more water would be absorbed by the sample. WSI provides information on the amount of soluble solids that pass from the material to an aqueous medium.

CONCLUSION

The ratio of IDF to SDF of the PPF was 3.14, and it may be considered as a balanced dietary fiber source. The powder showed good techno-functional properties, and therefore it could be used as a food additive in the food industry. The results revealed the high potential for recovery of waste derived from seed pumpkin to obtain a functional product. The richness of PPF in amino acids such as glutamine and asparagine, which enhance umami flavor, has revealed its potential use as a flavor enhancer in food. Additionally, its high water and oil retention capacities, along with increased emulsion stability and activity, indicate its suitability for use in emulsified products.

Conflict of Interests

There is not any conflict of interests.

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Research Paper / Araştırma Makalesi

Process Optimization for the Extraction of Phenolic Compounds from Pomegranate Peels: Response Surface Methodology-Desirability Function and Artificial Neural Network-Genetic Algorithm

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ABSTRACT

Valorization of agricultural wastes is ongoing topic in industry. Determining the best conditions by artificial neural networks based optimization techniques is the key step to extract valuable compounds efficiently and to obtain high quality extracts. In this study, the response surface methodology (RSM)-desirability function (DF) and artificial neural network (ANN)-genetic algorithm (GA) approaches were compared in modeling and optimization the extraction parameters (temperature, time and ethanol concentration (ratio of ethanol to water, % v/v)) of phenolic compounds in pomegranate peels. The ANN-GA approach providing higher coefficient of determination and lower root mean square deviation showed better predictive capability than the RSM. The optimum time (81.4 min) and ethanol concentration (15.7%) of RSM-DF approach shifted to the lower levels (78.8 min and 15.3%) with the ANN-GA approach while the optimum temperature (54.0°C) shifted to a higher level (59.3°C). The use of these values provided total phenolic content of >1000 mg GAE L^{-1} and the corresponding antioxidant activity was 11 mmol TE L^{-1}. As a result, increasing temperature up to a critical level decreased the extraction time and ethanol concentration, and it was determined that higher time-temperature combinations must be used for the complete water-based extraction of phenolic compounds from plant wastes in comparison to ethanol-water based extraction.

Keywords: Valorization pomegranate peel, Extraction, Response surface methodology, Artificial neural network, Genetic algorithm

Nar Kabuklarından Fenolik Bileşiklerin Ekstraksiyonunda Proses Optimizasyonu: Yanıt Yüzeyi Yöntemi-İstenebilirlik Fonksiyonu ve Yapay Sinir Ağı-Genetik Algoritma

ÖZ

Tarımsal atıkların değerlendirilmesi endüstride güncelliğini sürdüren bir konudur. Değerli bileşikleri verimli bir şekilde ekstrakte etmek ve yüksek kaliteli ekstraktlar elde etmek için en iyi koşulların yapay sinir ağları tabanlı optimizasyon teknikleri ile belirlenmesi önemli bir adımdır. Bu çalışmada, nar kabuklarındaki fenolik bileşiklerin ekstraksiyon parametrelerinin (sıcaklık, süre ve etanol konsantrasyonu (etanol/su oranı, % v/v)) modellenmesinde ve optimizasyonunda yanıt yüzeyi yöntemi (RSM)-istenebilirlik fonksiyonu (DF) ve yapay sinir ağı (YSA)-genetik algoritma (GA) yaklaşımları karşılaştırılmıştır. ANN-GA yaklaşımı daha yüksek determinasyon katsayısı ve daha düşük ortalama karekök sapması sağlayarak RSM'den daha iyi bir tahmin yeteneği göstermiştir. RSM-DF yaklaşımının optimum süresi (81.4 dakika) ve etanol konsantrasyonu (%15.7) ANN-GA yaklaşımı ile daha düşük seviyelere (78.8 dakika ve %15.3) kayarken optimum sıcaklık (54.0°C) ise daha yüksek bir seviyeye kaymıştır (59.3°C). Bu değerlerin kullanımı >1000 mg GAE L^{-1} toplam fenolik içerik ve 11 mmol TE L^{-1} antioksidan aktivite sağlamıştır. Sonuç olarak, sıcaklığın kritik bir seviyeye çıkarılması ekstraksiyon süresini ve etanol konsantrasyonunu azaltmıştır ve bitki atıklarından fenolik bileşiklerin tamamen su bazlı ekstraksiyonunda etanol-su bazlı ekstraksiyonuna göre daha yüksek zaman-sıcaklık kombinasyonlarının kullanılması gerektiği belirlenmiştir.

Anahtar Kelimeler: Nar kabuklarının değerlendirilmesi, Ekstraksiyon, Yanıt yüzey yöntemi, Yapay sinir ağı, Genetik algoritma

INTRODUCTION

Extraction is known as a general method used to extract phenolic compounds from pomegranate peel as well as other natural plant sources. In the first step of the method, the solute and solvent are contacted to occur a mass transfer between the two phases up to equilibrium state. Initially, the solute is dissolved from the solid surface and then dispersed into the solvent by diffusion. In this stage, diffusion coefficient and dissolution rate are the main factors affecting extraction yield until reaching equilibrium concentration. In the second step, the solid residue is separated from the solvent which contain rich solute in the system [1]. Totally, pH, physicochemical structure of solute and solvent, particle size, surface area, temperature and contact time are known as the main parameters which were evaluated based on extraction yield and quality [2]. There are studies in the literature to investigate the effects of different combinations of these parameters on extracting phenolic compounds from pomegranate peel. Amyrgialaki et al. [3] determined that optimum extraction parameters were ethanol concentration of 40%, pH of 2 and time of extraction of 1 h at room temperature for the maximum total polyphenolic content and 97% of overall effect is accounted by ethanol concentration. Sood et al. [4] determined that extraction parameters were solid to solvent ratio of 1:30, temperature of 50°C and time of extraction of 45 min for the maximum total polyphenolic content using 60% ethanol concentration. These studies show that some of the extraction parameters have more impact on the process than others and that they interact with each other. By evaluating these interactions, it is possible to increase the extraction efficiency of temperature-sensitive compounds and the amount of use of green solvents or shorten the extraction times.

Phenolic compounds contain one or more hydroxyl groups (polar part) in their structure, which are bonded to an aromatic ring (apolar part) and they are usually not free in plant tissues, but in the form of esters or glycosides [5]. Differences in the molecular structure of the phenolic compounds cause changes in their polarity. Flavonoid aglycones and phenolic acids are well soluble in solvents such as diethyl ether, ethyl acetate [6] while the hydrolysable tannins are better dissolved in polar protic solvents such as hydroethanolic mixtures [7]. The rise in temperature increases the solubility and diffusion coefficient of the soluble compounds and the permeability of the cell wall [8] and higher efficiency is obtained by using longer period. However, high-temperature and long-time combinations cause to deteriorate structure of the extracted compounds [9]. Therefore, not only the selection of the solvent mixture considering its suitability to substrate material, its toxicity and residual limit value but also performing the process in optimum conditions are important steps to obtain maximum process efficiency and higher quality of final extract.

According to the foreseen effects of time-temperature combinations and solvent type on the extraction yield, carrying out the process under the best conditions is key step to obtain high quality extracts. Statistical and mathematical techniques such as response surface method (RSM) are used to evaluate interactions of independent variables with individual effects on the response, which is the main difference between the RSM and a single-factor experimental design [10]. The RSM was used intensively for the development, improvement, modeling and optimization of new or existing products [11]. Artificial neural network (ANN) which is an information processing concept inspired by the biological nervous systems [12], can be also used modeling linear and non-linear problems and by associating the single or multivariable inputs and a single or multivariable outputs [13]. Genetic algorithm (GA) is a non-mathematical optimization technique that obtain the solution of single and multi-objective optimization problems inspired by the biological evolution theory [14]. In the literature, studies that predict and model processes such as drying, extrusion, sterilization, membrane separation, extraction using ANNs [15-17], moreover studies which optimize processes using ANNs hybridized with GA are increasing [18-20]. These methods have been used together for both modeling and optimizing several extraction processes and it was shown that strong correlations with experimental results can be associated via developed models. Using the RSM and optimization with desirability function (DF) and ANNs hybridized with GA in extraction of phenolic compounds from pomegranate peels take attention by shortening the experimental study time, comprehensively explaining the contribution of extraction parameters to the process and making it easier to determine the optimum condition. To the best of our knowledge, there is no comparative study to investigate predictive capabilities of the RSM-DF and the ANN-GA on extraction of phenolic compounds from pomegranate peels.

In view of the above-mentioned facts, objectives of this study were: (i) evaluation of individual and interaction effects of temperature, time and solvent (ethanol) concentration on total phenolic content (TPC) and antioxidant activity (AA) of pomegranate peel extracts by Box Behnken method, (ii) modeling and optimizing the process with RSM-DF and ANN-GA approaches while reducing ethanol concentration and process temperature for maximum TPC and AA and finally (iii) comparing predictive capabilities of the approaches based on optimization results.

MATERIALS and METHODS

Materials

Fresh pomegranates were purchased from a local market in Edirne (Turkey). Peels were manually separated from the whole fruits. Fresh and ground peels were kept at - 25°C until the extraction procedure.

The main chemicals such as 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-Ciocalteu phenol agent, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), gallic acid, sodium hydroxide, potassium persulfate, ethyl alcohol and sodium carbonate were purchased from Sigma (St. Louis, MO, USA). All the chemicals were of analytical grade.

Extraction Procedure

Outer peel (exocarp), spongy fleshy tissue (mesocarp) and white membrane layer (endocarp) parts of the pomegranate peels were separated from edible part (arils). These parts were chopped with electric chopper while fresh and stored at -25 degrees until extraction. Phenolic compounds from pomegranate peel were extracted in a shaking water bath (Memmert WNB22, Schawabach, Germany) with ±0.1°C adjustment precision. All samples were studied with the same shaking speed and 100 mL solvent volume. The extraction parameters were temperature (30, 55 and 80°C), time (10-50-90 min) and ethanol concentration (0-50-100%) and peel/solvent ratio was 1/30 (g mL^{-1}). The extracts were filtered by using a filter paper (Whatman Grade 5) and stored at - 25°C until the analysis.

Total Phenolic Content

Total phenolic content (TPC) was measured by the Folin Ciocalteu method [21]. The method based on the reduction of tungstate and/or molybdate in the Folin-Ciocalteu reagent by phenols. Blue product formation in alkaline medium by reduction is measured by a Shimadzu UV1800 spectrophotometer (Kyoto, Japan). 1 mL of sample, 5 mL of 0.2 N Folin-Ciocalteu reagent and 4 mL of 75 g L^{-1} sodium carbonate (Na_2CO_3) were mixed and the samples were incubated for 5 minutes at 50°C in a water bath (Memmert WNB22, Schawabach, Germany). Total phenolic content was calculated as gallic acid equivalent (mg GAE L^{-1}) by using absorbance values measured at 760 nm.

Antioxidant Activity

Antioxidant activity (AA) was determined by the Trolox Equivalent Antioxidant Capacity (TEAC) assay [22] which is based on scavenging of ABTS•+ radical and measuring of decolorization at 734 nm. The ABTS•+ radical cation was prepared via reaction of 7 mM ABTS and 2.45 mM potassium persulfate (1/1, v/v) for 16 hours in dark. Firstly, the solution was diluted by using ethanol in order to provide absorbance of 0.70 (± 0.02) at 734 nm. After that, 30 µL sample was added to 3 mL diluted ABTS•+ radical. Finally, absorbance at 734 nm was recorded for 6 min. Total AA was expressed as TEAC (mmol TE L^{-1}) calculated by using percent absorbance reduction.

Experimental Design

The effects of temperature (T, °C), time (t, min) and ethanol concentration (%) on the TPC (mg GAE L^{-1}) and total AA (mmol TE L^{-1}) were investigated by using three level, three factor Box Behnken design. In order to determine the factors and their levels, previous studies in the literature were taken into consideration [3, 23]. Design-Expert® 11 (Stat-Ease Inc., Minneapolis, USA) statistical software was used to experimental design, modeling and optimization. The results of 15 runs (3 runs in the central point) were summarized in the Table 1. These results were used to both the RSM and the ANN studies.

Table 1. Box-Behnken experimental design and results of TPC and AA

Run	Independent Variables			Responses	
	(A) Temperature (°C)	(B) Time (min)	(C) Ethanol concentration (%)	TPC* (mg GAE L^{-1})	AA** (mmol TE L^{-1})
1	55(0)	50(0)	50(0)	945	10.41
2	55(0)	10(-1)	100(+1)	410	4.49
3	55(0)	50(0)	50(0)	915	10.10
4	30(-1)	90(+1)	50(0)	899	9.79
5	80(+1)	90(+1)	50(0)	722	7.34
6	80(+1)	50(0)	0(-1)	862	9.59
7	55(0)	90(+1)	100(+1)	519	5.14
8	80(+1)	50(0)	100(+1)	510	4.14
9	30(-1)	50(0)	100(+1)	341	3.75
10	30(-1)	10(-1)	50(0)	638	6.71
11	55(0)	10(-1)	0(-1)	808	8.33
12	30(-1)	50(0)	0(-1)	791	7.62
13	55(0)	50(0)	50(0)	928	10.27
14	80(+1)	10(-1)	50(0)	900	9.63
15	55(0)	90(+1)	0(-1)	996	10.95

*TPC: total phenolic content, **AA: antioxidant activity

Modeling and Optimization

Response Surface Methodology and Desirability Function Approach

Correlation between dependent variable and independent variables were modeled by the quadratic polynomial equation which was shown in Equation 1:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

where Y is the dependent variable (TPC or AA), Xi and Xj are independent variables (temperature, time and ethanol concentration), β0 is the constant, βi is the linear, βii and βij are the interaction coefficient terms and ε is the error (residual) term.

Quadratic polynomial equation constitutes by least squares method. Least squares method is based on minimizing the sum of squares of residual terms. The least squares method assumes that the residual (ε) has zero mean and constant variance (σ^2) and is independently and normally distributed. The appropriate of the residuals to the assumptions in the evaluation of the model adequacy is tested by residual graphs [24].

ANOVA based on hypothesis testing was used to evaluation of models and model terms. In order to investigate models statistical data were analyzed with F-statistic. P-value that corresponds to the F-statistic was calculated and the significance of the model and model coefficient terms was examined at the 95% confidence level. In the 95% confidence level the significance of the model and model coefficients are less than 5% ($p<0.05$) indicating that the model and model coefficients are statistically significant. The DF approach was employed to maximize TPC and AA. The purpose of this approach is to transform all targeted responses into a collective response with the best fit and determine the independent variables that provide this response. In the case of more than one targeted response, the geometric mean of desirability functions is calculated [25].

Artificial Neural Network and Optimization by Genetic Algorithm

The ANN-GA studies were performed with MATLAB® 2017b (The MathWorks, Inc., Natick, USA). Neural network tool (nn-tool) was used to the creation of the network. Network type, training function, performance function and adaption learning function were feed forward back-prop, Levenberg-Marquardt backpropagation algorithm (trainlm), mean squared error (MSE) and gradient descent with momentum weight and bias learning function (learngdm) respectively. The 60%, 20% and 20% of data was used to for training, validation and test.

The network architecture consists of three layers which one layer was input layer with three neurons (temperature, time and ethanol concentration), one layer was hidden layer with seven neurons and one layer was output layer with two neurons (TPC and AA). The hyperbolic tangent sigmoid function (tansig) (Equation 2) was used for hidden layer and the linear function (purelin) (Equation 3) was used to output layer.

$$Tansig(n) = \frac{1}{1+e^{-2n}} - 1 \quad (2)$$

$$Purelin(n) = n \quad (3)$$

The relationship between output and input parameters and the role of weights and bias are defined by Equation 4.

$$\begin{bmatrix} TPC \\ AA \end{bmatrix} = purelin\left(LW\{2,1\} \times tansig\left(IW\{1,1\} \times \begin{bmatrix} Temperature \\ Time \\ Ethanol\ concentration \end{bmatrix} + b(1) \right) + b(2) \right) \quad (4)$$

where IW is the input weight matrix, LW is the layer weight matrix, the b is the bias.

GA optimization tool was used to maximize the output variables. The fitness function used for the GA optimization is given in Equation 5.

$$F = Y_{TPC} + Y_{AA} \quad (5)$$

where Y_{TPC} is the TPC variable and Y_{AA} is the AA variable from ANN model. The GA parameters for maximizing the optimization criteria TPC and AA were as follows: population types: double vector, creation function: feasible population, selection function: uniform, scaling function: rank, crossover function: scattered, mutation function: adaptive feasible. Since GA optimization is minimization based, fitness function was multiplied by -1 to turn the operation into a maximization problem.

Comparison of Predictive Capacities of the RSM-DF and the ANN-GA Approaches

The ability of RSM and ANN to predict the relationship between independent and dependent variables was evaluated using coefficient of determination (R^2), root mean square error (RMSE) and average absolute deviation (AAD%) as were given in Equation 6, 7 and 8, respectively.

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(Y_{exp}-Y_{pre})^2}{\sum_{i=1}^{n}(Y_{exp}-Y_{avg})^2} \quad (6)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(Y_{exp}-Y_{pre})^2}{n}} \quad (7)$$

$$AAD\% = \frac{\sum_{i=1}^{n}\frac{|(Y_{exp}-Y_{pre})^2|}{Y_{exp}}}{n} \times 100 \quad (8)$$

Where Y_{exp} is the experimental value, Y_{pre} is the predicted value (by RSM and ANN); Y_{avg} is the average of the experimental values, n is the number of experiments.

RESULTS and DISCUSSION

Modeling with Response Surface Methodology

The goal of statistical modeling of effects of temperature, time and solvent mixture on extraction efficiency of phenolic compounds from pomegranate peels was determining the best conditions providing the highest total phenolic content (TPC) and antioxidant activity (AA). The linearity between TPC and AA reported by Wang et al. [23] was also observed in this study (Table 1). There was strong correlation ($R^2 = 0.9690$) between the TPC and the AA of the pomegranate peel extracts (PPE). The statistical analysis of the linear, two-factor interaction (2FI) and quadratic models generated with the least squares method were done (Table 2) and quadratic model was found to be the most appropriate model to explain the TPC and the AA of the PPE according to both p-value (p) < 0.0500 and lack of fit (LF) > 0.0500. Quadratic models had lower standard deviation (SD) and higher coefficient of determination (R^2), adjusted coefficient of determination (A-R^2) and predicted coefficient of determination (P-R^2) as compared with the linear and 2FI models. R^2 is a measure of the fit of the model. It expresses to what extent the dependent variables can be explained by the independent variables in the regression. When a new variable is added to the model the R^2 increases, whether this variable is statistically significant or insignificant. This will result in poor predictive power in evaluating new observations or answers, even though the model has a high R^2. Therefore, it is recommended to use the A-R^2 instead of the R^2. The A-R^2 does not increase when a new variable is added to the model and decreases when a insignificant variable is added. The high difference between R^2 and A-R^2 indicates that there are insignificant terms in the model. P-R^2 is a measure of the variation in new data explained by the model. As suggested the difference between the R^2 and the A- R^2 and the A- R^2 and the P-R^2 were less than 0.2 was a measure of the adequacy of the quadratic model [10]. In addition, the predicted residual error sum of squares (PRESS) values of both quadratic models were lower than the other models confirming desirability of the quadratic model to evaluate its prediction ability [10]. Furthermore, the coefficient of precision (adequate precision) measuring the signal/noise ratio of the models were found to be 16.80 and 16.32. This coefficient which compares the range of predicted maximum and minimum values with the average prediction error was greater than 4.00 is another indication that the models can be used to navigate the design space [26]. The coefficients of variation (CV: relative standard deviation) which expresses the percentage of the standard deviation relative to the mean were 5.95% and 6.41% and were below 10% as desired.

Table 2. Statistical data of the obtained models for TPC model and AA model

Model	p-value	LF	SD	R^2	A-R^2	P-R^2	PRESS
TPC_{Linear}	0.0129	0.0083	148.8800	0.6109	0.5048	0.3280	421100
TPC_{2FI}	0.5648	0.0071	154.7900	0.6941	0.4647	0.0491	595800
$TPC_{Quadratic}$	0.0012	0.0682	44.3800	0.9843	0.9560	0.7585	151300
AA_{Linear}	0.0289	0.0054	1.9100	0.5455	0.4215	0.2182	68.8500
AA_{2FI}	0.5541	0.0046	1.9800	0.6454	0.3795	-0.0826	95.3500
$AA_{Quadratic}$	0.0007	0.0561	0.5054	0.9855	0.9594	0.7755	19.7700

TPC: total phenolic content, AA: antioxidant activity, 2FI: two factor interaction, LF: lack of fit, SD: standard deviation, R^2: coefficient of determination, A-R^2: adjusted coefficient of determination, P-R^2: predicted coefficient of determination, PRESS: the predicted residual error sum of squares

For the analysis of model appropriate, the residual graphs of the TPC model and the AA model were shown in the Figure 1. One of the assumptions of the regression model is that the residuals have a normal distribution. The residuals that conform to normal distribution should be distributed near the normal line. As shown in the Figure 1a and 1g, the residuals showed a small scattering and showed a very close distribution to the theoretical normal distribution line. Constant variance and zero mean assumption were investigated in the Figure 1b and 1c for the TPC model and Figure 1h and 1i for the AA model, as shown in residuals were randomly distributed around the $y = 0$ and did not exceed 95% confidence level boundaries in the residuals against predicted and runs graphs.

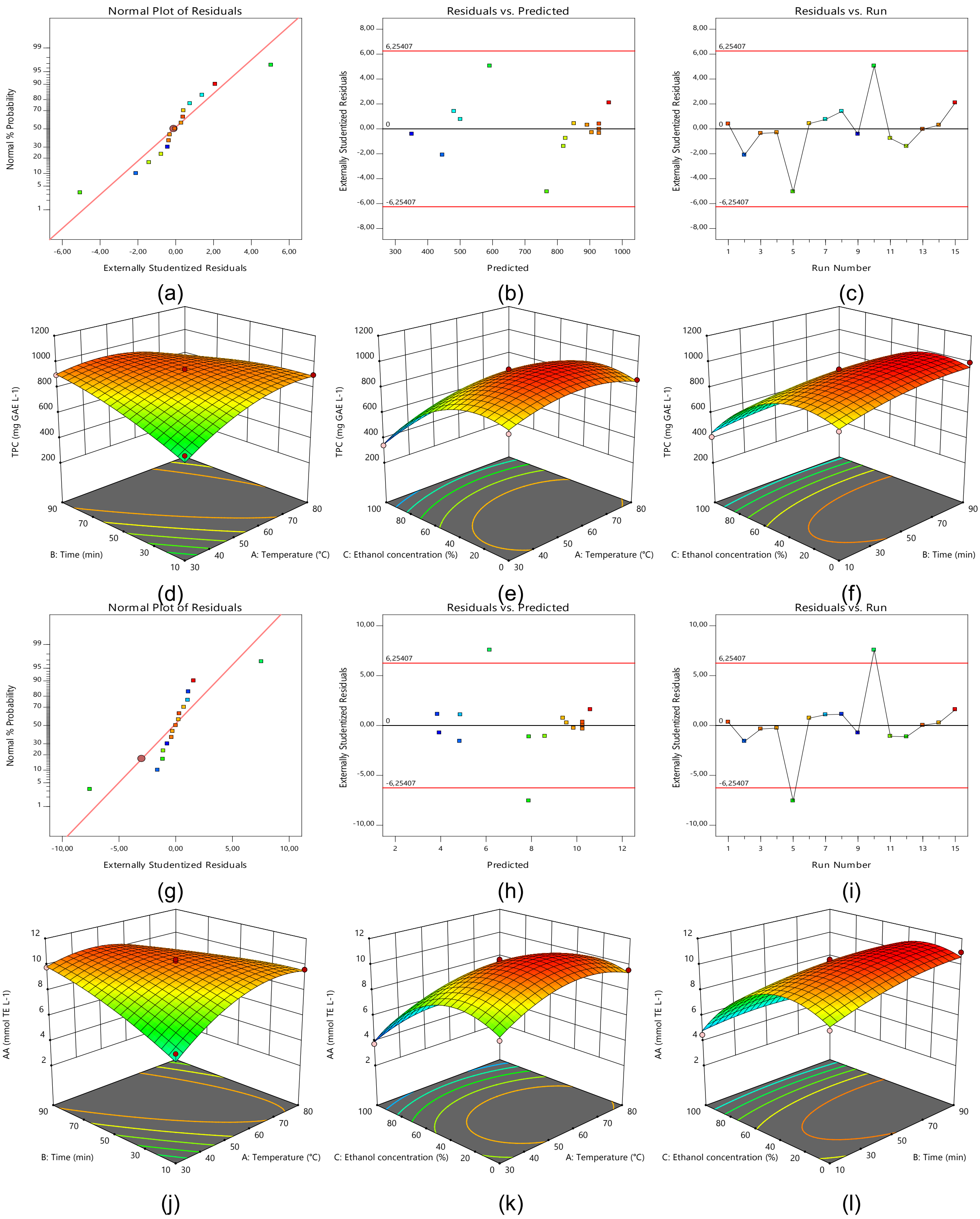


Figure 1. Residuals plots for **a-c** TPC, **g-i** AA, **a** and **g** normal plots of residuals, **b** and **h** residuals vs. predicted, **c** and **i** residuals vs. run, Response surface graphs for **d-f** TPC, **j-l** AA. **d** and **j** effect of temperature and time at constant ethanol concentration, **e** and **k** effect of temperature and ethanol concentration at constant time, **f** and **l** effect of time and ethanol concentration at constant temperature

ANOVA results for evaluating statistical significance of the model were reported in Table 3. The TPC model and the AA model was analyzed at 95% confidence level. The F-values of models were higher than F-critical values and the p-values of the models were determined less than 0.0500 (p=0.0006 and p=0.0005). Pure error was calculated by using 3 replicates at the center point. In lack of fit test, pure error can be used to test the significance of the residual variance sourced by the factors and their interactions that are currently in the model [27]. For lack of fit test, mean square of lack of fit and mean square of pure error calculated by dividing the sum of squares by the degrees of freedoms. F- values of lack of fit calculated by dividing mean square of lack of fit by mean square of pure error. The p-values for the lack of fit of the models were 0.0682 and 0.0561. In hypothesis testing, the lack of fit value was insignificant ($p>0.0500$) means that the responses can be explained by regression equation. These findings showing the TPC model and the AA model were statistically significant. The TPC and the AA models with actual values were shown in Equation 9 and 10. As the p-value of model term decreases, the importance of the term on the model increases. Accordingly, we observed that linear effects of temperature, time and ethanol concentration, quadratic effects of temperature and ethanol concentration and interaction effect of the temperature and time were significant in TPC model and linear effects of time and ethanol concentration, quadratic effects of temperature and ethanol concentration and interaction effect of the temperature and time were significant in AA model.

$$TPC\ (mg\ GA\ L^{-1}) = -27.96792 + 23.45383\mathrm{A} + 10.29042\mathrm{B} + 3.41992\mathrm{C} - 0.109750\mathrm{AB} + 0.019600\mathrm{AC} - 0.009875\mathrm{BC} - 0.157467A^2 - 0.025729\mathrm{B}^2 - 0.081967\mathrm{C}^2 \tag{9}$$

$$AA\ (mmol\ Trolox\ L^{-1}) = -4.13965 + 0.347435\mathrm{A} + 0.128213\mathrm{B} + 0.084768\mathrm{C} - 0.001343\mathrm{AB} - 0.000316\mathrm{AC} - 0.000246\mathrm{BC} - 0.002276\mathrm{A}^2 - 0.000294\mathrm{B}^2 - 0.001025\mathrm{C}^2 \tag{10}$$

where A, B and C is temperature, time and ethanol concentration, respectively.

Effect of Independent Variables on the TPC and the AA of Extracts

Three-dimensional (3-D) response surface graphs, which were formed by plotting the response variable against two independent variables while other independent variable was kept constant at a medium level, were given in the Figure 1. In these graphs, the response is shown gradually from red to green, from high to low, and with a contour graph at the base. At constant ethanol concentration (50%), increase in temperature and time increased the TPC and the AA up to a certain level (Figure 1d and 1j). After the maximum point, further increase in both temperature and time had a negative effect on the TPC and the AA, which can be attributed to the structural deterioration of phenolic compounds in long-term extraction processes at high temperatures [28]. Although it was mentioned that increase in temperature increased the TPC while extraction time was not significant before by Demir et al. [29], both the linear effect of temperature and time were found to be significant in this study. However, only the linear effect of temperature was not significant in the AA model (Table 3). Even Fourati et al. [30] stated that a longer extraction time presents a negative effect on the TPC. However, beside the linear effect of contact time was significant term of the quadratic TPC model, temperature-time interaction was also important to improve extraction of phenolic compounds from pomegranate peels. It was also determined that the similar TPC and AA values were obtained in high temperature-low time and low temperature-high time combinations, that means the extraction time decreases with increase of the extraction temperature. For example, TPC of 900 mg GAE L^{-1} was achieved in 88 min at 30°C while it was achieved in 11 min at 75°C. Consequently, increasing the extraction temperature up to a level having no deteriorative effects will cause to save time.

The effects of temperature and ethanol concentration in the medium level of time (50 min) are given in the Figure 1e and 1k. As in the results of ANOVA, the effect of ethanol concentration on both the TPC and the AA was higher than the effect of temperature. The effect of ethanol concentration on extraction efficiency was also reported by previous studies [29, 30]. The available range of ethanol concentration was determined 10-30% in the similar studies. In this study, at medium level of time, the maximum TPC (985 mg GAE L^{-1}) was obtained at 25% ethanol concentration and 59°C temperature while the maximum AA (10.86 mmol TE L^{-1}) was obtained at 26% ethanol concentration and 60°C temperature. The least TPC and AA values were obtained when 100% ethanol concentration was used. Tsakona et al. [31] also reported that the polarity of the solvent used in the extraction process had a significant effect on the TPC of the extracts and that the polar phenolic compounds were better dissolved in ethanolic mixtures containing ethanol in the 25-75% range compared to pure ethanol. The increase in temperature positively affected the response when the contact time kept constant. The positive effect of temperature on extraction efficiency was probably due to the increase in dissolution and diffusion rates of solutes [32].

The effects of time and ethanol concentration at constant temperature (55°C) are shown in the Figure 1f and Figure 1l. According to point prediction, the TPC and the AA reached 1003 mg GAE L^{-1} and 11.1 mmol TE L^{-1}, respectively, at using 22% ethanol concentration while reached 961 mg GAE L^{-1} TPC and 10.6 mmol TE L^{-1} using 0% ethanol (pure water) and reached 512 mg GAE L^{-1} and 5.1 mmol TE L^{-1} using 100% ethanol at the same temperature and time (55°C and 80 min). It was stated before by Wang et al. [23] that using an appropriate ethanol concentration in the solvent instead of pure ethanol or pure water approximately doubled TPC and AA of extracts. The TPC of 883 mg GAE L^{-1} and the AA of 9.5 mmol TE L^{-1} were obtained at 10 min when 22% ethanol was used in the solvent while the same values were obtained in 27.7 min when pure water was used at 55°C. This result showed that using a certain level of ethanol in the solvent shortened the extraction time.

Table 3. Results of statistical analysis for TPC model and AA model

Source	Total Phenolic Content (TPC)					Antioxidant Activity (AA)				
	Sum of squares	Df	Mean square	F-value	p-value	Sum of squares	Df	Mean square	F-value	p-value
Model	6.168E+05	9	68530.35	34.80	0.0006*	86.80	9	9.64	37.76	0.0005*
A	13203.12	1	13203.12	6.70	0.0489*	1.00	1	1.00	3.92	0.1046
B	18050.00	1	18050.00	9.17	0.0292*	2.06	1	2.06	8.07	0.0362*
C	3.515E+05	1	3.515E+05	178.51	< 0.0001*	44.98	1	44.98	176.11	< 0.0001*
AB	48180.25	1	48180.25	24.47	0.0043*	7.21	1	7.21	28.22	0.0032*
AC	2401.00	1	2401.00	1.22	0.3198	0.6241	1	0.6241	2.44	0.1788
BC	1560.25	1	1560.25	0.79	0.4142	0.9702	1	0.9702	3.80	0.1088
A^2	35763.10	1	35763.10	18.16	0.0080*	7.47	1	7.47	29.25	0.0029*
B^2	6257.33	1	6257.33	3.18	0.1348	0.8156	1	0.8156	3.19	0.1340
C^2	1.550E+05	1	1.550E+05	78.73	0.0003*	24.25	1	24.25	94.92	0.0002*
Residual	9846.42	5	1969.28			1.28	5	0.2554		
Lack of Fit	9393.75	3	3131.25	13.83	0.0682	1.23	3	0.4096	17.00	0.0561
Pure Error	452.67	2	226.33			0.0482	2	0.0241		
Cor Total	6.266E+05	14				88.08	14			

A: temperature (°C), B: time (min), C: ethanol concentration (%) Df: degrees of freedom, *significant ($p<0.05$)

Optimization with DF Approach

The extraction process was optimized for achieving the maximum TPC and AA. The lower and upper limits of the operating variables were kept at – 1 and + 1 levels in the experimental design. The lower and upper limits of the responses were equal to the lowest and highest values obtained in the experiments (341-996 mg GAE L^{-1} for TPC and 3.75-10.95 mmol TE L^{-1} for AA). While the importance level of all operating variables (temperature, time and ethanol concentration) was determined as 3, with the same importance, the importance level of the responses (TPC and AA) was determined as 5, with the same importance level and higher than the operating variables. Optimum conditions for the extracting phenolic compounds from pomegranate peels by solid-liquid method were temperature of 54.0°C, contact time of 81.4 min and ethanol concentration of 15.7% providing 1002 mg GAE L^{-1} TPC and 11.1 mmol TE L^{-1} AA.

Modeling with ANN

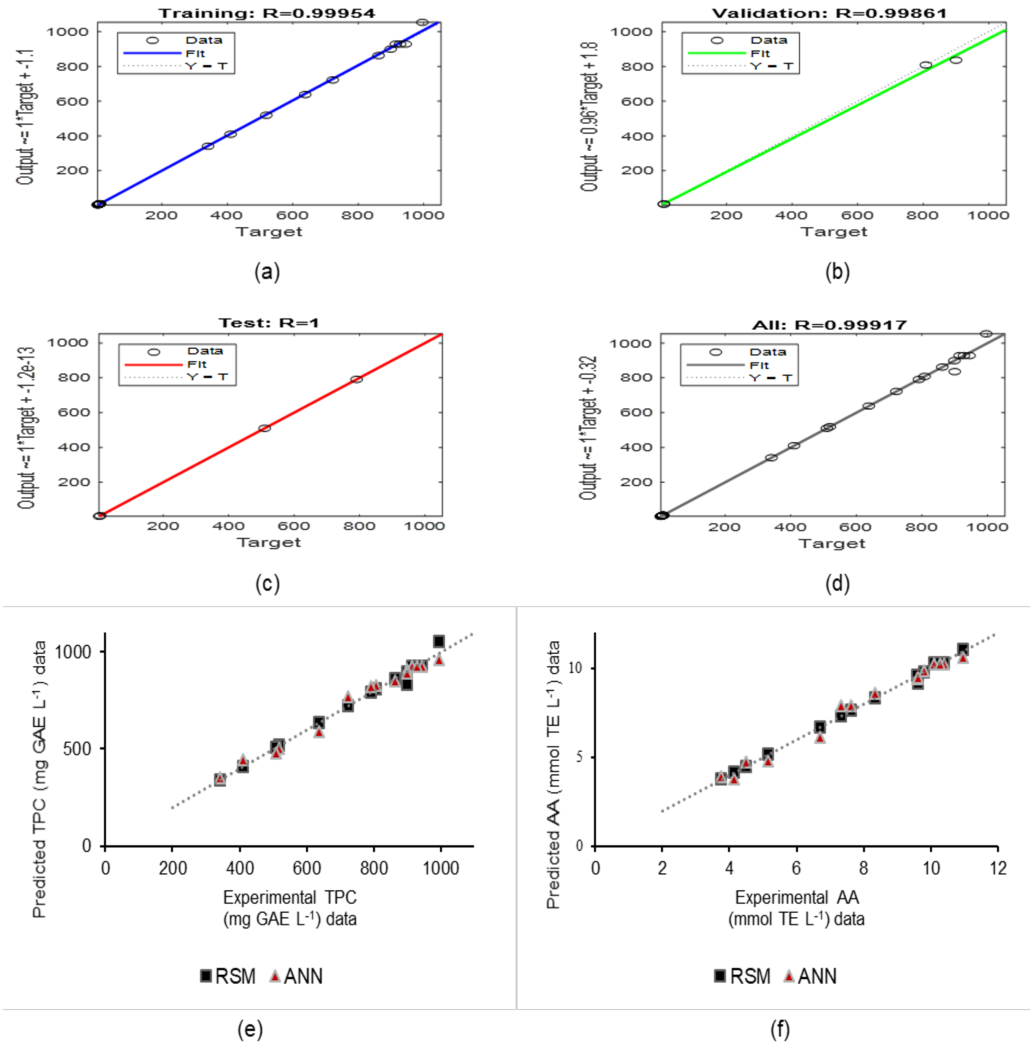
The ANN was also used to determine the relationship between the independent variables (temperature, time and ethanol concentration) and the dependent variables (TPC and AA). The experimental data of RSM study was used in the ANN study as input layer and output layer. Optimum neuron number of hidden layer was determined to be seven by trial and error method using the experimental data [33]. The testing of the model was carried out with the scatter plot between target and output data for training, validation, testing, and all data with the Pearson's correlation coefficient (R) were shown in the Figure 2a, 2b, 2c and 2d. The R value was 0.99954, 0.99861, 1.00000, 0.99917 for training, validation, testing, and all data, respectively. The approaching of the R to 1 indicates a strength and positive relationship between target and output.

$$IW\{1,1\} = \begin{bmatrix} 1.84448 & 2.50100 & 1.33054 \\ -0.55856 & 0.90620 & -1.69485 \\ -2.41696 & 0.97652 & -1.74415 \\ 0.25289 & 2.29764 & -1.80169 \\ -0.05689 & -1.79711 & -1.08251 \\ 0.25015 & 2.61598 & 3.54671 \\ 0.26070 & 1.46703 & 1.50553 \end{bmatrix} \tag{11}$$

$$LW\{2,1\} \begin{bmatrix} -0.10740 & -0.98973 & -0.11166 & 1.28154 & -0.97385 & -1.46273 & 0.04531 \\ -0.54899 & -0.4\mathrm{E}-05 & -0.28944 & 0.81503 & -1.16563 & -1.50773 & -0.26878 \end{bmatrix} \tag{12}$$

$$b(1) = \begin{bmatrix} -4.30325 \\ 2.74390 \\ -0.61207 \\ 3.26872 \\ 0.85750 \\ -2.47740 \\ -3.40283 \end{bmatrix} \tag{13}$$

$$b(2) = \begin{bmatrix} -0.39242 \\ -1.652989 \end{bmatrix} \tag{14}$$



(a) (b) (c) (d) (e) (f)

Figure 2. Scatter plot between experimental target and ANN output for **a** training, **b** validation, **c** testing, and **d** all data, scatter plot between experimental data and RSM and ANN predicted data for **e** TPC, **f** AA

The optimum weights and bias values used to predict the output data by minimizing the error between target and output data were given in Equation 11-14. The IW was the 7x3 weight matrix connecting the neurons of input layer to the hidden layer. The IW had 7 rows and 3 columns because the number of neurons was 7 and the number of independent variables was 3. The LW was the 2x7 weight matrix connecting the neurons of hidden layer to the output layer. The LW had 2 rows and 7 columns because the number of neurons was 7 and the number of dependent variables was 2. b(1) was the 7x1 bias column vector of the neurons of the hidden layer and b(2) was the 2x1 bias column vector of the neurons of the output layer.

Optimization with GA

The optimization was performed with three variables which lower bounds (30, 10 ,0), upper bounds (80, 90, 100). The TPC and the AA maximized after 51 iterations with GA. According to the optimization results performed with GA, the optimum conditions were temperature of 59.3°C, contact time of 78.8 min and ethanol concentration of 15.3% providing TPC of 1012 mg GAE L^{-1} and AA of 10.7 mmol TE L^{-1}.

Comparison of RSM and ANN Methods

The predictive capabilities of the techniques were compared with the values of coefficient of determination (R^2), root mean square (RMSE) and average absolute deviation (AAD%) values given in the Table 4. In both the methods, values of R^2 very closed to 1, values of RMSE and AAD% were low as required. The experimental data versus predicted data graphs for the RSM and the ANN were also shown in the Figure 2e and 2f. Accordingly, the values predicted to the model showed a linear distribution with the actual data so the ability to predict the fitted model was high. Depend on these results, the predicted data with the RSM and the ANN correspond to the experimental data, both the methods could be used for estimation. As in similar studies summarized in the Table 4, R^2 of ANN for both the TPC and the AA were closer to 1 than RSM, RMSE and AAD% of ANN for both responses were lower than RSM indicates that ANN has improved predictive capability [33-35].

Table 4. Statistical evaluation for RSM's and ANN's predictive capability

Responses	RSM Model			ANN model			Reference
	R^2	RMSE	AAD%	R^2	RMSE	AAD%	
TPC	0.9843	25.62	3.32	0.9878	22.85	1.07	This study
AA	0.9855	0.30	3.88	0.9965	0.14	0.62	
TPC	0.9148	0.35	6.37	0.9930	0.10	2.17	[33]
AA	0.8806	0.10	7.00	0.9887	0.03	2.15	
TPC	0.9443	23.45	8.29	0.9833	12.93	3.66	[34]
AA	0.9742	3.07	5.64	0.9803	2.69	3.38	
TPC	0.9791	17.10	1.86	0.9851	14.82	1.50	[35]
AA	0.9666	1.28	0.15	0.9764	1.20	0.14	

RSM: response surface methodology, ANN: artificial neural network, TPC: total phenolic content, AA: antioxidant activity, R^2: coefficient of determination, RMSE: root mean square error, AAD%: average absolute deviation

CONCLUSION

Solid-liquid extraction method has a good potential to recover phenolic compounds from pomegranate peels. The optimum levels of independent variables of the process were successfully predicted by the RSM-DF and the ANN-GA approaches. It was showed that the ANN can generate better predictions by processing experimental data although both the methods have similar functions. Additionally, the individual and interaction effects of the factors on the process efficiency were well described by the RSM. Based on these results, it is recommended to use various approaches in optimization studies and evaluate them according to the characteristics of the study. The experimental data were represented by a quadratic-second order model. Using ethanol (in the range of 15-25%) in the solvent mixture provided the highest extraction yield. On the other hand, higher time-temperature combinations were needed to obtain a sustainable water base extraction method of high value phenolic compounds compared to ethanol-water base extraction. Further studies are still needed to evaluate predictive capability of the ANN-GA and increase the diversity of studies on comparing the results of the ANN-GA method with the results of different modeling and optimization method in optimization of alternative green extraction processes of bioactive substances from several plant-based wastes.

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Research Paper / Araştırma Makalesi

Decontamination of *Salmonella* Enteritidis on Eggshell: Assessment of Efficiency of a Bacteriophage and Levulinic Acid-Sodium Dodecyl Sulfate

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ABSTRACT

The majority of food infections caused by consumption of egg and egg products are caused by *Salmonella enterica* subspecies *enterica* serovar Enteritidis. In this study, the inhibitory effect against *S.* Enteritidis was determined by dipping the eggshell into *Salmonella*-specific bacteriophage SE-P47 and levulinic acid (LVA) plus sodium dodecyl sulfate (SDS) solutions (0.5% LVA+0.05% SDS, 1% LVA+0.05% SDS and 2% LVA+0.5% SDS) separately for 10 minutes. The treatments of phage and 2% LVA+0.5% SDS reduced *S.* Enteritidis below the detectable level on eggshell (at 2.76, 3.22, 4.48 and 5.30 log CFU/cm^2 inoculum levels). After the treatment of 1% LVA+0.05% SDS, 1.94 and 0.89 log reductions were obtained at 4.48 and 5.30 log CFU/cm^2 inoculum levels, respectively, while *S.* Enteritidis decreased below the detectable number at 2.76, 3.22 log CFU/cm^2 inoculum levels. Although the lowest antibacterial activity was observed in the treatment of 0.5% LVA+%0.05 SDS, the decrease in the number of *S.* Enteritidis detected in all samples except 5.30 log CFU/cm^2 inoculum level was found to be significant compared to the control sample. The results indicated that the combination of LVA and SDS, and especially SE-P47 phage alone had good potential efficacy for *Salmonella* decontamination on eggshell.

Keywords: Bacteriophage, Decontamination, Eggshell, Levulinic acid, *Salmonella* Enteritidis

Yumurta Kabuğunda *Salmonella* Enteritidis'in Dekontaminasyonu: Bakteriyofaj ve Levülinik Asit-Sodyum Dodesil Sülfatın Etkinliğinin Değerlendirilmesi

ÖZ

Yumurta ve yumurta ürünlerinin tüketiminden kaynaklanan enfeksiyonların çoğu, *Salmonella enterica* subspecies *enterica* serovar Enteritidis kaynaklıdır. Bu çalışmada, yumurta kabukları *Salmonella*'ya özgü SE-P47 bakteriyofajı ve levülinik asit (LVA) ile sodyum dodesil sülfat (SDS) çözeltilerine (0.5% LVA+0.05% SDS, 1% LVA+0.05% SDS ve 2% LVA+0.5% SDS) ayrı ayrı 10 dk süreyle daldırılarak *S.* Enteritidis üzerindeki inhibitör etki belirlenmiştir. Faj ve %2 LVA+%0.5 SDS uygulamaları, yumurta kabuğu üzerinde *S.* Enteritidis'i tespit edilebilir seviyenin altına düşürmüştür (2.76, 3.22, 4.48 ve 5.30 log kob/cm^2 inokulum seviyelerinde). %1 LVA+%0.05 SDS uygulamasından sonra 4.48 ve 5.30 log kob/cm^2 inokulum seviyelerinde sırasıyla 1.94 ve 0.89 log azalma elde edilirken, 2.76, 3.22 log kob/cm^2 inokulum seviyelerinde *S.* Enteritidis, tespit edilebilir seviyenin altına düşmüştür. En düşük antibakteriyel aktivite %0.5 LVA+%0.05 SDS uygulamasında gözlemlenmesine rağmen, 5.30 log kob/cm^2 inokulum seviyesi hariç tüm örneklerde tespit edilen *S.* Enteritidis sayısındaki azalma, kontrol örneğine göre önemli bulunmuştur. Sonuçlar, yumurta kabuğunda *Salmonella* dekontaminasyonu için LVA ile SDS'nin kombinasyonu ve özellikle SE-P47 fajının tek başına iyi bir potansiyel etkinliğe sahip olduğunu göstermiştir.

Anahtar Kelimeler: Bakteriyofaj, Dekontaminasyon, Yumurta kabuğu, Levülinik asit, *Salmonella* Enteritidis

INTRODUCTION

Increasing urbanization and changes in consumer dietary trends (increased consumption of quality protein) lead to an increase in the demand for animal products and therefore more animal food products to be processed [1, 2]. The increase in production complicates food safety control and increases the risk of contamination of products with foodborne pathogens. Approximately 600 million cases of foodborne diseases and 420.000 deaths occur worldwide every year according to the World Health Organization. About 40% of foodborne diseases are particularly common among children under 5 years of age due to weak immune systems [3].

Insufficient food safety applications during production, packaging, transportation, and storage cause serious consequences such as foodborne illness and death, as well as socioeconomic and psychological problems in society. Unsafe food consumption is estimated to cause losses of 110 billion dollars each year, especially in low- and middle-income countries, through reductions in productivity, health expenditures, and mass destruction of food [4].

Salmonella enterica (non-typhoidal) is one of the main causes of foodborne diseases, especially diarrheal diseases [3, 5]. The Centers for Disease Control and Prevention (CDC) estimates there are approximately 1.35 million diseases, 26,500 hospitalizations, and 420 deaths from *Salmonella* in the United States every year [6]. *Salmonella* is a member of the *Enterobacteriaceae* family and has two main species, *S. enterica* and *S. bongori*. Approximately 2,600 serotypes have been identified for *Salmonella* species, of which less than 100 are known to cause human infections. *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) and *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) are the most frequently reported serovars responsible for *Salmonella* infections worldwide [1, 7, 8].

Salmonella species are found in the intestinal microflora of humans, domestic, and farm animals [2]. *Salmonella* is usually transmitted to humans by consuming contaminated food or water but can also be transmitted through contact with infected animals [7]. The poultry, egg and egg products, pork, beef, dairy products, fruits, vegetables, seafood, and water are the reservoirs of *Salmonella* species [9, 10]. It is well known that one of the most common sources of *Salmonella* outbreaks is the consumption of poultry and eggs, however, the most common serotypes isolated from poultry and egg products are *S.* Enteritidis [2, 11-13].

Egg is a frequently preferred food for human nutrition because it is nutritious and cheap compared to other protein sources [14]. Various egg products consumed in the world can be listed as shell eggs, egg whites, egg yolks, liquid, frozen or dried forms [15, 16]. In addition, the egg is included in the composition of many products such as bakery products, noodles, mayonnaise, ice cream, and desserts, due to the functional properties of its various components such as emulsifying and foaming ability [16, 17].

The inner part of eggs obtained from healthy poultry is considered sterile, however, it is known that there are a large number of microorganisms in the eggshell. Eggshells can be contaminated with microorganisms during production, processing, preparation, and packaging in the food chain [18]. Contamination of eggshell with *Salmonella* occurs due to contact of the eggshell with contaminated feces during or after laying. Other sources such as farmers, pets, and rodents play a role in the contamination of eggshells with *Salmonella.* Contamination of egg contents with *Salmonella* occurs through transfer from the eggshell (horizontal contamination) or direct contamination of the egg as a result of infected ovaries or oviduct tissue before shell formation (vertical contamination) [15, 19].

Various methods have been investigated to reduce or prevent *Salmonella* contamination on eggshells, including washing with chlorine-based surface sanitizers [20], hydrogen peroxide and sodium dodecyl sulfate [21], ozone [22], lactic acid [23], plant extracts with antimicrobial properties [18, 24], X-ray irradiation, chlorine dioxide, and the synergistic effect of the combined treatment [25], hot air treatment (pasteurization) [26], ultraviolet light [27], pulsed UV light [28, 29], far infrared [30], atmospheric plasma treatment [31, 32].

Organic acids are known to have antimicrobial effects. Among organic acids, levulinic acid (LVA) stands out because it can be produced with high efficiency from renewable raw materials [33]. LVA is also used as a flavoring agent in addition to its antimicrobial effect in the food industry. On the other hand, sodium dodecyl sulfate (SDS) which has inhibitory and lethal effects against foodborne microorganisms is used as an all-purpose food additive and surfactant. Both LVA and SDS have been generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA) [34, 35]. The bactericidal and virucidal activities of the combination of LVA with SDS are greater than that of LVA or SDS alone [36, 37]. Moreover, these antimicrobial activities can be achieved in lower LVA and SDS concentrations and shorter contact time. This is due to the synergistic effect between LVA and SDS. In previous studies, it has been shown that the combined use of LVA and SDS solution is effective in inactivating various pathogenic microorganisms in biofilms [37, 38], food contact surfaces [39], and food surfaces such as lettuce, poultry skin, cantaloupe, strawberries [33, 40, 41]. It was reported that the use of LVA with SDS is effective in the inactivation of microorganism groups such as bacteria, viruses, molds, and yeast, but not in foodborne parasites such as *Cryptosporidium* [36].

Bacteriophages (phages) are bacteria-specific viruses. Virulent phages infect bacterial cells and multiply intracellularly, causing host bacterial cells to lyse. Therefore, phages are potential biocontrol agents against foodborne pathogens [42]. They attract attention with their host specificity and environmentally friendly characteristics when compared to chemical compounds. In addition, the advantages of using phages as biocontrol agents are that they can eliminate biofilms, are effective even at low doses and have a relatively cost-effective and simple production process [43, 44].

Studies on the use of bacteriophages as biocontrol agents in various foods have focused on *Salmonella* serovars, *Listeria monocytogenes*, *Escherichia coli*, *Shigella* spp., *Campylobacter jejuni*, and *Staphylococcus aureus* pathogenic bacteria [42, 45-49]. FDA has approved the use in certain foods of several phage-based preparations designed for the control of foodborne bacterial pathogens such *as E. coli* (Secure Shield E1), *E. coli* O157:H7 (EcoShield™), *L. monocytogenes* (ListShield™, PhageGuard Listex™), *Salmonella* spp. (SalmoFresh™, PhageGuard S™, SalmoPro®), *Shigella* spp. (ShigaShield™) [46, 50].

In this study, phage or different concentrations of LVA plus SDS solutions were used for decontamination of *S.* Enteritidis on the surface of eggshells. It was aimed to evaluate the inhibition effects of phage and LVA plus SDS treatments against *S.* Enteritidis on the eggshell.

MATERIALS and METHODS

Bacterial Strain and Bacteriophage

S. Enteritidis MET-S1-411 and SE-P47 phage specific for *S.* Enteritidis used in the present study were obtained from our bacterial and phage culture collection. *S.* Enteritidis MET-S1-411 was cultured in Brain Heart Infusion (BHI) broth (Lab M, United Kingdom) and stored in BHI broth containing 20% glycerol at −80°C. For the preparation of the SE-P47 phage, isolated and characterized in previous studies [51, 52], nutrient broth (Lab M, United Kingdom) was used and phage samples were stored in 30% glycerol (in total solution) at −80°C.

Reproduction of Bacteriophage Sample

The phage samples were prepared with some modifications to the method of Yildirim et al. [51]. The phage sample (100 µL) and fresh culture of *S.* Enteritidis (300 µL) in 10 mL of nutrient broth were incubated at 37°C overnight at 120 rpm. Then, chloroform (50 µL/mL) was added to lyse the bacterial cells and the mixture was centrifuged at 7000×*g* for 15 minutes at 4°C. After the supernatant was filtered using a 0.45 µm pore size filter, the phage titer was determined by the double-layer agar plate method [53]. Briefly, 300 µL of host cells and 100 µL of phage dilution were added to nutrient soft agar (0.7% agar) at 45-50°C, mixed, and spread on petri dishes containing solidified nutrient agar (1.5% agar). After 24-48 hours incubation at 37°C, phage titer was expressed as a plaque forming unit per mL (PFU/mL). The activity of the phage used for decontamination was determined as 9.9 log PFU/mL.

Preparation of Levulinic Acid plus Sodium Dodecyl Sulfate Solutions

LVA (Merck, Germany) and SDS (≥98.5%, Sigma-Aldrich, USA) solutions were prepared with distilled water at concentrations of 0.5% LVA plus 0.05% SDS, 1% LVA plus 0.05% SDS, and 2% LVA plus 0.5% SDS before each experiment under aseptic conditions.

Determination of Antibacterial Activity of Treatment Solutions

The antibacterial activities of 0.5% LVA plus 0.05% SDS, 1% LVA plus 0.05% SDS, 2% LVA plus 0.5% SDS solutions, SE-P47 phage, and water against *S.* Enteritidis were determined using the disc diffusion method [54]. For this purpose, 20 µL of bacteria suspension (8 log CFU/mL) was spread on the soft nutrient agar (0.7% agar). Sterile filter paper discs (Oxoid, United Kingdom) with a diameter of 6 mm were immersed in washing solutions (1 mL) and left for 15 min to allow the solutions to penetrate the discs. Then, the paper discs were placed on the soft nutrient agar surface inoculated with *S.* Enteritidis. The diameter of the inhibition zones were measured after incubation at 37°C for 24-48 hours in aerobic conditions.

Preparation and Inoculation of Eggshell

Fresh eggs were purchased from a local market in Nigde, Turkey, and stored at 4°C to be used within a week. All of the eggs used in the study were of medium size (53-62 g). In the preparation of eggshells, the method of Rodriguez-Romo et al. [22] was used with some modifications. The shell parts were obtained by puncturing the tip of the eggshell and emptying the inside of the egg. Eggshells cut in 3x3 cm^2 dimensions were immersed in 70% ethanol solution and kept waiting for 5 minutes to disinfect. After the disinfection process, the shell pieces were washed with sterile distilled water and placed in sterile petri dishes and allowed to dry at room temperature under aseptic conditions (approximately 20 min).

For the preparation of inoculum solution, 100 µL of stock culture of *S.* Enteritidis was added to 5 mL of BHI broth and incubated at 37°C for 24 h. The absorbance of the bacterial solution was measured in a spectrophotometer (Evolution 300, Thermo Scientific, Waltham, USA) at 600 nm. When the inoculum solution had an optical density of approximately 0.3, the cell density was 7 to 8 log CFU/mL. The inoculum solution was diluted with 0.1% buffered peptone water (BPW) in the range of 4 to 8 log CFU/mL. The inoculum (100 µL) was spread on the eggshell (3x3 cm^2) using a pipette tip, and the samples were kept in the biosafety cabinet at room temperature for 20 min to ensure bacterial attachment onto the eggshell [55]. The count of the inoculated *S.* Enteritidis was determined by the spread plate method. A 100 µL of serial dilutions were spread on salmonella-shigella agar (1.5% agar) and colonies were counted after the incubation at 37°C for 24-48 h.

Determination of *S.* Enteritidis Inactivation on Eggshell

For each trial, five eggshell samples inoculated with *S.* Enteritidis were treated with LVA plus SDS solutions at three different concentrations, phage (9.9 log PFU/mL), and sterile distilled water. Briefly, the inoculated samples were immersed in washing solutions (20 mL) in sterile petri dishes. The inoculated eggshell surfaces were placed in direct contact with the washing solutions. The samples were kept in treatment solutions for 10 min at room temperature. Additionally, inoculated and untreated samples were used as positive control, and uninoculated and untreated samples were used as negative controls in each experiment. For bacterial count, eggshells were homogenized in a stomacher bag (VWR, West Chester, PA, USA) with 0.1% 10 mL BPW in a stomacher (IUL 707/470 Instruments, Spain) for 2 minutes. The bag fluid was serially diluted in 0.1% BPW and 100 µL from each dilution was plated in duplicate on salmonella-shigella agar (Merck, Germany) plates. After incubation at 37°C for 24-48 h, the colonies were counted and expressed as colony-forming units per cm^2 (CFU/cm^2).

Statistical Analysis

Samples were tested in triplicate for evaluating the inhibitory effect of the sanitizer washing on eggshells. The obtained data were analyzed using ANOVA-General Linear Model in MINITAB 17. Tukey's method was used to determine the mean significant differences between treatments at the 95% confidence interval ($p<0.05$).

RESULTS and DISCUSSION

Antibacterial Activities of Treatment Solutions

The antibacterial activity of SE-P47 phage (9.9 log PFU/mL), 0.5% LVA+ 0.05% SDS, 1% LVA+ 0.05% SDS, and 2% LVA+0.5% SDS solutions against *S.* Enteritidis was determined using disc diffusion method. In addition, it was examined whether sterile distilled water had an antibacterial effect when compared to the treatment solutions. The clear zones formed by the treatment solutions on the surface of the medium inoculated with *S.* Enteritidis are given in Figure 1. The clear zone diameters observed in 0.5% LVA+0.05% SDS, 1% LVA+0.05% SDS, and 2% LVA+0.5% SDS solutions were 6.72, 7.67, and 9.78 mm, respectively, and the difference between them was statistically significant ($p<0.05$) (Table 1). The clear zone formed by the use of SE-P47 phage was measured as 15.56 mm. As seen in Figure 1, a larger clear zone was obtained compared to other solutions and this value was statistically significant ($p<0.05$). A clear zone was not observed for the sterile distilled water.

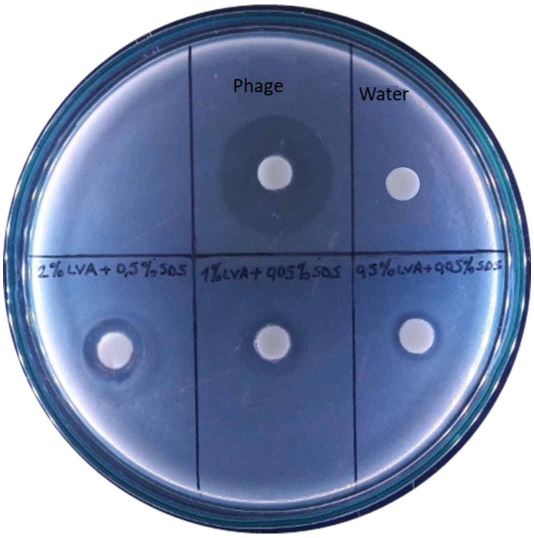

Figure 1. Clear zones observed by the agar disc diffusion method

Table 1. Diameters of clear zones obtained by disc diffusion method

Treatment solutions*	Clear zone diameter (mm)
Sterile distilled water	$0.00\pm0.00^{e**}$
0.5% LVA+0.05% SDS	6.72 ± 0.23^{d}
1% LVA+0.05% SDS	7.67 ± 0.28^{c}
2% LVA+0.5% SDS	9.78 ± 0.21^{b}
Bacteriophage SE-P47	15.56 ± 0.44^{a}

*LVA: levulinic acid, SDS: sodium dodecyl sulfate; **Different letters in the same column of treatment indicate statistically significant differences ($p<0.05$).

Inactivation of *S.* Enteritidis on Eggshell

Inoculum of different microbial concentrations was used to determine the antibacterial activity of LVA plus SDS solutions prepared at different concentrations and SE-P47 phage against *S.* Enteritidis on the eggshell surface. The *Salmonella* count of the inoculum solutions was determined separately as 4.7, 6.9, 7.88, and 8.3 log

CFU/mL. After inoculation, the *Salmonella* count on the eggshell was determined as 2.76, 3.22, 4.48, and 5.3 log CFU/cm^2, respectively. The microbial inactivation results are given in Table 2.

A 2-3 log difference was observed between the bacterial count of the *S.* Enteritidis inoculum solution and the bacterial count detected on the eggshell surface after inoculation. The fact that the bacterial count attached to the eggshell is less may be due to the physicochemical structure of the eggshell. The shell, the protective structure of the egg, contains the cuticle layer on the outside and the shell membrane on the inside. The cuticle layer is a proteinaceous layer covering the shell that has pores. On the other hand, the shell membranes consist of 3 different layers the inner membrane, the outer membrane, and the limiting membrane, and they are responsible for the bacterial defense system of the eggs [56]. In a study by Himathongkham et al. [57], the initial bacterial count of eggshells immersed in *S.* Enteritidis culture solution was approximately 7.5 log CFU/mL, while after 3 minutes incubation at 37°C and 30% RH, the *Salmonella* count decreased by about 2 logs. Himathongkham et al. [57] reported that a significant number of *S.* Enteritidis penetrated through the shell and on the shell membrane, based on the correlation between the bacterial count of the shell and the membrane. In a study investigating the penetration of *Salmonella* through the eggshell, it was reported that *Salmonella* translocated from the eggshell surface to the outer and inner membranes (shell membrane layers) [58]. Accordingly, in our study, the decrease count of *S.* Enteritidis on the eggshell after inoculation is attributed to bacterial penetration and displacement in/on the shell.

Table 2. Inhibitory effects of water, LVA plus SDS solutions, and bacteriophage treatments on *Salmonella* Enteritidis on the eggshell surface.

Treatments*	pH	*Salmonella* Enteritidis count on eggshell surface (log CFU/cm^2)			
SE	-	$2.76\pm0.05^{a**}$	3.22 ± 0.03^{a}	4.48 ± 0.07^{a}	5.30 ± 0.21^{a}
Water	8.65 ± 0.10	2.76 ± 0.05^{a}	3.10 ± 0.18^{ab}	4.15 ± 0.17^{a}	5.19 ± 0.24^{ab}
0.5% LVA+0.05% SDS	2.96 ± 0.01	2.34 ± 0.13^{b}	2.82 ± 0.07^{b}	2.93 ± 0.03^{b}	5.11 ± 0.20^{ab}
1% LVA+0.05% SDS	2.76 ± 0.02	$<1^{c}$	$<1^{c}$	2.54 ± 0.16^{b}	4.41 ± 0.19^{b}
2% LVA+0.5% SDS	2.69 ± 0.01	$<1^{c}$	$<1^{c}$	$<1^{c}$	$<1^{c}$
SE-P47	7.94 ± 0.16	$<1^{c}$	$<1^{c}$	$<1^{c}$	$<1^{c}$

*SE, the sample containing only *S.* Enteritidis MET-S1-411, control sample; <1, undetectable level, log CFU/cm^2 <1; LVA, levulinic acid; SDS, sodium dodecyl sulfate. **Different letters in the same column of treatment indicate statistically significant differences ($p<0.05$).

There was no reduction in the *S.* Enteritidis population on eggshells treated with sterile distilled water. This result supports that water does not form a clear zone in the agar disc diffusion method (Table 1). *S.* Enteritidis was inactivated ranged from 0.19 to 1.55 log CFU/cm^2 by treatment with 0.5% LVA+0.05% SDS solution. The difference between surviving *Salmonella* cells after the treatment of eggshells with 0.5% LVA+0.05% SDS and control samples is statistically significant, except inoculum level of 5.30 log CFU/cm^2 ($p<0.05$). However, there was no significant difference between water and 0.5% LVA+0.05% SDS treatments at inoculum levels of 3.22 and 5.30 log CFU/cm^2 ($p>0.05$). After treatment with 1% LVA+0.05% SDS solution, *S.* Enteritidis cell counts were undetectable at 2.76 and 3.22 log CFU/cm^2 inoculum levels. The *S.* Enteritidis population decreased by 1.94 and 0.89 log CFU/cm^2 at inoculum levels of 4.48 and 5.30 log CFU/cm^2, respectively ($p<0.05$). The highest log reductions in *S.* Enteritidis inactivation were achieved with 2% LVA+0.5% SDS and phage treatments at all inoculum levels, and viable cell counts were undetectable. The log reductions obtained in the 2% LVA+0.5% SDS and phage treatments were statistically significant compared to the control sample, water, 0.5% LVA+0.05% SDS, and 1% LVA+0.05% SDS treatments ($p<0.05$). It was observed that the decontamination results were consistent with the results obtained by the agar disc diffusion method (Table 1).

One of the microbiological criteria valid in many parts of the world for eggs defined as eggs in shell and egg products is the absence of *Salmonella* spp. in 25 g-mL [59, 60]. Also, according to the microbiological criteria of the Turkish Food Codex regulation, *Enterobacteriaceae* count should be less than 10^2 CFU/g-mL and *Salmonella* spp. should not be present in egg products (pasteurized and frozen eggs, egg powder, etc.) [61]. Since contamination of egg content with *Salmonella* can occur through transfer from the shell (horizontal contamination) [15], the microbiological safety of egg content and products is closely related to eggshells [62]. In our study, *S.* Enteritidis could not be detected in eggshells, especially after treatment with 2% LVA + 0.5% SDS or SE-P47 phage.

Levulinic acid causes the disruption of lipopolysaccharide in the outer membrane of Gram-negative bacteria. Depending on the increase in cell permeability, the absorption of both acid and SDS molecules into cells increases [36]. On the other hand, SDS can facilitate the contact of levulinic acid with bacterial cells by reducing the surface tension. It was reported that the effect of SDS to denature surface proteins and damage the cell membrane is higher between pH 1.5 and 3.0 [33, 36]. The average pH value of 0.05% SDS solution is 6.2. Therefore, the antimicrobial activity of SDS increases when used in combination with LVA. In this study, the pH values of LVA plus SDS solutions prepared at different concentrations decreased with increasing LVA concentration (Table 2). The reason for the lower log reductions in 0.5% LVA+0.05% SDS treatment compared to other LVA+SDS treatments can be attributed to the decrease in acid concentration, that is, the partial increase in pH value.

In a previous study, average log reductions in counts of influenza A H3N2 virus on eggshells individually treated with 0.5% LVA+0.5% SDS, 2% LVA+1% SDS, and 5% LVA+2 % SDS solutions for 1 minute at 21°C were 1.73, 1.90, and 2.33 log PFU/mL reductions, respectively [55]. The log reduction obtained in 0.5% LVA treatment was similar to our study. However, lower antimicrobial activity was observed in 2% LVA treatment compared to our study. This may be due to the diversity of the target microorganism and the short treatment time. Zhao et al. [33] reported that 0.5% LVA+0.05% SDS treatment (1 min) showed 4.4 and 4.5 log CFU/cm^2 reduction for *S.* Typhimurium and *E. coli* O157:H7 on the lettuce surface, respectively and 2.9 log CFU/cm^2 reduction for *S.* Enteritidis on chicken skin. After 5 minutes of treatment, approximately 7 log CFU/cm^2 reduction was obtained for the three pathogenic bacteria tested in both food samples. Maktabi et al. [21] investigated the inactivation of *S.* typhimurium on eggshell by immersion (5 min) in 1.5% SDS, 0.5% H_2O_2, and 1.0% citric acid solutions. After treatment with SDS, H_2O_2, and citric acid, the count of *S.* Typhimurium on eggshells decreased by 2.0, 2.1, and 0.4 log CFU/mL, respectively, compared to the control sample. They also reported that the antibacterial effect increased when citric acid or H_2O_2 combined with SDS.

Many studies have shown that the use of phages is effective in reducing the number of pathogenic bacteria in various food samples. However, there has been an increasing trend toward the use of phage and phage cocktails in eggs in recent years. To the best of our knowledge, there are limited studies that have applied phage to whole eggs or eggshells. Spricigo et al. [47] obtained 0.9 log CFU/cm^2 reduction for *S.* Enteritidis and *S.* Typhimurium in fresh eggs by spraying with the phage cocktail (10^{11} PFU/mL). In another study, approximately 3 log CFU/mL reduction was obtained in *S.* Typhimurium after 6 hours of phage cocktail (10^{10} PFU/mL) application on the eggshell while no viable cells could be detected in the samples after 24 hours of application [63]. In the same study, 1.7 log CFU/mL reduction was obtained after 72 hours of treatment of liquid egg with phage, and a lower antibacterial activity was observed compared to the eggshell sample. The difference in structure and composition between the eggshell and the liquid egg and the distribution of the microorganism on/in the sample can be effective on the results.

Controlling the colonization of pathogenic microorganisms in animals is one of the ways to prevent contamination of egg contents [15]. In the study of Henriques et al. [64], a phage cocktail (2×10^6 PFU/mL) was applied by aerosol spray on fertile eggs with *S.* Enteritidis to reduce horizontal contamination by *Salmonella*. Analysis of hatched chicks showed that the number of diseased chicks (arthritis and pasting) decreased. Furthermore, *S.* Enteritidis recovered from the chick ceca decreased after phage application to fertile eggs, while no significant reduction was observed for *S.* Enteritidis recovered from internal organs (pooled heart, liver, and spleen). This was attributed to the presence of high doses of bacteria by researchers. Therefore, the potential to use phages (phage therapy) to prevent pathogen colonization in poultry is thought to be quite high [64-66].

CONCLUSION

In this study, the effectiveness of LVA plus SDS and SE-P47 phage treatments was tested in the biocontrol of *S.* Enteritidis on eggshell. At all inoculum levels, the highest bactericidal effect was obtained with 2% LVA+0.5% SDS and phage treatments. Treatment of 1% LVA+0.05% SDS was very effective in preventing the growth of *S.* Enteritidis on the eggshells at lower inoculum levels. By reducing the microbial count on the eggshell, it is expected that the probability of horizontal contamination will also decrease. In other words, the egg content will be protected against contamination. Moreover, it will reduce the risk of cross-contamination of other foods in case of contact with whole eggs. In conclusion, it was demonstrated that levulinic acid plus SDS and SE-P47 phage can be effectively used decontamination of eggshell against the foodborne pathogen *S.* Enteritidis. In future studies, it will be very useful to investigate the effect of these treatments on the quality characteristics of eggs and eggshells.

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Research Paper / Araştırma Makalesi

Proximate Composition and Heavy Metal Contents of Edible Seaweeds *Kappaphycus alvarezii* and *Caulerpa* cf. *macrodisca* ecad *corynephora*

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ABSTRACTS

Edible seaweeds, such as *Kappaphycus* and *Caulerpa* genera, have been popularly consumed for centuries by the local people in the Tawi-Tawi, Philippines. This study examined the proximate composition and heavy metal contents of two edible seaweeds, namely *Kappaphycus alvarezii* (KA) (Rhodophyta) and *Caulerpa* cf. *macrodisca* ecad *corynephora* (CMC) (Chlorophyta), which are readily available in the public market of Bongao, Tawi-Tawi, Philippines. The results revealed significant differences in their proximate compositions. The moisture content was remarkably higher in KA (16.96±0.02 g.100^{-1}g) than that of CMC (10.49±0.08 g.100^{-1}g). CMC contained significantly more crude protein (7.14±0.80 g.100^{-1}g) than KA (2.73±0.40 g.100^{-1}g). The carbohydrate content of KA (44.82±0.34 g.100^{-1}g) was significantly higher than that of CMC (38.09±0.71 g.100^{-1}g). The ash content was notably greater in CMC (44.00±0.66 g.100^{-1}g) than in KA (34.91±0.39 g.100^{-1}g). The total fat content was substantially higher ($p \leq 0.05$) in KA (0.60±0.30 g.100^{-1}g) than in CMC (0.28±0.01 g.100^{-1}g). The heavy metal contents (mg.kg^{-1}) followed the order of K > Zn > Fe > Pb > Cu > Mn > Cd for KA and Zn > Mn > Pb > K > Fe > Cu > Cd for CMC. Fe, K, Cu, and Cd were significantly higher in KA than in CMC, while Zn, Mn, and Pb were remarkably higher in CMC than in KA. However, all these heavy metals were found to be within safe limits of WHO, the US (EPA and FDA), and EMA. These findings underscore the importance of considering these factors in seaweed consumption, affirming their enduring significance in human diets.

Keywords: Edible seaweeds, *Kappaphycus alvarezii, Caulerpa* cf. *macrodisca* ecad *corynephora,* Proximate composition, Heavy metals.

Kappaphycus alvarezii ve *Caulerpa* cf. *macrodisca* ecad *corynephora* Türlerine Ait Yenilebilir Deniz Yosunlarının Yaklaşık Bileşimi ve Ağır Metal İçeriği

ÖZ

Kappaphycus ve *Caulerpa* cinsleri gibi yenilebilir deniz yosunları, Filipinler'in Tawi-Tawi bölgesindeki yerel halk tarafından yüzyıllardır popüler bir şekilde tüketilmektedir. Bu çalışmada, yenilebilir iki deniz yosunu olan ve Filipinler'de Bongao, Tawi-Tawi'deki halk pazarlarında kolaylıkla bulunabilen *Kappaphycus alvarezii* (KA) (Rhodophyta) ve *Caulerpa* cf. *macrodisca* ecad *corynephora* (CMC) (Chlorophyta)'nin yaklaşık bileşimi ve ağır metal içeriği incelenmiştir. Sonuçlar, deniz yosunlarının bileşimlerinde önemli farklılıklar olduğunu ortaya çıkarmıştır. KA'daki nem içeriği (16.96±0.02 g.100^{-1}g), CMC'ye (10.49±0.08 g.100^{-1}g) göre oldukça yüksek bulunmuştur. CMC, KA'ya (2.73±0.40 g.100^{-1}g) göre anlamlı derecede daha fazla ham protein (7.14±0.80 g.100^{-1}g) içermektedir. KA'nın karbonhidrat içeriği (44,82±0,34 g.100^{-1}g), CMC'den (38,09±0,71 g.100^{-1}g) anlamlı derecede yüksek bulunmuştur. Kül içeriği CMC'de (44.00±0.66 g.100^{-1}g), KA'ya (34.91±0.39 g.100^{-1}g) göre belirgin şekilde daha yüksek olmuştur. Toplam yağ içeriği KA'da (0,60±0,30 g.100^{-1}g), CMC'ye (0,28±0,01 g.100^{-1}g) göre önemli ölçüde daha yüksek bulunmuştur ($p \leq 0,05$). Ağır metal içerikleri KA için K >

Zn > Fe > Pb > Cu > Mn > Cd ve CMC için Zn > Mn > Pb > K > Fe > Cu > Cd sırasını takip etti. Fe, K, Cu ve Cd, KA'da CMC'ye göre anlamlı derecede yüksekken, Zn, Mn ve Pb, CMC'de KA'ya göre oldukça yüksek olmuştur. Ancak tüm bu ağır metallerin güvenli sınırlar içerisinde olduğu görülmüştür. Bu bulgular, deniz yosunu tüketiminde bu faktörlerin dikkate alınmasının öneminin altını çizmekte ve bunların insan beslenmesindeki kalıcı önemini doğrulamaktadır.

Anahtar Kelimeler: Yenilebilir deniz yosunu, *Kappaphycus alvarezii*, *Caulerpa* cf. *macrodisca* ecad *corynephora*, Yaklaşık bileşim, Ağır metal

INTRODUCTION

Throughout Asian and Pacific cultures, seaweeds have been consumed as traditional food sources. Notably, edible seaweeds offer unique nutritional benefits compared to terrestrial crops, particularly in their high mineral and fiber content [1, 2]. Some popular dishes traditionally made with seaweeds include salads, sushi, pickled seaweed accompanied by condiments like relish or vinegar, and soups [3]. Scientific evidence has shown that the consumption of seaweeds provides a positive impact on global health, especially in addressing the rising prevalence of lifestyle-related diseases resulting from poor dietary habits [4]. Beyond food, seaweeds are significant contributors to aquaculture and biomass production, with applications in cosmetics, pharmaceuticals, animal feed, and biofuels [5-9].

Kappaphycus alvarezii (Rhodophyta) and *Caulerpa* (Chlorophyta) species are globally recognized edible seaweeds. K. *alvarezii*, cultivated worldwide for its carrageenan content and economic value, finds uses in both food and non-food industries [10, 11]. Local communities even consider it edible, and its high nutritional composition has food scientists exploring its potential as an alternative flour source [12, 13]. Additionally, *Kappaphycus* species can be processed into flour and used in crackers and animal feeds [14-16]. *Caulerpa*, harvested from the wild or through aquaculture, is a common sight in local markets across the Indo-Pacific region [12, 17, 18]. Popularly consumed fresh in salads or snacks, *Caulerpa* offers a delightful texture and boasts numerous nutritional benefits, including being a source of phenolics and antioxidants [5, 19, 20]. Tawi-Tawi, a haven for seaweed production in the Philippines, is a prime example. This region, known as the country's seaweed capital, is a major producer of red seaweed varieties like *Kappaphycus*, *Eucheuma*, and *Solieria* spp., alongside green seaweed Caulerpa spp. [12, 21]. The bustling Bongao Public Market showcases the importance of edible seaweeds to the local community. Here, *Caulerpa* is enjoyed fresh, while *Kappaphycus* is typically boiled before being incorporated into salads, often featuring a combination of tomatoes, onions, and vinegar [12].

While extensive research in Tawi-Tawi has explored seaweed farming practices [21-28], ethnobotany [12], taxonomy [29-32], and the microorganisms within the seaweed ecosystem [33-35, 18], a critical knowledge gap remains. Previous research has neglected to investigate the levels of heavy metals and the detailed nutritional makeup (proximate composition) of commonly consumed edible seaweeds in Tawi-Tawi. This is particularly concerning for *Kappaphycus alvarezii* and *Caulerpa* cf. *macrodisca* ecad *corynephora*, two edible seaweed species readily available year-round in the bustling Bongao Public Market, a central hub for the local community. Understanding the levels of heavy metals and the proximate composition of these popular edible seaweeds is crucial. This information can provide valuable insights into their nutritional value for consumers and raise awareness of potential health risks associated with heavy metal content. Therefore, this study breaks new ground by determining the levels of heavy metals and proximate composition in *Kappaphycus alvarezii* and *Caulerpa* cf. *macrodisca* ecad *corynephora* sold at the Bongao Public Market in Tawi-Tawi, Philippines.

MATERIALS and METHODS

Sampling Site

The sampling site is the Bongao Public Market (Figure 1), the central wet market situated in the capital of Tawi-Tawi, the Bongao municipality. It is among the busiest public markets in the province.

Sample Collection

Edible seaweeds available in the market during the sample collection were considered in the study. Initially identified based on morphological characteristics as described in the paper of Dumilag [12], *Kappaphycus alvarezii* and *Caulerpa* cf. *macrodisca* ecad *corynephora* are the common edible seaweeds available all throughout the year. Originally from Banaran, Sapa-Sapa municipality, these seaweeds were obtained from a vendor in the public market of Bongao.

Drying of Samples

Edible seaweed samples were transported to the Fish Processing Laboratory of the College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography. These were washed carefully and dried under the sun in a solar dryer for 3-4 days at a temperature ranging between 33 and 50°C. Dried seaweed samples were then sent to DOST-Region IX at Zamboanga City, Philippines, for analysis.

Heavy Metal Determination

Different heavy metals, including copper (Cu), cadmium (Cd), iron (Fe), manganese (Mn), lead (Pb), Zinc (Zn), and potassium (K), were determined by using the Flame Atomic Absorption Spectrophotometric (FAAS) method with dry ashing digestion technique at 500°C for 2 hr. A flame atomic absorption spectrophotometer (CAAM-2001, Hottine, China) was employed for the analysis [36].

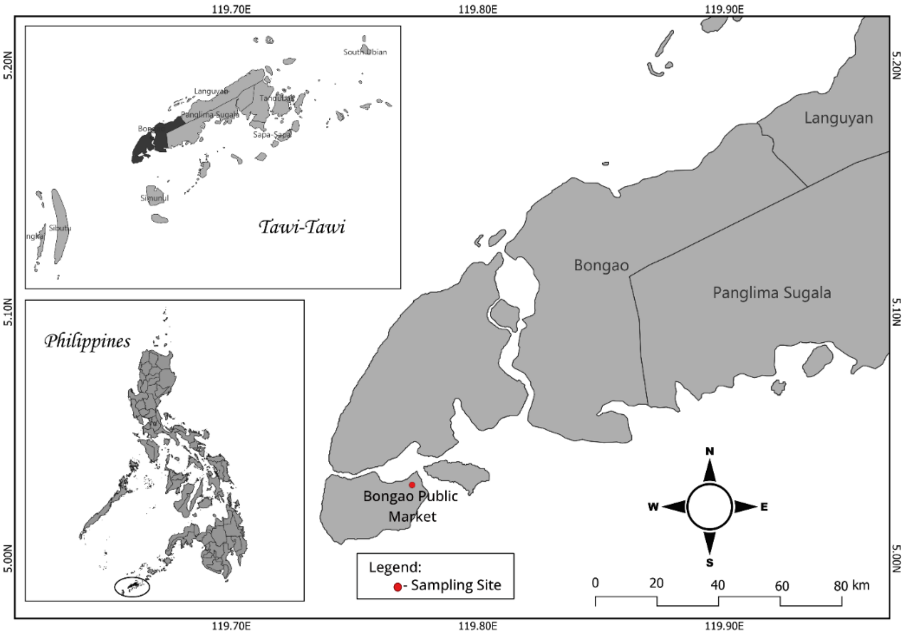

Figure 1. Sampling site for the present study

Proximate Composition

The proximate composition analysis of the edible seaweed samples included the assessment of moisture content, crude protein, total fat, ash, and carbohydrates. These components were quantified using the methods outlined in the AOAC guidelines. Specifically, the moisture content of the chosen edible seaweeds was determined through a gravimetric approach involving air-oven drying at a temperature of 65°C. The investigation of crude protein content employed the Kjeldahl method, which included steps such as block digestion and steam distillation. The analysis of total fat content utilized the Randall/Soxtec/Ether extraction-submersion method combined with acid hydrolysis. The ash content was evaluated via a gravimetric method involving furnace exposure at 600°C. Then, the determination of carbohydrate content was achieved by calculating the difference between 100 and the combined proportions of the previously mentioned components (moisture + ash + crude fiber + crude protein + total fat).

Statistical Analysis

Using the IBM SPSS version 20, an independent sample t-test was used to analyze the data. The significant difference was set at $p<0.05$.

RESULTS

The proximate composition of the edible seaweeds in the present study is shown in Table 1. The moisture content of *K. alvarezii* (16.96±0.02 $g.100^{-1}g$) was significantly greater ($p \leq 0.05$) than *Caulerpa* cf. *macrodisca* ecad *corynephora* (10.49±0.08 $g.100^{-1}g$). Crude protein was significantly higher ($p \leq 0.0.5$) in *Caulerpa* cf. *macrodisca* ecad *corynephora* (7.14±0.80 $g.100^{-1}g$) than in *K. alvarezii* (2.73±0.40 $g.100^{-1}g$). *K. alvarezii* contained a notable amount of carbohydrates (44.82±0.34 $g.100^{-1}g$) compared to *Caulerpa* cf. *macrodisca* ecad *corynephora* (38.09±0.71 $g.100^{-1}g$). Total fat content was remarkable in *K. alvarezii* (0.60±0.30 $g.100^{-1}g$) compared to *Caulerpa* cf. *macrodisca* ecad *corynephora* (0.28±0.01 $g.100^{-1}g$). Lastly, the ash content of *Caulerpa* cf. *macrodisca* ecad *corynephora (*44.00±0.66 $g.100^{-1}g$) was substantially higher ($p \leq 0.05$) than *K. alvarezii* (34.91±0.39 $g.100^{-1}g$).

Table 1. Proximate analysis of some edible seaweeds available in the Bongao public market, Tawi-Tawi, Philippines

Edible seaweeds	Moisture content ($g.100^{-1}g$)	Crude protein ($g.100^{-1}g$)	Carbohydrates ($g.100^{-1}g$)	Total fat ($g.100^{-1}g$)	Ash ($g.100^{-1}g$)
Kappaphycus alvarezii	$16.96 \pm 0.02^{a*}$	2.73 ± 0.40^{b}	44.82 ± 0.34^{a}	0.60 ± 0.30^{a}	34.91 ± 0.39^{b}
Caulerpa cf. *macrodisca* ecad *corynephora*	10.49 ± 0.08^{b}	7.14 ± 0.80^{a}	38.09 ± 0.71^{b}	0.28 ± 0.01^{b}	44.00 ± 0.66^{a}

*: Note: Superscript letters that are different imply significant differences at $p \leq 0.05$.

The determined heavy metal concentrations in the edible seaweeds are shown in Table 2. The average concentration of heavy metals followed the order of K > Zn > Fe > Pb > Cu > Mn > Cd for *K. alvarezii* and Zn >

Mn > Pb > K > Fe > Cu > Cd for *Caulerpa* cf. *macrodisca* ecad *corynephora*. When these two edible seaweeds were compared statistically, the t-test revealed that the Fe content (4.03±0.05 mg.kg^{-1}) of *K. alvarezii* was significantly higher than that of *C.* cf. *macrodisca* ecad *corynephora* (0.59±0.02 mg.kg^{-1}). The Mn content (1.30±0.04 mg.kg^{-1}) of *K. alvarezii* was significantly lower (p≤0.05) than that of *C.* cf. *macrodisca* ecad *corynephora* (7.98±0.03 mg.kg^{-1}). Pb content was notably higher (p≤0.05) in *C.* cf. *macrodisca* ecad *corynephora* (3.26±0.21 mg.kg^{-1}) than in *K. alvarezii* (2.05±0.04 mg.kg^{-1}). Zn content was substantially higher (p≤0.05) in *C.* cf. *macrodisca* ecad *corynephora* (987.59±25.86 mg.kg^{-1}) than in *K. alvarezii* (8.52±0.19 mg.kg^{-1}). K content was recorded to be notably higher (p≤0.05) in *K. alvarezii* (12.75±0.42 mg.kg^{-1}) than in *C.* cf. *macrodisca* ecad *corynephora* (1.42±0.01 mg.kg^{-1}). The Cu content was remarkably greater (p≤0.05) in *K. alvarezii* (1.92±0.04 mg.kg^{-1}) than in *C.* cf. *macrodisca* ecad *corynephora* (0.39±0.01 mg.kg^{-1}). Cd content was noted to be 0.40±0.02 mg/kg in *K. alvarezii*, while the Cd content in *C.* cf. *macrodisca* ecad *corynephora* was below the method detection level (MDL). The analytical characterization of heavy metal detection limits and calibration ranges using FAAS is shown in Table 3.

Table 2. Heavy metals of some edible seaweeds available in the Bongao public market, Tawi-Tawi, Philippines

Edible seaweeds	Heavy metals (mg.kg^{-1})						
	Fe	Mn	Pb	Zn	K	Cu	Cd
Kappaphycus alvarezii	4.03±0.05^{a*}	1.30±0.04^{b}	2.05±0.04^{b}	8.52±0.19^{b}	12.75±0.42^{a}	1.92±0.04^{a}	0.40±0.02
Caulerpa cf. *macrodisca* ecad *corynephora*	0.59±0.02^{b}	7.98±0.03^{a}	3.26±0.21^{a}	987.59±25.86^{a}	1.42±0.01^{b}	0.39±0.01^{b}	< MDL*

*: Note: Different superscript letters imply significant differences at p≤0.05. *<MDL = below the method detection level.

Table 3. Analytical characterization of heavy metal detection limits and calibration ranges using Flame Atomic Absorption Spectroscopy (FAAS)

Heavy metals	Limit of detection (mg.kg^{-1})	Limit of quantitation (mg.kg^{-1})	Linear concentration range (mg.kg^{-1})	Standard reference material (SRM)/Certified reference material (CRM)
Fe	0.0001	0.0003	0.001-1	NIST SRM 2780, NIST SRM 2781
Mn	0.00005	0.00015	0.0005-0.5	NIST SRM 3114, NIST SRM 3115
Pb	0.0001	0.0003	0.001-1	NIST SRM 1643d, NIST SRM 981a
Zn	0.00003	0.0001	0.0003-0.3	NIST SRM 3114, NIST SRM 3115
K	0.0002	0.0006	0.002-2	NIST SRM 1486, NIST SRM 1487
Cu	0.00005	0.00015	0.0005-0.5	NIST SRM 3114, NIST SRM 3115
Cd	0.00005	0.00015	0.0005-0.5	NIST SRM 3104a, NIST SRM 3113

*: Note: Different superscript letters imply significant differences at p≤0.05. *<MDL = below the method detection level.

DISCUSSION

The proximate composition of seaweeds varies according to species and type. In general, the Rhodophyta members contain high protein content (32%), whereas Chlorophyta members contain highest carbohydrate content (35%) [37]. However, in the present study, this was not the case; the crude protein of *Caulerpa* cf. *macrodisca* ecad *corynephora* (Chlorophyta) was higher than *K. alvarezii* (Rhodophyta). Additionally, carbohydrate content was greater in *K. alvarezii* (Rhodophyta) than in *Caulerpa* cf. *macrodisca* ecad *corynephora* (Chlorophyta). Apart from species, various factors, including spatial and seasonal variations, geographical distribution, reproductive status, and environmental parameters, have an impact on the seaweed's chemical composition [38, 39]. In the present study, the crude protein of *K. alvarezii* (2.73±0.40 g.100^{-1}g) was comparably lower than other studies utilizing the same species sampled in different areas, such as 4.13-5.09% in Antique, Philippines [40], 5.38 – 6.8% in Malaysia [41, 42], 12.69 – 23.61% in India [43], but it was higher than those determined in Indonesia with protein content of 1.03 – 1.94% [44, 39]. The crude protein of *Caulerpa* cf. *macrodisca* ecad *corynephora* (7.14±0.80 g.100^{-1}g) in the present study, although higher than *K. alvarezii*, it was relatively lower compared with other species, such as 10.41% [45], 13.24% [46], 14.76% [47], 17.28% [48], 20.54 – 21.52% in *C. macrodisa* [49, 50]. Carbohydrates, which constitute the majority portion of *K. alvarezii* (nearly 45%), were found greater than *Caulerpa* cf. *macrodisca* ecad *corynephora* (38%) in the present study. Compared with other studies, the carbohydrate content of the present study was higher than that found by Adharini et al. [39] in Indonesia (4.55 – 5.24%) and by Suresh Kumar et al. [43] in India (23%), suggesting that location is one of the factors influencing the carbohydrate content of this species. When the carbohydrate content (38%) of *Caulerpa* cf. *macrodisca* ecad *corynephora* of the present study was compared with other *Caulerpa* species, it revealed similar content with *C. lentillifera* with carbohydrate content of 38.66% [45] but higher than *C. macrodisca* with carbohydrate content of 37.66% [50]. However, it was relatively lower than in other studies, where *C. lentillifera* with 43-53% carbohydrate content [47, 48] and 50% in *C. racemosa* var. *laetevirans* [48]. The total fat of seaweeds is generally more than 0.4 g.100-1 g [51]. In the present study, the total fat was found higher in *K. alvarezii* (0.60±0.30 g.100^{-1}g) than in *C.* cf. *macrodisca* ecad *corynephora* (0.28±0.01 g.100^{-1}g). In comparison with other studies, the total oil of K. alvarezii was higher at 0.9-1.0% [42]. In terms of ash content, *C.* cf. *macrodisca* ecad *corynephora* contain a higher amount of ash (44.00±0.66 g.100^{-1}g) than *K. alvarezii* (34.91±0.39 g.100^{-1}g). This species of *Caulerpa* has greater ash content than other species obtained from previous studies, such as 27% in *C. racemosa* var. *laetevirans* [48], 14-31% in C. lentillifera [47, 48], and 29% in similar species of *C. macrodisca* [50]. The ash content (34.91±0.39 g.100^{-1}g) of *K. alvarezii* in the present study was greater than those of *K. alvarezii* (16.3 – 17.1%) as reported by Xiren and Aminah [42] in India, but was within the range of ash content (30.27- 36.46%) found in Antique, Philippines [40].

Heavy metals, recognized as highly potent environmental pollutants, are released into aquatic ecosystems via the effluents of various industries, thereby leading to a critical issue of aquatic pollution [50]. Although edible seaweeds offer a rich supply of micronutrients, macronutrients, and bioactive substances, serving as potential ingredients in various food items, nonetheless, these seaweeds have the capacity to accumulate substances that could pose risks to human health and animals, specifically heavy metals [51]. In the present study, the average concentration of heavy metals followed the order of K > Zn > Fe > Pb > Cu > Mn > Cd for *K. alvarezii* and Zn > Mn > Pb > K > Fe > Cu > Cd for *C.* cf. *macrodisca* ecad *corynephora*. However, all these determined heavy metals were within the safety limits of WHO, the US (EPA and FDA), and EMA [54, 55, 56].

Iron (Fe) is naturally abundant in seaweeds. In *K. alvarezii*, the concentration of Fe ranged from 250 to 301 mg.kg^{-1} [39]. However, the Fe content in the present study was lower (4.03 mg.kg^{-1}) than previously reported. Caulerpa species are known to have high mineral content; for example, *C. lentillifera* has been reported to contain 511-1973 g.100^{-1}g of Fe [48], which is significantly higher than what was observed in *C.* cf. *macrodisca* ecad *corynephora* in the present study. A higher manganese (Mn) content was observed in *C.* cf. *macrodisca* ecad *corynephora* (7.98 mg.kg^{-1}) in the present study, which was greater than those found in *C. lentillifera* (0.860 mg.kg^{-1}) and *C. racemosa* (0.453 mg.kg^{-1}) as reported by Kasmiati et al. [57]. *Caulerpa* species have the ability to accumulate lead (Pb) metal [58]. In the present study, *C.* cf. *macrodisca* ecad *corynephora* had a relatively higher Pb content (3.26 mg.kg^{-1}). The Pb content (2.05 mg.kg^{-1}) in *K. alvarezii* in the present study was higher than in a previous study, where it was found to be 0.761 mg.kg^{-1} [59]. Zinc (Zn) is the most abundant heavy metal in *C.* cf. *macrodisca* ecad *corynephora* (988 mg.kg^{-1}) compared to *K. alvarezii* in the present study, as well as higher than in *C. lentillifera* in previous study [48]. *Caulerpa* species, such as *C. taxifolia*, also have the ability to adsorb Zn [60]. On the other hand, the Zn content (8.52 mg.kg^{-1}) of K. alvarezii was higher than in another study [61]. The potassium (K) content of edible seaweed in the current study was higher in *K. alvarezii* (nearly 13 mg.kg^{-1}) than in *C.* cf. *macrodisca* ecad *corynephora* (1.42 mg.kg^{-1}). Caulerpa species have lower K content, typically around 2 – 4% [55]. The copper (Cu) content of the determined edible seaweeds in the present study was higher in *K. alvarezii* (nearly 2 mg.kg^{-1}) than in *C.* cf. *macrodisca* ecad *corynephora* (0.4 mg.kg^{-1}), and the cadmium (Cd) content was 0.4 mg.kg^{-1} in *K. alvarezii* while it was undetectable in *C.* cf. *macrodisca* ecad *corynephora*. These findings were higher than those reported by Tresnati et al. [59] for *K. alvarezii*.

The cell wall of seaweeds is composed of a diverse range of polysaccharides and proteins. Among these, certain molecules contain negatively charged groups like carboxyl, phosphate, or sulfate, which serve as highly effective sites for capturing metals [62]. Research conducted by Bryan [63] demonstrated that seaweeds have a robust capacity to bind metals, and there is minimal exchange between the metals bound by the seaweeds and the surrounding water. This binding phenomenon enables seaweeds to accumulate trace metals, reaching concentrations thousands of times higher than those found in the surrounding seawater [64]. It's important to note that seaweeds specifically bind to freely available metal ions, whose levels are influenced by the composition of suspended particulate matter. This particulate matter, as shown by studies by Seeliger and Edwards [65] and Volterra and Conti [66], consists of a combination of organic and inorganic complexes.

The accumulation of metals in seaweeds is contingent on various factors, with the most pertinent ones being the availability of metals in the surrounding water and the seaweeds' uptake capacity [67, 68]. Seaweed's metal uptake occurs through two distinct mechanisms. The first involves a surface reaction where metals are adsorbed by the seaweed surfaces through electrostatic attraction to negative sites. Interestingly, this process is independent of factors affecting metabolism, such as temperature, pH, light, or the age of the plant. However, it is influenced by the relative abundance of elements in the surrounding water. This surface reaction appears to be the primary uptake mechanism for Zn. The second mechanism is a slower and more active process in which metal ions are transported across the cell membrane and into the cytoplasm. This type of uptake relies more on metabolic processes and seems particularly relevant for the uptake of Mn and Cu. Importantly, this active uptake mechanism is subject to variations due to changes in light, temperature, or the age of the plant [67, 68].

CONCLUSION

In conclusion, this study provides valuable insights into the proximate composition and heavy metal content of two commonly consumed edible seaweeds, *Kappaphycus alvarezii* and *Caulerpa* cf. *macrodisca* ecad *corynephora*, available in the public market of Bongao, Tawi-Tawi, Philippines. The findings highlight significant differences in their nutritional profiles, with *K. alvarezii* exhibiting higher moisture content and total fat while *C.* cf. *macrodisca* ecad *corynephora* showed greater crude protein and ash content. Importantly, both seaweeds fell within safe limits for heavy metal concentrations, assuaging potential concerns about their consumption.

This research contributes to the broader understanding of seaweed's nutritional value and safety considerations, underscoring its historical and contemporary importance as a vital marine resource in human diets. Further studies may delve into specific health benefits and culinary applications of these seaweeds, expanding our knowledge base in this area.

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Research Paper / Araştırma Makalesi

Assessment of Health Risks Associated with the Presence of Phthalate Esters in Tea Samples: A Comprehensive Analysis

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ABSTRACT

This study presents novel insights into the occurrence of phthalate esters (PAEs) in tea samples consumed in Turkey. Employing multiple reaction monitoring (MRM) mode with precursor-product ion transitions, quantitative and qualitative analyses of PAEs were conducted using an LC-MS/MS system. A dispersive solid-phase extraction (dSPE) technique was utilized to determine the PAE contents in the tea samples. The limit of detection for all PAEs ranged from 0.350 to 1.882 ng/mL, while the limit of quantification ranged from 1.165 to 6.273 ng/mL. Moreover, strong correlations were observed with R^2 values exceeding 0.996 for all PAEs, indicating robustness. Recovery studies demonstrated satisfactory results, falling within the range of 92.20% to 97.24%, indicating effective retrieval. The relative standard deviation values for the target PAEs ranged from 3.22% to 5.54%. The study findings indicate that PAE levels in the tea samples generally comply with permissible limits set by EU regulations, except for DBP (1807.70±1478.86 ng/mL). Notably, DBP and DINP (941.44±852.80 ng/mL) were identified as the predominant plasticizers in the tea samples. Health risk assessment, conducted through hazard quotient and hazard index calculations across various age groups, consistently yielded values below 1 for DEP, DEHP, DINP, BBP, and DBP, suggesting minimal non-carcinogenic health risks associated with tea consumption across all age groups. Furthermore, the intake of individual phthalate esters, including DEP, DEHP, DINP, BBP, and DBP, remained below acceptable daily limits defined by the EFSA. Although some tea products exhibited slightly elevated PAE levels, overall, the study emphasizes the importance of continuous monitoring and regulations to ensure the safety of packaged tea and mitigate potential long-term exposure risks linked to PAEs.

Keywords: Tea bags, Health risk assessment, Phthalates, LC-MS/MS

Çay Örneklerindeki Fitalat Esterlerinin Varlığına İlişkin Sağlık Risklerinin Değerlendirilmesi: Kapsamlı Bir Analiz

ÖZ

Bu çalışma, Türkiye'de tüketilen çay örneklerinde fitalat esterlerinin (PAE’lerin) varlığına ilişkin yeni perspektifler sunmaktadır. Çoklu reaksiyon izleme (MRM) modu ve öncü-ürün iyon geçişleriyle, LC-MS/MS sistemi kullanılarak PAE'lerin nicel ve nitel analizleri gerçekleştirilmiştir. Çay örneklerindeki PAE içeriklerini belirlemek için dağıtıcı katı faz ekstraksiyonu (dSPE) tekniği kullanılmıştır. Tüm PAE’ler için saptama limiti (LOD) 0.350 ile 1.882 ng/mL arasında değişirken, nicemleme limiti (LOQ) 1.165 ile 6.273 ng/mL arasında değişmektedir. Ayrıca, tüm PAE'ler için 0.996'dan büyük R^2 değerleriyle güçlü korelasyonlar gözlemlenmiş, bu da sağlamlığı işaret etmektedir. Kurtarma çalışmaları, etkili kurtarma gösteren %92.20 ila %97.24 aralığında tatmin edici sonuçlar vermiştir. Hedef PAE'ler için göreceli standart sapma değerleri %3.22 ila %5.54 arasında değişmektedir. Çalışmanın bulguları, çay örneklerindeki PAE seviyelerinin genellikle AB düzenlemeleri tarafından belirlenen izin verilen sınırlarla uyumlu olduğunu göstermektedir, ancak DBP (1807.70±1478.86 ng/mL) için değil. Özellikle, DBP ve DINP (941.44±852.80 ng/mL) çay örneklerinde belirgin plastikleştiriciler olarak belirlenmiştir. Çeşitli yaş grupları arasında yapılan tehlike oranı (HQ) ve tehlike indeksi (HI) hesaplamalarıyla gerçekleştirilen sağlık riski değerlendirmesi, çay tüketiminin tüm yaş gruplarında minimal kanserojen olmayan sağlık riskleri taşıdığını gösteren DEP, DEHP, DINP, BBP ve DBP için 1'den düşük değerler sağlamıştır. Ayrıca, EFSA tarafından belirlenen kabul edilebilir günlük limitler dahilinde DEP, DEHP, DINP, BBP ve DBP gibi bireysel fitalat esterlerinin alımı devam etmektedir. Bazı çay ürünlerinin hafif yüksek PAE seviyeleri göstermesine rağmen, genel olarak, çalışma, paketli çayın güvenliğini sağlamak ve PAE'lerle ilişkilendirilen potansiyel uzun vadeli maruziyet risklerini en aza indirmek için sürekli izleme ve düzenlemelerin önemini vurgulamaktadır.

Anahtar Kelimeler: Çay poşetleri, Sağlık risk değerlendirmesi, Fitalatlar, LC-MS/MS

INTRODUCTION

Tea, originating from the leaves of the plant species *Camellia sinensis* L., is one of the most extensively consumed beverages on a global scale [1]. Despite lacking official endorsement from the US Food and Drug Administration (FDA), the consumption of hot water tea extracts, commonly known as tea infusions, is believed by some to confer beneficial effects on human health [2], while also being rich in proteins, amino acids, vitamins, and minerals [1], and showing potential in reducing the risk of chronic diseases such as heart disease [3], diabetes [4], arthritis [3], and cancer [6]. Due to its perceived therapeutic efficacy and the assumption of minimal or negligible toxic side-effects when consumed in significant quantities compared to synthetic drugs, consumers may regard tea as a safe beverage. However, concerns may arise regarding the presence of poly-fluoroalkyl substances (PFAS) [1], heavy metals [2], phthalate esters (PAEs) [7] in tea and their potential adverse health effects. PAEs, chemical compounds commonly employed as plastic softeners, have been well-established as endocrine disruptors [8]. Exposure to high doses of certain PAEs has been associated with reproductive and developmental toxicities in both males and females [9]. Several human studies have explored potential links between PAEs and alterations in semen quality [10], shortened gestation [11], reduced anogenital distance in newborn boys [12], and premature breast development in girls [13]. Although the inherent PAEs contamination in tea leaves is typically minimal, the tea production process, encompassing various stages such as harvesting, transportation, transformation, and packaging [14], can introduce PAEs into the final tea product [2]. Notably, the principal source of PAEs in commercial tea products appears to be the plastics used for packaging or the plastic lining present in filter paper-based tea bags [15]. In this context, the present study aims to investigate the presence of PAEs in tea products available in the Turkish market. The study aims to assess the potential migration of PAEs from the packaging materials into the tea and its potential implications for consumer health.

MATERIALS and METHODS

Chemicals and Reagents

In this research, we employed high-purity chemicals and standards for the analyses. Formic acid (>98%), acetic acid (100% glacial), and acetonitrile (>99.9%) were sourced from Merck (Darmstadt, Germany), while di-isodecyl phthalate (DIDP), methanol (≥99.9%), and di-isononyl phthalate (DINP) were obtained from Sigma-Aldrich (St. Louis, USA). The standard mixture, PAE 2000 µg/mL, was acquired from Dr. Ehrenstorfer (Augsburg, Bavaria, Germany). To facilitate filtration, a 0.45 µm PTFE filter from ISOLAB (Wertheim, Germany) was utilized. For the extraction process, Q-sep dSPE extraction salt and Q-sep dSPE tubes were provided by RESTEK (Bellefonte, USA).

Sampling Method

In this comprehensive research, our core objective was to investigate the occurrence of PAEs in various tea samples and assess their potential impact on consumer exposure. To accomplish this, we collected a total of 64 tea samples from both local and international markets, encompassing eight distinct types of tea: black, Import black, green, Import green, earl grey, Import earl grey, herbal, and fruit. All teas examined within the scope of the research are composed of teas packaged in tea bags for sale. The samples were analyzed for PAE content, focusing on potential variations among different tea products. The insights gained from this study provide valuable information regarding PAE exposure through tea consumption, aiding in the establishment of consumer safety measures. Understanding the presence and potential health implications of PAEs in tea is crucial for ensuring food safety and promoting public health.

Sample Extraction Procedure

In this study, we employed a dispersive solid-phase extraction (dSPE) technique to determine the PAE contents in the tea samples, following the protocol outlined by Isci et al [16]. The tea samples were first prepared by brewing them in glass beakers, following the instructions provided on the tea packaging. Subsequently, a combination of ultrapure water and acetonitrile, along with dSPE extraction salt, was added to the tea samples. After vigorous vortexing and centrifugation, the supernatant, primarily containing acetonitrile, was separated. Next, the extraction tube underwent a vacuum-assisted drying process, and PSA powder and cleaning salts were added. After another round of vortexing and centrifugation, the resulting supernatant was carefully filtered through a 0.45 µm filter before being injected into the LC-MS/MS system for analysis.

Instrument

The liquid chromatography (LC) system utilized in this study was an advanced triple quadrupole instrument

(Agilent Model no: K6460) manufactured by Agilent Technologies, a prominent company based in Loveland, CO, USA. This system was equipped with cutting-edge MS/MS (Mass Spectrometry) capabilities, ensuring precise and reliable analyses. The setup also incorporated other crucial components, including a highly efficient Vacuum Degasser (Agilent Model No: G1322A 1200 Series), a versatile Quaternary Pump (Agilent Model No: G4204A), a modern Infinity Autosampler 1260 Series (Agilent Model No: G4226A), and a thermostatted column oven 1200 Series (Agilent Model No: G1316A). To achieve optimal separation and detection performance, we carefully selected a Poroshell 120 SB-C18 column (3.0 mm, 100 mm, 2.7 µm) from Agilent Technologies as the chromatographic column.

LC-MS/MS Analysis

In this study, the quantitative and qualitative analysis of PAEs was conducted using the multiple reaction monitoring (MRM) mode with precursor-product ion transitions. The LC-MS/MS system employed in this analysis utilized a mobile phase A, composed of 0.1% formic acid and 5 mM ammonium formate in water, with an injection volume of 5 µL and a flow rate of 0.3 mL/min. Additionally, mobile phase B, containing 0.1% formic acid in methanol, was also used. To establish the calibration curve, a series of known concentrations of PAEs ranging from 1 to 250 ng/mL were prepared and injected twice into the LC-MS/MS system. To ensure the reliability of the method, we evaluated several important parameters, including the relative standard deviation (RSD) to measure precision, the limit of detection (LOD) to establish the lowest detectable concentration, the limit of quantification (LOQ) to determine the lowest accurately quantifiable concentration, the correlation coefficient (R^2) to assess calibration curve linearity, and the recovery (%) to measure the accuracy of the method by determining the amount of analyte successfully recovered from the samples. The results of these evaluations are presented in Table 1, demonstrating the method's robustness and suitability for analyzing PAEs in tea samples.

Study Population

The Turkey Nutrition and Health Survey (TBSA) is a resource prepared with the aim of ensuring that individuals living in Turkey have a healthy and balanced diet. This guide includes information on dietary habits and nutritional status in Turkey. The TBSA was jointly prepared by the Ministry of Health. This guide has been created based on scientific research and the opinions of nutrition experts [17]. According to TBSA, the daily tea consumption for males is as follows: 221.5 ± 261.9 mL day^{-1} for ages 15-18, 570.3 ± 473.2 mL day^{-1} for ages 19-64, and 423.5 ± 308.5 mL day^{-1} for ages >65. For females, the daily tea consumption is 142.6 ± 174.2 mL day^{-1} for ages 15-18, 459.1 ± 401.6 mL day^{-1} for ages 19-64, and 316.52 ± 261.7 mL day^{-1} for ages >65. The exposure calculations in the study were based on these recommended consumption values (Table 1).

Table 1. Parameters used for EDI and HQ calculation in different age groups (mean±standard deviation)

Age (years)	Body weight (kg)	Tea consumption (mL/day)
	Male	
15-18	66.30±14.50	221.50±261.90
19-64	81.20±15.30	570.30±473.20
>65	78.80±13.50	423.50±308.50
	Female	
15-18	58.60±14.30	142.60±174.20
19-64	71.60±15.80	459.10±401.60
>65	73.60±16.00	316.20±261.70

Non-carcinogenic Risk Assessment of PAEs

In order to evaluate the potential health risks associated with PAEs in tea, we focused on estimating the daily intake (EDI) of these compounds based on average concentration levels (C) in ng/mL. To achieve this, we took into consideration various essential factors, including the daily volume of tea consumed (IV) in milliliters (mL), the individual's body weight (Bw) in kilograms (kg), and their respective age groups, as specified in the study population. Utilizing the established Equation (1), we calculated the estimated daily intake (EDI) of PAEs, providing a comprehensive assessment of the exposure levels related to tea consumption and its potential health implications.

$$EDI = (C \times IV)/B_W \qquad \text{Eq. (1)}$$

To determine the estimated daily intake (EDI) of PAEs, a comprehensive calculation was conducted, yielding results expressed in µg/kg Bw/day.

Hazard Index (HI) and Hazard Quotient (HQ)

The assessment of potential health risks, including both carcinogenic and non-carcinogenic effects, related to tea consumption involved the computation of the HQ for individual PAEs, as detailed in Equation (2). Additionally, the HI was calculated for multiple PAEs using Equation (3), following the methodology established by the US Environmental Protection Agency [18].

$$HQ = EDI/RfD \qquad \text{Eq. (2)}$$

An HQ and HI values below 1 indicates a safe level of health risk, while an HQ and HI values equal to or greater than 1 suggests a potential non-carcinogenic health risk [18].

$$HI = \sum HQ\text{DEP} + HQ\text{BBP} + HQDBP + HQDEHP + HQ\text{DINP} \qquad \text{Eq. (3)}$$

The RfD (Reference Dose) values in (Eq. 2) are $2x10^{-1}$,810^{-1}, $1x10^{-1}$, $8x10^{-1}$, $2x10^{-2}$ and mg/kg Bw/day for BBP, DINP, DBP, DEP, DEHP, respectively [12].

Carcinogenic Risk Assessment of PAEs

Within the scope of this study, our assessment of cancer risk (CR) was specifically centered on DEHP and BBP. We utilized the corresponding cancer slope factor (CSF) values provided by the USEPA, which were reported as $1.4x10^{-2}$ mg/kg/day for DEHP and $1.9x10^{-3}$ mg/kg/day for BBP. This approach allowed us to delve into the potential cancer risks associated with these specific compounds and their exposure levels [19]. The daily intake (EDI) value, expressed in μg/kg Bw/day, represents the calculated intake of PAEs resulting from tea consumption.

$$CR = CSF \times EDI \qquad \text{Eq. (4)}$$

The CR assessment, following US EPA guidelines, evaluates potential oral health risks related to specific substances. CR values are classified into three levels: high cancer risk ($CR>1\times10^{-4}$), indicating significant health risk; acceptable cancer risk (CR: $1x10^{-6}$ to $1x10^{-4}$), within an acceptable range; and negligible cancer risk ($CR<1x10^{-6}$), indicating minimal risk [19].

Statistical Analysis

The analysis of the obtained data involved statistical procedures such as ANOVA, analysis of variance, performed using SPSS version 26.0 (IBM, Chicago, IL, USA). In the case of tea samples, ANOVA was utilized to examine the average PAE values, with a significance level set at $p<0.05$. To ascertain the statistical variances among tea samples, Duncan's multiple tests were applied. Integral percentiles, including the mean, P5, P50 (median), and P95, were pivotal in evaluating the data.

RESULTS and DISCUSSION

Quality Control and Quality Assurance

As shown in Table 2, the LOD for all PAEs ranged from 0.350 to 1.882 ng/mL, while the LOQ ranged from 1.165 to 6.273 ng/mL. Moreover, the R^2 exceeded 0.996 for all PAEs, indicating a strong correlation. Additionally, the recovery studies demonstrated satisfactory results, falling within the range of 92.20% to 97.24%, thus indicating effective recovery. The RSD values for the target PAEs ranged from 3.22% to 5.54%, further affirming the suitability of the extraction method within the tea matrix.

Table 2. Method verification parameters

Analytes	Retention time (min)	Spiking level (ng/mL)	Linearly range		Recovery (%)	RSD (%)	Quantification	
			(ng/mL)	R^2	Tea	Tea	LOD (ng/mL)	LOQ (ng/mL)
DMP	2.62	100	1-100	0.998	94.75	5.27	1.882	6.272
DEP	3.58	100	1-100	0.998	95.44	4.57	0.350	1.165
BBP	5.16	100	1-100	0.998	94.40	3.22	0.517	1.723
DBP	5.25	100	1-100	0.999	92.20	4.78	0.567	1.891
DEHP	7.92	100	1-100	0.999	97.24	5.27	1.176	3.920
DNOP	8.02	100	1-100	0.999	95.42	5.27	1.347	4.490
DINP	8.24	100	1-100	0.996	95.74	5.54	1.064	3.546
DIDP	8.51	100	1-100	0.999	95.42	4.70	0.474	1.581

PAEs Levels in Samples

PAE levels of BBP, DBP, DEHP, DMP, DIDP, DNOP, DEP, and DINP of 64 tea samples with different types which are available in Turkey are shown in Table 3. Among the samples, all PAEs were detected. The mean DEHP levels across the different types were detected at 743.29 (ND-4507.84) ng/mL. Statistical analysis indicated that there were no significant differences in DEHP levels among the various types (P>0.05). The highest average DEHP levels was found in I. Black (1199.62 ng/mL), while the lowest average level was found in herbal (327.35 ng/mL). Under Commission Regulation (EU) No 10/ (2011) [20], maximum allowable specific migration limit (SML) value of 1.5 mg/kg has been set for DEHP. The mean DEHP levels in all samples do not exceed the established SML value. Contrary to this study, the literature lacks research on the migration of PAEs from tea bags. However, there are some studies related to tea and tea cultivation. In comparison, Tang et al. [21] reported lower DEHP levels in Chinese black tea samples, ranging from 11.73 μg kg^{-1} to 28.33 μg/kg. Additionally, Troisi et al.[2] observed a median DEHP concentration of 9.4 (8–10.8) ng/mL in black tea and 8.6 (8.3–10.4) ng/mL in green tea from Southern Italy. Liu et al.[22] reported an average DEHP range of 10.7 μg kg^{-1} to 353.3 μg kg^{-1} in fresh tea leaves sourced from China. Similarly, Li et al. [23] detected mean DEHP concentrations spanning from ND to 9.34 mg/kg in Chinese tea plantation soils. The utilization of nylon or polyethylene terephthalate (PET) tea bags is linked to the migration of micro- and nanoparticles [24]. These PET tea bags may contribute to the migration of PAEs. The analysis revealed that the mean concentrations of DEP across various tea types ranged from 26.52 (ND-143.36) ng/mL. Among the different tea types, herbal tea samples exhibited the highest average DEP levels (38.50 ng/mL), while e. green tea samples showed the lowest average levels (13.15 ng/mL). Despite these variations, statistical analysis indicated no significant difference in DEP levels among the different tea types (P>0.05). Notably, Alnaimat et al. [15] conducted a study in which DEP was not detected in any of the tea bag infusions that were analyzed. In our study, we found that the average concentrations of DBP varied among various types of tea, ranging from 1807.70 (60.98-5905.38) ng/mL. Notably, herbal tea samples displayed the highest average DBP levels (2400.83 ng/mL), whereas I. Earl Grey tea samples exhibited the lowest average levels (1436.37 ng/mL). The DBP was determined as the most abundant plasticizer in tea samples. It is important to highlight that Commission Regulation No 10/(2011) sets the SML for DBP at 0.3 mg/kg. However, upon thorough assessment of the tea samples, it was observed that the average migration of DBP from tea bags significantly exceeded the established SML value. Alnaimat et al. [15] reported significantly lower values compared to the findings of this study, indicating the presence of DBP in all analyzed tea bag infusions, with concentrations ranging from 12.6 to 51.7 ng/mL. The observed disparity between these two studies is believed to originate from the variance in the materials used to produce tea bags. In this study, the mean DINP levels across distinct tea types were determined to be 941.44 (19.11-2682.09) ng/mL. Among the different tea types, fruit tea samples exhibited the highest average DINP levels (1121.93 ng/mL), while e. black samples showed the lowest average levels (788.93 ng/mL). Similarly, the mean DIDP levels across the various tea types were detected at 104.23 (1.23-526.74) ng/mL. Notably, black tea samples displayed the highest average DIDP levels (145.99 ng/mL), while I. black samples exhibited the lowest average levels (79.89 ng/mL). Despite these variations, the statistical analysis indicated no significant difference in DINP and DIDP levels among the different tea types (P>0.05). According to Commission Regulation No 10/ (2011), the SML for both DINP and DIDP is established at 9 mg kg^{-1}. In this study, the levels of DINP and DIDP in the tea samples were found to be below the established limits. The mean DNOP levels among different tea types were measured at 383.43 (ND-2558.91) ng/mL. The statistical analysis demonstrated no significant variations in DNOP levels across the different types (P>0.05). The highest average DNOP concentration was observed in I. black tea samples (638.96 ng/mL), while the lowest average level was identified in herbal tea samples (147.69 ng/mL). The analysis showed no significant differences in DMP and BBP levels among the different types (P>0.05). The mean DMP and BBP concentrations across various tea types were 3.15 (ND-122.26) ng/mL and 2.19 (ND-61.30) ng/mL, respectively. Notably, the levels of BBP in the tea samples remained below the established limit of 30 mg/kg according to Commission Regulation No 10/(2011). The study highlights the significance of considering sample collection methods, analytical techniques, and regional differences when interpreting results. Variations in PAE concentrations among samples may be attributed to factors like production methods, additives, machinery, and packaging materials. Notably, except for DBP, all tested tea samples in this study remained below the SML defined by Commission Regulation (EU) No 10/2011 for PAEs, confirming their safety for consumers. Nonetheless, continuous monitoring of PAE levels is vital, especially for food-contact plastics, to ensure compliance with safety regulations and minimize potential risks associated with PAE exposure.

Table 3. The PAE's level of different tea samples (ng/mL) (mean±standard deviation)

Tea Types	BBP	DMP	DBP	DEHP	DEP	DINP	DIDP	DNOP
Black	1.27±3.35 (ND-9.55)	<LOD	2249.77±2009.14 (372.30-5905.38)	1015.15±1528.42 (ND-4507.84)	20.79±15.57 (ND-45.36)	1067.54±1116.42 (29.24-2682.09)	145.99±184.04 (6.57-526.74)	553.72±872.40 (ND-2558.91)
I. Black	2.56±4.98 (ND-14.41)	17.19±42.69 (ND-122.26)	1499.84±1560.80 (60.98-4493.06)	1199.62±1164.25 (ND-3267.07)	25.15±49.62 (ND-143.36)	788.93±757.61 (19.32-2066.27)	79.89±101.67 (1.65-258.15)	638.96±679.83 (18.19-1846.78)
Green	7.85±21.60 (ND-61.30)	<LOD	1479.98±1013.33 (299.38-2826.26)	834.80±1289.17 (ND-3930.93)	31.28±39.60 (ND-101.44)	842.01±845.49 (25.51-2265.40)	87.33±106.30 (1.23-261.70)	429.06±739.00 (ND-2231.16)
I. Green	2.01±2.85 (ND-6.79)	<LOD	1685.39±1186.79 (175.81-3097.68)	833.48±932.76 (10.64-3001.58)	13.15±20.36 (ND-61.76)	893.78±828.27 (116.09-2080.09)	99.07±110.02 (5.26-265.31)	426.31±539.29 (0.24-1701.87)
Earl Grey	0.92±0.86 (ND-2.11)	<LOD	2055.37±1742.92 (121.78-4117.24)	806.58±1102.86 (8.07-3311.63)	26.23±19.98 (2.39-58.83)	940.32±845.70 (53.15-2332.40)	105.63±112.05 (2.05-303.20)	420.43±635.14 (1.07-1878.42)
I. Earl Grey	1.12±1.78 (ND-5.32)	2.12±4.01 (ND-9.95)	1436.37±1455.50 (113.17-4287.38)	327.91±313.13 (ND-771.97)	38.01±40.90 (ND-106.71)	895.68±881.01 (19.11-2192.18)	92.33±113.29 (6.23-264.51)	166.58±155.89 (ND-431.34)
Herbal	1.18±1.71 (ND-4.42)	2.69±3.41 (ND-8.02)	2400.83±1725.56 (306.76-4370.22)	327.35±377.29 (ND-866.21)	38.50±33.68 (ND-104.64)	981.31±928.59 (89.23-2333.21)	108.40±116.34 (14.67-281.88)	147.69±159.33 (ND-332.11)
Fruit	0.59±0.86 (ND-2.17)	<LOD	1654.06±1230.09 (292.34-3720.87)	601.42±763.06 (34.02-2333.87)	19.05±27.75 (ND-81.63)	1121.93±932.96 (137.14-2362.95)	115.22±119.86 (5.31-286.08)	284.67±429.04 (18.36-1319.69)
Mean± SD (min. - max.)	2.19±7.91 (ND-61.30)	3.15±15.41 (ND-122.26)	1807.70±1478.86 (60.98-5905.38)	743.29±1001.46 (ND-4507.84)	26.52±32.17 (ND-143.36)	941.44±852.80 (19.11-2682.09)	104.23±117.50 (1.23-526.74)	383.43±571.51 (ND-2558.91)

ND, Not Detected; I, Import

Health Risk Assessment

Hazard Quotient (HQ) and Hazard Index (HI)

The analysis presented in Table 4 and Table 5 offers valuable insights into the non-carcinogenic health risks linked to tea consumption across different age groups. Notably, all calculated HQ and HI values for BBP, DEP, DBP, DEHP, and DINP were found to be below 1, indicating that the levels of these substances in tea do not pose significant non-carcinogenic health risks to the population (USEPA, 2019). Population exposure to DEP, DBP, DEHP, and DINP from various tea brands and types in Turkey was assessed using both average and P95 sample amounts, as shown in Table 4. When comparing the results with the TDI values recommended by EFSA [25] 0.5, 0.5, 0.15, 0.05, and 0.01 mg/kg/day for BBP, DEP, DINP, DEHP, and DBP, respectively. it becomes evident that the exposure levels for all age groups remain below the established TDI values. These findings suggest that consuming tea available in the Turkish market does not pose a health risk. While limited researchers have explored tea consumption-related exposure assessments, a study by Li et al.[23] revealed that the mean HQ associated with DEHP, DiBP, DOP, DEP, and DMP were all below 1. Additionally, Alnaimat et al. [15] reported that HQ values were consistently below 1 as well. This comprehensive evaluation contributes to a more accurate understanding of potential health risks related to PAE exposure from various food sources.

Table 4. Consumers exposure to PAEs from different tea consumption (mean±standard deviation)

Analytes	Age (years)	Female				Male			
		Exposure (µg/kg Bw/day)	P95	P50 (Median)	P5	Exposure (µg/kg Bw/day)	P95	P50 (Median)	P5
BBP	15-18	0.01±0.00	0.02	2.96E-03	6.18E-03	0.01±0.00	0.03	4.10E-03	0.00
	19-64	0.01±0.01	0.05	0.01	8.10E-03	0.02±0.01	0.06	0.01	8.94E-03
	>65	0.01±0.01	0.03	0.01	8.58E-03	0.01±0.01	0.04	0.01	6.82E-03
DBP	15-18	4.36±0.85	5.80	4.03	3.47	6.05±1.18	8.04	5.59	4.81
	19-64	11.52±1.24	15.31	10.64	9.16	12.72±2.47	16.89	11.75	10.11
	>65	7.72±1.50	10.25	7.13	6.13	9.70±1.89	12.89	8.96	7.71
DEHP	15-18	1.79±0.48	2.90	1.98	0.79	2.49±0.67	4.02	2.75	1.10
	19-64	4.74±1.28	7.65	5.23	2.09	5.23±1.41	8.44	5.77	2.30
	>65	3.17±0.86	5.12	3.50	1.40	3.99±1.08	6.44	4.40	1.76
DEP	15-18	0.06±0.02	0.09	0.06	0.03	0.09± 0.03	0.13	0.09	0.06
	19-64	0.17±0.05	0.24	0.16	0.08	0.19±0.05	0.27	0.18	0.03
	>65	0.11±0.03	0.16	0.11	0.06	0.14±0.04	0.20	0.14	0.07
DINP	15-18	2.27±0.21	2.71	2.21	1.90	3.15±0.29	3.75	3.07	2.64
	19-64	6.00±0.55	7.15	5.85	5.02	6.62±0.60	7.89	6.46	5.55
	>65	4.02±0.37	4.79	3.92	3.36	5.05±0.46	6.02	4.92	4.23

Table 5. Estimation of HQ, HI and CR values of PAEs exposure due to tea consumption

Analytes	Age groups (years)	Consumers exposure			
		Female		Male	
		HQ	CR	HQ	CR
BBP	15-18	2.64E-05	1.00E-05	3.66E-05	1.39E-05
	19-64	6.97E-05	2.65E-05	7.70E-05	2.92E-05
	>65	4.67E-05	1.77E-05	5.87E-05	2.23E-05
DEHP	15-18	8.97E-02	2.51E-02	0.12	3.48E-02
	19-64	2.37E-01	6.63E-02	0.26	7.32E-02
	>65	1.59E-01	4.44E-02	1.99E-01	5.59E-02
DEP	15-18	7.95E-05	*	1.10E-04	*
	19-64	2.10E-04	*	2.32E-04	*
	>65	1.41E-04	*	1.77E-04	*
DINP	15-18	2.84E-03	*	3.94E-03	*
	19-64	7.50E-03	*	0.01	*
	>65	5.02E-03	*	6.31E-03	*
DBP	15-18	4.36E-02	*	0.06	*
	19-64	1.15E-01	*	0.13	*
	>65	7.72E-02	*	0.10	*
HI	15-18	0.14		0.19	
	19-64	0.36		0.40	
	>65	0.24		0.30	

*Not calculated for CR; HQ, Hazard Quotient

Carcinogenic Risk (CR) Assessment

CR indicates a risk assessment and classification system developed by the US Environmental Protection Agency [19]. In this system, grade A (CR>1x10^{-4}) indicates high cancer risk, grade B (CR: 1x10^{-6} to 1x10^{-5}) indicates acceptable cancer risk, and grade C (CR<1x10^{-6}) indicates a negligible cancer risk. In this context, carcinogenic risk assessment among age groups were calculated for DEHP and BBP, whose CSF values were given by the authorities. The carcinogenic risk assessment results for tea consumers of different ages are summarized in Table 5. Carcinogenic risk factors for BBP in age and gender groups consuming tea are in the range of 1.00x10^{-5}- 2.92x10^{-5}. So, the carcinogenic risk factor for BBP was determined at the acceptable cancer risk level (grade B) for all age and gender groups. Furthermore, the carcinogenic risk assessment for DEHP among different age and gender groups consuming tea revealed a range of 2.51x10^{-2} to 7.32x10^{-2}. As a result, the carcinogenic risk factor for DEHP was classified at a high cancer risk level (Grade A) across all age and gender categories. In the context of the existing literature, only one study was identified concerning the estimated cancer risk factor in tea samples. Li et al. [23] conducted a study where they determined that the average cancer risk (CR) value for DEHP through dietary exposure surpassed the threshold of 10^{-6}, measuring specifically at 7.20×10^{-6} for adults and 2.84×10^{-6} for children. These findings raise concerns regarding the potential carcinogenic risk associated with DEHP exposure. In contrast, the present study focused on the consumption of steeped tea from tea bags and indicated an acceptable level of cancer risk.

CONCLUSION

This study provides valuable insights into the presence of plasticizers (specifically, phthalate esters or PAEs) and their potential impact on consumers in tea samples within Turkey. The findings indicated detectable levels of PAEs in the tea samples, although tested compounds except for DBP remained below the SML established by the EU Regulation. Among the analyzed PAEs, DINP, and DBP were identified as the most prevalent plasticizers in the tea samples. To assess potential health risks, researchers calculated HQ and HI values based on tea consumption patterns across various age and gender groups. The results revealed that all HQ and HI values for DEHP, DINP, DEP, and DBP were below 1, indicating minimal non-carcinogenic health risks associated with tea consumption across different age groups. Furthermore, the dietary intake levels of each phthalate ester, including BBP, DEHP, DINP, DEP, and DBP, were found to be well below the TDI values established by the EFSA. The study offers reassurance that the detected concentrations of PAEs in tea samples, whether from local or international brands in Turkey, do not pose a health risk to consumers. However, it does raise concerns about the possibility of long-term consumption of packaged tea leading to PAE exposure exceeding TDI values. Notably, packaged tea products generally exhibited slightly elevated PAE levels, resulting in marginally increased exposure for consumers of such products. In conclusion, this research underscores the significance of ongoing monitoring and regulatory measures to ensure the safety of packaged tea and mitigate potential risks associated with long-term PAE exposure.

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Araştırma Makalesi / Research Paper

Ketencik Proteinlerinin Bazı Tekno-Fonksiyonel Özellikleri Üzerine Ultrasonikasyon Uygulamasının Etkisi

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ÖZ

Bu araştırmada, farklı güçlerdeki ultrasonikasyon uygulamasının ketencik (*Camelina sativa* (L.) Crantz) proteinlerinin tekno-fonksiyonel özelliklerine etkisinin belirlenmesi amaçlanmıştır. Alkali ekstraksiyon (pH 12) ve izoelektrik noktada çöktürme (pH 4.5) yöntemi kullanılarak elde edilen ketencik proteinlerine beş farklı ultrasonikasyon (20 kHz) gücünde (0, 50, 100, 150 ve 200 W) işlem uygulanmış ve proteinlerin su bağlama, yağ bağlama, köpürme ve emülsiyon özelliklerindeki değişimler incelenmiştir. Uygulanan ultrasonikasyon işlemlerinin ketencik proteinlerinin su bağlama kapasitelerinde %251.07-500.03 aralığında gerçekleşen bir azalışa, yağ bağlama kapasitelerinde ise %14.10-21.21 aralığında gerçekleşen bir artışa neden olduğu belirlenmiştir. Ayrıca en yüksek köpürme kapasitesi (%53.59) ve köpürme stabilitesi değerleri (15. ve 30. dakikalar için sırasıyla %42.20 ve %40.71) 200 W gücünde ultrasonikasyon işlemi ile üretilen protein örneğinde tespit edilmiştir. Elde edilen sonuçlara göre ketencik proteinlerinin bazı tekno-fonksiyonel özelliklerinin geliştirilmesinde ultrasonikasyon işleminin kullanım potansiyeli ortaya koyulmuştur.

Anahtar Kelimeler: *Camelina sativa*, Ultrasonikasyon, Köpürme, Emülsiyon

Effect of Ultrasonication on Some Techno-functional Properties of *Camelina sativa* Proteins

ABSTRACT

In this study, it was aimed to determine the effect of ultrasonication at different powers on the techno-functional properties of *Camelina sativa* proteins. Five different ultrasonication (20 kHz) powers (0, 50, 100, 150, and 200 W) were applied to the proteins of *Camelina sativa* (*Camelina sativa* (L.) Crantz), that were obtained by alkaline extraction (pH 12.0) and isoelectric point precipitation (pH 4.5) methods, and the changes in water holding, oil holding, foaming and emulsification properties of proteins were determined. It was determined that the ultrasonication process caused a decrease in the water binding capacity of *Camelina sativa* proteins between 251.07-500.03%, and an increase in the oil binding capacity between 14.10-21.21%. Moreover, the highest foaming capacity (53.59%) and foaming stability values (42.20% for the 15 min and 40.71% for the 30 min) were determined in the protein sample produced with 200 W ultrasonication. According to the results, it was determined that ultrasonication could be potentially used to improve some techno-functional properties of *Camelina sativa* proteins.

Keyword: *Camelina sativa*, Ultrasonication, Foaming, Emulsification

GİRİŞ

Brassicaceae familyasına ait bir protein ve yağlı tohum bitkisi olan ketencik (*Camelina sativa* (L.) Crantz) [1] türlerine, yetiştirildiği ortamdaki toprak bileşimi ve çevresel faktörlere bağlı olarak yaklaşık %30-38 yağ ve %23-30 protein içermektedir [2].

Beslenmede çok önemli bir rol oynayan proteinlerin farklı kaynaklardan izole edilmeleri ile ilgili çeşitli çalışmalar yapılmakta olup, günümüzde bitkisel proteinlerin daha kolay elde edilebilmeleri, ekonomik ve sürdürülebilir olmaları gibi nedenlerle hayvansal kaynaklı proteinlere göre daha fazla ilgi gördüğü belirtilmektedir [3]. Yapısında albüminler, globulinler ve glutelinler dahil olmak üzere çeşitli çözünürlüklere sahip protein fraksiyonlarını bulunduran [4] ve yağı ayrıldıktan sonra protein içeriği yaklaşık %45 değerine kadar artış gösteren ketencik tohumlarının ise bitkisel bir protein kaynağı olarak gıda endüstrisinde değerlendirilme potansiyeli bulunmaktadır [3]. Ayrıca protein kaynakları gıda ürünlerinin besleyici değerinde artış sağlarken, ilave edildiği ürünün bazı fonksiyonel özellikleri üzerinde de etkili olmaktadır. Proteinlerin sahip olduğu su ve yağ bağlama kapasiteleri, çözünürlükleri, jelleşme, emülsiyon ve köpük oluşturma gibi fonksiyonel özellikleri ilave edildiği ürünler açısından önemli olabilmektedir. Bu özellikler proteinlerin konformasyonel yapılarından etkilenmekte olup, genel olarak doğal bitki proteinleri yapısal özellikleri nedeniyle gıda ürünlerinde uygun jelleşme veya emülsiyon oluşumu sağlayamamaktadır. Bu nedenle bitkisel kaynaklı proteinlerin belirtilen özelliklerinin geliştirilmesi amacıyla farklı fiziksel, kimyasal ve enzimatik işlemler uygulanmaktadır [5].

Ultrasonikasyon tekniği proteinlerde yapısal ve fonksiyonel değişikliklere neden olan ve bu sayede proteinlerin tekno-fonksiyonel özelliklerinin iyileştirilmesinde kullanılan bir uygulamadır [5]. Ultrason teknolojisi, insan duyma eşiğinin üzerinde frekanslar üreten akustik dalgalara dayalı bir teknoloji olup, 1 W/cm^2 değerinin altında güce sahip düşük yoğunluklu (100 kHz-1 MHz) ve 10-1000 W/cm^2 aralığında güce sahip yüksek yoğunluklu (20-100 kHz) ultrason olmak üzere iki farklı uygulama şekli bulunmaktadır [6]. Yüksek yoğunluklu ultrasonikasyon uygulamasının moleküller arası hidrofilik ve hidrofobik etkileşimleri değiştirdiği, proteinlerin uzamsal konformasyonunu açabildiği, protein agregratlarını parçalayabildiği, parçacık boyutunu azaltabildiği ve akustik kavitasyon tarafından üretilen fiziksel, kimyasal ve termal etkilere bağlı olarak proteinlerin ikincil ve üçüncül yapılarını tahrip edebildiği bildirilmiştir. Bu değişimlere bağlı olarak ise proteinlerin fonksiyonel özelliklerinde farklılaşma meydana geldiği ve proteinlerin su ve yağ arayüzüne kolayca adsorbe olabildiği ve yağ damlacıklarını birleşmeye karşı stabilize edebildiği belirtilmiştir [5].

Literatürde kolza tohumu [7], ayçiçeği [6], siyah mercimek [8], soya [9] ve demirhindi [10] gibi farklı bitkisel tohumlardan izole edilen proteinlerin ultrasonikasyon tekniği ile tekno-fonksiyonel özelliklerinin geliştirilmesini konu alan çeşitli çalışmalar gerçekleştirilmiştir. Ancak bu uygulamanın ketencik tohumu proteinleri açısından etkilerinin araştırıldığı bir araştırmaya rastlanmamıştır. Bu nedenle ilgili çalışmada; ketencik tohumu proteinlerinin izole edilmesi ve izole edilen proteinlerin farklı güçlerde uygulanan ultrasonikasyon işlemi ile su ve yağ bağlama, köpük ve emülsiyon oluşturma gibi tekno-fonksiyonel özelliklerindeki değişimin belirlenmesi amaçlanmıştır. Böylece çeşitli gıda ürünlerinde kullanılmak üzere gıda endüstrisi için önemli olan yağ bağlama kapasitesi, köpürme kapasitesi ve köpürme stabilitesi gibi tekno-fonksiyonel özellikleri geliştirilmiş alternatif bir bitkisel protein kaynağı elde edilmiş olacaktır.

MATERYAL ve METOT

Materyal

Araştırmada kullanılan ketencik tohumları ve ayçiçek yağı piyasadan temin edilmiştir. Kimyasallar malzemeler ise analitik saflıkta Honeywell (Hessen, Almanya) ve Sigma-Aldrich (Taufkirchen, Almanya) firmalarından temin edilerek kullanılmıştır.

Metot

Ketencik Tohumundan Protein Eldesi

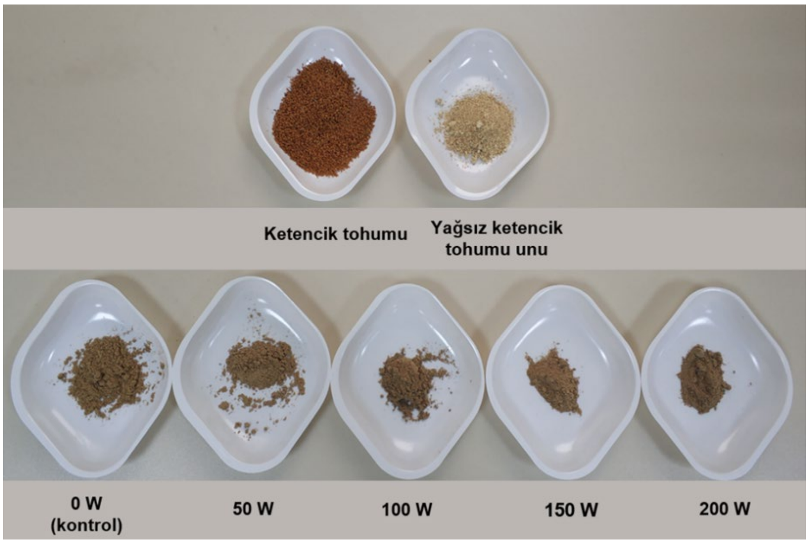
Ketencik tohumlarından protein eldesi Ngo ve Shahidi [3] tarafından bildirilen alkali ekstraksiyon ve izoelektrik noktada çöktürme metodunda bazı modifikasyonlar yapılarak gerçekleştirilmiştir. Ketencik tohumları protein ekstraksiyonu işleminden önce öğütülerek (SCM-2910, Sinbo, Çin) 1 mm gözenek çapına sahip elekten geçirilmiştir. Elenen materyalin yağının uzaklaştırılması amacıyla materyal üzerine 1:5 oranında hekzan ilave edilmiş ve oda sıcaklığında 1 saat süresince 150 rpm hızla bir çalkalayıcıda (Open air shaker OS-4000, Jeio Tech, Güney Kore) dairesel olarak karıştırılmıştır. Süre sonunda filtre kağıdından süzülen materyalin katı kısmı ayrılmış ve katı kısımda kalan hekzan buharlaştırılarak uzaklaştırılmıştır. Katı kısımda yağ içeriğinin varlığının anlaşılması amacıyla Soxhlet yöntemi ile yağ ekstraksiyonu yapılarak (AACC Metot 30-25) örneklerin yağının tamamen uzaklaştırılmış olduğu doğrulanmıştır [11]. Elde edilen yağsız kısım protein ekstraksiyonunda kullanılmıştır. Ketencik tohumunun protein içeriği %29.78 ve yağının uzaklaştırılması sonucu elde edilen ketencik küspesinin protein içeriği ise %36.97 olarak belirlenmiştir.

Öğütülen ve yağı ayrıştırılan ketencik tohumlarından 25 g tartılarak üzerine 750 mL saf su ilave edilmiş ve pH 12.0 değerine 2 M NaOH çözeltisi ile pH metre (Orion 3-Star, Thermo Scientific, ABD) kullanılarak ayarlanmıştır. Bu karışım 1 saat süresinde manyetik karıştırıcı üzerinde 800 rpm hızla oda sıcaklığında karıştırılmış ve süre sonunda 5800 rpm hızla 10 dakika santrifüj (Benchtop centrifuges C2006, Centurion, İngiltere) edilerek sıvı kısım toplanmıştır. Ayrılan sıvı kısmın pH değeri 4.5 olarak 2 M HCl çözeltisi ile yeniden ayarlanmıştır. pH değerindeki değişime bağlı olarak çöken protein fraksiyonunun ayrılması amacıyla karışım 5800 rpm hızla 10 dakika santrifüj edilmiş ve üstteki sıvı kısım uzaklaştırılarak çöken katı kısımlar birleştirilmiştir. Katı kısım üzerine 100 mL saf su ilave edilmiş ve pH değeri 2 M NaOH çözeltisi kullanılarak 7.0 değerine ayarlanmıştır.

Elde edilen protein ekstraktlarından kontrol örneği olarak kullanılan örneklere ultrasonikasyon uygulaması yapılmamış ve bu örneklere doğrudan kurutma işlemi uygulanmıştır. Bu amaçla protein ekstraktları tepsi üzerine yayılarak vakum kurutucuda (Oven ON-11E, Jeio Tech, Güney Kore) 45°C sıcaklıkta ve 200 mbar basınçta kurutulmuştur. Kurutma işleminin ardından elde edilen örnekler öğütülmüş ve analiz edilinceye kadar -18°C sıcaklıkta hava geçirmeyen amber renkli bir kap içerisinde tutulmuştur. Belirtilen şartlarda elde edilen ketencik protein tozlarının protein içeriği %64.01 olarak tespit edilmiştir.

Ultrasonikasyon Uygulaması

Protein ekstraktlarına yapılan ultrasonikasyon işleminin koşulları literatürde belirtilen farklı bitkisel kaynaklı proteinlere uygulanan koşullar dikkate alınarak ve yapılan ön denemeler sonucunda belirlenmiştir. Buna göre pH değeri 7.0 olarak ayarlanan örneklere 4°C sıcaklığındaki ortam içerisinde 45 dakika süresince 50, 100, 150 ve 200 W olmak üzere 4 farklı düzeyde 13 mm çapında prob aracılığıyla ve probun örnek içerisine 3 cm derinliğinde olacak şekilde daldırılmasıyla ultrasonikasyon (Sonopuls HD 2200.2, Bandelin, Almanya) işlemi uygulanmıştır [5, 6, 12]. Sıcaklık ultrasonikasyon işlemi süresince takip edilmiş ve sıcaklığın sabit tutulması amacıyla soğutucu sistem (RW3-0525, Lab Companion, Jeio Tech, Güney Kore) kullanılmıştır. Ultrasonikasyon işleminden sonra elde edilen örneklerde bulunan suyun uzaklaştırılması amacıyla kontrol örneğine uygulanan koşullarda vakum altında kurutma işlemi gerçekleştirilmiştir. Kurutma işleminin ardından elde edilen örnekler öğütülmüş ve analiz edilinceye kadar -18°C sıcaklıkta hava geçirmeyen amber bir kap içerisinde tutulmuştur. Araştırma kapsamında kullanılan ketencik tohumu, yağsız ketencik tohumu unu ile ultrasonikasyon işlemi uygulanmamış (kontrol) ve farklı ultrasonikasyon güçlerinde işlem uygulanmış ketencik proteinlerine ait görsel Şekil 1'de verilmiştir.



Şekil 1. Ketencik tohumu, yağsız ketencik tohumu unu ve ketencik proteinleri
Figure 1. Camelina sativa seed, Camelina sativa meal and Camelina sativa proteins

Ham Protein Analizi

Örneklerin ham protein içerikleri, AACC Metot 46-12'de belirtilene göre Kjeldahl yöntemi ile belirlenmiştir [11].

Su ve Yağ Bağlama Kapasitesi Analizleri

Örneklerin su bağlama kapasitesini belirlemek amacıyla 1 g örnek tartılmış ve üzerine 10 mL su ilave edilerek 5 dakika süresince girdap karıştırıcı (Vortex mixer M10110002, Four E'S Scientific, Çin) ile karıştırılmıştır. Bu karışım oda sıcaklığında 30 dakika bekletildikten sonra 4500 rpm hızla 30 dakika santrifüj edilmiş ve sıvı kısım uzaklaştırılmıştır. Elde edilen katı kısım tartılarak aşağıda verilen eşitlik yardımıyla örneklerin su bağlama kapasiteleri % olarak hesaplanmıştır [6].

Örneklerin yağ bağlama kapasitesini belirlemek amacıyla 1 g örnek tartılmış ve üzerine 10 mL ayçiçek yağı ilave edilerek 5 dakika süresince girdap karıştırıcı ile karıştırılmıştır. Bu karışım oda sıcaklığında 30 dakika bekletildikten sonra 4500 rpm hızla 30 dakika santrifüj edilmiş ve ayrılan yağ kısmı uzaklaştırılmıştır. Elde edilen katı kısım tartılarak aşağıda verilen eşitlik yardımıyla örneklerin yağ bağlama kapasiteleri % olarak hesaplanmıştır [6].

$$Su\ veya\ yağ\ bağlama\ kapasitesi\ (\%) = \frac{Son\ tartım - Dara}{Örnek\ miktarı} x\ 100 \qquad \text{Eşitlik 1}$$

Köpürme Kapasitesi ve Stabilitesi Analizleri

Köpürme kapasitesi ve stabilitesi analizleri için %3 konsantrasyonundaki protein çözeltisi saf su kullanılarak hazırlanmıştır. Hazırlanan bu çözelti bir mezüre aktarılarak başlangıç hacmi (V_1) belirlendikten sonra bir karıştırıcı (8011ES, Blender, ABD) içerisinde 5 dakika süresince karıştırılmış ve hemen bir mezüre aktarılarak toplam hacmi (V_2) kaydedilmiştir. Ardından örneklerin 15. ve 30. dakikalardaki köpük hacimleri (V_3) belirlenmiştir. Elde edilen veriler kullanılarak aşağıdaki eşitlikler yardımıyla örneklerin köpürme kapasite ve stabilite değerleri % olarak hesaplanmıştır [6].

$$Köpürme\ kapasitesi\ (\%) = \frac{V_2-V_1}{V_1} x\ 100$$ Eşitlik 2

$$Köpürme\ stabilitesi\ (\%) = \frac{V_3}{V_1} x\ 100$$ Eşitlik 3

Emülsiyon Kapasitesi ve Stabilitesi Analizleri

Emülsiyon kapasitesi ve stabilitesi analizleri için %3 konsantrasyonundaki protein çözeltisi saf su kullanılarak hazırlanmış ve üzerine ayçiçek yağı (1:1) ilave edilerek toplam hacim (V_1) belirlenmiştir. Hazırlanan bu karışım 90 saniye süresince karıştırıcı içerisinde karıştırılmıştır. Karıştırma işlemi sonunda karışıma 3500 rpm hızla 5 dakika santrifüj uygulanmış ve emülsiyon kısmının hacmi (V_2) ölçülmüştür. Bu işlemden sonra karışımlar 80°C sıcaklıktaki etüv içerisinde 30 dakika bekletilmiş ve 3500 rpm hızla 5 dakika santrifüj işlemi uygulanarak emülsiyon kısmının hacmi yeniden (V_3) ölçülmüştür. Elde edilen veriler kullanılarak aşağıdaki eşitlikler yardımıyla örneklerin emülsiyon kapasite ve stabilite değerleri % olarak hesaplanmıştır [6].

$$Emülsiyon\ kapasitesi\ (\%) = \frac{V_2}{V_1} x\ 100$$ Eşitlik 4

$$Emülsiyon\ stabilitesi\ (\%) = \frac{V_3}{V_2} x\ 100$$ Eşitlik 5

İstatistiksel Analiz

Araştırmadaki üretimler ve uygulamalar tekerrürlü ve analizler ise paralelli olarak gerçekleştirilmiştir. Elde edilen veriler örnek kuru maddesi üzerinden hesaplanmış ve Minitab (ver. 17.0) kullanılarak verilere varyans analizi ve Tukey Çoklu Karşılaştırma Testi uygulanmıştır. Sonuçlar ise “ortalama ± standart hata” şeklinde düzenlenmiştir

BULGULAR ve TARTIŞMA

Ultrasonikasyon Uygulamasının Ketencik Proteinlerinin Su ve Yağ Bağlama Kapasitelerine Etkisi

Ultrasonikasyon uygulamasının ketencik proteinlerinin su ve yağ bağlama kapasitesi değerleri üzerine etkisi Tablo 1’de gösterilmiştir. Su bağlama kapasitesi proteinlerin yerçekimine karşı suyu bağlama yeteneğinin bir göstergesi olup, bu değer proteinlerin gıda sistemleri içerisinde sağladıkları viskozite ve tekstür özellikleriyle yakından ilişkili olması nedeniyle önem taşımaktadır [13, 14]. Proteinler için önemli bir tekno-fonksiyonel özellik olan su bağlama kapasitesi değeri ketencik proteinleri için %691.07 olarak belirlenmiştir. Ngo ve Shahidi [3] tarafından benzer bir ekstraksiyon yöntemi kullanılarak elde edilen ketencik proteinlerinin su bağlama kapasiteleri %562 olarak tespit edilmiştir. Protein ekstraksiyonundan önce tohumundaki musilaj yapısının ekstraksiyonunun gerçekleştirildiği farklı bir araştırmada ise ketencik proteinlerinin su bağlama kapasitesinin %158 olduğu bildirilmiştir [2]. Ketencik proteinlerinin su bağlama kapasitelerindeki bu farkın kullanılan hammaddeler arasındaki farklılıklardan ve protein ekstraksiyonundan önce tanedeki musilaj yapısının ekstraksiyonu sayesinde protein örneklerinde bulunan yüksek su bağlama kapasitesine sahip musilaj kalıntısının daha az olmasından kaynaklanabileceği değerlendirilmiştir.

Uygulanan ultrasonikasyon işlemleri ketencik proteinlerinin su bağlama kapasitesinde önemli bir azalmaya neden olmuştur (p<0.05). Benzer şekilde, Malik ve ark. [6] da ayçiçeği küspesi proteinlerine 20 kHz frekansa sahip (500 W ve %25 genlik) ultrasonik prob ve 40 kHz (500 W) frekanstaki ultrasonik banyo kullanarak farklı sürelerde (5, 10, 20 ve 30 dakika) uyguladıkları işlemlerin örneklerin su bağlama kapasitesinde önemli bir azalmaya neden olduğunu bildirmişlerdir. Ayrıca ketencik proteinlerinin su bağlama kapasitesindeki söz konusu azalmanın ultrasonikasyon işleminin gücünün artmasıyla beraber (50, 100, 150 ve 200 W) sırasıyla %251.07, %354.59, %443.09 ve %500.03 düzeyinde olduğu tespit edilmiştir. Resendiz-Vazquez ve ark. [15] tarafından jak meyve çekirdeği proteinlerine 0, 200, 400 ve 600 W güçlerinde 15 dakika (5 saniye açık, 25 saniye kapalı) süresince uygulanan ultrasonikasyon işlemi neticesinde proteinlerin %335 olan su tutma kapasitesinin ultrasonikasyon gücünün artmasıyla beraber azalan bir eğilim (200 W için %320, 400 W için %199, 600 W için %188) gösterdiği bildirilmiştir [15]. Araştırmacılar, uyguladıkları ultrasonikasyon işlemlerinin proteinlerin molekül yapısında değişime neden olarak yüzeydeki hidrofobik yapıların artması sonucu örneklerin su tutma kapasitesini azaltabileceğini belirtmişlerdir [6–16].

Proteinlerin yağ bağlama özelliği, formüle gıdalarda (mayonez ve salata sosları, et anologları, çorbalar vd.) lezzetin artmasını ve muhafaza edilmesini sağlaması sebebiyle büyük bir öneme sahiptir [17–19]. Buna göre işlem görmemiş ketencik proteinlerinin yağ bağlama kapasitesi %110.48 olarak belirlenmiştir (Tablo 1). Yapılan bir araştırmada alkali ekstraksiyon ile izoelektrik noktada çöktürme yöntemi kullanılarak elde edilen ketencik proteinlerinin yağ bağlama kapasitesinin %107 olduğu bildirilmiştir [2].

Tablo 1. Ketencik proteinlerinin su bağlama kapasitesi ve yağ bağlama kapasitesi değerleri

Table 1. Water holding capacity and oil holding capacity values of Camelina sativa proteins

Ultrasonikasyon gücü (W)	Su bağlama kapasitesi	Yağ bağlama kapasitesi
0 (kontrol)	691.07± 9.72 ^{A}	110.48±2.97 ^{B}
50	440.00±17.50 ^{B}	124.58±5.74 ^{AB}
100	336.48±5.26 ^{C}	131.69±1.85 ^{A}
150	257.98±1.33 ^{D}	129.78±3.98 ^{A}
200	191.84±3.42 ^{E}	128.90±1.16 ^{A}

*Aynı sütun içindeki farklı harfler, ortalamalar arasındaki farkın istatistiksel açıdan önemli olduğunu göstermektedir (p<0.05).

** Capital letters show statistically significant difference between values with different ultrasonication power (p<0.05).*

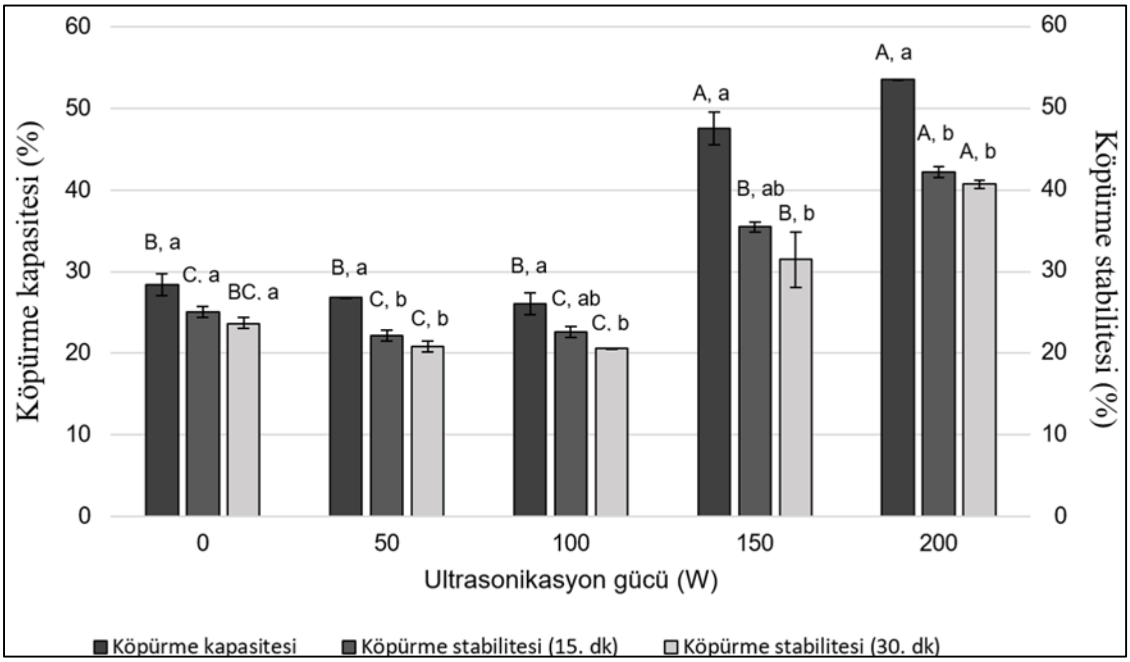
Ultrasonikasyon uygulamasının ketencik proteinlerinin yağ bağlama kapasitelerinde %14.10-21.21 aralığında gerçekleşen istatistiksel olarak önemli bir artışa neden olduğu değerlendirilmiş ($p<0.05$), ancak bu artışın uygulanan ultrasonikasyon gücüyle ilişkilendirilebilir bir düzeyde olmadığı belirlenmiştir ($p>0.05$). Resendiz-Vazquez ve ark. [15] farklı güçlerde uyguladıkları ultrasonikasyon işleminin jak meyve çekirdeği proteinlerinin yağ bağlama kapasitesini %192 değerinden %322 (600 W) ile %401 (400 W) aralığına artırdığını bildirmişlerdir. Bir diğer araştırmada ise ayçiçeği küspesi proteinlerine uygulanan ultrasonikasyon işleminin örneklerin yağ bağlama kapasitelerinde önemli bir artışa neden olduğu rapor edilmiştir [6]. Araştırmacılar, ultrasonikasyon uygulamasının proteinlerin kümeleşmesine neden olarak yüzeyde açığa çıkan hidrofobik grupların fiziksel olarak daha fazla yağın tutulmasını sağlayabileceğini belirtmişlerdir [6, 15]. Ayrıca protein yapısında oluşabilecek bu değişimlerin; proteinin doğal özellikleri ile ultrasonikasyon kaynağına, yoğunluğuna, frekansına, süresine, ve uygulama esnasındaki pH, sıcaklık ve iyonik kuvvetlere bağlı olarak gerçekleşebileceğini bildirmişlerdir [15, 20]. Benzer şekilde Ngo ve Shahidi [3] tarafından ketencik küspesinden protein eldesinde ultrasonikasyon destekli ekstraksiyon uygulaması (180 W, 40 kHz ve 20 dakika) ile elde edilen proteinlerin yağ bağlama kapasitesinde %29.52 düzeyinde bir artış meydana geldiği rapor edilmiştir. Araştırmacılar bu artışın ultrasonikasyon uygulaması sonucu protein yüzeyinde hidrofobik grupların açığa çıkmasından kaynaklanabileceğini ifade etmişlerdir [3].

Ultrasonikasyon Uygulamasının Ketencik Proteinlerinin Köpürme Özelliklerine Etkisi

Proteinlerin su ve hava arayüzündeki yüzey gerilimini azaltma yeteneği ile ilişkili olan köpürme kapasitesi ve stabilitesi özellikleri proteinlerin yüzey hidrofobikliği, parçacık boyutu ve yapısal esnekliği gibi özellikleriyle güçlü bir şekilde bağlantılıdır [3, 6]. Çeşitli gıda ürünlerine (çırpılmış tatlılar ve soslar gibi) arzu edilen dokusal ve duyusal özellikleri kazandırması nedeniyle proteinlerin köpürme özellikleri ve bu özelliklerin geliştirilmesi oldukça önemlidir [21]. Buna göre ultrasonikasyon uygulamasının ketencik proteinlerinin köpürme kapasitesi ve stabilitesi değerleri üzerine etkisi Şekil 2'de gösterilmiştir. Ultrasonikasyon işlemi uygulanmamış ketencik proteinlerinin köpürme kapasitesi değeri %28.39 olarak belirlenirken, 15. ve 30. dakikadaki köpürme stabilitesi değerleri ise sırasıyla %25.00 ve %23.65 olarak tespit edilmiştir. Ayrıca ketencik proteinlerinin köpürme kapasitesi değerinde 30 dakikalık süre içerisinde meydana gelen değişim istatistiksel olarak önemli bulunmamıştır ($p>0.05$). Yapılan bir araştırmada da benzer ekstraksiyon yöntemi kullanılarak elde edilen ketencik proteinlerinin köpürme kapasitesi ve 10. dakikadaki köpürme stabilitesi değerlerinin %40 ile %50 aralığında değiştiği tespit edilmiştir [3]. Kullanılan hammaddeler arasındaki farklılıklar ile analiz yöntemi farklılıklarının ketencik proteinlerinin köpürme özelliklerindeki söz konusu farka neden olabileceği öngörülmektedir.

Düşük güçlerde (50 ve 100 W) uygulanan ultrasonikasyon işleminin ketencik proteinlerinin köpürme kapasitesi ve köpürme stabilitesi değerlerinde önemli bir değişime neden olmadığı ($p>0.05$) belirlenmiştir. Ayrıca 150 ve 200 W güçlerinde ultrasonikasyon uygulaması ile birlikte ketencik proteinlerinin köpürme kapasitesinin sırasıyla %47.51 ve %53.59 değerlerine yükseldiği görülmüştür ($p<0.05$) (Şekil 2). Benzer şekilde ketencik proteinlerinin 15. ve 30. dakikalardaki köpürme stabilitesi değerlerinin de yüksek güçlerdeki ultrasonikasyon uygulaması ile önemli düzeyde arttığı belirlenmiştir ($p<0.05$). En yüksek köpürme stabilitesi değeri, 200 W gücünde ultrasonikasyon işlemi uygulanmış protein örneğinde tespit edilmiş olup, bu değerler 15. dakika için %42.20 ve 30. dakika için ise %40.71 olarak tespit edilmiştir. Ayrıca işlem görmemiş protein örneği ile karşılaştırıldığında, 200 W gücünde ultrasonikasyon uygulamasının ketencik proteinlerinin köpürme stabilitesinde %17.20 ve %17.06 düzeylerinde bir artışa neden olduğu belirlenmiştir. Diğer yandan ultrasonikasyon işlemi ile ketencik proteinlerinin 30 dakikalık süre içerisinde köpürme kapasitesi değerinin istatistiksel olarak önemli düzeyde azaldığı ve bu azalmanın %5.49-16.06 aralığında gerçekleştiği tespit edilmiş ($p<0.05$), ancak bu değişimin uygulanan ultrasonikasyon gücüyle ilişkilendirilebilir düzeyde olmadığı değerlendirilmiştir ($p>0.05$).

Jambrak ve ark. [12], 20 kHz frekansa sahip ultrasonik prob ile 30 dakika süresince uyguladıkları ultrasonikasyon işlemi sonucu soya proteinlerinin köpürme kapasitesi değerinin %95'ten %104'e arttığını tespit etmişlerdir. Morales ve ark. [22] ise soya proteinlerine farklı sürelerde (5, 10, 15 ve 20 dakika) uyguladıkları ultrasonikasyon işlemi (20 kHz, 750 W ve %20 genlik) ile örneklerin köpürme kapasitesi değerlerinin %62 (5. dakika) ile %75 (20. dakika) aralığında arttığını belirtmiştir. Benzer şekilde jak meyve çekirdeği proteinlerinin de köpürme kapasitesi değerlerinin ultrasonikasyon uygulaması sonucu önemli düzeyde arttığı bildirilmiştir [15]. Nazari ve ark. [23] darı proteinlerine farklı ultrasonikasyon güçleri (0, 20, 60 ve 100 W) ve sürelerinde (5, 12.5 ve 20 dakika) ultrasonikasyon işlemi uygulamış ve 100 W gücündeki işlemlerin örneklerin köpürme kapasitesi ve stabilitesi değerlerinde önemli bir artış sağladığını belirlemişlerdir. Malik ve ark. [6] ayçiçeği küspesi proteinlerine uyguladıkları ultrasonikasyon işleminin örneklerin hem köpürme kapasitesi hem de köpürme stabilitesi değerlerini önemli düzeyde arttırdığını bildirmişler ve uygulanan ultrasonikasyon işleminin protein örneklerinin yüzey hidrofobikliğinin artmasına ve parçacık boyutunun azalmasına neden olarak proteinlerin köpürme özelliklerinin gelişmesine neden olabileceğini ifade etmişlerdir [6]. Farklı bir çalışmada ise 3 farklı ultrasonikasyon gücünde (540, 720 ve 900 W) 10 dakika süresince uygulanan işlemlerin buğday gluteninin köpürme kapasitesi ve stabilitesi değerleri üzerine etkisi incelendiğinde, ultrasonikasyon gücünün artmasıyla birlikte örneklerin hem köpürme kapasitesi hem de köpürme stabilitesi değerlerinin önemli düzeyde arttığı rapor edilmiştir [24]. Buğday gluteninin köpürme özelliklerindeki bu gelişme ultrasonikasyon uygulamaları ile birlikte oluşan protein denatürasyonu sonucu yapıda hidrofobik bölgelerin açığa çıkması ile ilişkilendirilmiş olup, bu hidrofobik bölgelerin köpük yapısındaki su ve hava arayüzünde adsorpsiyonu geliştirerek köpürme özelliklerini iyileştirebileceği belirtilmiştir [24].



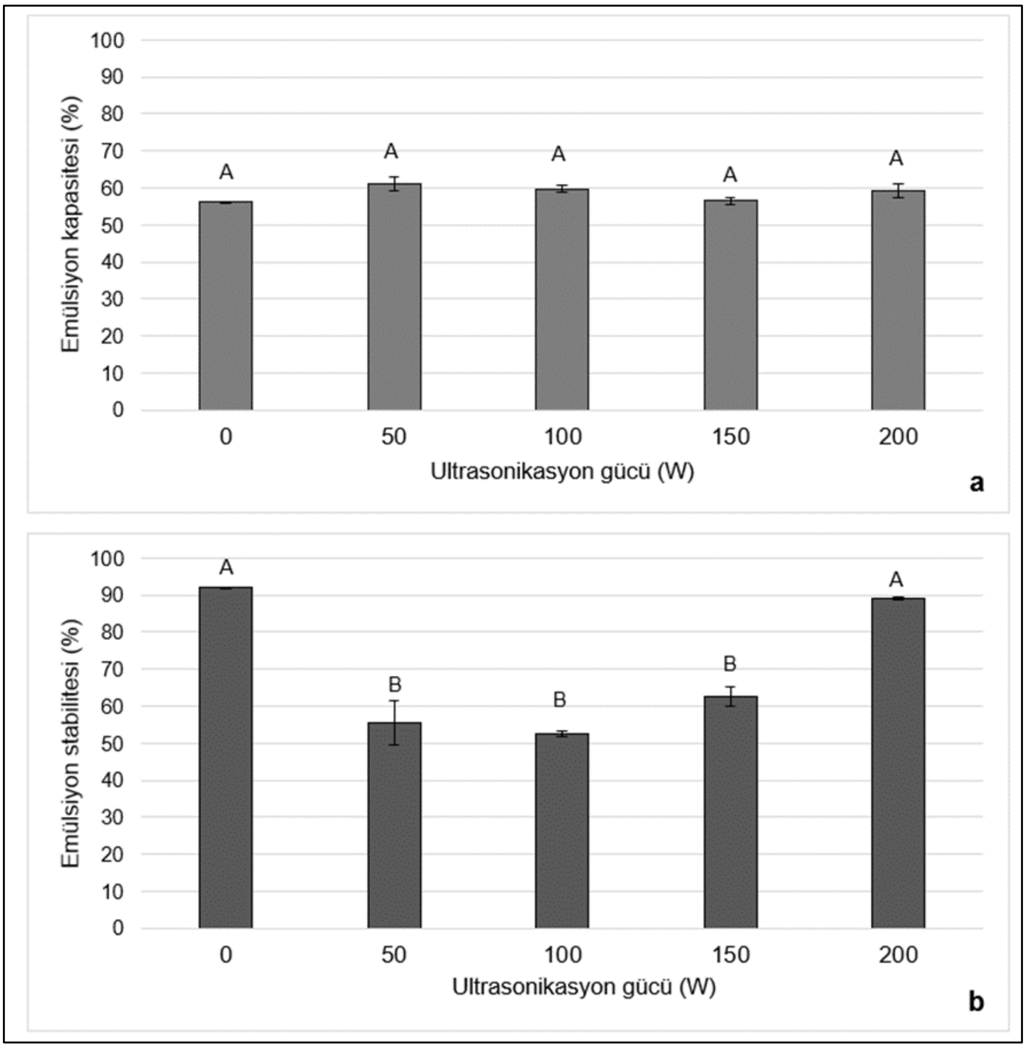
Şekil 2. Ketencik proteinlerinin köpürme kapasitesi ve köpürme stabilitesi değerleri. Büyük harfler, farklı ultrasonikasyon gücünde işlem görmüş örnekler arasındaki istatistiksel olarak önemli düzeydeki farkı göstermektedir (p<0.05). Küçük harfler, aynı ultrasonikasyon gücünde işlem görmüş örneklerin farklı bekleme sürelerindeki stabiliteleri arasındaki istatistiksel olarak önemli düzeydeki farkı göstermektedir (p<0.05)

Figure 2. Foaming capacity and foaming stability values of Camelina sativa proteins. Capital letters show statistically significant difference between values with different ultrasonication power (p<0.05). Small letters show statistically significant difference between stability values at different waiting times with same ultrasonication power (p<0.05)

Ultrasonikasyon Uygulamasının Ketencik Proteinlerinin Emülsiyon Özelliklerine Etkisi

Proteinlerin yağ damlacıklarının çevresini sararak bir film tabakası oluşturup faz ayrımını engelleyerek emülsiyon oluşturma özelliği bulunmaktadır. Proteinlerin emülsiyon özellikleri değerlendirilirken emülsiyon kapasitesi ve emülsiyon stabilitesi parametreleri incelenmektedir [25]. Emülsiyon kapasitesi proteinlerin su ve yağ arayüzünde adsorbe olma yeteneğini gösteren bir değer olup, emülsiyon stabilitesi ise oluşturulan emülsiyon yapılarının depolanması veya ısıtılmasından sonra proteinlerin su ve yağ arayüzünde kalma kararlılığını göstermektedir [6]. Bu emülsiyon özellikleri proteinlerin moleküler yapısının esnekliğine ve stabilitesine bağlı olarak değişmektedir [15, 26]. Proteinlerin emülsiyon oluşumuna ve oluşan emülsiyonların stabilizasyonunu katkı sağlaması, formülasyonunda yer aldığı birçok gıda ürünün (soslar, kremalar ve mayonez gibi) kalite özelliklerini etkilemesi nedeniyle oldukça önemlidir [27]. Bu bağlamda ultrasonikasyon uygulamasının ketencik proteinlerinin emülsiyon kapasitesi ve stabilitesi değerleri üzerine etkisi Şekil 3'te gösterilmiştir. Ketencik proteinlerinin %56.23 olan emülsiyon kapasitesi değeri ultrasonikasyon işlemi sonrasında %56.54-61.09 aralığında değişmekte olup, bu değişim istatistiksel olarak önemli bulunmamıştır (p>0.05). Diğer yandan ketencik proteinlerinin emülsiyon stabilitesi değerinin 200 W gücünde uygulanan ultrasonikasyon işlemi ile önemli düzeyde değişmediği (p>0.05), ancak diğer ultrasonikasyon güçlerinde (50, 100 ve 150 W) emülsiyon stabilitesinin önemli düzeyde azaldığı (p<0.05) belirlenmiştir.

Yapılan araştırmalarda ultrasonikasyon gücüne ve işlem süresine bağlı olarak uygulanan işlemlerin jak meyve çekirdeği [15], ayçiçeği küspesi [6], darı [23] ve ceviz [28] proteinlerinin emülsiyon özelliklerinin gelişmesini sağlayabileceği belirtilmiştir. Malik ve ark. [6] ultrasonikasyon uygulaması sonucu meydana gelen yapısal değişimlerin, proteinlerin moleküler esnekliğini ve yüzey hidrofobikliğini etkileyebileceğini ve dolayısıyla proteinlerin emülsiyon özelliklerini değiştirebileceğini ifade etmişlerdir. Ayrıca araştırmacılar emülsiyon özelliklerinin protein çözünürlüğünden doğrudan etkilendiğini, protein çözünürlüğünün artmasının yağ ve su arayüzünde daha fazla protein bulunmasını sağlayarak emülsiyon yapısını etkileyebileceğini belirtmişlerdir [6]. Resendiz-Vazquez ve ark. [15] ve Jambrak ve ark. [12] ise ultrasonikasyon uygulaması ile üçüncül ve dördüncül yapılarda oluşan değişimlerin proteinlerin su ve yağ arayüzünde adsorbe olma özelliklerini etkileyebileceğini ve dolayısıyla emülsiyon özelliklerinin de değişebileceğini ifade etmişlerdir.



Şekil 3. Ketencik proteinlerinin emülsiyon kapasitesi (a) ve emülsiyon stabilitesi (b) değerleri
Figure 3. Emulsifying capacity (a) and emulsifying stability (b) values of Camelina sativa proteins

SONUÇ

Son yıllarda protein ihtiyacının artmasıyla beraber yeni ve alternatif protein kaynaklarına olan ilgi de artmaktadır. Bitkisel proteinler daha kolay elde edilebilmeleri, ekonomik ve sürdürülebilir olmaları sebepleriyle büyük önem kazanmakla birlikte doğal halleriyle kullanıldıklarında gıda endüstrisinin ihtiyaç duyduğu bazı fonksiyonel özellikleri karşılamayabilmektedir. Bu nedenle çeşitli uygulamalar ile bitkisel proteinlerin tekno-fonksiyonel özelliklerinin geliştirilmesi önem kazanmakta ve bu amaçla en yaygın kullanılan yöntemlerden birisi ise ultrasonikasyon uygulaması olarak karşımıza çıkmaktadır. Bu doğrultuda gerçekleştirilen çalışmada, ketencik proteinlerine 5 farklı ultrasonikasyon gücünde (0, 50, 100, 150 ve 200 W) işlem uygulanmış ve proteinlerin su bağlama, yağ bağlama, köpürme ve emülsiyon özelliklerindeki değişimler incelenmiştir. Buna göre ultrasonikasyon uygulamasıyla ketencik proteinlerinin su bağlama kapasitesinin azaldığı, ancak yağ bağlama kapasitesi, köpürme kapasitesi ve köpürme stabilitesinin ise arttığı tespit edilmiştir. Sonuç olarak bu çalışma ile ketencik proteinlerinin tekno-fonksiyonel özelliklerinin geliştirilmesi amacıyla ultrasonikasyon uygulamasının kullanılabileceği ortaya konulmuştur. Bu değişimler göz önüne alındığında, 200 W gücünde ultrasonikasyon uygulamasının ketencik proteinlerinin yağ bağlama kapasitesi (%18.42), köpürme kapasitesi (%25.20) ve stabilitesi (%17.20-17.06) değerlerinde sağladığı artışın yanı sıra emülsiyon özelliklerinde önemli bir değişime neden olmaması sonucu ketencik proteinlerinin tekno-fonksiyonel özelliklerinin geliştirilmesi amacıyla kullanılabileceği ortaya konulmuştur.

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Araştırma Makalesi / Research Paper

Katık Keşinin Fizikokimyasal, Biyokimyasal, Mikrobiyolojik ve Duyusal Özellikleri Üzerine Üretim Yöntemi ile Depolama Sıcaklığının Etkisi

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ÖZ

Bu çalışmada, yoğurdun süzülmesi (S yöntemi) ve ısıtılıp süzülmesi (I yöntemi) yöntemleri ile üretilen Katık Keşi peynir örnekleri iki gruba ayrılarak; birinci grup 4°C'de, ikinci grup ise 24°C'de 90 gün boyunca depolanmıştır. Depolama süresince örneklerin bazı fizikokimyasal, biyokimyasal ve mikrobiyolojik özelliklerinde meydana gelen değişimler belirlenmiştir. Örneklerin duyusal testleri ise sadece depolama sonunda gerçekleştirilmiştir. Elde edilen sonuçlara göre yöntem farklılığı Katık Keşi örneklerinin kuru madde, yağ, protein, tuz ve pH değerlerini etkilemiştir ($p<0.05$). Depolama sıcaklıkları kuru madde, yağ ve protein değerleri üzerinde etkili olmazken ($p>0.05$), tuz, asitlik ve pH değerleri üzerinde etkili olmuştur ($p<0.05$). Üretim yöntemi her iki örneğin proteoliz ve lipoliz değerlerini etkilemezken, en yüksek proteoliz ve lipoliz değerleri 24°C'de saklanan örneklerden elde edilmiştir. Yöntem ve depolama sıcaklığındaki farklılık su aktivitesi, *Lactobacillus* spp., küf ve koliform sayılarına etkide bulunmazken, *Streptococcus* spp. ve maya sayılarını etkilemiştir ($p<0.05$). Öte yandan, depolama süresi boyunca Katık Keşi örneklerinde *Lactobacillus* spp., *Streptococcus* spp. ve maya sayılarındaki değişim önemli ($p<0.05$) bulunmuştur. S yöntemiyle üretilen numuneler daha yüksek L* renk değerlerine sahiptir. Duyusal özellikler açısından en fazla S yöntemi ile üretilen ve 4°C'de depolanan örnekler tercih edilmiştir. Sonuç olarak, yoğurdun süzülmesi, tuzlanması ve ardından kurutulması (S yöntemi) ile üretilen ve 4°C'de depolanan örnekler, Katık Keşi üretimi için en uygun üretim ve depolama yöntemi olarak ortaya çıkmıştır.

Anahtar Kelimeler: Keş, Geleneksel üretim, Kurut, Yoğurt

Effect of Production Method and Storage Temperature on Physicochemical, Biochemical, Microbiological and Sensory Properties of Katık Keş (Yoghurt-based Cheese)

ABSTRACT

In this study, Katık Keş cheese samples produced by directly straining (method S) and heating & straining (method I) of yogurt were divided into two groups; the first group was stored at 4°C and the second group was stored at 24°C for 90 days. Changes in some physicochemical, biochemical and microbiological properties of the samples during storage were determined. The sensory evaluation of the samples was completed only at the end of storage. The result of the study showed that the production method influenced the dry matter, fat, protein, salt, and pH values of the Katık Keş samples ($p<0.05$). Storage temperature had no effect on dry matter, fat, and protein values ($p>0.05$) while it affected the salt, acidity and pH values ($p<0.05$). The proteolysis and lipolysis values of both samples was not influenced by production method; however, the highest proteolysis and lipolysis values were obtained from all the samples stored at 24°C. While the difference in production method and storage temperature did not change the water activity, *Lactobacillus* spp., mold and coliform counts, they changed the *Streptococcus* spp. and yeast numbers ($p<0.05$). On the other hand, the change in the counts of *Lactobacillus* spp., *Streptococcus* spp. and yeasts in Katık Keş samples during the storage period was found to be significant ($p<0.05$). Samples produced by the method S had higher L* values than the methods I. In terms of sensory properties, the samples produced by the method S and stored at 4°C were preferred the most. As a result, the samples produced by straining, salting and then drying (S method) of yogurt and stored at 4°C have emerged as the most suitable production and storage method for the production of Katık Keş cheeses.

Keywords: Keş, Traditional production, Kurut, Yogurt

GİRİŞ

Keş, diğer yaygın adıyla Kurut, Türkler tarafından yüzlerce yıldır üretilip tüketilen, hammaddesi yoğurt olan bir peynir türüdür [1, 2]. Bu ürün, Orta Asya'da ve Orta Asya'dan Anadolu'ya göç eden Türklerin geleneklerini korumasıyla birlikte Anadolu'da geleneksel olarak üretilmeye devam edilmektedir [3]. Literatürde "yumru şeklindeki, süzülmüş yoğurttan yapılan kuru, tuzlu yiyecek" anlamına gelen kurut kelimesine karşılık; Kazakça, Uygurca, Kırgızca, Özbekçe ve Türkmence'de *qurut* ve/veya benzeri bir kelime mevcuttur [4]. Bu ürün İran'da Kashk, Lübnan'da Kishk, Suriye'de Jub-Jub ve Irak'ta Kuşuk adıyla bilinmektedir [1, 5]. Keş veya Kurut, ülkemizin farklı bölgelerinde dikdörtgen, konik, topaç, armut, soğan başı ve yassı topaklar şeklinde farklı büyüklükte üretilmekte olup; Geşk, Keşk, Kesük, Kiş, Çörten, Çortan, Torak, Terne, Çökelek ve Sürk gibi farklı isimlerle de anılmaktadır [6, 7].

Geleneksel olarak Keş yapımında, süt yağı oranı kısmen azaltılmış inek sütünden yoğurt üretilmektedir. Soğutulan yoğurt bez torbaya aktarılıp bir gece süzülmesi sağlanmaktadır. Süzülen yoğurda tuz katılıp değişik şekiller verilerek açık havada kurutulmaktadır. Başka bir geleneksel yöntemde ise, yayık tereyağı üretiminde yoğurdun yayıklanmasından sonra geriye kalan ayranın ısıtılmasıyla çökelek elde edilmektedir. Çökeleğin benzer şekilde süzülmesi, tuzlanması ve şekil verilerek kurutulmasıyla Keş üretilmektedir [8, 9].

Keş, protein içeriğinin fazla olması dolayısıyla besin değeri yüksek bir süt ürünüdür. Ordu ilinde piyasada satılan Keş örneklerinin sırasıyla en düşük ve en yüksek ortalama kuru madde değeri %60.69-78.40, yağ değeri %4.50-23.50, protein değeri %31.22-50.68, toplam kül değeri %4.36-14.23, tuz değeri %2.84-13.19, titrasyon asitliği değeri %1.49-3.26 ve pH değeri 3.37-4.24 olarak belirlenmiştir [8]. Burdur piyasasından toplanan Keş örnekleri üzerinde yürütülen bir çalışmada; örneklerin kuru madde değerleri %40.56-83.10 arasında bulunmuştur [10]. Bolu'da yerel pazarlarda satışa sunulan Keş üzerine yapılan bir çalışmada; örneklerin kuru madde değerleri %53.10-81.45 arasında ve ortalama %61.59 olduğu rapor edilmiştir. Ayrıca, örneklerin ortalama protein değeri %34.42, yağ değeri %6.30 ve tuz değeri %13.26 olarak bulunmuştur [1].

Bolu'da Makarnalık Keş, Kızartmalık Keş ve Katık Keş olmak üzere üç farklı keş üretimi yapılmaktadır. Makarnalık Keş, yağsız sütten üretilen yoğurdun işlenmesiyle üretilir. Makarnalık Keş, tuzlu ve sert bir yapıya sahiptir [9]. Kızartmalık Keş üretiminde ise diğer keş peynirlerine göre nispeten yağ oranı daha fazla olan süt ya da yoğurt kullanılmaktadır. Ayrıca Kızartmalık Keşin kurutma işlemi daha kısa olup, Makarnalık Keş kadar sert değildir. Tüketim öncesi Kızartmalık Keş dilimlenmekte ve yağsız tavaya konularak her iki yüzeyi kızartılarak servis yapılmaktadır [7]. Hem Makarnalık Keşin [11] hem de Kızartmalık Keşin [7] üretim parametreleri endüstriyel üretim için optimize edilmiştir.

Katık Keşinin üretim şekli Makarnalık Keş ile hemen hemen aynıdır. Katık Keşi yapımında farklı uygulamalar olmakla birlikte sütün veya yoğurdun yağı alınmamakta ve istenirse çörek otu katılmaktadır [9]. Katık Keşi, geçmişte çiftçiler tarafından tarlalarda çalışırken veya çobanlar tarafından meralarda hayvan otlatırken ekmeğine katık ettiği keş olarak bilinmektedir. Bugün halen pazarlarda ve yöresel ürün satan yerlerde bulmak mümkündür [5].

Bolu'da yerel pazarlarda satılan Katık Keşi örneklerine ait özelliklerin belirlenmesi üzerine yapılan bir çalışmada; örneklerin sırasıyla en düşük ve en yüksek ortalama kuru madde değeri %44.90-85.12, yağ değeri %1.50-28.00, protein değeri %29.09-47.79, toplam kül değeri %6.55-15.25, tuz değeri %2.80-6.93, titrasyon asitliği değeri %0.80-2.30 ve pH değeri 3.46-4.74 olarak belirlenmiştir. Ayrıca örneklerin maya ve küf sayısı 2.00-8.01 log kob/g, koliform sayısı 0-8.65 log kob/g ve su aktivitesi değeri 0.67-0.90 olarak bulunmuştur. Üstelik örneklerin duyusal özelliklerinde de farklılıklar olduğu bildirilmiştir [5]. Örneklerin fizikokimyasal özelliklerindeki bu geniş aralık ve duyusal özelliklerdeki farklılık, geleneksel olarak üretilip satılan Katık Keşlerinin standart özellikte olmadığını göstermektedir. Çalışmada bazı örneklerin yüksek tuz oranına sahip olduğu da dikkati çekmiştir. Katık Keşi üretiminde kuru madde ve yağ içeriğinin optimizasyonu üzerine yürütülen bir çalışmada; Katık Keşinin 42 saat kurutulmasıyla standart oranda kuru maddeye sahip Katık Keşi üretmenin mümkün olduğu ve Katık Keşinin yağlı sütten yapılmasının daha iyi sonuçlar verdiği ortaya konulmuştur [12].

Geleneksel olarak üretimi yapılan Katık Keşi, standart ve sürdürülebilir bir endüstriyel üretimle geleceğe taşıyabilmek için bu çalışma yapılmıştır. Bu çalışmada; iki farklı üretim yönteminin ve iki farklı depolama sıcaklığının 90 günlük depolama süresince Katık Keşi örneklerinin kimyasal, biyokimyasal, mikrobiyolojik, fiziksel ve duyusal özelliklerinde meydana getirdiği değişmeler incelenmiştir. Elde edilen bulgular, ürüne katkı maddesi ilave etmeden dayanıklı hale getirilmesi ve tüm bunlar neticesinde Katık Keşinin modern şartlarda işletme düzeyinde üretilmesinin sağlanmasında alt yapı oluşturması bakımından önemlidir.

MATERYAL ve METOT

Materyal

Katık Keşi üretiminde tam yağlı çiğ inek sütü kullanılmıştır. Çiğ sütün ortalama kuru madde oranı %12.40±0.566, yağ oranı %3.68±0.318 ve pH değeri ise 6.60±0.035'tır. Yoğurt üretiminde *Lactobacillus bulgaricus* ve *Streptococcus thermophilus* suşlarını içeren ticari starter kültürden (Chr. Hansen) işletme kültürü hazırlanmış ve %2 oranında süte katılmıştır. Üretim ve analizler, Bolu Abant İzzet Baysal Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü laboratuvarlarında gerçekleştirilmiştir.

Katık Keşlerin Üretimi

Katık keşi örneklerinin üretiminde Özcan ve Coşkun [12] tarafından tespit edilen kuru madde ve yağ oranları dikkate alınmıştır. Katık Keşi yapımında kullanılan sütler öncelikle çelik süzgeç üzerine yerleştirilen süzme bezinden geçirilerek süzülmüştür. Süt, 85ºC'de 20 dakika pastörize edilip inkübasyon sıcaklığına (44ºC) soğutulmuştur. Pastörize edilmiş süte daha önceden aktive edilen yoğurt starter kültüründen %2 oranında inoküle edilmiştir. İnoküle edilen sütler, 44°C'de pH 4.8 değerine ulaşana kadar inkübasyona bırakılmıştır. Daha sonra yoğurdun sıcaklığını düşürmek için buzdolabında (7ºC) bir gece dinlendirilmiştir. Ertesi gün yoğurt iki kısma ayrılmıştır.

Katık keşi üretiminde dikkate alınan birinci yöntemde; yoğurt, santrifüj (Erba Makine, Türkiye) tamburunun içindeki süzme bezine aktarılarak bir müddet kendi halinde süzülmesi için beklenmiştir. Sonra santrifüj 200 rpm'de 2 saat, 400 rpm'de 1.5 saat ve 600 rpm'de 1 saat çalıştırılarak ortamdan su uzaklaştırılmıştır. Bu şekilde yoğurdun doğrudan süzülmesiyle elde edilen örnekler "S" ile kodlanmıştır.

Hem ısıtma hem süzme işleminin uygulandığı 2. yöntemde; yoğurt, devamlı karıştırılmak suretiyle 85ºC'de 10 dk ısıtılmış ve çökmesi sağlanmıştır. Ardından tank içerisinde soğutularak sıcaklığın yaklaşık 10ºC'ye düşürülmesi sağlanmıştır. Daha sonra 1. yöntemde uygulanan parametrelerde santrifüjle süzme işlemi yapılmıştır. Bu şekilde ısıtıldıktan sonra süzülerek elde edilen örnekler "I" olarak kodlanmıştır.

Her iki yöntemde de santrifüjleme işleminden sonra geriye kalan kısma, %3 oranında kuru tuz ilave edilip karıştırılmıştır. Daha sonra tuzlanmış örnekler kalıplara (7 cm çapında, 4 cm yükseklikte silindirik kalıp) yaklaşık 156 g olacak şekilde sıkıca doldurulmuştur. Ardından kalıplar oda sıcaklığında yaklaşık 12 saat dinlendirilmiştir. Katık Keşi örnekleri, sıcaklığı 25°C ve fan hızı 0.5 m/s olan endüstriyel kurutucuda (Eksis, TK-LAB, Türkiye) toplam 42 saat kurutulmuştur. Kurutucuda örnekler altı saatte bir alt-üst yapılmıştır. Ancak Katık Keşi örneklerinde daha sonra çatlama olmaması için kurutma işlemi tamamlanana kadar 12 saat kurutma, 12 saat streçlenerek buzdolabında dinlendirme şeklinde uygulama yapılmıştır. Kurutma aşaması toplamda 78 saat sürmüştür. Kurutma sonrası örnekler, vakum paketleme makinesi (Lipovak, Türkiye) ile paketlenmiştir.

Her iki yöntemle üretilen Katık Keşi örnekleri kendi arasında tekrar ikiye ayrılarak birinci grup +4°C'de, ikinci grup +24°C'de 3 ay süreyle depolanmıştır. Depolamanın 0., 30., 60. ve 90. günlerinde hedeflenen analizler yapılmıştır. Çalışma iki farklı üretim yöntemi, iki farklı depolama sıcaklığı ve 4 farklı depolama süresi olacak şekilde tasarlanmıştır. Her üretim iki tekerrürlü yapılmıştır.

Katık Keşi Örneklerinde Yapılan Analizler

Örneklerin kuru madde tayini standart gravimetrik yöntemle, yağ tayini Gerber yöntemiyle, tuz tayini Mohr yöntemiyle, laktik asit cinsinden titrasyon asitliği titrimetrik yöntemle belirlenmiştir [13]. Örneklerin protein analizi Kjeldahl yöntemiyle gerçekleştirilmiştir [14]. Örneklerinin pH değerleri Hanna marka pH metre kullanılarak ölçülmüştür. Ölçüm yapmadan önce örnekler rendelenerek 1:1 oranında sulandırılmıştır [13]. Suda çözünür azot oranının belirlenmesi Bütikofer ve ark. [15] tarafından verilen yöntem esas alınarak yapılmıştır. Asitlik derecesi tayini (ADV), Salji ve Kroger [16] ile Case ve ark. [17]'nın önerdiği metotla yapılmıştır. Örneklerin L*, a* ve b* değerleri CIE (International Commission on Illumination) renk ölçüm sistemine göre Konica Minolta CR-400 (Osaka, Japonya) renk tayin cihazı ile tespit edilmiştir. Hem depolama öncesi ile depolama sonrası örnekler arasındaki renk değişimini ve hem de depolama boyunca örneklerin renk değişimini belirlemek amacıyla, depolamanın 0. gününde analiz edilen örnekler dikkate alınarak, ΔE değerleri hesaplanmıştır.

$$\Delta E=\sqrt{\Delta L^2+\Delta a^2+\Delta b^2}$$

Streptococcus spp. sayımı M17 agar ve *Lactobacillus* ssp. sayımı MRS agar kullanılarak yapılmıştır [18]. Koliform grubu bakterilerin sayımında VRBA kullanarak Frank ve ark. [19] göre; maya ve küf sayımında YGC Agar kullanılarak Halkman [20]'a göre yapılmıştır. Sonuçlar log kob/g olarak kaydedilmiştir. Çalışmada üretilen örneklerin duyusal analizlerinde Sıralama (Tercih) Testi kullanılmıştır. Duyusal testler 90 günlük depolama süresi sonunda 12-14 kişilik yarı eğitilmiş panelistler tarafından gerçekleştirilmiştir. Panelistler tarafından örnekler; renk ve görünüş, yapı ve kıvam, lezzet ve genel beğeni açısından sıralanmıştır [21].

İstatistiksel Analizler

Çalışmada üretim yöntemleri ve depolama sıcaklıkları arasındaki fark t-testi ile analiz edilmiştir. Depolama süresi boyunca meydana gelen değişmeler ise varyans analizi ve Duncan çoklu karşılaştırma testi ile $p<0.05$ düzeyinde karşılaştırılmıştır [22]. İstatistiksel analizler SPPS Statistics 25.0 programı kullanılarak yapılmıştır.

BULGULAR ve TARTIŞMA

Kimyasal Özellikler

Katık Keşi örneklerinin kuru madde, yağ, protein, tuz, asitlik ve pH özellikleri Tablo 1'de bir araya getirilmiştir. Yoğurdun süzülmesiyle üretilen (S) ve yoğurdun ısıtılıp süzülmesiyle üretilen (I) Katık Keşi örneklerinin kuru madde değerleri kurutma işleminden önce sırasıyla %41 ve %49 civarındayken, kurutma işleminden sonra %70 ve %73 civarına çıkmıştır. Üretim yöntemi, Katık Keşi üretiminde son ürünün kuru madde değeri üzerinde önemli bir fark oluşturmuştur ($p<0.05$). Bundan dolayı, I örneklerinin kuru madde değeri S örneklerinin kuru madde değerinden daha yüksek bulunmuştur. Bu durum, I örneklerinde ısıtmayla birlikte daha fazla su kaybı ile açıklanabilir. Örneklerin 4°C ve 24°C'de depolanması sonucu elde edilen kuru madde değerleri birbirine yakın çıkmıştır ($p>0.05$). Depolamanın başı ile sonu arasındaki kuru madde değerindeki değişim, S 4°C örneği hariç, önemli çıkmamıştır ($p>0.05$). Elde edilen kuru madde değerleri Makarnalık Keş [11] ve Kızartmalık Keş [23] için elde edilen değerlerden yüksekken; Akyüz ve Gülümser [24] tarafından bildirilen kurut örneklerinin ortalama kuru madde değerinden düşüktür. Ancak Bolu ilinde geleneksel olarak üretilip satılan Keş örneklerinin kuru madde değerleriyle benzerdir [1, 5]. Kuru maddedeki farklılık, üretimde kullanılan sütün bileşimi, ilave edilen tuz miktarı ve kurutma süresine bağlı olarak değişmektedir.

Tablo 1. Farklı yöntemlerle üretilen ve farklı derecelerde depolanan Katık keşi örneklerinin kimyasal özelliklerinde meydana gelen değişmeler

Table 1. The changes in the chemical properties of Katık Keş samples produced by different methods and stored at different temperatures

Örnekler		Depolama süresi (Gün)	N	Kimyasal Özellikler ($\bar{x}$±SD)					
Yöntem	Depolama sıcaklığı			Kuru madde (%)	Yağ (%)	Protein (%)	Tuz (%)	Asitlik (%)	pH
Süzme (S)	4°C	Kurutma öncesi	2	41.16 ± 0.051	17.75 ± 0.707	16.52 ± 0.442	3.63 ± 0.165	0.45 ± 0.011	4.43 ± 0.021
		0	2	70.02 ± 0.042^{b}	32.00 ± 0.707^{a}	27.75 ± 1.987^{a}	5.85 ± 0.000^{a}	0.78 ± 0.064^{a}	4.41 ± 0.071^{a}
		30	2	70.19 ± 0.050^{b}	29.00 ± 1.414^{a}	28.23 ± 1.138^{a}	5.56 ± 0.248^{a}	0.75 ± 0.014^{a}	4.40 ± 0.021^{a}
		60	2	70.90 ± 0.396^{a}	30.25 ± 1.768^{a}	28.14 ± 0.375^{a}	5.56 ± 0.247^{a}	0.71 ± 0.000^{a}	4.41 ± 0.071^{a}
		90	2	69.38 ± 0.035^{c}	30.25 ± 1.768^{a}	28.08 ± 1.640^{a}	5.60 ± 0.354^{a}	0.75 ± 0.042^{a}	4.37 ± 0.021^{a}
		Ortalama	8	70.12 ± 0.600^{Y}	30.25 ± 1.908^{Y}	28.05 ± 1.091^{Y}	5.64 ± 0.229^{Z}	0.75 ± 0.042^{Z}	4.40 ± 0.044^{Z}
Süzme (S)	24°C	Kurutma öncesi	2	41.16 ± 0.051	17.75 ± 0.707	16.52 ± 0.442	3.63 ± 0.165	0.45 ± 0.011	4.43 ± 0.021
		0	2	70.02 ± 0.042^{a}	32.00 ± 0.707^{a}	27.75 ± 1.987^{a}	5.85 ± 0.000^{a}	0.78 ± 0.064^{a}	4.41 ± 0.071^{a}
		30	2	70.23 ± 0.275^{a}	29.50 ± 0.707^{a}	28.85 ± 1.895^{a}	6.14 ± 0.255^{a}	0.90 ± 0.071^{a}	4.45 ± 0.014^{a}
		60	2	69.86 ± 1.174^{a}	30.00 ± 0.000^{a}	29.03 ± 1.259^{a}	6.55 ± 0.495^{a}	0.85 ± 0.071^{a}	4.53 ± 0.064^{a}
		90	2	69.61 ± 0.403^{a}	30.75 ± 1.768^{a}	28.50 ± 1.273^{a}	6.53 ± 0.460^{a}	0.92 ± 0.064^{a}	4.50 ± 0.134^{a}
		Ortalama	8	69.93 ±0.540^{Y}	30.56 ± 1.266^{Y}	28.53 ± 1.344^{Y}	6.26 ± 0.413^{Y}	0.86 ± 0.077^{Y}	4.47 ± 0.078^{Y}
Isıtma (I)	4°C	Kurutma öncesi	2	48.83 ± 2.578	17.13 ± 0.177	26.17 ± 2.842	3.74 ± 0.331	0.39 ± 0.029	4.54 ± 0.028
		0	2	73.20 ± 0.820^{a}	27.13 ± 0.177^{a}	37.45 ± 1.039^{a}	4.74 ± 0.247^{a}	0.54 ± 0.014^{a}	4.57 ± 0.028^{a}
		30	2	74.14 ± 0.672^{a}	26.00 ± 0.000^{ab}	36.40 ± 2.213^{a}	4.39 ± 1.075^{a}	0.57 ± 0.071^{a}	4.54 ± 0.028^{a}
		60	2	72.97 ± 1.683^{a}	25.50 ± 0.707^{ab}	35.70 ± 1.987^{a}	4.50 ± 0.077^{a}	0.63 ± 0.092^{a}	4.36 ± 0.092^{b}
		90	2	72.14 ± 0.240^{a}	25.30 ± 0.990^{b}	36.04 ± 2.333^{a}	5.03 ± 0.155^{a}	0.60 ± 0.028^{a}	4.45 ± 0.007^{ab}
		Ortalama	8	73.11 ±1.073^{Y}	25.98 ± 0.888^{Y}	36.40 ± 1.638^{Y}	4.67 ± 0.496^{Z}	0.58 ± 0.057^{Z}	4.48 ± 0.098^{Y}
Isıtma (I)	24°C	Kurutma öncesi	2	48.83 ± 2.578	17.13 ± 0.177	26.17 ± 2.842	3.74 ± 0.331	0.39 ± 0.029	4.54 ± 0.028
		0	2	73.20 ± 0.820^{a}	27.13 ± 0.177^{a}	37.45 ± 1.039^{a}	4.74 ± 0.247^{b}	0.54 ± 0.014^{a}	4.57 ± 0.028^{a}
		30	2	74.58 ± 2.567^{a}	25.00 ± 0.000^{b}	37.51 ± 1.895^{a}	5.85 ± 0.163^{ab}	1.01 ± 0.127^{a}	4.56 ± 0.007^{ab}
		60	2	73.34 ± 1.697^{a}	25.00 ± 0.000^{b}	37.52 ± 0.629^{a}	6.32 ± 0.665^{a}	0.94 ± 0.071^{a}	4.65 ± 0.064^{a}
		90	2	72.75 ± 2.553^{a}	25.00 ± 0.707^{b}	37.59 ± 0.014^{a}	6.32 ± 0.658^{a}	0.99 ± 0.375^{a}	4.47 ± 0.007^{b}
		Ortalama	8	73.47 ±1.704^{Y}	25.53 ± 1.022^{Y}	37.52 ± 0.853^{Y}	5.80 ± 0.784^{Y}	0.87 ± 0.255^{Y}	4.56 ± 0.073^{Y}
Süzme (S)			16	70.03 ± 0.560^{B}	30.41 ± 1.573^{A}	28.29 ± 1.208^{B}	5.95 ± 0.457^{A}	0.80 ± 0.083^{A}	4.43 ± 0.072^{B}
Isıtma (I)			16	73.29 ± 1.388^{A}	25.76 ± 0.953^{B}	36.95 ± 1.387^{A}	5.23 ± 0.865^{B}	0.73 ± 0.231^{A}	4.52 ± 0.094^{A}
	4°C		16	71.62 ± 1.758^{A}	28.12 ± 2.632^{A}	32.22 ± 4.515^{A}	5.15 ± 0.627^{B}	0.67 ± 0.096^{B}	4.44 ± 0.085^{B}
	24°C		16	71.70 ± 2.196^{A}	28.05 ± 2.826^{A}	33.02 ± 4.764^{A}	6.04 ± 0.651^{A}	0.86 ± 0.182^{A}	4.51 ± 0.086^{A}
		0	8	71.61 ± 1.756^{AB}	29.56 ± 2.635^{A}	32.60 ± 5.316^{A}	5.29 ± 0.611^{B}	0.66 ± 0.130^{A}	4.49 ± 0.095^{A}
		30	8	72.28 ± 2.440^{A}	27.38 ± 2.134^{B}	32.75 ± 4.730^{A}	5.48 ± 0.831^{AB}	0.81 ± 0.187^{B}	4.49 ± 0.072^{A}
		60	8	71.77 ± 1.847^{AB}	27.69 ± 2.712^{B}	32.60 ± 4.457^{A}	5.73 ± 0.915^{AB}	0.78 ±0.140^{AB}	4.48 ± 0.131^{A}
		90	8	70.97 ± 1.957^{B}	27.70 ± 3.113^{B}	32.55 ± 4.746^{A}	5.87 ± 0.718^{A}	0.81 ± 0.216^{B}	4.44 ± 0.073^{A}

^{a,b,c}: Aynı sütunda farklı küçük harf taşıyan ortalamalar birbirinden farklı ($p<0.05$), aynı harf taşıyanlar farksızdır ($p>0.05$); ^{A,B}: Her bir faktör için aynı sütunda farklı büyük harf taşıyan ortalamalar birbirinden farklı ($p<0.05$), aynı harf taşıyanlar farksızdır ($p>0.05$). ^{Y,Z}: Her bir kimyasal özellikte süzme yönteminde 4°C ile 24°C veya ısıtma yönteminde 4°C ile 24°C'de ortalama değerleri için; farklı büyük harf taşıyan ortalamalar birbirinden farklı ($p<0.05$), aynı harf taşıyanlar farksızdır ($p>0.05$).

Her iki yöntemle üretilen Katık keşi örneklerinin (S ve I) yağ değerleri kurutmadan önce birbirine yakın çıkmıştır. Ancak hem kurutma öncesi örneklerde hem de kurutma sonrası örneklerde, süzme yöntemi ile elde edilen örneklerin yağ değeri daha yüksektir ($p<0.05$). Isıtma yöntemiyle elde edilen örneklerde yağ oranının düşük çıkmasının sebebi süzme esnasında yağ kaybı ile açıklanabilir. Depolama sıcaklığının, örneklerde yağ oranı üzerinde etkisi olmamıştır ($p>0.05$). Depolama süresi boyunca yağ değerindeki değişim, S örneklerinde önemsiz (p>0.05); I örneklerinde önemlidir (p<0.05). Elde edilen yağ oranları Coşkun ve ark. [9] ile Çakır ve ark. [1] tarafından tespit edilen ortalama yağ değerlerinden yüksektir. Kızartmalık Keş örneklerinde yaklaşık %24 yağ içerdiği bildirilmiştir [23]. Coşkun ve ark. [5] tarafından yapılan çalışmada ise geleneksel olarak üretilip pazarlarda satılan 15 adet Katış Keşi örneklerinden sadece bir tanesi %28 oranında yağ içeriğine sahip olduğu, diğerlerin %1.5-12 arasında değiştiği tespit edilmiştir. Bu durum, Keş üretiminde kullanılan hammaddenin yağ içeriği ile ilişkilidir.

Katık Keşi örneklerinin protein değerleri üzerinde üretim yönteminin etkisi önemli (p<0.05) bulunmuştur. S örneklerinin protein değerleri kurutma öncesinde %16.52, kurutma sonrasında ortalama %28.29; I örneklerinde ise protein değerleri kurutma öncesinde %26.17, kurutma sonrasında ortalama %36.95 olarak belirlenmiştir. Zira santrifüjleme sırasında, ısıtılan örneklerde serum ayrılması daha yüksek oranda gerçekleşmiştir, bu durum kuru madde değerlerinde de gözükmektedir. Hem depolama sıcaklığı hem de depolama süresi, örneklerin protein oranlarında bir değişikliğe neden olmamıştır (p>0.05). S örneklerinin protein değeri Kalender ve Güzeler [25] ve Yaman ve Coşkun [11] tarafından bildirilen ortalama protein değerlerine benzerdir. I örnekleri için tespit edilen genel ortalama protein değeri ise Çakır ve ark. [1] ve Coşkun ve ark. [5] tarafından rapor edilen protein değerleri ile uyumludur. Bu değerler, Tarakçı ve ark. [8] tarafından elde edilen protein değerlerinden düşüktür.

S örneklerinin genel ortalama tuz değerleri (%5.95), I örneklerinkinden (%5.23) yüksek çıkmıştır. Aradaki fark istatistiksel olarak önemli (p<0.05) bulunmuştur. Hem S ve hem de I örneklerinde, 24°C'de depolanan örneklerin tuz değerleri daha yüksek çıkmıştır (p<0.05). Depolama boyunca, I 24°C örneği hariç, tuz değerlerinde pek bir değişim olmamıştır (p>0.05). Ancak örneklerin depolama günlerindeki genel ortalama değerine bakıldığında, 0. güne göre 90. günde tuz miktarındaki artış önemli (p<0.05) bulunmuştur. Elde edilen tuz değerleri, Kalender ve Güzeler [25], Emirmustafaoğlu ve Coşkun [23] ve Kırdar ve ark. [26] tarafından bildirilen tuz değerlerinden yüksek, Tarakçı ve ark. [8] ve Coşkun ve ark. [5] tarafından bildirilen tuz değerleri ile uyumludur. Elde edilen veriler ile diğer araştırmacıların bulmuş olduğu değerlerin farklı olması, ilave edilen tuz miktarına, üretim yöntemlerine ve ürünün su-tuz tutma kapasitesine bağlı olarak değiştiği düşünülmektedir.

Katık Keşi örneklerinin asitlik değeri üzerinde üretim yönteminin etkisi önemsiz (p>0.05) çıkmıştır. S ve I örneklerinin asitlik değerleri genel ortalaması sırasıyla %0.80 ve %0.73 olarak belirlenmiştir. Hem S hem de I örneklerinde 24°C'de depolanan örneklerin asitlik değerleri daha yüksektir (p<0.05). 4°C'de depolanan örneklerinin asitlik değerleri genel ortalaması %0.67; 24°C'de depolanan örneklerinin genel ortalama asitlik değerleri ise %0.86 bulunmuştur. Farkın 24°C'de gelişebilen mikroorganizmalardan kaynaklandığı düşünülmektedir. S ve I örneklerinin asitlik değerindeki değişim depolama boyunca önemsiz (p>0.05) bulunmuştur. Ancak örneklerin depolama günlerindeki genel ortalama asitlik değerlerindeki değişim önemli (p<0.05) bulunmuştur. Elde edilen veriler Tarakçı ve ark. [8] ve Coşkun ve ark. [5] tarafından elde edilen değerlerden düşük, Emirmustafaoğlu ve Coşkun [23] tarafından bildirilen değerlere benzerdir. Araştırmacılar, sonuçlarımıza benzer şekilde, asitlik değerini 30. günde arttığını ve depolama sonuna kadar önemli bir değişim olmadığını bildirmiştir [23].

S örneklerinin ortalama pH değeri (4.43), I örneklerinden (4.52) daha düşüktür. Örneklerin pH değerleri arasındaki fark önemlidir (p<0.05). I örneklerinde pH değerinin yüksek olması; proteinlerin tamponlama kapasitesi ile ilişkili olarak protein oranının yüksek olmasından ve ısıtmanın mikroorganizmalar üzerindeki etkisinden dolayı mikroflora farklılığından kaynaklı olduğu düşünülmektedir. Depolama sıcaklığının ortalama pH değerlerine etkisi I örneklerinde önemsiz (p>0.05) bulunurken, S örneklerinde önemli (p<0.05) bulunmuştur. 4 ve 24°C'de depolanan örneklerin genel ortalama pH değeri sırasıyla 4.44 ve 4.51 olup, iki depolama sıcaklığı arasındaki fark anlamlıdır (p<0.05). Örneklerin depolama süresince genel ortalama pH değerlerindeki değişim önemsiz (p>0.05) çıkmıştır. Dervişoğlu ve ark. [27], piyasadan satın alarak inceledikleri Keş örnekleri pH değerlerinin 3.43-5.81 aralığında değiştiğini ve ortalama 4.75 olduğunu rapor etmişlerdir. Elde edilen değerler, geleneksel olarak üretilip pazarlarda satılan 15 adet Katış Keşi örneklerinden sadece iki tanesinin pH değeriyle benzerdir [5]. Hem bu çalışmada hem de diğer çalışmalarda elde edilen pH değerleri çok daha düşüktür [8, 11, 23].

Biyokimyasal Özellikler

Lipoliz, süt ve ürünlerinde depolama boyunca yağda meydana gelen parçalanmayı ifade eder [28]. S örneklerinin (0.84 meq KOH/100 g yağ) I örneklerine (0.68 meq KOH/100 g yağ) göre genel ortalama lipoliz değeri daha yüksek çıkmıştır (Tablo 2). Bu durum, I örneklerinde uygulanan ısıtma işleminin lipaz salgılayan mikroorganizmalar ve ortamdaki serbest lipazlar üzerine inaktivasyon etkisinden kaynaklanmış olabilir [29]. Diğer yandan, örneklerin ADV değeri 24°C'de depolananlarda daha yüksektir. Ancak hem üretim yönteminin hem de depolama sıcaklığının lipoliz değeri üzerindeki etkisi istatistiksel olarak önemli bulunmamıştır (p>0.05). Örneklerin lipoliz değeri, depolama boyunca artış göstermiştir. Örneklerin depolama süresince genel ortalama ADV değerlerindeki değişim önemli (p<0.05) çıkmıştır. Çakır ve ark. [1] Keş örneklerinde lipoliz değerlerini (1.62-8.79 meq KOH/100 g yağ) oldukça yüksek bulmuştur. Kızartmalık Keş örneklerinin lipoliz değeri, depolama sırasında 0.70-1.03 meq KOH/100 g yağ aralığında olduğu ve depolama boyunca arttığı rapor edilmiştir [23]. Süt ve süt ürünlerinde kabul edilebilir bir eşik değerine kadar lipoliz makul sayıldığı halde, aşırı miktarlarda lipoliz gelişimi ürünün tat ve aromasını olumsuz yönde etkilemektedir. Lipoliz gelişimi ortamda bulunan starter ve starter olmayan mikroorganizma varlığına, lipaz varlığına, ortamın pH değerine ve depolama sıcaklığına bağlı olarak değişebilmektedir [28].

Tablo 2. Farklı yöntemlerle üretilen ve farklı derecelerde depolanan Katık keşi örneklerinin biyokimyasal özelliklerinde meydana gelen değişmeler

Table 2. The changes in the biochemical properties of Katık Keş samples produced by different methods and stored at different temperatures

Örnekler		Depolama süresi (Gün)	N	Biyokimyasal Özellikleri ($\bar{x}$±SD)	
Yöntem	Depolama sıcaklığı			Lipoliz (ADV) (meq KOH/100 g yağ)	Suda çözünen azot (%)
Süzme (S)	4°C	Kurutma öncesi	2	1.16 ± 0.105	0.03 ± 0.012
		0	2	0.58 ± 0.050^{a}	0.06 ± 0.011^{a}
		30	2	0.78 ± 0.283^{a}	0.07 ± 0.005^{a}
		60	2	0.81 ± 0.064^{a}	0.08 ± 0.020^{a}
		90	2	0.88 ± 0.813^{a}	0.08 ± 0.035^{a}
		Ortalama	8	0.76 ± 0.348^{Y}	0.07 ± 0.017^{Z}
Süzme (S)	24°C	Kurutma öncesi	2	1.16 ± 0.105	0.03 ± 0.012
		0	2	0.58 ± 0.050^{b}	0.06 ± 0.011^{a}
		30	2	1.31 ± 0.269^{a}	0.12 ± 0.042^{a}
		60	2	0.88 ± 0.050^{ab}	0.29 ± 0.109^{a}
		90	2	0.95 ± 0.233^{ab}	0.31 ± 0.165^{a}
		Ortalama	8	0.93 ± 0.311^{Y}	0.20 ± 0.137^{Y}
Isıtma (I)	4°C	Kurutma öncesi	2	0.58 ± 0.000	0.004 ± 0.005
		0	2	0.49 ± 0.092^{a}	0.06 ± 0.016^{a}
		30	2	0.67 ± 0.127^{a}	0.07 ± 0.005^{a}
		60	2	0.69 ± 0.113^{a}	0.08 ± 0.006^{a}
		90	2	0.76 ± 0.021^{a}	0.08 ± 0.011^{a}
		Ortalama	8	0.65 ± 0.130^{Y}	0.07 ± 0.012^{Z}
Isıtma (I)	24°C	Kurutma öncesi	2	0.58 ± 0.000	0.004 ± 0.005
		0	2	0.49 ± 0.092^{b}	0.06 ± 0.016^{b}
		30	2	0.72 ± 0.099^{ab}	0.25 ± 0.138^{ab}
		60	2	0.79 ± 0.007^{a}	0.51 ± 0.074^{a}
		90	2	0.89 ± 0.134^{a}	0.53 ± 0.170^{a}
		Ortalama	8	0.72 ± 0.173^{Y}	0.34 ± 0.226^{Y}
Süzme (S)			16	0.84 ± 0.331^{A}	0.13 ± 0.114^{A}
Isıtma (I)			16	0.68 ± 0.152^{A}	0.21 ± 0.207^{A}
	4°C		16	0.70 ± 0.260^{A}	0.07 ± 0.014^{B}
	24°C		16	0.82 ± 0.266^{A}	0.27 ± 0.195^{A}
		0	8	0.53 ± 0.074^{A}	0.06 ± 0.010^{B}
		30	8	0.87 ± 0.318^{B}	0.13 ± 0.097^{AB}
		60	8	0.79 ± 0.088^{B}	0.24 ± 0.199^{A}
		90	8	0.87 ± 0.332^{B}	0.25 ± 0.220^{A}

^{a,b,c}: Aynı sütunda farklı küçük harf taşıyan ortalamalar birbirinden farklı (p<0.05), aynı harf taşıyanlar farksızdır (p>0.05); ^{A,B}: Her bir faktör için aynı sütunda farklı büyük harf taşıyan ortalamalar birbirinden farklı (p<0.05), aynı harf taşıyanlar farksızdır (p>0.05). ^{Y,Z}: Her bir kimyasal özellikte süzme yönteminde 4°C ile 24°C veya ısıtma yönteminde 4°C ile 24°C'de ortalama değerleri için; farklı büyük harf taşıyan ortalamalar birbirinden farklı (p<0.05), aynı harf taşıyanlar farksızdır (p>0.05).

Proteoliz, proteinlerin parçalanma seviyesini göstermekte ve peynir gibi ürünlerde depolama boyunca artmaktadır. Proteinlerin parçalanması üründe tat ve aromaya olumlu yönde etki etmektedir [28]. Suda çözünen azot (WSN) olarak ölçülen proteoliz değerleri, I örneklerinde (%0.21), S örneklerine (%0.13) göre daha yüksek çıkmıştır (Tablo 2). Örneklerin genel ortalama WSN değerleri üzerinde üretim yönteminin etkisi önemsiz (p>0.05) çıkarken, depolama sıcaklığı ve süresinin etkisi önemli (p<0.05) bulunmuştur. S ve I örneklerinin 24°C'de depolanması WSN değerini arttırmıştır. Ayrıca 24°C'de depolanan örneklerin depolama boyunca WSN değerinde artış gözlemlenmiştir. Artış, sadece 24°C'de depolanan I örnekleri için önemli (p<0.05) bulunmuştur. Yapılan bir çalışmada, Keş örneklerinin WSN değeri %0.02-0.12 aralığında değişmiştir [1]. Bu çalışmada elde edilen veriler, Emirmustafaoğlu ve Coşkun [23] tarafından bildirilen değerlerden yüksekken, Yaman ve Coşkun [11] tarafından elde edilen değerlere benzerdir. Araştırmacılar keş örneklerinde depolama sıcaklığının artmasıyla WSN değerinin arttığını rapor etmişlerdir [11]. Aydın [28], ortamdaki su miktarı, depolama sıcaklığı, ortamın pH'sı, depolama süresi, ısı uygulaması gibi faktörlerin WSN değerini etkileyebildiğini bildirmiştir.

Mikrobiyolojik Özellikler

Katık Keşi örneklerinin mikrobiyolojik özellikleri Tablo 3'te bir araya getirilmiştir. Örneklerde kurutma işlemi sonrası su aktivitesinde azalma tespit edilmiştir. Örneklerin genel ortalama su aktivitesi değerlerine üretim yöntemi ile depolama sıcaklığı etkili olamazken (p>0.05), depolama süresi etkili olmuştur (p<0.05). Su aktivitesi, mikroorganizmaların aktivitelerini devam ettirebilmeleri açısından önemlidir. Keş peynirinin veya diğer adıyla kurutların su aktivitesi diğer süt ürünlerine göre oldukça düşüktür. Coşkun ve ark. [9], başlangıçta su aktivite değeri 0.96 olan örneklerin 20 günlük kurutma sonrası su aktivite değerini 0.75 olarak bulmuşlardır. Elde edilen değerler, diğer araştırmacıların belirlediği değerlerden düşüktür [11, 23, 26]. Geleneksel olarak üretilen ve piyasan temin edilen Keş peynirlerinin su aktivite değeri 0.67-0.90 arasında değişmektedir [1, 5].

Katık Keş örneklerinin *Lactobacillus* spp. sayısı üzerinde üretim yönteminin ve depolama sıcaklığının etkisi önemsiz ($p>0.05$), depolama süresinin etkisi önemli bulunmuştur ($p<0.05$). Örneklerin *Lactobacillus* spp. sayısındaki azalış depolama boyunca devam etmiştir. Uygulanan kurutma işlemi ve I örneklerinde uygulanan ısıtma işlemi *Lactobacillus* spp. sayısında azalmaya sebep olmuştur. Elde edilen sonuçlar, Kırdar ve ark. [26] tarafından bildirilen laktobasil sayısından düşüktür. Piyasadan toplanan Keş örneklerinde yapılan çalışmalarda laktobasil sayısı; Tarakçı ve ark. [8] tarafından 3.61-5.34 log kob/g, Çakır ve ark. [1] tarafından 2.17-5.85 log kob/g ve Kırdar [10] tarafından 5.20-8.93 log kob/g olarak bulunmuştur.

Örneklerin *Streptococcus* spp. sayısı üzerinde üretim yöntemi, depolama sıcaklığı ve depolama süresinin etkili olduğu ($p<0.05$) belirlenmiştir. *Streptococcus* spp. sayısı hem kurutmadan önce hem de kurutmadan sonra I örneklerinde daha düşük çıkmıştır. Bu durum, ısıtmanın etkisiyle ilişkilidir. *Streptococcus* spp. genel ortalama sayısı, 24°C'de depolanan örneklere göre, 4°C'de depolanan örneklerde yaklaşık 2 log birim daha fazladır. Hem S örneklerinde hem de genel ortalama değerleri açısından depolamanın 30. günündeki azalış önemlidir ($p<0.05$). Elde edilen sonuçlar, Kırdar ve ark. [26] tarafından bildirilen streptokok sayısından düşüktür. Geleneksek olarak üretilip satılan Keş örneklerinde *Streptococcus* spp. sayısı; Coşkun ve ark. [9] tarafından 5.25 log kob/g olarak bulunurken, Çakır ve ark. [1] tarafından 2.39-5.20 log kob/g, Kırdar [10] tarafından ise 4.38-8.78 log kob/g arasında belirlenmiştir.

Tablo 3. Farklı yöntemlerle üretilen ve farklı derecelerde depolanan Katık keşi örneklerinin mikrobiyolojik özelliklerinde meydana gelen değişmeler

Table 3. The changes in the microbiological properties of Katık Keş samples produced by different methods and stored at different temperatures

Örnekler		Depolama süresi (Gün)	N	Mikrobiyolojik Özellikler ($\bar{x}$±SD)					
Yöntem	Depolama sıcaklığı			Su aktivitesi (aw)	*Lactobacillus* spp. (log kob/g)	*Streptococcus spp.* (log kob/g)	Maya sayısı (log kob/g)	Küf sayısı (log kob/g)	Koliform sayısı (log kob/g)
Süzme (S)	4°C	Kurutma öncesi	2	0.92 ± 0.000	4.84 ± 0.980	8.02 ± 0.242	6.24 ± 0.175	4.00 ± 2.828	2.67 ± 1.937
		0	2	0.81 ± 0.002^{ab}	2.98 ± 0.460^{a}	7.79 ± 0.050^{a}	5.11 ± 0.722^{a}	1.59 ± 2.249^{a}	1.60 ± 2.263^{a}
		30	2	0.82 ± 0.005^{a}	2.95 ± 0.495^{a}	7.03 ± 0.014^{b}	5.11 ± 0.299^{a}	3.50 ± 0.283^{a}	0.00 ± 0.000^{a}
		60	2	0.79 ± 0.001^{c}	1.69 ± 0.550^{b}	6.66 ± 0.260^{b}	4.93 ± 0.033^{a}	2.69 ± 0.127^{a}	0.00 ± 0.000^{a}
		90	2	0.81 ± 0.001^{b}	1.32 ± 0.163^{b}	6.75 ± 0.071^{b}	4.84 ± 0.110^{a}	2.70 ± 2.687^{a}	0.00 ± 0.000^{a}
		Ortalama	8	0.81 ± 0.011^{Y}	2.23 ± 0.861^{Y}	7.06 ± 0.487^{Y}	5.00 ± 0.325^{Y}	2.62 ± 1.515^{Y}	0.40 ± 1.131^{Y}
Süzme (S)	24°C	Kurutma öncesi	2	0.92 ± 0.000	4.84 ± 0.980	8.02 ± 0.242	6.24 ± 0.175	4.00 ± 2.828	2.67 ± 1.937
		0	2	0.81 ± 0.002^{a}	2.98 ± 0.460^{a}	7.79 ± 0.050^{a}	5.11 ± 0.722^{a}	1.59 ± 2.249^{a}	1.60 ± 2.263^{a}
		30	2	0.82 ± 0.002^{a}	2.22 ± 0.262^{ab}	1.65 ± 2.333^{b}	3.30 ± 0.707^{b}	2.06 ± 1.782^{a}	0.00 ± 0.000^{a}
		60	2	0.75 ± 0.001^{b}	1.88 ± 0.566^{ab}	2.24 ± 1.047^{b}	2.10 ± 0.071^{b}	2.10 ± 1.273^{a}	0.00 ± 0.000^{a}
		90	2	0.81 ± 0.001^{a}	1.15 ± 0.213^{b}	2.24 ± 1.047^{b}	3.35 ± 0.636^{b}	2.05 ± 0.495^{a}	0.00 ± 0.000^{a}
		Ortalama	8	0.80 ± 0.031^{Y}	2.06 ± 0.764^{Y}	3.40 ± 2.907^{Z}	3.47 ± 1.235^{Z}	1.95 ± 1.222^{Y}	0.40 ± 1.131^{Y}
Isıtma (I)	4°C	Kurutma öncesi	2	0.91 ± 0.008	1.15 ± 1.626	4.12 ± 0.598	6.24 ± 0.175	0.00 ± 0.000^{a}	0.00 ± 0.000
		0	2	0.82 ± 0.030^{a}	3.34 ± 0.007^{a}	3.05 ± 0.000^{a}	5.68 ± 0.000^{a}	0.00 ± 0.000^{a}	0.00 ± 0.000
		30	2	0.83 ± 0.005^{a}	2.94 ± 0.000^{a}	2.90 ± 1.061^{a}	5.62 ± 0.291^{a}	0.76 ± 1.068^{a}	0.00 ± 0.000
		60	2	0.80 ± 0.001^{a}	1.80 ± 0.281^{b}	2.49 ± 1.393^{a}	5.00 ± 0.168^{a}	1.65 ± 2.333^{a}	0.00 ± 0.000
		90	2	0.79 ± 0.009^{a}	2.94 ± 0.156^{a}	3.24 ± 0.463^{a}	5.02 ± 0.361^{a}	1.67 ± 0.262^{a}	0.00 ± 0.000
		Ortalama	8	0.81 ± 0.020^{Y}	2.75 ± 0.625^{Y}	2.91 ± 0.746^{Y}	5.33 ± 0.390^{Y}	1.89 ± 1.446^{Y}	0.00 ± 0.000^{Y}
Isıtma (I)	24°C	Kurutma öncesi	2	0.91 ± 0.008	1.15 ± 1.626	4.12 ± 0.598	6.24 ± 0.175	1.30 ± 0.920	0.00 ± 0.000
		0	2	0.82 ± 0.030^{a}	3.34 ± 0.007^{a}	3.05 ± 0.000^{a}	5.68 ± 0.000^{a}	3.50 ± 0.000^{a}	0.00 ± 0.000^{a}
		30	2	0.82 ± 0.005^{a}	1.59 ± 0.156^{b}	3.14 ± 0.767^{a}	5.17 ± 0.727^{ab}	2.30 ± 0.424^{a}	0.60 ± 0.849^{a}
		60	2	0.75 ± 0.002^{b}	1.54 ± 0.337^{b}	2.38 ± 0.050^{a}	4.46 ± 0.095^{b}	2.50 ± 1.697^{a}	0.15 ± 0.071^{a}
		90	2	0.80 ± 0.004^{a}	0.98 ± 0.523^{b}	2.10 ± 0.134^{a}	3.27 ± 0.016^{c}	2.15 ± 1.351^{a}	0.05 ± 0.071^{a}
		Ortalama	8	0.80 ± 0.031^{Y}	1.86 ± 0.976^{Z}	2.66 ± 0.558^{Y}	4.64 ± 1.006^{Y}	2.61 ± 1.008^{Y}	0.20 ± 0.411^{Y}
Süzme (S)			16	0.80 ± 0.023^{A}	2.14 ± 0.792^{A}	5.23 ± 2.762^{A}	4.23 ± 1.178^{B}	2.29 ± 1.374^{A}	0.40 ± 1.093^{A}
Isıtma (I)			16	0.80 ± 0.026^{A}	2.31 ± 0.916^{A}	2.79 ± 0.650^{B}	4.99 ± 0.818^{A}	2.25 ± 1.260^{A}	0.10 ± 0.300^{A}
	4°C		16	0.81 ± 0.016^{A}	2.49 ± 0.775^{A}	4.99 ± 2.222^{A}	5.17 ± 0.387^{A}	2.26 ± 1.479^{A}	0.20 ± 0.800^{A}
	24°C		16	0.80 ± 0.030^{A}	1.96 ± 0.853^{A}	3.03 ± 2.056^{B}	4.06 ± 1.247^{B}	2.28 ± 1.135^{A}	0.30 ± 0.829^{A}
		0	8	0.82 ± 0.016^{A}	3.16 ± 0.312^{A}	5.42 ± 2.534^{A}	5.40 ± 0.491^{A}	2.55 ± 1.577^{A}	0.80 ± 1.481^{A}
		30	8	0.82 ± 0.007^{A}	2.42 ± 0.644^{B}	3.68 ± 2.380^{B}	4.80 ± 1.036^{AB}	2.15 ± 1.319^{A}	0.15 ± 0.424^{A}
		60	8	0.77 ± 0.025^{C}	1.73 ± 0.368^{C}	3.44 ± 2.098^{B}	4.12 ± 1.271^{B}	2.24 ± 1.267^{A}	0.04 ± 0.074^{A}
		90	8	0.80 ± 0.010^{B}	1.60 ± 0.870^{C}	3.50 ± 2.114^{B}	4.12 ± 0.914^{B}	2.14 ± 1.222^{A}	0.01 ± 0.035^{A}

^{a,b,c}: Aynı sütunda farklı küçük harf taşıyan ortalamalar birbirinden farklı ($p<0.05$), aynı harf taşıyanlar farksızdır ($p>0.05$); ^{A,B}: Her bir faktör için aynı sütunda farklı büyük harf taşıyan ortalamalar birbirinden farklı ($p<0.05$), aynı harf taşıyanlar farksızdır ($p>0.05$). ^{Y,Z}: Her bir kimyasal özellikte süzme yönteminde 4°C ile 24°C veya ısıtma yönteminde 4°C ile 24°C'de ortalama değerleri için; farklı büyük harf taşıyan ortalamalar birbirinden farklı ($p<0.05$), aynı harf taşıyanlar farksızdır ($p>0.05$).

Örneklerin maya sayısı üzerinde üretim yöntemi, depolama sıcaklığı ve depolama süresinin etkili olduğu (p<0.05) belirlenmiştir. Maya sayıları I örneklerinde genel ortalama olarak 4.99 log kob/g iken, S örneklerinde 4.23 log kob/g olarak belirlenmiştir. Maya sayısı, 24°C'de depolanan örneklerde, 4°C'de depolanan örneklere göre yaklaşık 1 log birim düşüktür. S ve I örneklerindeki depolama boyunca maya sayısındaki değişim, 4°C'de depolanan örneklerde önemli (p>0.05) bulunmazken; 24°C'de depolanan örneklerde önemli (p<0.05) bulunmuştur. Depolama boyunca maya sayısında düşüş olduğu belirlenmiştir. Ancak Kırdar ve ark. [26], maya sayısında depolamanın 60. gününe kadar artış, 90. gününde ise azalış tespit etmiş ve depolama sırasında 5.47-7.20 log kob/g arasında tespit etmişlerdir.
Genel ortalama küf sayıları dikkate alındığında, Katık Keşi örneklerinde yaklaşık olarak 2.3 log kob/g küf sayısı belirlenmiştir. Küf sayısını, üretim yöntemi, depolama sıcaklığı ve depolama süresi etkilememiştir. Yapılan bir çalışmada küf sayısı 4.76-5.65 log kob/g arasında belirlenmiş ve depolama boyunca arttığı rapor edilmiştir [26]. Benzer sonuç Emirmustafaoğlu ve Coşkun [23] tarafından yapılan çalışmada da bulunmuş ve depolama sonunda 5.92 log kob/g değerine ulaşmıştır. Piyasadan toplanan Keş örneklerinde yapılan çalışmalarda toplam maya-küf sayısı; Tarakçı ve ark. [8] tarafından 3.28-5.38 log kob/g, Çakır ve ark. [1] tarafından 1.69-6.86 log kob/g ve Kırdar [10] tarafından 2-9 log kob/g olarak bulunmuştur.

Bu çalışmada, üretim yöntemi, depolama sıcaklığı ve depolama süresinin koliform sayısını önemli düzeyde (p>0.05) etkilemediği saptanmıştır. S örneklerinde koliform bakteri grubu, kurutma öncesi ve depolama başında tespit edilirken, depolamanın diğer günlerinde tespit edilmemiştir. I örneklerinde ise kurutma öncesi ve depolama başında koliform grubu bakteri tespit edilmemiştir. Üstelik depolamanın sonunda tüm örneklerde koliform sayısı sıfır ya da sıfıra yakındır. Keşin kurutulmasından dolayı su aktivitesinin düşük olması, ürünün asidik olması ve ayrıca I örneklerinin ısıtılarak üretilmiş olması, koliform sayılarının sıfır veya sıfıra yakın çıkmasında etkili olduğu düşünülmektedir. Kırdar [10] tarafından koliform sayısı 2.00-5.53 log kob/g arasında tespit edilirken, diğer bir çalışmada hiçbir örnekte koliform grubu bakteriye rastlanılmamıştır [1]. Emirmustafaoğlu ve Coşkun [23] da yaptıkları çalışmada koliform grubu bakteri tespit etmemişlerdir.

Renk Özellikleri

Katık Keşi örneklerinin iç ve dış yüzey L*, a* ve b* değerleri Tablo 4'te bir araya getirilmiştir. Katık Keşi örneklerinde kurutma işlemi sonrası L* (beyazlık) değerlerinde azalış belirlenirken, a* değerinde negatif (yeşil) yönde b* değerinde ise pozitif (sarı) yönde artış tespit edilmiştir.

S örneklerinin ortalama L* değerleri (89.25), I örneklerine (85.08) göre daha yüksektir ve aradaki fark önemlidir (p<0.05). Benzer durum, kurutma öncesi alınan örneklerde de ortaya çıkmıştır. Bu fark, ısıtma işlemi uygulanan (I) örneklerde, ısıtmanın etkisiyle yoğurtta meydana gelen değişimden kaynaklanmış olabilir. Ayrıca her iki yöntemle üretilen örneklerde (S ve I) 24°C'de depolananlar daha düşük L* değerine sahip olmuşlardır. Depolama sıcaklığı, S örneklerinde ve genel ortalama dış yüzey L* değeri üzerinde etkili olmuştur (p<0.05). Dış yüzey L* değerlerindeki değişim, depolama boyunca sadece I 24°C örneklerinde istatistiksel olarak önemli (p<0.05) bulunmuştur. Bu sonuçlar neticesinde, Katık Keşi örneklerinde L* değerleri kurutma, ısıtma ve yüksek derecelerde depolamadan etkilendiği söylenebilir. Makarnalık Keş örneklerinde L* değerlerinin depolama boyunca azaldığı rapor edilmiştir [11]. Elde edilen değerler, Kızartmalık Keş [23] örneklerinde elde edilen değerlerden düşük; Makarnalık Keş [11] örneklerinde elde edilen değerlere benzerdir.

Genel ortalama dış yüzey a* değeri S örneklerinde -2.88 iken, I örneklerinde -2.02 olarak bulunmuştur. Ancak her iki genel ortalama değerleri arasında istatistiksel olarak fark saptanmamıştır (p>0.05). Her iki üretim yönteminde 4°C'de depolanan örneklerin a* değeri negatif yönde daha yüksek (daha yeşil) çıkmıştır. Hem S örneklerinde hem de genel ortalama değerleri açısından farklı depolama sıcaklıklarında saklamanın a* değerlerine etkisi önemli (p<0.05) bulunmuştur. Depolama boyunca a* değerinde negatif yönde azalma olmuş ve değişim tüm örneklerde önemli (p<0.05) çıkmıştır. Benzer sonuç Emirmustafaoğlu ve Coşkun [23] tarafından da kaydedilmiştir.

I örneklerinin genel ortalama b* değerleri S örneğininkinden yüksek olsa da aralarındaki fark önemsizdir (p>0.05). Ancak her iki örnekte 24°C'de depolama işlemi b* dış yüzey değerlerini artırmıştır (p<0.05). Genel ortalama dış yüzey b* değeri; 4°C'de depolanan örneklerde 20.52 şeklinde belirlenirken, 24°C'de depolanan örneklerde 25.81 bulunmuştur. Depolama süresi de b* dış yüzey değerleri üzerinde etkili olmuştur (p<0.05). Bu çalışmada belirlenen b* değerleri, diğer çalışmalarda kaydedilen b* değerlerinden yüksektir [11, 23].

I örneklerinin genel ortalama iç yüzey L* değerleri (83.96) S örneklerininkinden (89.16) daha düşük ve aradaki fark önemli (p<0.05) bulunmuştur. Ancak her iki örnekte de depolama sıcaklıkları arasında fark oluşmamıştır (p>0.05). Depolama boyunca meydana gelen değişim, I 24°C hariç diğer örneklerde önemsiz (p>0.05) bulunmuştur. Genel ortalama iç yüzey L* değerleri dikkate alındığında; depolamanın 30. günündeki düşüş önemliyken (p<0.05), 30. gün ile 90. gün arasındaki değişim önemsiz (p>0.05) bulunmuştur. Elde edilen iç yüzey L* değerleri, Özcan ve Coşkun [12] tarafından Katık Keşi için rapor edilen iç yüzey L* değerinden (82.40) yüksek; Emirmustafaoğlu ve Coşkun [23] tarafından belirlenen Kızartmalık Keş için belirlenen değerlerden düşüktür.

Genel ortalama iç yüzey a* değerleri; S örnekleri I örneklerine göre, 4°C'de depolanan örnekler 24°C'de depolanan örneklere göre negatif yönde daha yüksek (daha yeşil) bulunmuştur. Aradaki farklar istatistiksel olarak önemli ($p<0.05$) çıkmıştır. Ancak depolama süresi I örneklerinde önemsiz ($p>0.05$), S örneklerinde önemli ($p>0.05$) bulunmuştur. Depolamanın başına göre depolama sonundaki değişim S-24°C örneğinde ve genel ortalamada önemli bulunmuştur. Çalışmadan elde edilen değerler, Emirmustafaoğlu ve Coşkun [23] tarafından rapor edilen değerlerden negatif yönde daha yüksektir.

Tablo 4. Farklı yöntemlerle üretilen ve farklı derecelerde depolanan Katık keşi örneklerinin renk özelliklerinde meydana gelen değişmeler

Table 4. The changes in the color properties of Katık Keş samples produced by different methods and stored at different temperatures

Örnekler		Depolama süresi (Gün)		Renk Özellikleri ($\bar{x}$±SD)					
Yöntem	Depolama sıcaklığı		N	L* Dış yüzey	a* Dış yüzey	b* Dış yüzey	L* İç yüzey	a* İç yüzey	b* İç yüzey
Süzme (S)	4°C	Kurutma öncesi	2	95.82 ± 0.049	-2.95 ± 0.019	13.85 ± 0.301	95.11 ± 0.316	-3.27 ± 0.074	7.83 ± 9.857
		0	2	90.76 ± 0.629^{a}	-4.22 ± 0.262^{b}	21.49 ± 1.344^{a}	91.01 ± 2.722^{a}	-3.75 ± 0.127^{a}	19.55 ± 0.742^{a}
		30	2	90.21 ± 0.226^{a}	-3.22 ± 0.106^{a}	17.55 ± 0.120^{b}	85.38 ± 3.932^{a}	-3.62 ± 0.014^{a}	19.01 ± 0.636^{a}
		60	2	89.79 ± 1.393^{a}	-3.70 ± 0.092^{ab}	19.23 ± 0.354^{ab}	87.83 ± 0.226^{a}	-3.75 ± 0.071^{a}	19.37 ± 0.092^{a}
		90	2	91.12 ± 0.580^{a}	-3.13 ± 0.580^{a}	18.65 ± 1.372^{b}	89.59 ± 1.789^{a}	-3.63 ± 0.057^{a}	19.97 ± 0.460^{a}
		Ortalama	8	90.47 ± 0.829^{Y}	-3.56 ± 0.525^{Z}	19.23 ± 1.707^{Z}	88.45 ± 2.961^{Y}	-3.69 ± 0.089^{Z}	19.47 ± 0.551^{Y}
Süzme (S)	24°C	Kurutma öncesi	2	95.82 ± 0.049	-2.95 ± 0.019	13.85 ± 0.301	95.11 ± 0.316	-3.27 ± 0.074	7.83 ± 9.857
		0	2	90.76 ± 0.629^{a}	-4.22 ± 0.262^{b}	21.49 ± 1.344^{a}	91.01 ± 2.722^{a}	-3.75 ± 0.127^{c}	19.55 ± 0.742^{a}
		30	2	87.91 ± 0.636^{a}	-2.09 ± 0.304^{a}	25.41 ± 1.542^{a}	89.94 ± 1.195^{a}	-3.30 ± 0.368^{bc}	19.58 ± 1.414^{a}
		60	2	86.51 ± 0.248^{a}	-1.36 ± 0.460^{a}	30.91 ± 2.496^{a}	88.89 ± 1.450^{a}	-2.93 ± 0.007^{ab}	18.97 ± 0.502^{a}
		90	2	86.97 ± 4.101^{a}	-1.16 ± 0.997^{a}	24.72 ± 9.051^{a}	89.67 ± 0.113^{a}	-2.54 ± 0.021^{a}	19.69 ± 0.099^{a}
		Ortalama	8	88.04 ± 2.374^{Z}	-2.20 ± 1.369^{Y}	25.63 ± 5.127^{Y}	89.87 ± 1.491^{Y}	-3.13 ± 0.503^{Y}	19.45 ± 0.702^{Y}
Isıtma (I)	4°C	Kurutma öncesi	2	93.75 ± 1.299	-2.70 ± 0.377	14.64 ± 0.293	93.04 ± 1.055	-3.03 ± 0.645	16.96 ± 0.217
		0	2	86.69 ± 2.885^{a}	-4.31 ± 0.240^{b}	25.23 ± 2.418^{a}	86.34 ± 1.874^{a}	-3.17 ± 0.686^{a}	19.38 ± 0.078^{a}
		30	2	87.44 ± 1.881^{a}	-2.47 ± 0.813^{ab}	20.35 ± 1.124^{b}	84.39 ± 2.539^{a}	-3.35 ± 0.573^{a}	21.81 ± 1.174^{a}
		60	2	85.35 ± 1.075^{a}	-2.84 ± 0.905^{ab}	22.26 ± 0.658^{ab}	83.29 ± 0.990^{a}	-3.15 ± 1.131^{a}	20.11 ± 1.987^{a}
		90	2	84.74 ± 1.032^{a}	-1.38 ± 0.870^{a}	19.43 ± 1.909^{b}	82.28 ± 1.351^{a}	-2.52 ± 0.283^{a}	19.58 ± 0.099^{a}
		Ortalama	8	86.06 ± 1.820^{Y}	-2.75 ± 1.260^{Y}	21.82 ± 2.689^{Z}	84.07 ± 2.100^{Y}	-3.05 ± 0.648^{Y}	20.22 ± 1.345^{Y}
Isıtma (I)	24°C	Kurutma öncesi	2	93.75 ± 1.299	-2.70 ± 0.377	14.64 ± 0.293	93.04 ± 1.055	-3.03 ± 0.645	16.96 ± 0.217
		0	2	86.69 ± 2.885^{a}	-4.31 ± 0.240^{b}	25.23 ± 2.418^{ab}	86.34 ± 1.874^{a}	-3.17 ± 0.686^{a}	19.38 ± 0.078^{a}
		30	2	85.01 ± 0.156^{ab}	-1.00 ± 1.040^{a}	26.28 ± 1.414^{ab}	84.69 ± 1.966^{ab}	-2.82 ± 1.174^{a}	19.65 ± 2.539^{a}
		60	2	80.91 ± 0.997^{b}	0.56 ± 1.697^{a}	30.80 ± 1.718^{a}	80.36 ± 0.509^{b}	-2.24 ± 0.304^{a}	20.73 ± 2.037^{a}
		90	2	83.83 ± 1.591^{ab}	-0.40 ± 0.679^{a}	21.68 ± 3.882^{b}	84.03 ± 1.973^{ab}	-1.89 ± 0.339^{a}	20.80 ± 0.085^{a}
		Ortalama	8	84.11 ± 2.605^{Y}	-1.29 ± 2.115^{Y}	26.00 ± 3.975^{Y}	83.85 ± 2.665^{Y}	-2.53 ± 0.758^{Y}	20.14 ± 1.406^{Y}
Süzme (S)			16	89.25 ± 2.128^{A}	-2.88 ± 1.224^{A}	22.43 ± 4.956^{A}	89.16 ± 2.381^{A}	-3.41 ± 0.453^{B}	19.46 ± 0.610^{A}
Isıtma (I)			16	85.08 ± 2.392^{B}	-2.02 ± 1.844^{A}	23.91 ± 3.925^{A}	83.96 ± 2.321^{B}	-2.79 ± 0.732^{A}	20.18 ± 1.330^{A}
	4°C		16	88.26 ± 2.657^{A}	-3.16 ± 1.024^{B}	20.52 ± 2.553^{B}	86.26 ± 3.356^{A}	-3.37 ± 0.557^{B}	19.84 ± 1.065^{A}
	24°C		16	86.07 ± 3.148^{B}	-1.74 ± 1.785^{A}	25.81 ± 4.436^{A}	86.86 ± 3.744^{A}	-2.83 ± 0.695^{A}	19.79 ± 1.131^{A}
		0	8	88.72 ± 2.686^{A}	-4.26 ± 0.197^{B}	23.36 ± 2.487^{AB}	88.67 ± 3.058^{A}	-3.46 ± 0.487^{B}	19.46 ± 0.409^{A}
		30	8	87.64 ± 2.114^{AB}	-2.19 ± 0.998^{A}	22.40 ± 3.955^{AB}	86.10 ± 3.105^{B}	-3.27 ± 0.598^{B}	20.01 ± 1.662^{A}
		60	8	85.64 ± 3.485^{C}	-1.83 ± 1.882^{A}	25.80 ± 5.647^{A}	85.09 ± 3.750^{B}	-2.64 ± 0.690^{AB}	19.79 ± 1.312^{A}
		90	8	86.66 ± 3.468^{BC}	-1.52 ± 1.228^{A}	21.12 ± 4.582^{B}	86.39 ± 3.701^{B}	-2.79 ± 0.732^{A}	20.01 ± 0.543^{A}

^{a,b,c}: Aynı sütunda farklı küçük harf taşıyan ortalamalar birbirinden farklı ($p<0.05$), aynı harf taşıyanlar farksızdır ($p>0.05$); ^{A,B}: Her bir faktör için aynı sütunda farklı büyük harf taşıyan ortalamalar birbirinden farklı ($p<0.05$), aynı harf taşıyanlar farksızdır ($p>0.05$). ^{Y,Z}: Her bir kimyasal özellikte süzme yönteminde 4°C ile 24°C veya ısıtma yönteminde 4°C ile 24°C'de ortalama değerleri için; farklı büyük harf taşıyan ortalamalar birbirinden farklı ($p<0.05$), aynı harf taşıyanlar farksızdır ($p>0.05$).

Tablo 4'ten örneklerin iç yüzey b* değerleri incelendiğinde; I örneklerinin b* değeri daha yüksek çıkmış, ancak fark istatistiksel olarak önemli ($p>0.05$) değildir. Diğer yandan S ve I örneklerinin farklı derecelerde depolanması iç yüzey b* değerlerini etkilememiştir ($p>0.05$). Benzer şekilde depolama boyunca tüm örneklerde iç yüzey b* değerlerinde önemli bir değişim meydana gelmemiştir ($p>0.05$). Kurutma öncesinde S ve I örneklerinin b* değerlerindeki fark kurutma sonrasında ortadan kaybolmuş ve benzer sonuçlar kaydedilmiştir. Netice olarak söylenebilir ki, örneklerin iç yüzey b* değerleri kurutma işleminden etkilenmiş, ancak yöntem farklılığı ve depolama sıcaklığı ile süresinden etkilenmemiştir. Çalışmadan elde edilen iç yüzey b* değerleri, Emirmustafaoğlu ve Coşkun [23] tarafından bildirilen iç yüzey b* değerlerinden (12.09) yüksekken, Özcan ve Coşkun [12] tarafından yapılan çalışmadaki sonuçlarla benzerdir.

Depolama boyunca elde edilen renk değerleri dikkate alınarak örneklerin ΔE değerleri hesaplanmıştır. Kurutma öncesi ile kurutma sonrası örneklerin dış ve iç yüzey renk değerleri arasındaki ΔE değeri sırasıyla süzme yönteminde 9.3 ve 12.4; ısıtma yönteminde 12.8 ve 7.1 olarak belirlenmiştir. Katık Keşi örneklerinin depolama boyunca dış yüzey rengi 2.5-10.7; iç yüzey rengi ise 1.2-6.2 arasında değişmiştir. Örneklerin dış yüzey rengi açısından en fazla ΔE değeri 24°C'de depolanan I ve S örneklerinde belirlenmiştir. ΔE değeri 3'ten büyükse farkın gözle rahatlıkla ayırt edilebildiği, 1.5-3 arasındaysa belirgin bir farklılık olduğu, 1.5'tan küçükse farkın az olduğu rapor edilmiştir [30].

Duyusal Özellikler

Duyusal analizlerde panelistlere renk ve görünüş, yapı ve kıvam, lezzet ve genel beğeni kategorisinde Sıralama (Tercih) Testi uygulanmıştır. Duyusal analize katılan panelistlerin büyük çoğunluğu tüm kategorilerde S örneklerini daha çok beğenmişlerdir. Benzer şekilde 4°C'de depolanan örnekler daha çok tercih edilmiştir. Panelistler tarafından tüm kategorilerde en çok tercih oranı sırasıyla, S 4°C örneği, S 24°C örneği, I 4°C örneği ve I 24°C örneği şeklinde olmuştur. I 24°C örneği, panelistler tarafından lezzet ve genel beğeni kategorisinde hiç tercih edilmemiştir.

SONUÇ

Keş veya kurut, iki farklı geleneksel yöntemle üretilmektedir. Bu çalışmada dikkate alınan; birinci geleneksel yöntemde (S) üretim basamakları yoğurdun süzülmesi, tuzlanması, şekil verilmesi ve kurutulması şeklindedir (S). İkinci geleneksel yöntemde (I) ise yoğurdun yayıklanması sonrası geriye kalan ayranın ısıtılıp çöktürülmesi, süzülmesi, tuzlanması, şekil verilmesi ve kurutulması şeklindedir. Yapılan literatür incelemesinde, yoğurdu süzerek veya ısıtıp çöktürdükten sonra süzerek kurutulması ve ortaya çıkan ürünlerin karşılaştırmasına yönelik çalışmaya rastlanmamıştır. Bu çalışmada, geleneksel yöntemler dikkate alınarak Katık Keşi üretiminde iki farklı yöntem arasından en uygun yöntemin belirlenmesine çalışılmıştır. Her iki yöntemle üretilen örnekler 4°C ve 24°C olmak üzere 2 farklı depolama sıcaklığında 90 gün muhafaza edilmiştir. Çalışmadan elde edilen veriler Katık Keşinin işletme düzeyinde üretilebilmesi bakımından önemli bilgiler sunmaktadır. Tüm elde edilen veriler topluca değerlendirildiğinde yoğurdun süzülmesi, tuzlanması ve ardından kurutulması (S yöntemi) ile üretilen ve 4°C'de depolanan (S 4°C) örnekler Katık Keşi üretimi için en uygun üretim ve depolama yöntemi olarak ortaya çıkmıştır. Katık Keşi üretiminin halihazırda geleneksel düzeyde kalması ve tam manasıyla geniş kitlelere aktarılamaması dolayısıyla özellikle üretim ve pazarlama konuları üzerine daha fazla çalışma yapılmasına ihtiyaç vardır.

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Çalışmaların Hazırlanması

1. Çalışmalar A4 boyutunda hazırlanmalı, üstten 2.45 cm, alttan 2.45 cm, sağ ve soldan 1.75 cm boşluk bırakılmalı ve tek kolon olarak hazırlanmalıdır. Metin çift satır aralıklı yazılmalı, paragraflar arasında tek satır boşluk bırakılmalıdır. Metinde bütün satırlar (sürekli) numaralandırılmalıdır.

2. Çalışma başlığı 14 punto Arial, koyu, küçük harflerle ve ortalanmış olarak yazılmalıdır. Başlıktan sonra bir satır boşluk bırakılmalı (11 punto); yazar isimleri (yalnızca ilk harfler büyük) 10 punto Arial ve ortalanmış olarak verilmelidir. Yazarların adresleri, telefon ve faks bilgileri ile yazışmalardan sorumlu yazarın e-posta adresi hemen alt satırda 9 punto Arial, ilk harfler büyük olacak şekilde ve ortalanmış olarak yazılmalıdır. Yazarların çalıştıkları kuruluşlar (ve/veya adresler) farklı ise her bir yazar isminin sonuna rakamlarla üst indis konulmalıdır.

3. Metin içindeki kısımların başlıkları (ÖZ, ABSTRACT, GİRİŞ vb.) 10 punto Arial ve koyu olarak büyük harflerle yazılmalı, başlıktan sonra bir satır boşluk bırakılarak metine geçilmelidir. Alt başlıklarda ilk harfler büyük, 10 punto Arial ve koyu yazı karakteri kullanılmalıdır. ÖZ’ün altına bir satır boşluk bırakıldıktan sonra en fazla 5 adet Anahtar Kelime konmalıdır. Anahtar Kelimelerden sonra bir satır boşluk bırakılarak İngilizce başlık ve altına ABSTRACT ve Keywords yazılmalıdır. Bir satır boşluk bırakılarak ana metine geçilmelidir.

4. Ana metin 9.5 punto Arial olarak hazırlanmalıdır.

5. Çalışma başlıca şu kısımlardan oluşmalıdır: Başlık, Yazar İsimleri, Adresleri, İletişim Bilgileri, Yazışmalardan Sorumlu Yazarın E-posta adresi, Öz, Abstract, Ana Metin (Giriş, Materyal ve Metot, Bulgular ve Tartışma, Sonuç), Teşekkür (gerekiyorsa), Kısaltmalar (gerekiyorsa), Kaynaklar.

6. Öz ve Abstract 250 kelimeyi geçmemeli, çalışmanın amacını, metodunu ve önemli sonuçlarını içermelidir. Öz tek paragraf olarak yazılmalı ve öz içinde kaynaklara atıf yapılmamalıdır.

7. Çalışma içerisinde geçen mikroorganizma isimleri ile Latince ifade ve isimler italik olarak yazılmalı ve kısaltmalarda uluslararası yazım kuralları göz önünde bulundurulmalıdır.

8. Tablo başlıkları tablonun üstüne, şekil başlıkları ise şeklin altına yazılmalı ve numaralandırılmalıdır. Kullanılan tablo ve şekillere metin içinde mutlaka atıf yapılmalıdır. Metin içinde geçen veriler tablo ve şekillerin tekrarı olmamalıdır. Tablo ve şekillerin başlıkları içerikleriyle uyumlu ve anlaşılabilir olmalıdır. Şekiller ve resimlerin yüksek çözünürlükte olmasına dikkat edilmelidir. Resimler (ve gerekiyorsa Şekiller) *.jpg formatında metin içerisinde yer almalıdır.

9. Metin içerisinde atıflar köşeli parantez içerisinde rakamlarla yapılmalı [1] ve Kaynaklar bölümünde bu numara sırasıyla detayları yazılmalıdır. Kaynakların numaralandırılması MS Word Numaralandırma Kitaplığı kullanılarak yapılmalıdır.

10. Kullanılan matematiksel denklemler numaralandırılmalı ve metin içerisinde bu denklemlere atıf yapılmalıdır.

11. Kaynaklar kısmı APA yazım stili kullanılarak hazırlanmalıdır. Kaynakların yazımında aşağıdaki örnek yazım biçimleri kullanılmalı ve makalelerin yayınlandığı dergi isimleri kısaltma kullanılmadan ve italik olarak yazılmalıdır. Web adreslerine atıf yapılacağında (mümkün olduğunca Resmi web sayfalarına atıf yapılmalıdır) mutlaka ilgili web adresine erişim tarihi verilmelidir.

Makale

[1] Bozkurt, H., İçier, F. (2009). İnegöl köfte üretiminde ohmik pişirmenin uygulanabilirliğinin incelenmesi. *Akademik Gıda,* 9(1), 6-12.

Kitap

[2] Kılıç, S. (2001). Süt Endüstrisinde Laktik Asit Bakterileri. Ege Üniversitesi Ziraat Fakültesi Yayınları, Ege Üniversitesi Matbaası, Bornova, İzmir.

Kitap Bölümü

[3] Gibson, G.R., Saavedra, J.M., MacFarlane, S., MacFarlane, G.T. (1997). Probiotics and Intestinal Infections. In Probiotics 2: Applications and Practical Aspects, Edited by R. Fuller, Chapman & Hall, 2-6 Boundary Row, London SE1 8HN, England, 212p.

Kongre-Sempozyum Bildirisi

[4] Gürsoy, O., Akdemir, O., Hepbaşlı, A., Kınık, Ö. (2004). Recent situation of energy consumption in Turkey dairy industry. *International Dairy Symposium: Recent Developments in Dairy Science and Technology*, May 24-28, 2004, Isparta, Turkey, Book of Proceedings, 10-16p.

12. Hakem görüşleri doğrultusunda düzeltilmek üzere yazar(lar)a gönderilen çalışmaların gerekli düzeltmeleri yapılarak yayın ofisine ulaştırılması gereklidir. Editörler tarafından belirtilen süre zarfında gönderilmeyen çalışmalar “ilk defa gönderilmiş çalışma” olarak değerlendirilecektir.

13. Yukarıdaki kurallara uygun olarak hazırlanmamış çalışmalar değerlendirmeye alınmaz.

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1. Manuscripts should be prepared in A4 size, and the text must be prepared in a single column format. The text must be double-spaced, and a single space should be left between paragraphs. All lines and pages must be continuously numbered.

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3. The headings (e.g. Abstract, Introduction, Materials and Methods etc.) must be 10pt Arial, and should be typed in bold capital letters. Each heading should appear on its own separate line. A blank line should be left after each heading. A list of keywords, a maximum of 5, should be provided below the abstract section of the manuscript.

4. The main text should be prepared in 9.5pt Arial.

5. Typical articles mainly consist of the following divisions: Title, Author Names, Addresses, Contact Information, Corresponding author's e-mail address, Abstract, Main text (Introduction, Materials and Methods, Results and Discussion, Conclusions), Acknowledgements (if necessary), Abbreviations (if necessary) and References.

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8. Table headings should be on the top of each table and figure captions below each figure. Each table or figure must be numbered consecutively in accordance with their appearance in the text. All figures and tables should be cited in the text. The data presented in the tables and figures should not be repeated in the text. Table headings and figure captions should be self-explanatory. Figures and pictures must be provided in high resolution, and pictures (and, if necessary figures) should be included in the text as *. jpg format.

9. References in the text should be cited in numbers in square brackets [1] and details of the citations must be provided in the Literature or References section with their respective numbers.

10. Mathematical equations should be numbered and cited in the text.

11. References should be given according to the APA manual of style. The following formats should be used for the details of cited references, and the journal names must be typed in italics. References to the Web addresses (if necessary, the official web pages should be preferred) must include full web address and the date of access.

Article

[1] Güzeler, N., Kaçar, A., Say, D. (2011). Effect of milk powder, maltodextrin and polydextrose use on physical and sensory properties of low calorie ice cream during storage. *Akademik Gıda,* 9(2), 6-12.

Book

[2] Kilic, S. (2001). Lactic Acid Bacteria in Dairy Industry. Ege University Faculty of Agriculture Publications, Ege University Press, Bornova, Izmir, Turkey.

Book Chapter

[3] Gibson, G.R., Saavedra, J.M., MacFarlane, S., MacFarlane, G.T. (1997). Probiotics and Intestinal Infections. In Probiotics 2: Applications and Practical Aspects, Edited by R. Fuller, Chapman & Hall, 2-6 Boundary Row, London, England, 212p.

Proceedings of the Congress-Symposium

[4] Gursoy, O., Akdemir, O., Hepbasli, A., Kinik, O. (2004). Recent situation of energy consumption in dairy industry in Turkey. *International Dairy Symposium: Recent Developments in Dairy Science and Technology*, May 24-28, 2004, Isparta, Turkey, Book of Proceedings, 10-16p.

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Dergiye gönderilen tüm makaleler, bilimsel içeriklerinin özgünlüğü ve kalitesi ölçütlerine göre değerlendirilir.

- Dergiye gönderilen tüm yazılar, ilk olarak yayın ofisindeki (teknik ve genel kalite değerlendirilmesi açısından) eleme işleminden geçer ve ardından teknik ve bilimsel editörler tarafından değerlendirilir.
- İlk değerlendirmeden sonra, editörler (i) dergi kapsamı dışında kalan bir konu hakkında hazırlanmış makaleleri (ii) teknik olarak eksik/yetersiz makaleleri, (iii) kısmi ve marjinal artan sonuçları içeren makaleleri veya (iv) kötü yazılmış makaleleri reddetme hakkına sahiptir.
- İlk inceleme sonucunda makalenin ileri değerlendirme için uygun olduğuna karar verilirse, dergide yayımlanmak üzere kaliteli makalelerin seçimini yapmak amacıyla, makaleler çift-körlü (hakemin ve yazar/yazarların birbirlerini görmedikleri) değerlendirme sistemi ile en az iki bağımsız hakemden oluşan bir değerlendirme sürecinde bilimsel incelemeye alınır.
- Hakemler tarafından talep edilirse, makalenin hakem görüşleri doğrultusunda yazarlar tarafından revize edilmiş versiyonu orijinal hakemler tarafından tekrar değerlendirilir. Değerlendirmelerin ardından editörler hakem önerileri doğrultusunda makale hakkındaki nihai kararlarını verirler. Gerekirse editörler, hakemlerin istedikleri tüm şartların yerine getirilmesi için yazarlardan ilave revizyon isteyebilir.
- Kabul edilen makalelerin son versiyonu, yayın öncesi taslağın (galley proof) hazırlanması için teknik editörlere gönderilir. Yazarlardan, makalelerinin dizgisi hazırlanmış taslaklarını son kontrol için yayın öncesinde incelemeleri istenir.
- Tüm makaleler, nihai formlarında DOI numarası almış ve çevrimiçi olarak pdf dosyaları halinde yayımlanır. İlgili veritabanlarında bu şekilde indekslenir.

Gizlilik

Editörler, Akademik Gıda'ya gönderilen tüm makaleleri tam bir gizlilikle ele alır. Editörler, hakemler haricinde, COPE tavsiyelerine uyulmadığı takdirde, üçüncü şahıslara makale ile ilgili hiçbir bilgi vermezler. Yayınlanmak üzere dergiye gönderilen makaleler hakemler için de gizlidir ve bilimsel değerlendirme için aldıkları makalelerin herhangi bir bölümünü üçüncü şahıslarla paylaşmalarına veya dağıtmalarına izin verilmez. Suiistimal şüphesi olduğunda, hakemlerin derhal gizli bir şekilde yayın ofisine başvurmaları önerilir. Hakemler ayrıca, Dergi Editörleri İçin Davranış Kuralları ve En İyi Uygulama Kuralları ile Dergi Yayıncıları için Davranış Kuralları'nı (Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers) takip ederek editöre gizli yorumlarında belirli bir eylem önerebilirler.

Akademik Gıda, çift-kör bir hakem inceleme süreci yürütür, yani çalışmanın eleştirel değerlendirmesini sağlamak için hakemlerin isimleri gizlidir. Hakemlerden, raporlarında adlarını veya irtibat bilgilerini açıklamamaları istenir. Hakem raporları yazarlara gönderilemeden önce bu açıdan kontrol edilir.

Yazarlık

Bir yazar, bir araştırmanın fikrine veya tasarımına, verilerin elde edilmesine, verilerin analizine veya yorumlanmasına büyük ölçüde katkıda bulunan, makalenin hazırlanmasında, yazılmasında veya gözden geçirilmesinde entelektüel içeriğe eleştirel katkı yapan bireydir. Katkıda bulunanlar diğer kişiler makalenin Teşekkür bölümünde belirtilmelidir ve çalışmanın yazarı olarak kabul edilemez. Tüm yazarların doğru ve tam isimleri ile ORCID kimlikleri dergiye gönderilen makalenin başlık sayfasında yer almalıdır. Yazarların isimlerinin yanında çalıştıkları kurumlar ve yazışmalardan sorumlu yazarın geçerli bir adresi verilmelidir. Yazışmalardan sorumlu yazarın telefon ve faks numaraları ile e-posta adresi makalenin ilk sayfasında belirtilmelidir. Tüm yazarlar, gönderilen makalenin daha önce herhangi bir yerde yayınlanmadığını ve makale hakkında Akademik Gıda dergisi nihai bir karar vermeden önce makaleyi başka bir dergiye göndermeyeceklerini garanti etmelidir.

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Makalenin Akademik Gıda'ya sunulmasından sonra yazar isimlerinde değişiklik ancak revizyon sırasında gerekli olan ek çalışmalar durumunda olabilir. Makalenin yayına kabul edilmesinden sonra herhangi bir değişikliğe izin verilmez. Yazarlıktaki değişiklik, hakem görüşlerine verilen cevaplar sırasında yazışmalarda belirtilmeli ve tüm yazarlar tarafından kabul edilmelidir. Yazışmalardan sorumlu yazar, yazarların sırası da dahil olmak üzere makalenin revize edilmiş versiyonundaki değişikliklerden sorumludur.

Çalışma Verilerinde Düzeltme

Yayınlanan verilerin doğruluğundan tüm yazarlar sorumlu olmalıdır. Verilerin düzeltilmesi için, yazışmalardan sorumlu yazardan yayın öncesi taslağı (galley proof) incelemesi ve makalenin yayınlanmasından 4 gün önce dikkatlice düzeltmesi istenir.

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Bir makalenin geri çekilmesi, gönderim veya yayın hatalarını düzeltmek için kullanılır. Yazarlar makaleyi geri çekebilir ve bu durumda Yayın Etiği Komitesi (COPE) Geri Çekme Kurallarına [(COPE) retraction guidelines] uymalıdır. Tekrarlanan veya benzerlik oranı yüksek bir yayın, verilerin hileli kullanımı, intihal veya etik dışı araştırma yapılması durumunda, makale editör tarafından geri çekilecek ve geri çekilen makale linklerine bağlantı korunacak ancak elektronik veri tabanına (makale sayfasına) bir geri çekme bildirimi eklenecektir.

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İnsan denekleri, hayvan veya bitki içeren araştırmalar

- Araştırmanın insan denekleri veya hayvanları içermesi durumunda, yazarların Uluslararası Tıp Dergisi Editörleri Komitesinin (the International Committee of Medical Journal Editors) yönergelerini izlemeleri önerilir.
- İnsan denekleri içeren çalışmalarda, deneklerin çalışmaya katılmak için imzaladıkları onamlar yazarlar tarafından sağlanmalıdır. 18 yaşın altındaki deneklerin çalışmaya katılmaları için ebeveyn veya velileri tarafından izin verilmelidir.
- Test edilen tüm denekler için, makalenin, ilgili kurallara ve/veya uygun izinlere veya lisanslara uyumunu gösteren belgelerin sunulması gerekir.
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- Bitki materyallerinin toplanması dahil, bitkiler üzerinde yapılan deneysel araştırmalar, kurumsal, ulusal veya uluslararası kurallara uygun olmalıdır.
- Saha çalışmaları yerel mevzuata uygun olarak yapılmalı ve uygun izinleri ve/veya lisansları belirten bir açıklama makalede yer almalıdır.

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- Akademik Gıda dergisi, Dergi Editörleri İçin Davranış Kuralları ile En İyi Uygulama Kılavuzları ve Dergi Yayıncıları İçin Davranış Kurallarını (Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers) takip eder.
- Makalenin aynı anda birden fazla dergiye gönderilmesi, intihal, yayınlanmış makalenin yeniden yayınlanması, etik kuralların ihlali vb. şüpheli bir suiistimal durumunda, araştırmacılar, hakemler veya okuyucular Yayın Ofisi (ogursoy@yahoo.com) ile iletişime geçmeye teşvik edilir.
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