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Saygılarımla.

Prof. Dr. Oğuz Gürsoy
Baş Editör

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4. Uluslararası Türk Tarım - Gıda Bilimi ve Teknolojileri Kongresi

4. Uluslararası Türk Tarım - Gıda Bilimi ve Teknolojileri Kongresi (TURJAF 2025) **28-30 Nisan 2025** tarihleri arasında Niğde Ömer Halisdemir Üniversitesi ev sahipliğinde çevrimiçi ve yüz yüze olarak gerçekleştirilecektir. Kongre ile ilgili bilgilere **https://www.turjaf.com/index.php/TURSTEP** adresinden ulaşılabilir.

4. Uluslararası Gıda Kimyası Kongresi

Kimyagerler Derneği tarafından düzenlenen 4. Uluslararası Gıda Kimyası Kongresi **8-11 Mayıs 2025** tarihlerinde araştırmacı ve akademisyenlerin katılımıyla Antalya'da gerçekleştirilecektir. Kongre ile ilgili bilgilere **https://gidakimyasikongresi.org/** adresinden ulaşılabilir.

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8. Uluslararası Gıda, Tarım ve Veteriner Bilimleri Kongresi

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Türkiye 15. Gıda Kongresi

Gıda Teknolojisi Derneği tarafından 1978 yılından bu yana düzenlenmekte olan Türkiye Gıda Kongresinin on beşincisi, Sakarya Üniversitesi Gıda Mühendisliği Bölümü ev sahipliğinde **8-10 Ekim 2025** tarihlerinde Sakarya'da ülkemizden alanında uzman birçok değerli bilim insanının katılımı ile gerçekleştirilecektir. Kongre ile ilgili bilgilere **https://gidakongresi2025.org/** adresinden ulaşılabilir.

14. Gıda Mühendisliği Kongresi

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Research Paper / Araştırma Makalesi

Use of Hemp Seed Milk in Ice Cream and Its Effect on Physicochemical, Rheological, Bioactive and Sensorial Properties

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ABSTRACT

The nutritional and positive effects of plant-based milks on human health are increasingly studied, and their integration into various food formulations has become essential in new product development. In this study, hemp seed milk was added to the ice cream mix as a functional ingredient and a replacement for cow's milk (0-25-50-75-100%) to investigate its effect on the physicochemical, rheological, textural, functional, and sensory properties of ice cream. As the ratio of hemp seed milk with a high protein content increased in the mix, ice cream samples with higher protein content were obtained compared to the control sample. The use of hemp seed milk reduced the first dripping time and increased the melting rate of ice creams, causing them to melt in a greater mass. However, statistically insignificant changes in the hardness parameter were observed as their textural properties. The apparent viscosity values of ice creams decreased, and the difference between elastic (G') and viscous (G") properties also reduced compared to the control ice cream. Among ice creams with hemp seed milk, the sample containing 25% hemp seed milk received the highest sensory score. Considering the functional properties, as the hemp seed milk ratio increased, ice creams with higher total phenolic contents and antioxidant activities were obtained compared to the control sample. In conclusion, taking into account its favorable sensory properties, using a 25% hemp seed milk ratio can be suggested as a plant-based milk option that delivers desirable physicochemical and functional characteristics to ice cream.

Keywords: Hemp seed milk, Seed-based milk, Vegetable milk, Ice cream, Functional

Dondurmada Kenevir Tohumu Sütü Kullanımı ve Fizikokimyasal, Reolojik, Biyoaktif ve Duyusal Özellikleri Üzerine Etkisi

ÖZ

Bitki bazlı sütlerin insan sağlığı üzerindeki besleyici ve olumlu etkileri giderek daha fazla araştırılmakta ve bunların çeşitli gıda formülasyonlarına entegrasyonu, yeni ürün geliştirmede önemli bir hale gelmektedir. Bu çalışmada, kenevir tohumu sütü, fonksiyonel bir bileşen ve inek sütü yerine (0-25-50-75-100%) kullanılmak üzere dondurma karışımına eklenerek, dondurmanın fizikokimyasal, reolojik, tekstürel, fonksiyonel ve duyusal özellikleri üzerindeki etkileri araştırılmıştır. Dondurma karışımında protein içeriği yüksek kenevir tohumu sütü oranı arttıkça kontrol dondurmaya göre daha yüksek protein içeriğine sahip dondurmalar elde edilmiştir. Kenevir tohumu sütü kullanımı, dondurmaların ilk damlama süresini kısaltıp erime hızını artırarak daha büyük kütlede erimeye neden olmuştur. Ancak tekstürel özelikler incelendiğinde sertlik parametresi üzerinde istatistiksel olarak anlamlı bir değişiklik gözlenmemiştir. Dondurmaların görünür viskozite değerleri azalmış, elastik (G') ve viskoz (G") özellikleri arasındaki fark da kontrol dondurmasına göre azalmıştır. Kenevir tohumu sütü ilave edilen dondurmalar arasında %25 kenevir tohumu sütü içeren dondurma en yüksek duyusal değerlendirme puanını almıştır. Fonksiyonel özellikler dikkate alındığında ise kenevir tohumu sütü oranı arttıkça kontrol dondurmaya göre daha yüksek toplam fenolik ve antioksidan aktiviteye sahip dondurmalar elde edilmiştir. Sonuç olarak %25 kenevir tohumu sütü oranı dondurmanın hem fizikokimyasal ve

fonksiyonel özelliklerini geliştiren hem de duyusal açıdan beğenilen bitki bazlı süt olarak önerilebilir.

Anahtar Kelimeler: Kenevir tohumu sütü, Tohum bazlı sütler, Bitkisel sütler, Dondurma, Fonksiyonel

INTRODUCTION

Milk and dairy products constitute an important part of our diet. However, interest in plant-based products is increasing due to reasons such as the adverse effects of animal food production on the environment, the danger of insufficient animal food sources for the increasing population, lactose intolerance, adverse health effects, and new lifestyles including vegetarian and vegan diets [1-3]. Plant-based milk substitutes, or plant extracts, are water-soluble extracts of cereals (oats, rice), legumes (chickpea, soybeans), nuts (almonds, coconut, cashew nut, hazelnuts), seeds (sesame, sunflower, hemp, flaxseed) and pseudocereals (quinoa, teff) [4, 5]. Plant-based milks are not only consumed as beverages but also used as ingredients in food formulations. There are many studies in the literature investigating the use of plant-based milks in a fermented beverage [6, 7], yoghurt [8, 9], pudding [1, 10], cheese [10, 11], and ice cream [12-14] formulations. Since the sugar used in ice cream can mask the unfamiliar taste of plant-based ingredients, ice cream is a suitable food matrix in which plant-based ingredients can be included [15].

The cultivation and production of hemp (Cannabis sativa L.) were under control for years due to the psychoactive substance (delta-9 tetrahydrocannabinol, THC) it contains [16]. However, in the production of hemp-based food products, materials obtained from the industrial hemp plant containing 0.3% to 1.5% THC are used. Therefore, unlike Marijuana, which has psychoactive properties including 5%-10% or more THC, industrial hemp can be used legally [17]. Hemp seed is a nutritional valuable seed due to its protein (20-25%), lipids (25-35%), carbohydrate (20-30%), insoluble fiber (10-15%), and ash (4-7%) content, which varies depending on the variety. Hemp seed is also vital for human nutrition, mainly due to its total polyphenols and essential fatty acids (linoleic acid-LA, C18:2, ω-6 and α-linolenic acid-ALA, C18:3, ω-3) [18, 19]. Hemp seeds are also a rich source of antioxidants and phytochemicals that positively affect health [20]. Therefore, hemp-based materials such as protein, oil, flour, and milk can be used as functional ingredients in producing functional food products [20, 21].

Hemp seed milk is one of seed-based plant milk and is an oil-in-water emulsion resulting from the extraction of hemp seeds with water [22]. There are studies examining hemp seed milk only as a beverage [23-25] or examining the effects of some technological processes, such as ultrasound [26, 27], homogenization [16, 26], enzyme applications [28] and heat treatment [29], on the stability of hemp seed milk. Studies also investigate its use as an ingredient in different food formulations to develop new products. The possibilities of using hemp seed milk in the production of products such as ayran [9], yoghurt [30], ice cream [31] and kefir [32] was investigated.

In this study, because the ice cream mix already included stabilizers and emulsifiers, it was considered a food matrix that did not require any further processing to stabilize hemp seed milk. Differing from previous research, this study focused on evaluating the impact of varying hemp seed milk to cow's milk ratios, rather than just a single formulation, by analyzing how these ratios influenced the structure of the resulting ice cream. The research investigated the use of hemp seed milk as a replacement for cow's milk in ice cream formulations - without the need for additional processing- and examined its effects on the physicochemical, rheological, sensory, and functional properties of the ice cream.

MATERIALS and METHODS

Materials

The raw materials used in ice cream production were purchased from the following companies in Türkiye: skim milk powder (Enka Süt, Adana), sugar (Doğuş Çay, Afyonkarahisar), vanilla (Dr. Oetker, İzmir), emulsifier and stabilizer mixture (Danisco, İstanbul) and milk cream with 35% milk fat content (Şok Market, Samsun). Hemp seeds were supplied from Kenevir Araştırmaları Enstitüsü (Ondokuz Mayıs University, Samsun). To obtain hemp seed milk, hemp seeds (20%, w/v) were mixed with distilled water and mixed with Ultra Turrax homogenizer (Ika Homogenizer T-25 basic Ultra Turrax, Staufen im Breisgau, Baden-Württemberg, Germany) at 10000 rpm for 10 min. Then, hemp seed milk passed through a 120-mesh sieve.

Methods

Ice Cream Production

The ice cream mix was calculated to contain 30% total dry matter and 8% fat. The ingredients used in the ice cream mix were as listed: 15% sugar, 0.75% stabilizer, and the remainder were cow milk (CM) or hemp seed milk (HM) and various combinations of them (CM-HM ratios: 100-0 (Control), 75-25, 50-50, 25–75, 0–100%). Besides, to achieve 8 g/100 g fat content in ice cream formulations, milk cream with 35% milk fat content was used. The milk was heated to approximately 45°C, and powder ingredients were added. The mixture was then heated and homogenized at 10000 rpm for 10 minutes (Ika Homogenizer T-25 basic Ultra Turrax, Staufen im Breisgau, Baden-Württemberg, Germany). The homogenized mix was pasteurized in a water bath at 80°C for 10 minutes. Ice cream mixes were cooled to 4°C and then aged overnight at 4°C.

Physicochemical Analyses of Samples

The dry matter and ash content of the ice cream samples were determined gravimetrically by keeping them in an oven at 105 °C and a furnace at 550°C, respectively [33]. Kjeldahl method was applied to determine protein content [34]. After the total nitrogen amount was found, the protein amount was calculated by multiplying it by 6.38. Fat content was determined using the Gerber assay [35]. The pH values of the ice cream mixes were measured with a pH meter (Eutech Instruments, pH 700, Waltham, Massachusetts, USA).

Color values of ice cream samples were determined with a color measuring device (Minolta Chroma Meter CR-400, Tokyo, Japan) using CIELAB system. The "L*" value represents brightness (0: black, 100: white), the "a*" value represents red/green (+: red, -: green), and the "b*" value represents yellow/blue (+: yellow, -: blue). The color difference value (ΔE^*) value, which shows the difference of the ice creams with hempseed milk (L_2, a_2, b_2) from the control ice cream (L_1, a_1, b_1) in terms of color, was calculated according to Equation 1 given below;

$$\Delta E^* = \sqrt{(L_2 - L_1{}^2) + (a_2 - a_1)^2 + (b_2 - b_1)^2} \quad (1)$$

Rheological Properties of Ice Cream Mix

The rheological properties of ice cream samples were determined using a Haake Mars III (Thermo Scientific, Waltham, Massachusetts, USA) device with conical and plate geometry (35 mm diameter, 0.104 mm gap, 2° angle) according to Kurt and Atalar [35]. The steady and viscoelastic properties of the ice creams were measured at +4°C. In addition, the ice cream samples were loaded onto the geometric probe and waited for 2 minutes for structure recovery and temperature equilibrium before each measurement. Shear rates from 0.1 s^{-1} to 100 s^{-1} (increasing) were applied to the samples for 3 min to determine the flow behavior graphs and apparent viscosity values (at a 50 s^{-1} shear rate). The flow behavior was analyzed by using the Herschel–Bulkley model based on the following Equation 2:

$$\tau = \tau_Y + K\dot{\gamma}^n \quad (2)$$

where τ is the shear stress (Pa), τ_y is the yield stress (Pa), K is the consistency coefficient (Pa s), γ is the shear rate (s^{-1}) and n is the flow behavior index (dimensionless).

Dynamic rheological measurements were made to determine ice cream samples' viscoelastic properties, and storage (G') and loss modulus (G") values were obtained. For this purpose, measurements were made at a shear stress of 0.2 Pa (linear viscoelastic area determined by stress sweep analysis) and a frequency range of 0.1-10 Hz.

Textural Properties of Ice Cream Mix

A texture analyzer (TA-XT2 Stable Micro Systems Co., Ltd., Surrey, UK) was used to determine the hardness value of ice creams stored in a plastic container at -18°C for 24 h. The analysis was carried out using a cylindrical probe with a diameter of 5 mm, penetration depth of 5 mm, and penetration speed of 1.0 mm/s. The hardness is expressed as the peak pressure force (N) during penetration [35].

Melting Properties of Ice Cream Mix

The first dripping time (min) and melting rate (g/min) were determined according to the method reported by Aboulfazli, Baba [36]. The hardened ice cream sample (~30 g) was left to melt on a 1.34 mm sieve on top of a pre-tared beaker in a cabinet set at 25°C, and the amount of ice cream melted into the beaker was recorded at a 5-minute interval for 45 min. The percentage of ice cream drip-off was calculated using the following Equation 3 [37]:

$$\text{Drip-off (\%)} = (m_d/m_s) \times 100 \quad (3)$$

where m_d is the weight of the ice cream drip-off (g) at the end of 30 min, and m_s is the weight of the ice cream sample (g) at the beginning of the analysis.

Functional Properties of Ice Cream Samples

Bioactive compound extraction from ice creams was carried out according to the method of Gul, Atalar [8] with some modifications. The samples were mixed with 80% methanol at a ratio of 1:1 and shaken for 1 hour at ambient temperature. Then samples were centrifuged (Nüve-Bench Top Centrifuge, NF 1200R, Ankara, Türkiye) at 8,000x*g* for 30 min at 4°C. Suspensions were filtered with Whatman filter paper no. 1 and the filtrate was used for total phenolic content (TPC) and 1,1-diphenyl-2-picrylhydrazil (DPPH) analysis.

The filtrates were mixed with Folin reagent to determine total phenolic component determination, and then 2.5 mL of 7.5% (w/v) Na_2CO_3 was added. After keeping them in a dark condition for 30 min, absorbance was measured at 760 nm using a UV/VIS spectrophotometer (Shimadzu UV-1800, Tokyo, Japan). Results were calculated as Gallic acid equivalent using the calibration curve (R^2:0.9998) given below (Equation 4);

$$\text{TPC (mg GAE/100g)} = [(\text{Absorbance} - 0.179)/0.0123] \times \text{Dilution factor} \quad (4)$$

To determine DPPH activity, 4.9 mL of DPPH agent was added to 0.1 ml of filtrate, and the absorbance was read at 517 nm using a UV/VIS spectrophotometer (Shimadzu UV-1800, Japan) after waiting in the dark for 30 min. Results were calculated using the calibration

curve (R^2:0.9997) below based on the Trolox® equivalent (Equation 5);

$$\text{Trolox (mM)} = [(\text{Absorbance} + 0.5998)/(56.608)] \times \text{Dilution factor} \quad (5)$$

Sensorial Properties of Ice Cream Samples

Ice cream samples stored at -18°C for 25 days were evaluated using a hedonic scaling method for color/ appearance (1=undesired, 5=desired), texture (1=undesired, 9=desired), taste and aroma (1=undesired, 9=desired) and general acceptability [35]. Thirty panelists (academic staff and graduate students) at Ondokuz Mayis University Food Engineering Department participated in the sensorial evaluation test.

Statistical Analysis

The data was analyzed using IBM SPSS software (Version 20, Chicago, USA). The one-way ANOVA and Duncan's post-hoc test at a 5% significance level were conducted.

RESULTS and DISCUSSION

Physicochemical Properties of Samples

The physicochemical compositions of cow and hemp seed milk used for ice cream making are given in Table 1.

Table 1. Physicochemical properties of cow and hemp seed milk

Physicochemical properties	Cow milk	Hemp seed milk
Dry matter, %	11.94±0.09	12.13±0.04
Ash, %	1.70±0.28	0.73±0.08
Protein, %	2.50±0.44	4.67±0.03
Fat, %	3.45 0.07	9.30±0.14
pH	6.32±0.01	6.66±0.01
L* (lightness/ darkness)	90.10±0.20	87.70±0.22
a *(red/green)	-2.09±0.01	-0.71±0.23
b* (blue/yellow)	4.10±0.17	9.28±0.39

* L*: light (100) to dark (0), a*: red (+a*) to green (-a*), b*: blue (-b*) to yellow (+b*)

The dry matter content of hemp seed milk, prepared with 20% hemp seeds to obtain the dry matter similar to cow's milk (11.94%±0.09), was determined as 12.13%. In the study by Gram and Mortas (2023), the dry matter of hemp seed milk prepared at a 1:5 (seed: distilled water) ratio was around 10.3-10.46%. Protein, fat, and pH values were higher in hemp seed milk than cow milk. On the contrary, the ash amount and L* value were found to be higher in cow's milk. Since the composition of hemp milk varies depending on the amount of hemp seeds used, hemp milk with different compositions has been obtained in the literature. The protein, fat and ash contents of the hempseed milk samples used were found at 0.83-6.96%, 1.25–18.02% and 0.47–0.664%, respectively in studies [9, 16, 25]. Considering these minimum and maximum values in the literature, the chemical composition of the hemp seed milk produced was within the specified range.

The physicochemical properties of ice cream samples are given in Table 2.

Table 2. Some physicochemical parameters of control and HM added ice creams

Parameters	Hemp Seed Milk Concentration				
	Control	25%	50%	75%	100%
Dry matter, %	30.13±0.08^{bc}	29.89±0.04^{b}	29.52±0.10^{a}	29.98±0.10^{b}	30.42±0.02^{c}
Ash, %	0.58±0.06^{a}	0.54±0.09^{a}	0.56±0.01^{a}	0.55±0.04^{a}	0.70±0.01^{a}
Protein, %	2.47±0.37^{a}	2.81±0.07^{ab}	3.10±0.03^{ab}	3.50±0.10^{bc}	3.94±0.04^{c}
Fat, %	8.40±0.28^{a}	8.50±0.14^{a}	8.30±0.14^{a}	8.60±0.01^{a}	8.70±0.14^{a}
pH	6.29±0.01^{a}	6.66±0.01^{b}	6.70±0.01^{bc}	6.72±0.00^{c}	6.78±0.02^{d}
L* (Lightness)	89.42±0.23^{c}	87.64±0.03^{b}	86.75±0.10^{a}	87.72±0.00^{b}	87.76±0.01^{b}
a* (redness)	-1.26±0.08^{a}	-1.40±0.01^{a}	-1.31±0.01^{a}	-0.75±0.01^{b}	-0.59±0.01^{c}
b* (yellowness)	8.75±0.36^{a}	8.40±0.11^{a}	8.26±0.08^{a}	9.84±0.03^{b}	10.06±0.02^{b}
ΔE* (Color difference)	-	1.82±0.05^{a}	2.08±0.01^{b}	2.21±0.02^{b}	2.71±0.10^{c}
Hardness (N)	10.32±1.65^{a}	15.48±1.43^{a}	13.71±2.47^{a}	11.48±4.28^{a}	13.37±2.01^{a}

* Values are means ± standard deviation. a-c means within the same line with different letters are significantly different at p<0.05

The dry matter content of all ice creams was around 30% since the mix formulation was calculated to be 30% solids. Ice creams containing similar dry matter were produced in a study examining quince seeds' effect on ice cream's characteristics [35]. There was no statistical difference between the ash amount of the ice cream samples (p>0.05). The amount of protein increased as the hemp seed milk ratio increased. This was because the protein content of hemp seed milk was higher than cow's milk. It has been reported that plant milk generally has a higher protein content than cow's milk [38]. Since the fat content in the ice cream mix was calculated as

8%, the fat percentages were around this. According to the Turkish Food Codex (Number: 2022/13) the ice creams produced were included in the fatty ice cream category. The pH value of the samples increased as the hemp seed milk ratio in the ice cream mix increased since the pH value of hemp seed milk was higher than cow milk. The L* values of the samples with hemp milk added were statistically different from the control ice cream sample ($p<0.05$). The L* value of ice creams with hemp seed milk was lower than control ice cream because of the chlorophyll naturally found in hemp seeds [39]. Some studies also indicate that the L* value of ice cream prepared from plant milk was lower than ice cream prepared from cow's milk [12, 31, 36].

When the hardness values of the ice cream samples were examined, no statistically significant difference was found between the samples ($p>0.05$). The texture of ice cream varies according to ingredients and processing conditions [40]. The hardness values of the samples vary between 10.32±1.65 and 15.48±1.43. Some studies in the literature have similar results [40, 41]. The increase in the hardness value of the ice cream containing 25% hemp seed milk compared to the control sample can be attributed to increased protein content with adding hemp seed milk. The rich protein content may have interacted with other ingredients in the ice cream mixture and contributed to increased water immobilization in the ice cream due to its water-binding properties, resulting in the formation of harder ice cream [42].

However, a decrease was observed in the hardness value of the ice creams containing 50, 75 and 100% hemp milk compared to the ice cream containing 25% hemp milk, although it was not statistically significant. The reason for this decrease may be that as the hemp milk ratio increases, the unsaturated fat ratio increases as well as the protein content. The fat droplets emulsified by the proteins in the colloidal structure of the ice cream may have lost their stability due to the increase in the unsaturated fat ratio. The destabilized oil droplets may have also caused the viscosity to decrease as the shear rate increased [35, 43].

Rheological Properties of Ice Cream Mix

Rheological properties are important in terms of sensory evaluation in ice cream consumption. Furthermore, rheological parameters are important in mechanical transportation and convey the mix through lines during the process [31]. While the viscosity at low shear stress is associated with the consistency felt in the mouth, the viscosity at high shear rate can be understood by the flow characteristics in process operations such as pumping and spraying [44]. Apparent viscosities at 50 s^{-1} and Herschel-Bulkley model parameters are given in Table 3.

Table 3. The apparent viscosity and Herschel-Bulkley model parameters of ice creams

Samples	Apparent viscosity at 50 s^{-1}, η_{50} (Pa.s)	Herschel-Bulkley Model Parameters		
		Consistency index, K (Pa.s^n)	Flow behavior index, n	R^2
Control	0.59±0.01^{c}	9.98±2.28^{c}	0.31±0.05^{a}	0.9999
25%	0.39±0.00^{b}	5.94±0.39^{bc}	0.34±0.01^{ab}	0.9998
50%	0.29 0.02^{a}	1.19±0.45^{a}	0.65±0.07^{cd}	0.9999
75%	0.27±0.01^{a}	1.87±0.04^{ab}	0.52±0.00^{bc}	0.9999
100%	0.25±0.01^{a}	0.64±0.14^{a}	0.75±0.05^{d}	0.9998

*Values are means ± standard deviation. a-e means within the same column with different letters are significantly different at $p<0.05$

Herschel-Bulkley model was applied to the data obtained as a result of rheological analysis ($R^2>0.99$). Similar to our study, in many types of research in the literature, the flow behavior properties of ice creams produced were found to be suitable for the Herschel-Bulkley model [35, 54]. All ice cream mixes had lower (0.31-0.75) flow behavior index values than 1 (one) and thus exhibited pseudo-plastic or shear-thinning flow, meaning that their viscosity decreased as the shear rate increased. As the hemp seed milk ratio increased, the apparent viscosity (50 s^{-1}) and consistency index (K) decreased, while the flow behavior index (n) increased ($p<0.05$). High K value represents high consistency [45]. The increase in n and the decrease in K may result from the structural rupture of the protein network of the ice cream due to shearing [36]. Samples with a low flow behavior index were reported to be easier to pump and more effective in providing the desired mouthfeel and texture [31]. At the same time, the apparent viscosity values of the ice cream samples decreased as the hemp seed milk ratio increased. The reason for this may be that the milk fat in cow's milk is of animal origin, while the fat in hemp milk is of vegetable origin. An animal-based partially coalesced fat provides firmness and structure to ice cream [46]. Although an optimum mix viscosity can not be defined, the apparent viscosity range at 50 shear ratio after aging is suggested to be 0.1-0.8 Pas. In this study, the viscosity values of all prepared ice cream mixes were within this range [31].

Dynamic rheological properties of ice cream samples were examined to determine their viscoelastic properties. The storage (G') modulus and loss (G'') modulus and fluid behavior curves of ice cream mixes are given in Figure 1.

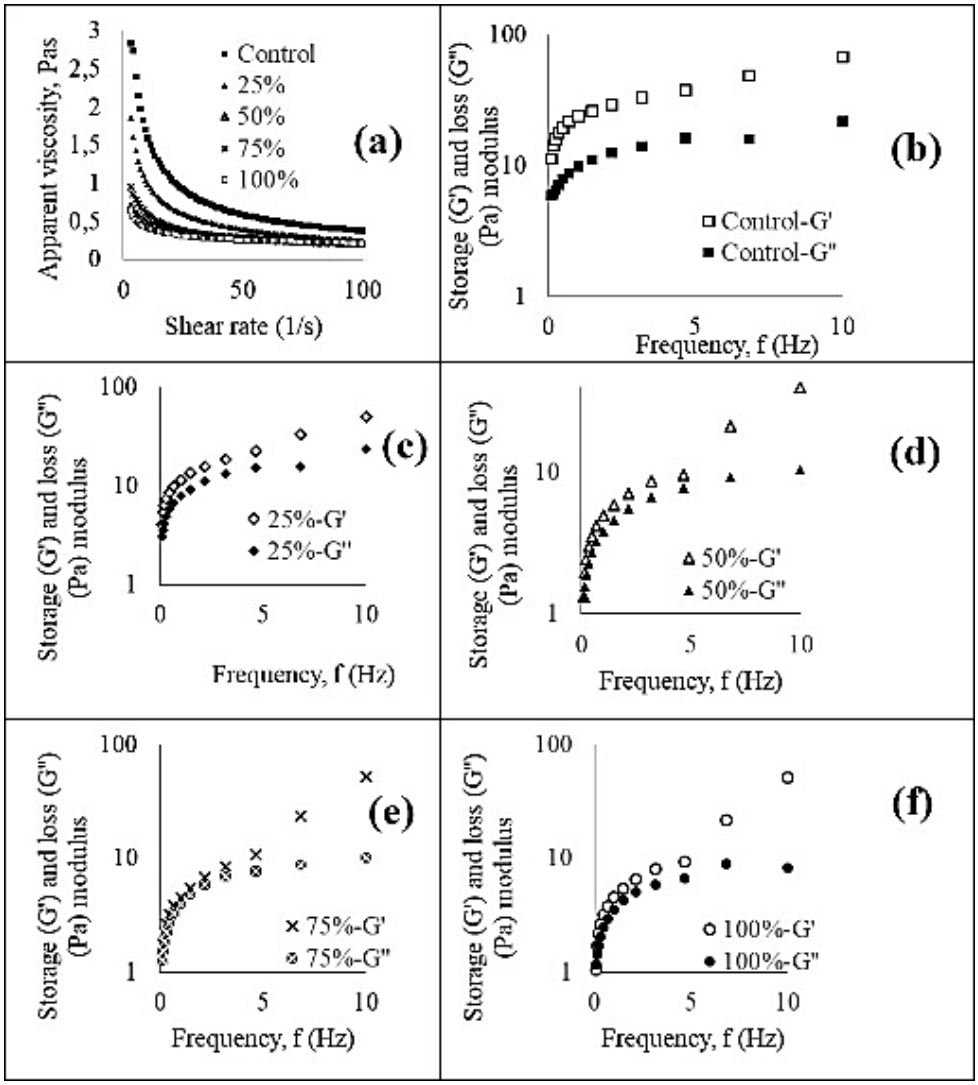

Figure 1. (a) Apparent viscosity vs. shear rate for ice cream mixes containing 0-100% hemp seed milk; (b-c-d-e-f-) Plots of storage (*G'*) and loss (*G"*) modulus as a function of frequency for ice cream mixes containing 0–0.100% hemp seed milk

The G' values of all ice cream samples were higher than the G'' values. This indicates an elastic (solid-like) structure of the ice cream mix rather than a viscous (liquid-like) structure. While the storage modulus (G') is associated with solid body-like behavior and therefore the hardness of ice cream, the loss modulus (G'') describes viscous behavior and fluidity and is associated with the sensation of the creaminess of ice cream [47]. As the hemp milk ratio increased, viscous and elastic behavior values became closer. This situation was supported by the fact that as you add hemp milk, the viscosity decreased, and it became more liquid. Moreover, the reason why the loss modulus value of the ice cream mix increased as the hemp milk ratio increased could be shown to be that hemp seeds contain long-chain fatty acids, which are known to be liquid [46]. Similar to this study, ice creams with storage modulus values higher than loss modulus values have been produced in the literature [48, 49]

Melting Properties of Ice Cream Samples

The chemical composition and physical structure of ice cream affect its melting properties [37]. The melting rate (g/min), drip-off (%) and first dripping time (min) of ice cream samples are given in Table 4. Also, images of ice cream samples at 0, 20, 30, and 45 minutes are shown in Figure 2.

As the hemp milk ratio increased, the melting rate and dripping amount of the ice cream samples also increased. This may be because hemp seed milk's fat melted faster than cow's milk fat. Similar results were obtained in a study using plant-based coconut milk [14]. Similar to this study, in a study where walnut milk was used in ice cream making, it was determined that the energy required to melt ice cream made with walnut milk was less than the control sample [13]. Unlike this study, the study conducted by Atalar and Kurt [12] stated that hazelnut milk improved the melting properties of ice cream samples due to its high protein content, which melted later.

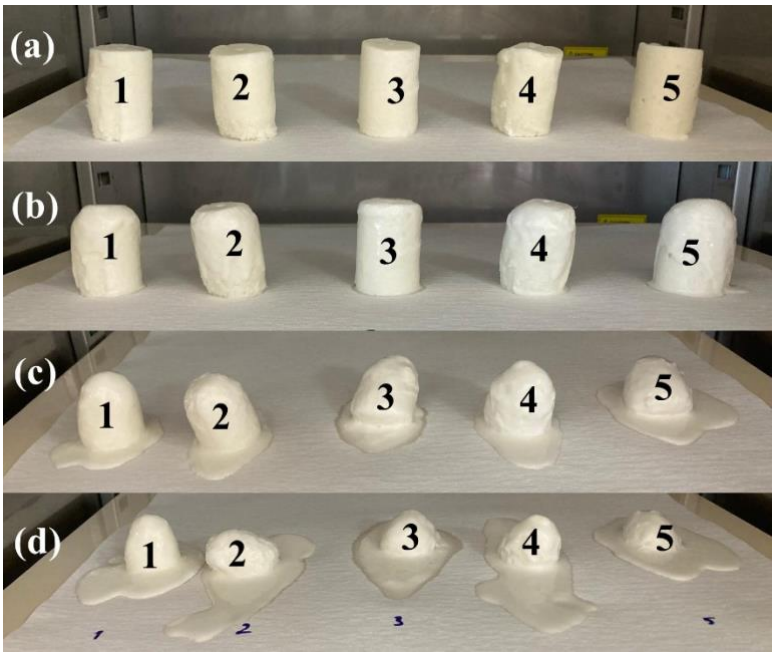

Figure 2. Melting behavior of ice cream containing 0-100% hemp seed milk (HM) 0 (a), 20 (b), 30 (c) and 45 (d) min later at room temperature (25±2 °C)

Milk fat globules affect the melting properties of ice cream by surrounding air bubbles and contributing to air phase stabilization. It has been reported that the more unsaturated the vegetable oil used in the ice cream mix and the longer the fatty acid chain, the more pronounced the destabilization of the fat globules [50]. The presence of polyunsaturated fatty acids (linoleic acid, C18:2ω6 and α-linolenic acid, C18:2ω3) in hempseed milk can explain the negative effect on melting properties as the hemp milk ratio increases in the ice cream mix [51]. Similar to this study, in the study conducted by Güven and Kalender [52], it was observed that the use of vegetable oils such as hazelnut oil and olive oil negatively affected and accelerated the melting of ice cream.

Table 4. Melting properties of ice creams

Sample	Melting rate (g/min)	Drip-off (%)	First dripping time (min)
Control	0.27±0.08^{a}	17.67±5.66^{a}	3.32±0.19^{a}
25%	0.40±0.13^{ab}	26.67±8.48^{ab}	2.85±0.46^{a}
50%	0.59±0.16^{ab}	39.58±10.48^{ab}	2.74±0.51^{a}
75%	0.73±0.03^{b}	48.50±2.12^{b}	2.57±0.01^{a}
100%	0.74±0.06^{b}	49.50±4.00^{b}	2.30±0.04^{a}

*Values are means ± standard deviation. a-b means within the same column with different letters are significantly different at $p < 0.05$.

No statistical difference was observed between the ice cream samples regarding the first dripping times (p: 0.149). A high negative correlation exists between initial drip time and melting (r: -0.93) rate. Additionally, a high negative correlation was also calculated between apparent viscosity values and melting rate (r: -0.94). A high positive correlation was also found between the first drip time and viscosity (r: 0.95). These results revealed the importance of viscosity on the melting properties of ice cream. In the study conducted by [35], it was stated that viscosity is important in ice cream samples with high correlation parameter.

Functional Properties of Ice Cream Samples

Among the functionalization strategies of ice cream, the presence of bioactive components is important [53]. Plant-based milk stand out with their bioactive components (for example, soy-based beverages contain isoflavones and phytosterols; almond-based beverage contains tocopherol and arabinose; oat-based beverage contains glucan; hemp seed milk contains polyunsaturated fatty acids (PUFAs) and essential fatty acids (EFAs), are therefore used to produce functional products [38, 54-56]. Bioactive compounds in ice creams were determined by total phenolic content and antioxidant activity analysis, and the results are shown in Figure 3.

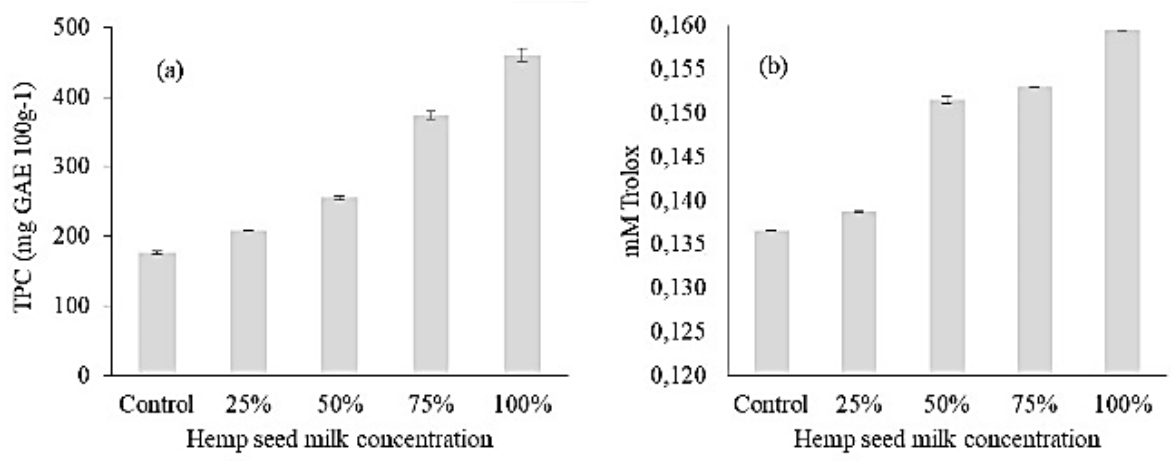

Figure 3. The total phenolic contents (TPC) (a) and antioxidant activity levels of ice cream mixes containing 0-100% hemp seed milk (b)

As the ratio of hemp seed milk in the ice cream mix increased, both the total phenolic content and antioxidant activity of the samples increased. Bioactive compounds such as linoleic acid, γ-tocopherol, cannabidiol acid, lignan-amides, and terpenes contained in hemp seed can be shown as the reason why the phenolic and antioxidant content increased as the hemp seed milk ratio increased [25]. A study conducted by Beşir and Mortaş [9] determined that the antioxidant and phenolic component content of ayran drink with increased hemp milk content was higher than that of ayran prepared entirely from cow's milk. Similar to this study, phenolic and antioxidant contents increased in ice creams produced from hazelnut milk in a study researched by Atalar and Kurt [12]. In another study, the effect of non-dairy ingredients (coconut milk and coconut sugar) on total phenolic content was found to be highly significant [14].

Sensorial Properties of Ice Cream Samples

Sensorial parameters evaluated for ice cream samples are given in Table 5.

Table 5. Sensory parameters of control and HM added ice creams

Parameters	Hemp seed milk concentration				
	Control	25%	50%	75%	100%
Appearance	8.59±0.62^{a}	8.53±0.72^{a}	8.29±0.92^{a}	7.88±0.99^{a}	7.94±1.03^{a}
Taste and aroma	8.58±0.62^{b}	7.88±0.93^{ab}	7.23±0.97^{a}	6.88±1.32^{a}	7.53±1.46^{a}
Texture	8.12±0.86^{b}	7.59±1.12^{ab}	7.47±0.80^{ab}	7.18±0.88^{ab}	7.12±1.41^{a}
General acceptance	8.56±0.55^{b}	7.91±0.94^{ab}	7.56±0.74^{a}	7.23±0.97^{a}	7.44±1.25^{a}

* Values are means ± standard deviation. (n = 25 panelists for each ice cream type). a-b means within the same line with different letters are significantly different at $p<0.05$. Sensory attributes and their scales: 1 = undesired, 9 = desired. Values are means ± standard deviation. a-b means within the same line with different letters are significantly different at $p<0.05$

There was no statistical difference between the ice cream samples regarding appearance (p: 0.058). For the taste-aroma parameter, ice cream containing 25% hemp milk was scored similarly to control ice cream. Taste and aroma differed statistically from control ice cream, starting from a 50% hemp seed milk ratio. Regarding texture, except 100% hemp seed milk all ratios were evaluated as close to control ice cream ($p>0.05$). When general acceptability was assessed, the most liked ice cream with hemp seed milk added by the panelists was the ice cream containing 25% hemp seed milk. While the addition of plant-based milk increased the acceptability compared to the control sample in some studies [12, 13], it decreased the acceptability in some studies [56-58]. In a study where ice cream was made using soy and sweet bean milk, as the proportion of plant milk increased, the panelists' approval score decreased, and they determined that the best concentration of plant-based milk was 25% [57]. In the sensory analysis performed on ice creams produced using hemp seed milk and almond milk, ice cream produced from hemp seed milk was less liked due to the high density and slightly unpleasant aroma specific to hemp seed milk [56]. Like hemp seed milk, ice cream made from soy milk was also disliked due to its intense flavor [58].

CONCLUSION

This study investigated the usability of hemp milk in ice cream production. Thanks to the high protein content of hemp seed and its rich phenolic-antioxidant content, hemp seed milk-based ice creams with higher protein content and improved functional properties were obtained compared to the control ice cream sample made from cow's milk. Using hemp milk instead of cow's milk did not positively affect viscosity and melting properties. When the hardness value was examined, ice creams similar to the control ice cream were obtained. As the hemp milk ratio increased, the apparent viscosity of the ice cream decreased due to the hemp seeds containing long-chain fatty acids, which are known to be

liquid. In addition, the loss modulus increased, and the viscous and elastic behavior values became closer to each other. From a sensory perspective, considering the general acceptability parameter, the acceptable hemp milk rate was determined to be 25%. If the results of this study are supported by future studies examining its effects on health, hemp seed milk can be recommended as an alternative plant-based milk that can be used in the production of functional ice cream, as it increases the total phenolic and antioxidant content of ice cream.

CONFLICT of INTEREST

The authors declare no conflict of interest.

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REFERENCES

[1] Karimidastjerd, A., Gulsunoglu-Konuskan, Z., Olum, E., Toker, O.S. (2024) Evaluation of rheological, textural, and sensory characteristics of optimized vegan rice puddings prepared by various plant-based milks. *Food Science & Nutrition,* 12(3), 1779-91.

[2] Pingali, P., Boiteau, J., Choudhry, A., Hall, A. (2023) Making meat and milk from plants: A review of plant-based food for human and planetary health. *World Development*, 170106316.

[3] McClements, D.J., & Grossmann, L. (2021). The science of plant-based foods: Constructing next-generation meat, fish, milk, and egg analogs. *Comprehensive Reviews in Food Science and Food Safety*, 20(4), 4049-4100.

[4] Silva, A.R.A., Silva, M.M.N., Ribeiro, B.D. (2020) Health issues and technological aspects of plant-based alternative milk. *Food Research International*, 131108972.

[5] Reyes-Jurado, F., Soto-Reyes, N., Dávila-Rodríguez, M., Lorenzo-Leal, A., Jiménez-Munguía, M., Mani-López, E., López-Malo, A. (2023) Plant-based milk alternatives: Types, processes, benefits, and characteristics. *Food Reviews International*, 39(4), 2320-51.

[6] Wang, S, Chelikani, V, Serventi, L. (2018) Evaluation of chickpea as alternative to soy in plant-based beverages, fresh and fermented. *LWT,* 97570-2.

[7] Deziderio, MA, de Souza, HF, Kamimura, ES, Petrus, RR. (2023) Plant-based fermented beverages: Development and characterization. *Foods*, 12(22), 4128.

[8] Gul, O., Atalar, I., Mortas, M., Saricaoglu, F.T., Besir, A., Gul, L.B., Yazici, F. (2022) Potential use of high pressure homogenized hazelnut beverage for a functional yoghurt-like product. *Anais da Academia Brasileira de Ciências*, 94e20191172.

[9] Beşir, A., Mortaş, M. (2022) Investigation properties of Ayran (yoghurt drink) produced from different ratio of cow and hemp seed milk mixtures. *European Food Science and Engineering*, 3(1), 5-10.

[10] Ayah, M., Ahmed, S., Sohair, F.N., El-Sawy, N. (2022) Formulation and evaluation of some novel food products containing plant (vegan) milks. *Alexandria Journal of Food Science and Technology,* 18(2), 13-22.

[11] Verma, P., Agrawal, U., Sharma, A., Sarkar, B., Sharma, H. (2005) Optimization of process parameters for the development of a cheese analogue from pigeon pea (*Cajanus cajan*) and soy milk using response surface methodology. *International Journal of Dairy Technology*, 58(1), 51-58.

[12] Atalar, I., Kurt, A., Gul, O., Yazici, F. (2021) Improved physicochemical, rheological and bioactive properties of ice cream: Enrichment with high pressure homogenized hazelnut milk. *International Journal of Gastronomy and Food Science*, 24100358.

[13] Bekiroglu, H., Goktas, H., Karaibrahim, D., Bozkurt, F., Sagdic, O. (2022) Determination of rheological, melting and sensorial properties and volatile compounds of vegan ice cream produced with fresh and dried walnut milk. *International Journal of Gastronomy and Food Science*, 28100521.

[14] Beegum, P.S., Nair, J.P., Manikantan, M., Pandiselvam, R., Shill, S., Neenu, S., Hebbar, K. (2022) Effect of coconut milk, tender coconut and coconut sugar on the physico-chemical and sensory attributes in ice cream. *Journal of Food Science and Technology,* 1-12.

[15] Jaeger, S.R., Dupas de Matos, A., Frempomaa Oduro, A., Hort, J. (2024) Sensory characteristics of plant-based milk alternatives: Product characterisation by consumers and drivers of liking. *Food Research International*, 180114093.

[16] Wang, Q., Jiang, J., Xiong, Y.L. (2018) High pressure homogenization combined with pH shift treatment: A process to produce physically and oxidatively stable hemp milk. *Food Research International,* 106487-94.

[17] Vahanvaty, U.S. (2009) Hemp seed and hemp milk: the new super foods? *ICAN: Infant, Child, & Adolescent Nutrition,* 1(4), 232-4.

[18] Callaway, J. (2004) Hemp seed production in Finland. *Journal of Industrial Hemp*. 9(1), 97-103.

[19] House, J.D., Neufeld, J., Leson, G. (2010) Evaluating the quality of protein from hemp seed (*Cannabis sativa* L.) products through the use of the protein digestibility-corrected amino acid score Method. *Journal of Agricultural and Food Chemistry*, 58(22), 11801-7.

[20] Rizzo, G., Storz, M.A., Calapai, G. (2023) The Role of hemp (*Cannabis sativa* L.) as a functional food in vegetarian nutrition. *Foods*, 12(18), 3505.

[21] Apostol, L. (2017) Studies on using hemp seed as functional ingredient in the production of functional food products. *Journal of EcoAgriTourism*, 13(1), 12-17.

[22] Paul, A.A., Kumar, S., Kumar, V., Sharma, R. (2020) Milk Analog: Plant based alternatives to conventional milk, production, potential and health

concerns. *Critical Reviews in Food Science and Nutrition*, 60,(18), 3005-23.
[23] Szparaga, A., Tabor, S., Kocira, S., Czerwińska, E., Kuboń, M., Płóciennik, B., Findura, P. (2019) Survivability of probiotic bacteria in model systems of non-fermented and fermented coconut and hemp milks. *Sustainability*, 11(21), 6093.
[24] Nissen, L., di Carlo, E., Gianotti, A. (2020) Prebiotic potential of hemp blended drinks fermented by probiotics. *Food Research International*, 131109029.
[25] Beşir, A., Awad, N., Mortaş, M. (2019) A Plant-based milk type: Hemp seed milk. *Akademik Gıda*, 20(2),170-81.
[26] Gram, S., Mortas, M. (2023) The effects of ultrasound and high pressure homogenization processes on physiochemical properties of hemp seed milk. *Food Chemistry Advances*, 3100477.
[27] Rojas, M.L., Kubo, M.T., Miano, A.C., Augusto, P.E. (2022) Ultrasound processing to enhance the functionality of plant-based beverages and proteins. *Current Opinion in Food Science*, 48100939.
[28] Aluko, R.E. (2017). Hemp seed (*Cannabis sativa* L.) proteins: composition, structure, enzymatic modification, and functional or bioactive properties. In Sustainable protein sources (pp. 121-132). Academic Press.
[29] Zhu, X-Q., Wang, Z-Y., Li, M-Y., Wang, Y., Li, Z-M., Yang, H-Z., Zhang, N., Sun, B-Y. (2021) Effect of heat treatment on the stability of hemp seed milk and characterization of protein structure. *Shipin Kexue / Food Science*, 42(7), 68-73.
[30] Hashemian, Z., Nouri, M. (2023) Enrichment of camel yogurt using hemp seeds as a rich source of omega 3 and physicochemical evaluation. *International Journal of Food Science & Technology,* 58(6), 3439-47.
[31] Hidas, K.I., Nyulas-Zeke, I.C., Szepessy, A., Romvári, V., Gerhart, K., Surányi, J., Laczay, P., Darnay, L. (2023) Physical properties of hemp drink-based ice cream with different plant proteins guar gum and microbial transglutaminase. *LWT,* 182114865.
[32] Li, X., He, Y., Xie, Y., Zhang, L., Li, J., Liu, H. (2023) Effects of fermentation with Kefir grains on nutrient composition, flavor volatiles, and product physical stability of a hemp seed (*Cannabis sativa* L.) beverage. *LWT*, 183114934.
[33] AOAC. (1990) Association of Official Analytical Chemists, Official methods of analysis (15th ed.). *Washington, DC*.
[34] AOAC. (2010) Official methods of analysis of AOAC international, 18th ed. *AOAC Int*.6,(382),90022-5.
[35] Kurt, A., Atalar, I. (2018) Effects of quince seed on the rheological, structural and sensory characteristics of ice cream. *Food Hydrocolloids,* 82186-95.
[36] Aboulfazli, F., Baba, A.S., Misran, M. (2014) Effect of vegetable milks on the physical and rheological properties of ice cream. *Food Science and Technology Research*, 20(5), 987-96.
[37] Markowska, J., Tyfa, A., Drabent, A., Stępniak, A. (2023) The Physicochemical properties and melting behavior of ice cream fortified with multimineral preparation from red algae. *Foods*, 12(24), 4481.
[38] Fructuoso, I., Romão, B., Han, H., Raposo, A., Ariza-Montes, A., Araya-Castillo, L., Zandonadi, R.P. (2021) An overview on nutritional aspects of plant-based beverages used as substitutes for cow's milk. *Nutrients*, 13(8), 2650.
[39] Tănase Apetroaei, V., Pricop, E.M., Istrati, D.I., Vizireanu, C. (2024) Hemp seeds (*Cannabis sativa* L.) as a valuable source of natural ingredients for functional foods - A review. *Molecules*, 29(9), 2097.
[40] Meneses, R.B., Silva, M.S., Monteiro, M.L.G., Rocha-Leão, M.H.M., Conte-Junior, C.A. (2020) Effect of dairy by-products as milk replacers on quality attributes of ice cream. *Journal of Dairy Science*, 103(11), 10022-35.
[41] Sert, D., Mercan, E., Kılınç, M. (2021) Development of buffalo milk ice-cream by high pressure-homogenisation of mix: Physicochemical, textural and microstructural characterisation. *LWT,* 150112013.
[42] Čolić, M.L., Jukić, M., Nakov, G., Lukinac, J., Antunović, M. (2024) Sustainable Utilization of Hemp Press Cake Flour in Ice Cream Production: Physicochemical, Rheological, Textural, and Sensorial Properties. *Sustainability*, 16(19), 1-23.
[43] Ranaweera, H., Krishnan, P., Martínez-Monteagudo, S.I. (2022) Rheological behavior of ice-cream mixes: Impact of temperature and protein concentration. *Journal of Food Process Engineering*, 45(3), e13989.
[44] Kurt, A., Cengiz, A., Kahyaoglu, T. (2016) The effect of gum tragacanth on the rheological properties of salep based ice cream mix. *Carbohydrate Polymers*, 143116-23.
[45] Mohamad, R., Agus, B., Hussain, N. (2019) Changes of phytosterols, rheology, antioxidant activity and emulsion stability of cocoa butter-based salad dressing during storage. *Food Technology and Biotechnology,* 57(1), 59-67.
[46] Zaaboul, F., Tian, T., Borah, P.K., Di Bari, V. (2024) Thermally treated peanut oil bodies as a fat replacer for ice cream: Physicochemical and rheological properties. *Food Chemistry*, 436137630.
[47] Toker, O.S., Karaman, S., Yuksel, F., Dogan, M., Kayacier, A., Yilmaz, M.T. (2013) Temperature dependency of steady, dynamic, and creep-recovery rheological properties of ice cream mix. *Food and Bioprocess Technology*, 62974-85.
[48] Kasapoglu, M.Z., Sagdic, O., Avci, E., Tekin-Cakmak, Z.H., Karasu, S., Turker, R.S. (2023) The potential use of cold-pressed coconut oil by-product as an alternative source in the production of plant-based drink and plant-based low-fat ice cream: The rheological, thermal, and sensory properties of plant-based ice cream. *Foods*, 12(3), 650.
[49] Jin, Y., Gu, Z., Cheng, L., Li, C., Li, Z., Hong, Y. (2024) Physicochemical characterization of debranched waxy rice starches and their effect on the quality of low-fat ice cream mixtures. *Food Bioscience*, 57103485.
[50] Nazaruddin, R., Syaliza, A., Wan Rosnani, A. (2008) The effect of vegetable fat on the physicochemical

characteristics of dates ice cream. *International Journal of Dairy Technology*, 61(3), 265-9.

[51] Baldino, N., Carnevale, I., Mileti, O., Aiello, D., Lupi, F.R., Napoli, A., Gabriele, D. (2022) Hemp seed oil extraction and stable emulsion formulation with hemp protein isolates. *Applied Sciences,*12(23), 11921.

[52] Güven, M, Kalender, M, Taşpinar, T. (2018) Effect of using different kinds and ratios of vegetable oils on ice cream quality characteristics. *Foods*, 7(7), 104.

[53] Genovese, A, Balivo, A, Salvati, A, Sacchi, R. (2022) Functional ice cream health benefits and sensory implications. *Food Research International*, 161111858.

[54] Kot, A, Kamińska-Dwórznicka, A, Galus, S, Jakubczyk, E. (2021) Effects of different ingredients and stabilisers on properties of mixes based on almond drink for vegan ice cream production. *Sustainability*, 13(21), 12113.

[55] Bocker, R, Silva, EK. (2022) Innovative technologies for manufacturing plant-based non-dairy alternative milk and their impact on nutritional, sensory and safety aspects. *Future Foods*, 5100098.

[56] Leahu, A, Ropciuc, S, Ghinea, C. (2022) Plant-based milks: Alternatives to the manufacture and characterization of ice cream. *Applied Sciences*, 12(3), 1754.

[57] Asres, AM, Woldemariam, HW, Gemechu, FG. (2022) Physicochemical and sensory properties of ice cream prepared using sweet lupin and soymilk as alternatives to cow milk. *International Journal of Food Properties,* 25(1), 278-87.

[58] Ghaderi, S, Mazaheri Tehrani, M, Hesarinejad, MA. (2021) Qualitative analysis of the structural, thermal and rheological properties of a plant ice cream based on soy and sesame milks. *Food Science & Nutrition,* 9(3), 1289-98.

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Research Paper / Araştırma Makalesi

Antioxidant Activity and Phytochemical Profile of Eight Wild Edible Plants Grown in Afyonkarahisar, Türkiye

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ABSTRACT

In this study, the antioxidant activities, total phenolic content (TPC), total flavonoid content (TFC), and phenolic compounds in the leaves of wild edible plants, grown in Afyonkarahisar, Turkey were investigated. Antioxidant activities were measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2´-azine-bis (3-ethylbenz-thiazoline-6-sulfonic acid) diammonium salt (ABTS), and ferric reducing antioxidant power (FRAP) assays, while phenolic acids and flavonoids were identified and quantified by high performance liquid chromatography. The plants included *Lactuca serriola* L. (bitter lettuce), *Thymus vulgaris* L. (thyme), *Sinapis arvensis* L. (mustard), *Malva neglecta* L. (hibiscus), *Amaranthus retroflexus* L. (redroot pigweed), *Tragopogon longirostris bisch* (goat's beard), *Taraxacum officinale* (dandelion), and *Chenopodium album* (baconweed or lamb's quarters). Phenolic acids, including gallic acid, ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid and flavonoids including catechin, apigenin, naringin, rutin and quercetin amounts in plant leaves were determined. All plants showed antioxidant properties but *Tragopogon longirostis bisch*, *Sinapis arvensis* L., and *Thymus vulgaris* L. had higher antioxidant activity than the rest. The highest TPC (2.69 mg/g) belonged to *Tragopogon longirostis bisch*, and the highest TFC (1.84mg/g) belonged to *Amaranthus retroflexus*. *Amaranthus retroflexus* L. had the highest gallic acid and vanillic acid levels. *Malva neglecta* L. had the highest ferulic, chlorogenic, ellagic, and cinnamic acid contents. *Tragopogon longirostris bisch* had the highest *p*-coumaric acid, 4-hydroxybenzoic acid, and 2,5 dihydroxybenzoic acid levels. It was observed that *Malva neglecta* L. had the highest catechin, apigenin, and quercetin contents while *Thymus vulgaris* L. had the highest naringin and rutin levels. These results suggested these leaves could be consumed as the sources of natural antioxidants in human diet.

Keywords: Phenolic, Antioxidant, DPPH, ABTS, FRAP

Afyonkarahisar'da Yetişen Sekiz Yabani Yenilebilir Bitkinin Antioksidan Aktivitesi ve Fitokimyasal Profili

ÖZ

Bu çalışmada, Türkiye'nin Afyonkarahisar ilinde yetişen yabani yenilebilir bitkilerin yapraklarının antioksidan aktiviteleri, toplam fenolik içerikleri (TPC), toplam flavonoid içerikleri ve fenolik bileşenler araştırılmıştır. Antioksidan aktiviteler 2,2-difenil-1-pikrilhidrazil (DPPH), 2,2´-azin-bis (3-etilbenz-tiazolin-6-sülfonik asit) diamonyum tuzu (ABTS) ve demir indirgeyici antioksidan güç (FRAP) analizleri ile ölçülürken, fenolik asitler ve flavonoidler HPLC ile tanımlanıp miktarları belirlenmiştir. Bu bitkiler *Lactuca serriola* L. (acı marul), *Thymus vulgaris* L. (kekik), *Sinapis arvensis* L. (hardal), Malva neglecta L. (ebegümeci), *Amaranthus retroflexus* L. (kızılbacak), *Tragopogon longirostris bisch*

(tekesakalı), *Taraxacum officinale* (acıgünek) ve *Chenopodium album*'dur (sirken). Bitki yapraklarında fenolik asitler (gallik asit, ferulik asit, klorojenik asit, *p*-kumarik asit, ellagik asit, vanilik asit, kafeik asit, sinnamik asit, 4-hidroksibenzoik asit, 2,5-dihidroksibenzoik asit) ile flavonoidlerin (kateşin, apigenin, naringin, rutin ve kuersetin) miktarları belirlenmiştir. Tüm bitkiler antioksidan özellik göstermiş fakat *Tragopogon longirostis bisch*, *Sinapis arvensis* L. ve *Thymus vulgaris* L. diğerlerinden daha yüksek antioksidan aktivite değerine sahip olmuştur. En yüksek toplam fenolik madde içeriği (2.69mg/g) *Tragopogon longirostis bisch*'e, en yüksek toplam flavonoid içeriği (1.84mg/g) *Amaranthus retroflexus'*a aittir. *Amaranthus retroflexus* L. en yüksek gallik asit ve vanilik asid içeriğine sahip iken *Malva neglecta* L. en yüksek ferulik, klorojenik, ellagik ve sinamik asit içeriğine sahip olmuştur. *Tragopogon longirostris bisch* en yüksek *p*-kumarik asit, 4-hidroksibenzoik asit ve 2,5 dihidroksibenzoik asidi düzeyini sergilemiştir. *Malva neglecta* L.'nin en yüksek kateşin, apigenin ve kuersetin düzeyi, *Thymus vulgaris* L.'nin ise en yüksek naringin ve rutin içeriğine sahip olduğu görülmüştür. Bulgular, bu bitki yapraklarının insan diyetinde doğal antioksidan kaynakları olarak tüketilebileceğini göstermiştir.

Anahtar Kelimeler: Fenolik, Antioksidan, DPPH, ABTS, FRAP

INTRODUCTION

Medicinal plants contain healthcare components [1]. Phenolic compounds are found in most plants and have antioxidant activity. Their redox properties sustain the antioxidant properties of phenolics. Therefore, phenolic compounds can take a role as reducing agents, oxygen quenchers, and metal chelators [2]. Plants need phenolic compounds for growth, pigmentation, and resistance to pathogens. Plants are exposed to UV-B (280-320nm) radiation, adversely affecting DNA. Plants protect themselves from this radiation by producing phenolic compounds [3]. Natural antioxidants like phenolic compounds can replace synthetic antioxidants against oxidative degradation caused by free radicals [4]. Flavonoids are given as examples of phenolic compounds. Flavonoids, which have high absorption at 250-270nm and 335-360nm, act as good UV screens [5]. The important flavonoid component is quercetin, one of the medicinal plants' most active antioxidants. Phenolics in these plants prevent cancer, cardiovascular diseases, and asthma [6]. Al-Laith et al. [7] studied the antioxidant properties of three wild medicinal plants from Bahrain (*Aizoon canariense* L., *Asphodelus tenuifolius* Cav., and *Emex spinosus* L. Campdera). *E. spinosus* was ranked the highest antioxidant and antiradical activities with an average FRAP value of 1.84 mmol/g and IC_{50} of 10.7 and 7.75 mg/mL for DPPH and ABTS assays, respectively. Günbatan et al. [8] reported that DPPH, ABTS, and TFC of *Malva neglecta* L. were 87.59%, 17.57 mg gallic acid equivalent/g and 42.93 mg rutin equivalent/g, respectively. Kolar et al. [9] demonstrated that *Chenopodium album* had 91.5% DPPH, 18.5 mg ascorbic acid equivalent/g FRAP, 14.9 mg tannic acid equivalent/g TPC, and 0.37 mg quercetin equivalent/g TFC. Ivanov [10] showed that DPPH, FRAP, and TPC of *Taraxacum officinale* grown in Bulgaria were 130.3 mM Trolox® equivalent/g, 131.5 mM Trolox® equivalent/g, and 33.90 mg gallic acid equivalent/g, respectively. Ao and Deb [11] investigated the antioxidant potential of 10 wild edible mushrooms of Nagalan in India. They reported that the highest phenolic content was 18.7 g/100 g, and the highest flavonoid content was 9.3 g/100 g. Shen et al. [12] reported that okra fruit shows antioxidant capacity. Somkuwar et al. [13] reported that gallic acid, vanillic acid, ellagic acid, chlorogenic acid, sorbic acid, coumaric acid, catechin, rutin, quercetin, and kaempferol were major phenolic compounds of red wine grape. White wine grapes contain lower phenolics than red wine grapes. Jagtap et al. [14] reported that *Carica papaya* L. leaves showed antioxidant activity. Yalcin and Schreiner [15] reported that the main phenolics of olive oil were tyrosol and hydroxytyrosol. Free radical scavenging activity of curry leaf (*Murraya koenigii* L.) was approximately 90% [16]. Perea-Dominguaz et al. [17] reported that the TPC of two tomatoes was 91.47 and 57.41 mg gallic acid equivalent/g dry samples. Fellah et al. [18] reported that the highest TPC (152.6 and 125.8 mg gallic acid equivalent/100 g) was recorded in Nabil flowers and Gabsi peels. Infrared treatment caused an increase in the phenolic content of soy [19]. Hassanzadeh and Hassanpour [20] investigated TPC, TFC, DPPH, and FRAP values of thirty-eight genotypes of *Elaeagnus angustifolia* L. grown in Iran. They reported that the TPC of peel and pulp was 268.38-1179 mg gallic acid/100 g and 250.57-820.85 mg gallic acid/100 g, respectively. The TFC of peel and pulp was 23.50-327.50 mg catechin/100 g and 16.50-318.75 mg catechin/100 g, respectively. DPPH of peel and pulp was 49.22-93.11% and 28.59-93.15%, respectively, while FRAP of peel and pulp was 57.00-128.67 mg Fe_2SO_4/100 g and 86.33-160.67 mg Fe_2SO_4/100 g, respectively. Orak [21] investigated phenolics of sixteen red grape cultivars grown in Tekirdağ, Turkey, and reported that TPC ranged from 817 to 3062 µg gallic acid equivalent/mL. Hassanpour and Alizadeh [22] investigated berberry genotypes' antioxidant capacity, TPC, and TFC (*Berberis vulgaris* and *Berberis integerrima)*. They reported that DPPH and FRAP values of genotypes ranged between 20.69-68.33% and 20.2-70.39 TE mmol/L, respectively. Genotypes' TPC and TFC ranged between 263.35-623.07 mg gallic acid equivalent/100 g and 158.33-280.00 mg catechin/100 g, respectively.

This study compares the antioxidant activities in terms of DPPH, ABTS, and FRAP, TPC, TFC, and phenolic compounds of wild edible plants collected in Afyonkarahisar, Turkey, on 25 May 2017. These plants were *Lactuca serriola* L., T*hymus vulgaris* L., *Sinapis arvensis* L., *Malva neglecta* L., *Amaranthus retroflexus* L., *Tragopogon longirostris bisch*, *Taraxacum officinale* and *Chenopodium album*. A comparison of the antioxidant activities, TPC, TFC, and phenolic compounds of the leaves of these plants has not been

reported. This study will contribute to literature. The data obtained will be a resource for researchers who will work in this field.

MATERIALS and METHODS

Chemicals

HPLC grade methanol, formic acid, sodium carbonate (purity≥99%), 2,4,6-tri(2-pyridyl)-s-triazine ferric chloride hexahydrate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) (98% purity), ABTS (98% purity), DPPH (95% purity), 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ) (98% purity), potassium persulfate, $AlCl_3$, $NaNO_2$, H_2O_2, $CuCl_2$ and $FeSO_4$ were purchased from Merck (Darmstadt, Germany). Folin-Chiocalteu reagent and phenolic standards (*p*-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, gallic acid, ellagic acid, cinnamic acid, vanillic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, catechin, apigenin, naringin, rutin and quercetin) were purchased from Sigma-Aldrich (St, Louis, MO, USA).

Materials

Lactuca serriola L., T*hymus vulgaris* L., *Sinapis arvensis* L., *Malva neglecta* L., *Amaranthus retroflexus* L., *Tragopogon longirostris bisch*, *Taraxacum officinale* and *Chenopodium album* L. were collected from their natural habitats (Latitude: 38,5917, Longitude: 31,0286 38° 35′ 30″ North, 31° 1′ 43″ East, Altitude: 1050m) in Afyonkarahisar on 25 May 2017. The leaves of these plants were separated from the plants and used for analysis.

For sample extraction, 1 g of leaves was extracted with 10 mL of methanol in a homogenizer (Daihan WiseTis HG-15D Digital Homogenizer, Seoul, South Korea) for 1 min at 20000 rpm. The homogenate was centrifuged at 3500 rpm for 10 min (Daihan Scientific Co., Ltd., WiseSpin® CF-10 Microcentrifuge, Seoul, South Korea). The extract was separated and dried by a vacuum rotary evaporator (Scilogex RE 100-Pro, USA) at 40°C. The dry residues were dissolved in 90% methanol (10 mL) before analysis.

DPPH Radical Scavenging Activity Assay

DPPH radical scavenging activity assay was performed according to the method reported by Brand-Williams et al. [23]. The determination of antioxidant activity with the DPPH assay is based on the ability of the reaction of the DPPH free radical with hydrogen donors. DPPH radical solution is decolorized after reduction with an antioxidant. So, color difference was calculated to determine antioxidant activity [24].

DPPH was dissolved in 100% methanol to obtain a solution with a concentration at 4.1075 mol/L. The sample extract (400 µL) was added to the DPPH solution (1.6 mL). After incubation in a dark place at room temperature for 30 min, the decrease in absorbance was measured at 517 nm. The DPPH solution (4.1075 mol/L) was used as a control for all samples. The DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\text{absorbance}_{\text{sample}}}{\text{absorbance}_{\text{control}}}\right) \text{x } 100$$

ABTS Radical Cation Decolorization Assay

ABTS radical cation decolorization of samples was determined according to the study reported by Re et al. [25] with some modifications. The determination of antioxidant activity by the ABTS radical cation decolorization assay is based on the neutralization of a radical cation after the one-electron oxidation of the synthetic chromophore ABTS. Antioxidant activity is determined by the change in the absorption spectrum after the reaction [24].

ABTS (1.8 mM) and potassium persulfate (0.63 mM) were mixed and stored in the dark for 24h at room temperature for reaction. This solution was mixed with methanol until an absorbance of 0.70 at 732 nm was obtained. Then, the mixture (1.98mL) was added to the sample extract (20 µL). After 30 min, the absorbance was measured using a spectrophotometer (Optizen pop Uv-Vis Spectrophotometer, South Korea) at 732 nm. A standard curve was prepared by plotting the percentage of radical cation decolorization of Trolox® (standard antioxidant) versus its concentration (0.1-2.5mM). The ABTS radical cation decolorization was expressed as mg Trolox® equivalent (TE) per g sample.

Ferric Reducing/Antioxidant Power (FRAP) Assay

The reducing capacity of samples was performed according to the method reported by Benzie and Strain [26]. Determination of antioxidant activity by the FRAP assay is based on the forming of blue color after reaction of 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ) with ferric chloride hexahydrate [24]. FRAP reagent was prepared for blank reading at 593 nm. 10 mL of sample was then added to FRAP reagent (300 mL), along with 30 mL H_2O; the final dilution of the sample in the reaction mixture was 1/34. Absorbance readings were taken after 0.5 s and every 15 s. The change in absorbance was calculated for each sample. 100-1000 mmol/L $FeSO_4 7H_2O$ were used for calibration. The results were expressed as mg $FeSO_4$ per g of sample.

Total Phenolic Content

The TPC of samples was determined by using the Folin-Chiocalteu method [27]. Sample extract (300 µL) and Folin-Chiocalteu reagent (750 µL) were mixed and incubated for 5 min. Then 750 µL of Na_2CO_3 (60g/L) was added, and the mixture was incubated in the dark for 90 min at room temperature. The absorbance was measured at 725 nm. Analysis was performed with

working solutions in the 0.0-0.1 mg/mL concentration range prepared from the catechin standard, and a calibration curve was prepared. TPC was expressed as mg catechin equivalents per g of the sample through the calibration curve of catechin.

Total Flavonoid Content

The TFC of samples was determined according to the method of Dewanto et al. [28]. 0.25 mL of the extract was mixed with 1.25 mL of distilled water in a tube, followed by the addition of 75 µL of a 5% $NaNO_2$ solution. After 6 min, 150 µL of a 10% $AlCl_3 6H_2O$ solution was added and allowed to stand for 5 min. Then, 0.5 mL of 1 M NaOH was added. The mixture was brought to 2.5 mL with distilled water and mixed. The absorbance was measured against the blank at 510 nm using a spectrophotometer. TFC was expressed as mg catechin equivalents per g of sample.

Analysis of Phenolic Compounds

The dried sample was dissolved in 1 mL of 100% methanol and filtered through a 0.45 µm nylon filter. Phenolic compounds of samples were analyzed according to the method of Caponio et al. [29] with some modification by using HPLC (Shimadzu Prominence, Kyoto, Japan) equipped with a diode array detector (SPD-M20A) and Zorbax Eclipse C18 column (250 × 4.6 mm, 5 µ). The mobile phase was 3% formic acid (A) and methanol (B). The elution gradient was 95% A/5% B for 3 min, 80%A/20%B in 15 min and isocratic for 2 min, 60%A/40%B in 10 min, 50%A/50%B in 10 min, and 100%B in 10 min until the end of the run. The flow rate was 1 mL/min. The eluates were detected at 278 nm. Quantitative phenolic compound evaluation was performed by using calibration curves of standards. Gallic acid, ferulic acid, chlorogenic acid, coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, catechin, apigenin, naringin, rutin, and quercetin were determined. The amount of phenolic compounds was expressed as µg per g of sample.

Statistical Analysis

Statistical differences between plant leaves were evaluated using a one-way analysis of variance (ANOVA) followed by the Duncan test. The difference between groups was significant at $P<0.05$. All data were analyzed using IBM Statistics SPSS 24 (Armonk, New York, USA). The analysis was done in duplicate.

RESULTS and DISCUSSION

DPPH Radical Scavenging Activity of Plant Leaves

The DPPH radical scavenging activities (%) of plant leaves are given in Table 1. Significant differences were found between DPPH radical scavenging activities of plant leaves. % Inhibition ranged from 15.9% (*Chenopodium album)* to 80.7% (*Tragopogon longirostris bisch*). *Tragopogon longirostris bisch* had the highest antioxidant activity, while *Chenopodium album* had the lowest. The antioxidant activity of T*hymus vulgaris* L. was statistically similar to that of *Sinapis arvensis L*. The result of *Lactuca serriola* L. was compatible with that recorded by Liu et al. [30]. Liu et al. [30] reported that the DPPH value of lettuce (*Lactuca serriola* L.*)* grown in Colorado was 69.6-81.6%. DPPH value of *Thymus vulgaris* L. was higher than those (22-55% and 27.5%) reported by Chizzola et al. [31] and Dauqan et al. [32]. DPPH value of *Malva neglecta* L. was lower than that (97.59%) reported by Günbatan et al. [8]. DPPH value *of Chenopodium album* was lower than that (91.5%) reported by Kolar et al. [9].

Table 1. Antioxidant activities of plant leaves (mean ± standard deviation)

Samples	DPPH (%)	ABTS (mg TE/g)	FRAP (mg $FeSO_4$/g)
Lactuca serriola L.	70.2±0.03d	2.13±0.018d	0.94±0.001h
T*hymus vulgaris* L.	77.9±0.15b	6.14±0.018c	8.77±0.001a
Sinapis arvensis L.	76.4±0.15b	10.01±0.018a	2.48±0.008f
Malva neglecta L.	69.5±0.59d	9.52±0.028b	7.74±0.057b
Amaranthus retroflexus L.	29.9±0.48e	8,92±0.030c	7.16±0.011c
Tragopogon longirostris bisch	80.7±1.73a	9.74±0.072ab	6.60±0.004e
Taraxacum officinale	74.3±0.15c	3.30±0.018d	2.43±0.007g
Chenopodium album	15.9±0.01f	8.63±0.035c	7.00±0.006d

*Values followed by the same letter in the same column are not significantly different ($p<0.05$)

ABTS Radical Scavenging Capacity of Plant Leaves

The ABTS radical scavenging capacities of plant leaves are given in Table 1. Significant differences were found between ABTS radical scavenging capacities of plant leaves. ABTS values of plant leaves ranged from 2.13 mg TE/g (*Lactuca serriola* L.) to 10.01 mg TE/g (*Sinapis arvensis* L.). Antioxidant capacity of *Lactuca serriola* L. was statistically similar to that of *Taraxacum officinale* and *Malva neglecta* L. The antioxidant capacity of *Thymus vulgaris* L. was not significantly different from that of *Amaranthus retroflexus* L. and *Chenopodium album.* Antioxidant capacity of *Sinapis arvensis* L. was the highest, while that of *Lactuca serriola* L. was the lowest. Compared with ABTS radical scavenging capacity of *Malva neglecta* L. (17.57 mg gallic acid equivalent/g) reported by Günbatan et al. [8], leaves of *Malva neglecta* L. used in this study had lower ABTS radical scavenging capacity.

FRAP of Plant Leaves

The FRAP values of plant leaves are given in Table 1. Significant differences were found between FRAP

values of plant leaves. FRAP values of plants ranged from 0.94 mg $FeSO_4$/g (*Lactuca serriola* L.) to 8.77 mg $FeSO_4$/g (*Thymus vulgaris* L.). *Thymus vulgaris* L. had the highest FRAP value, while *Lactuca serriola* L. had the lowest FRAP value. Lower FRAP value of T*hymus vulgaris* L. was 121.1-339.2 µmol TE/g obtained by Dauqan et al. [32]. FRAP value of *Malva neglecta* L. (7.74mg $FeSO_4$/g) was lower than that (190.3 µmol Fe^2/g) of *Malva neglecta* L. which was grown in Van, Turkey [33]. FRAP value of *Taraxacum officinale* (2.43 mg $FeSO_4$/g) was lower than that of *Taraxacum officinale* (131.5 mM TE/g) reported by Ivanov [10]. FRAP value of *Chenopodium album* (7.00 mg $FeSO_4$/g) was lower than that of *Chenopodium album* (18.5 mg ascorbic acid equivalent/g) reported by Kolar et al. [9].

Total Phenolic Content of Plant Leaves

The TPCs of plant leaves are given in Table 2. A significant difference was found between the TPC of plant leaves. TPC of plants ranged from 0.20 mg catechin/g (*Sinapis arvensis* L.) to 2.69 mg catechin/g (*Tragopogon longirostris bisch*). *Tragopogon longirostis bisch* had significantly higher TPC than other plant leaves. TPC of *Sinapis arvensis* L. was the lowest in all plant leaves. TPC of T*hymus vulgaris* L. was found statistically similar to that of *Malva neglecta* L. and *Amaranthus retroflexus* L. Higher TPC in *Lactuca serriola* L., *Malva neglecta* L., *Amaranthus retroflexus* L., *Taraxacum officinale and Chenopodium album* was reported by some researchers. TPC of *Lactuca serriola* L., grown in Netherlands, was 69.67-70.98 mg gallic acid equivalent/g [34], that of *Malva neglecta* L. leaf was 17 mg gallic acid equivalent/g [35], that of *Amaranthus retroflexus* L., grown in India, was 39.636 mg gallic acid equivalent/g [36], that of *Taraxacum officinale* was 33.90 mg gallic acid equivalent/g [10] and 41.47-691.6 mg gallic acid equivalent/g [37], that of *Chenopodium album was* 14.9 mg tannic acid equivalent/g [9] and 18.44 mg gallic acid equivalent/g [38]. Lower TPC (0.219 mg gallic acid equivalent/g) in T*hymus vulgaris* L. was obtained by Dauqan et al. [32].

Table 2. Total phenolic (TPC) and total flavonoid contents (TFC) of plant leaves (mean ± standard deviation)

Samples	TPC (mg catechin/g)	TFC (mg catechin/g)
Lactuca serriola L.	0.46±0.000e	0.06±0.011g
T*hymus vulgaris* L.	2.14±0.003b	1.22±0.006c
Sinapis arvensis L.	0.20±0.000f	0.18±0.000f
Malva neglecta L.	2.17±0.078b	1.39±0.057b
Amaranthus retroflexus L.	2.12±0.039b	1.84±0.001a
Tragopogon longirostris bisch	2.69±0.064a	1.38±0.029b
Taraxacum officinale	0.72±0.002d	0.36±0.011e
Chenopodium album	2.00±0.002c	1.04±0.011d

*Values followed by the same letter in the same column are not significantly different ($p<0.05$)

Total Flavonoid Content of Plant Leaves

The TFCs of plant leaves are given in Table 2. A significant difference was found between the TFC of plant leaves. TFC of plants ranged from 0.06 mg catechin/g (*Lactuca serriola* L.) to 1.84 mg catechin/g (*Amaranthus retroflexus* L.), *Amaranthus retroflexus* L. had significantly higher TFC compared to other plant leaves. TFC of *Malva neglecta* L. was statistically similar to that of *Tragopogon longirostris bisch*. Higher TFC was reported by some researchers. TFC of *Thymus vulgaris* L. was 36.6-44.2 µg quercetin/mg [39], that of *Malva neglecta* L. was 7.21 mg rutin equivalent/g [34] and 42.93 mg rutin equivalent/g [8] and that of *Amaranthus retroflexus* L., grown in India was 25.3 mg quercetin/g [35]. Lower TFC (0.37 mg quercetin equivalent/g) in *Chenopodium album* was reported by Kolar et al. [9].

Phenolic Compounds of Plant Leaves

The phenolic compounds of plant leaves are given in Tables 3 and 4. Chromatogram of phenolic compounds is shown in Figure 1. Gallic acid, ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid and 2,5-dihydroxybenzoic acid of plant leaves ranged between n.d.-0.625, n.d.-2.398, 0.002-1.446, 0.176-2.897, n.d.-28.093, n.d.-10.675, n.d.-73.951, 0.122-71.000, n.d.-45.432 and 0.249-7.407, respectively, Gallic acid was not determined in *Lactuca serriola* L., *Sinapis arvensis* L., *Malva neglecta* L. and *Chenopodium album.* Ferulic acid was not determined in T*hymus vulgaris* L. Catechin, apigenin, naringin, rutin and quercetin of plant leaves ranged between n.d.-2.398, n.d.-7.677, n.d.-0.247, 0.051-0.856 and 0.074-0.676, respectively, Naringin was not determined in *Lactuca serriola* L., *Sinapis arvensis* L., *Malva neglecta* L., *Amaranthus retroflexus* L., *Tragopogon longirostris bisch* and *Chenopodium album.* 3,4- dihydroxybenzoic acid and epicatechin were not found in plant leaves.

Lactuca serriola L. had ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, rutin and quercetin.
Thymus vulgaris L. had gallic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, catechin, apigenin, naringin, rutin and quercetin.

Sinapis arvensis L. had ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, catechin, apigenin, rutin and quercetin.

Malva neglecta L. had ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 2,5-dihydroxybenzoic acid, catechin, apigenin, rutin and quercetin.

Amaranthus retroflexus L. had gallic acid, ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, apigenin, rutin and quercetin.

Tragopogon longirostris bisch had gallic acid, ferulic acid, chlorogenic acid, *p*-coumaric acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, catechin, apigenin, rutin and quercetin.

Taraxacum officinale had gallic acid, ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, catechin, apigenin, naringin, rutin and quercetin.

Chenopodium album had ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, apigenin, rutin and quercetin.

Table 3. Phenolic acids of plant leaves (µg/g) (mean ± standard deviation)

Samples	Gallic acid	Ferulic acid	Chlorogenic acid	*p*-Coumaric acid	Ellagic acid	Vanillic acid	Caffeic acid	Cinnamic acid	4-hydroxy benzoic acid	2,5-dihydroxy benzoic acid
Lactuca Serriola L.	n.d.	0.055±0.001c	0.014±0.001d	0.176±0.007d	2.586±0.106c	0.108±0.017e	n.d.	0.122±0.015c	2.510±0.010c	0.249±0.013e
Thymus Vulgaris L.	0.016±0.003c	n.d.	0.832±0.023b	0.591±0.015c	0.591±0.009d	0.240±0.023e	0.839±0.070e	0.400±0.007b	1.097±0.017d	1.042±0.017d
Sinapis Arvensis L.	n.d.	0.002±0.000d	0.053±0.005d	0.715±0.017c	4.030±0.174b	7.708±0.371b	73.951±0.998a	0.384±0.093b	3.103±0.031c	1.506±0.039c
Malva Neglecta L.	n.d.	2.398±0.039a	1.446±0.030a	1.446±0.035b	28.093±0.903a	1.208±0.103c	25.584±0.071b	71.000±0.127a	n.d.	2.398±0.091b
Amaranthus retroflexus L.	0.625±0.005a	0.058±0.003c	0.038±0.001d	1.395±0.071b	4.156±0.103b	10.675±0.564a	28.036±0.067b	0.123±0.075c	5.370±0.059b	1.786±0.087c
Tragopogon longirostris bisch	0.134±0.013b	0.004±0.000d	0.187±0.013c	2.897±0.097a	n.d.	1.924±0.317c	9.333±0.097d	0.141±0.091c	45.432±0.307a	7.407±0.107a
Taraxacum officinale	0.024±0.007c	2.113±0.015a	0.074±0.009d	0.257±0.003d	1.936±0.098c	n.d.	0.313±0.017e	0.064±0.009d	0.024±0.009e	2.113±0.019b
Chenopodium album	n.d.	0.160±0.005b	0.002±0.000d	1.136±0.051b	0.018±0.003e	0.679±0.039d	18.217±0.873c	0.129±0.013c	3.007±0.019c	0.664±0.035d

*Values followed by the same letter in the same column are not significantly different (p<0.05)

Table 4. Flavonoids of plant leaves (µg/g) (mean ± standard deviation)

Samples	Catechin	Apigenin	Naringin	Rutin	Quercetin
Lactuca serriola L.	n.d.	n.d.	n.d.	0.051±0.009d	0.074±0.007c
T*hymus vulgaris* L.	0.015±0.001c	1.431±0.097c	0.247±0.074a	0.557±0.013b	0.102±0.009c
Sinapis arvensis L.	0.078±0.009c	0.648±0.075d	n.d.	0.076±0.007d	0.080±0.003c
Malva neglecta L.	2.398±0.073a	7.677±0.105a	n.d.	0.125±0.035c	0.676±0.103a
Amaranthus retroflexus L.	n.d.	0.601±0.056d	n.d.	0.051±0.007d	0.088±0.007c
Tragopogon longirostris bisch	0.444±0.051b	2.307±0.093b	n.d.	0.548±0.073b	0.548±0.091b
Taraxacum officinale	2.113±0.093a	0.074±0.009e	0.137±0.093b	0.856±0.091a	0.083±0.007c
Chenopodium album	n.d.	0.002±0.000e	n.d.	0.079±0.003d	0.094±0.005c

*Values followed by the same letter in the same column are not significantly different (p<0.05)

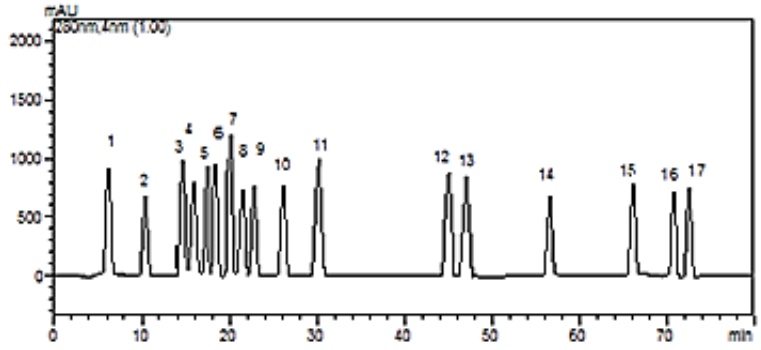

Figure 1. Chromatogram of phenolic compounds (1. gallic acid, 2. 3,4- dihydroxybenzoic acid, 3. 4-hydroxybenzoic acid, 4. 2,5-dihydroxybenzoic acid, 5. chlorogenic acid, 6. vanillic acid, 7. epicatechin, 8. caffeic acid, 9. *p*-coumaric acid, 10. ferulic acid, 11. rutin, 12. ellagic acid, 13. apigenin, 14. cinnamic acid, 15. quercetin. 16. naringin, 17. catechin).

Correlation

Pearson correlation coefficients between DPPH, ABTS, FRAP, TPC, and TFC in plant leaves are given in Table 5. There were high correlation coefficients between ABTS and FRAP, ABTS and TPC, and FRAP and TPC. This result indicated that ABTS could be used as a replacement of FRAP for determining antioxidant activity. Furthermore, ABTS and FRAP could be analyzed for determining TPC.

Table 5. Pearson correlation coefficients between antioxidant activity, total phenolic content, and total flavonoid content

	DPPH	ABTS	FRAP	TPC	TFC
DPPH	1.000	0.567	0.504	0.578	0.182
ABTS	0.567	1.000	0.910 **	0.898 **	0.291
FRAP	0.504	0.910 **	1.000	0.919 **	0.576
TPC	0.578	0.899 **	0.919 **	1.000	0.598
TFC	0.182	0.291	0.576	0.598	1.000

**p<0.01

CONCLUSION

Tragopogon longirostris bisch had the highest radical scavenging activity, TPC, *p*-coumaric acid, 4-hydroxybenzoic acid and 2,5-dihydroxybenzoic acid. *Sinapis arvensis* L. had the highest radical caution decolorization and caffeic acid. *Thymus vulgaris* L. had the highest ferric reducing antioxidant power, as well as naringin and rutin. *Amaranthus retroflexus* L. had the highest TFC, gallic acid and vanillic acid levels. The antioxidant potential of plants was positively correlated with TPC. *Malva neglecta* L. was a rich source of phenolic compounds with high antioxidant properties. The high yield of ferulic acid, chlorogenic acid, ellagic acid, cinnamic acid, catechin, apigenin, and quercetin could make this plant a valuable source of commercial production. Results indicated that *Tragopogon longirostris bisch, Sinapis arvensis* L.*,* T*hymus vulgaris* L.*, Amaranthus retroflexus* L. *and Malva neglecta* L. had a high capacity to prevent diseases caused by the overproduction of radicals.

REFERENCES

[1] Ivanova, D., Gerova, D., Chervenkov, T., Yankova, T. (2005). Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology*, 96(1-2), 145-150.

[2] Rice-Evans, C., Miller, N., Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science*, *2(4),* 152-159.

[3] Winkel-Shirley, B. (2002). Biosynthesis of flavonoids and effects of stress. *Current Opinion in Plant Biology*, *5*(3), 218-223.

[4] Moure, A., Cruz, J M., Franco, D., Domínguez, J.M., Sineiro, J., Domínguez, H., Parajó, J.C. (2001). Natural antioxidants from residual sources. *Food Chemistry*, 72(2), 145-171.

[5] Carletti, P., Masi, A., Wonisch, A., Grill, D., Tausz, M., Ferretti, M. (2003). Changes in antioxidant and pigment pool dimensions in UV-B irradiated maize seedlings. *Environmental and Experimental Botany*, 50(2), 149-157.

[6] Mohammedi, Z., Atik, F. (2012). HPLC-UV Analysis and antioxidant potential of phenolic compounds from endemic shrub of arid environment *Tamarix pauciovulata* J. Gay. *Journal of Life Sciences*, 6(8), 883-891.

[7] Al-Laith, A. A., Alkhuzai, J., Freije, A. (2019). Assessment of antioxidant activities of three wild medicinal plants from Bahrain. *Arabian Journal of Chemistry*, 12(8), 2365-2371.

[8] Günbatan, T., Salihoğlu, E. M., Akaydın, S., Akaydın, G., Gurbuz, İ. (2023). *Malva neglecta* Wallr. bitkisinin kimotripsin, üreaz inhibe edici ve antioksidan aktivitesi. *Mersin Üniversitesi Tıp Fakültesi Lokman Hekim Tıp Tarihi ve Folklorik Tıp Dergisi*, 13(2), 459-468.

[9] Kolar, F. R., Kambhar, S. V., Kamble, V. S., Daphedar, A. B. (2024). Comparative examination of phenolic content, flavonoid content, and antioxidant efficacy of Chenopodium album L. and *Chenopodium pumilio* R. Br. *İstanbul Journal of Pharmacy*, 54(2), 165-174.

[10] Ivanov, I.G. (2014). Polyphenols content and antioxidant activities of Taraxacum officinale FH Wigg (dandelion) leaves. *International Journal of Pharmacognosy and Phytochemical Research,* 6, 889-893.

[11] Ao, T., Deb, C.R. (2019), Nutritional and antioxidant potential of some wild edible mushrooms of Nagaland, India. *Journal of Food Science and Technology,* 56(2), 1084-1089.

[12] Shen, D.D., Li, X., Qin, Y.L., Li, M.T., Han, Q.H., Zhou, J., Wu, D.T. (2019). Physicochemical properties, phenolic profiles, antioxidant capacities, and inhibitory effects on digestive enzymes of okra (*Abelmoschus esculentus*) fruit at different maturation stages. *Journal of Food Science and Technology,* 56(3), 1275-1286.

[13] Somkuwar, R.G., Bhange, M.A., Oulkar, D.P., Sharma, A.K., Shabeer, T.A. (2018). Estimation of polyphenols by using HPLC–DAD in red and white wine grape varieties grown under tropical conditions of India. *Journal of Food Science and Technology,* 55(12), 4994-5002.

[14] Jagtap, N.S., Wagh, R.V., Chatli, M.K., Kumar, P., Malav, O.P., Mehta, N. (2019). Optimisation of extraction protocol for *Carica papaya* L, to obtain phenolic rich phyto-extract with prospective application in chevon emulsion system. *Journal of Food Science and Technology,* 56(1), 71-82.

[15] Yalcin, S., Schreiner, M. (2018). Stabilities of tocopherols and phenolic compounds in virgin olive oil during thermal oxidation. *Journal of Food Science and Technology, 55,* 244-251.

[16] Ningappa, M. B., Dinesha, R., Srinivas, L. (2008). Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (*Murraya koenigii* L.) extracts. *Food Chemistry*, 106(2), 720-728.

[17] Perea-Domínguez, X.P., Hernández-Gastelum, L.Z., Olivas-Olguin, H.R., Espinosa-Alonso, L.G., Valdez-Morales, M., Medina-Godoy, S. (2018). Phenolic composition of tomato varieties and an industrial tomato by-product: free, conjugated and bound phenolics and antioxidant activity. *Journal of Food Science and Technology*, *55*, 3453-3461.

[18] Fellah, B., Bannour, M., Rocchetti, G., Lucini, L., Ferchichi, A. (2018). Phenolic profiling and antioxidant capacity in flowers, leaves and peels of Tunisian cultivars of *Punica granatum* L. *Journal of Food Science and Technology*, *55*, 3606-3615.

[19] Yalcin, S., Basman, A. (2016). Effects of infrared treatment on tocopherols, total phenolics and antioxidant activity of soybean samples. *Quality Assurance and Safety of Crops & Foods,* 8(2),1-10.

[20] Hassanzadeh, Z., Hassanpour, H. (2018). Evaluation of physicochemical characteristics and antioxidant properties of *Elaeagnus angustifolia* L. *Scientia Horticulturae*, 238, 83-90.

[21] Orak, H.H. (2007). Total antioxidant activities, phenolics, anthocyanins, polyphenoloxidase activities of selected red grape cultivars and their correlations. *Scientia Horticulturae, 111(3),* 235-241.

[22] Hassanpour, H., Alizadeh, S. (2016). Evaluation of phenolic compound, antioxidant activities and antioxidant enzymes of barberry genotypes in Iran. *Scientia Horticulturae*, *200*, 125-130.

[23] Brand-Williams, W., Cuvelier, M.E., Berset, C.L.W.T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, 28(1), 25-30.

[24] Perez-Jimenez, J., Saura-Calixto, F. (2006). Effect of solvent and certain food constituents on different antioxidant capacity assays. *Food Research International,* 39, 791-800.

[25] Re, R., Pellegrini, N., Proteggente, N., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine,* 26, 1231-123.

[26] Benzie, I.F., Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1), 70-76.

[27] Singleton, V.L., Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture,* 16, 144-158.

[28] Dewanto, V., Wu, X., Adom, K.K., Liu, R.H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry*, 50, 3010-3014.

[29] Caponio, F., Alloggio, V., Gomes, T. (1999). Phenolic compounds of virgin olive oil: influence of paste preparation techniques. *Food Chemistry*, 64(2), 203-209.

[30] Liu, X., Ardo, S., Bunning, M., Parry, J., Zhou, K., Stushnoff, C., Kendall, P. (2007). Total phenolic content and DPPH radical scavenging activity of lettuce (*Lactuca sativa* L,) grown in Colorado. *LWT,* 40(3), 552-557.

[31] Chizzola, R., Michitsch, H., Franz, C. (2008). Antioxidative properties of *Thymus vulgaris* leaves: comparison of different extracts and essential oil chemotypes. *Journal of Agricultural and Food Chemistry,* 56(16), 6897-6904.

[32] Dauqan, E., Abdullah, A., Muin, N.M. (2017). Antioxidants and antibacterial activities of extract from thyme and mint leaves. *Journal of Chemical and Pharmaceutical Research,* 9(12), 104-110.

[33] Acıkara, Ö.B., Çitoglu, G.S., Çoban, T, (2013). Evaluation of antioxidant properties of some tragopogon species growing in Turkey. *The Turkish Journal of Pharmaceutical Sciences*, 10(3), 377-384.

[34] Zdravković, J., Aćamović-Đoković, G., Mladenović, J., Pavlović, R., Zdravković, M. (2014). Antioxidant capacity and contents of phenols, ascorbic acid, β-carotene and lycopene in lettuce. *Hemijska Industrija*, 68(2), 193-198.

[35] Dalar, A., Türker, M., Konczak, I. (2012). Antioxidant capacity and phenolic constituents of *Malva neglecta* Wallr, and *Plantago lanceolata* L, from Eastern Anatolia Region of Turkey. *Journal of Herbal Medicine,* 2(2), 42-51.

[36] Jaina, G.J., Settyb, S.R. (2018). Gallic acid and flavonoids of *Amaranthus retroflexus* MIT. International *Journal of Pharmacy and Pharmaceutical Sciences,* 4(2), 82–85.

[37] Khan, A.S., Arif, K., Munir, B., Kiran, S., Jalal, F., Qureshi, N., Tahir, M.A. (2019). Estimating total phenolics in *Taraxacum officinale* (L,) extracts. *Polish Journal of Environmental Studies,* 28(1), 497-501.

[38] Pandey, S., Gupta, R.K. (2014). Screening of nutritional, phytochemical, antioxidant and antibacterial activity of *Chenopodium album* (Bathua). *Journal of Pharmacognosy and Phytochemistry,* 3(3), 1-9.

[39] Köksal, E., Bursal, E., Gülçin, İ., Korkmaz, M., Çağlayan, C., Gören, A.C., Alwasel, S.H. (2017). Antioxidant activity and polyphenol content of Turkish thyme (*Thymus vulgaris*) monitored by liquid chromatography and tandem mass spectrometry. *International Journal of Food Properties*, 20(3), 514-525.

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Research Paper / Araştırma Makalesi

Changes in Color and Some Bioactive Properties of Cape Gooseberry Leathers Dried Under Different Conditions During Storage

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ABSTRACT

Fruit leathers are considered a type of alternative confectionery and are regarded as healthy snack options. In this study, cape gooseberry leathers were prepared using different drying methods, and the changes in their color, browning index, phenolic composition, sugar content, as well as levels of α-carotene and β-carotene, were monitored over a six-month storage period. The production technique for cape gooseberry leathers followed the traditional method used for making pestil, a well-known Turkish fruit leather, utilizing starch, sugar, and lemon. Immediately after production, the browning index of the samples ranged from 1.25 to 1.73 A_{420}/g dry weight (dw). However, a significant increase in browning index values was found in all samples during the final two months of storage, reaching between 2.762 and 3.278 A_{420}/g dw by the end of the six months. It was found that β-carotene levels were significantly higher than α-carotene levels in the samples. Both α-carotene and β-carotene contents decreased significantly during storage, with sun-dried samples experiencing the greatest reduction in β-carotene. Analysis of the phenolic profile revealed the presence of rutin, gallic acid, vanillic acid, *p*-coumaric acid, ferulic acid, and cinnamic acid in the fruit leathers. Some phenolic compounds decreased in concentration after six months of storage.

Keywords: Fruit leather, Pestil, Color, Bioactive properties

Farklı Koşullarda Kurutulan Altınçilek Pestillerinin Depolama Boyunca Renk ve Bazı Biyoaktif Özelliklerindeki Değişimler

ÖZ

Meyve pestilleri, alternatif şekerleme türlerinden biri olarak kabul edilir ve sağlıklı atıştırmalık seçenekleri arasında görülür. Bu çalışmada, farklı kurutma yöntemleri kullanılarak altın çilek pestilleri hazırlanmış ve altı aylık depolama süresi boyunca renk, kahverengileşme indeksi, fenolik bileşim, şeker içeriği ile α-karoten ve β-karoten seviyelerindeki değişimler izlenmiştir. Altın çilek pestillerinin üretim tekniği, nişasta, şeker ve limon kullanılarak yapılan, Türkiye'nin tanınmış meyve pestillerinden pestil yapımında kullanılan geleneksel yöntemi takip etmiştir. Üretimden hemen sonra örneklerin kahverengileşme indeksi 1,25 ile 1,73 A_{420}/g kuru ağırlık (ka) arasında değişmiştir. Ancak, depolamanın son iki ayında tüm örneklerde kahverengileşme indeksi değerlerinde önemli bir artış gözlemlenmiş ve altı ayın sonunda bu değerler 2,762 ile 3,278 A_{420}/g ka arasında değişmiştir. Örneklerde β-karoten seviyelerinin α-karoten seviyelerinden anlamlı derecede daha yüksek olduğu bulunmuştur. Depolama süresince hem α-karoten hem de β-karoten içerikleri önemli ölçüde azalmış, güneşte kurutulan örnekler β-karoten miktarında en büyük düşüşü göstermiştir. Fenolik profil analizi, meyve pestillerinde rutin, gallik asit, vanilik asit, *p*-kumarik asit, ferulik asit ve sinnamik asit gibi bileşiklerin varlığını ortaya koymuştur. Bazı fenolik bileşiklerin konsantrasyonlarının, altı aylık depolama süresi sonunda azaldığı tespit edilmiştir.

Anahtar Kelimeler: Pestil, Renk, Biyoaktif özellikler

INTRODUCTION

Cape gooseberry (*Physalis peruviana L.*) is an exotic fruit that originated region of Amazon and Andes Mountains from South America [1, 2]. It has important potential for healthy nutrition, especially owing to its bioactive functional compounds such as phenolic compounds, vitamin C and carotenoids and high fiber contents [3-5].

Fruit leathers can be made from wide variety of fruits including apple, apricot, banana, cherry, fig, grape, mulberry, orange, peach, pear, pineapple, plum, pomegranate strawberry, tangerine [6, 7]. Fruit leathers are easy to consume, convenient to pack and that can be consumed anywhere [8]. It is easy to store and shipping that due to their lightness, and they do not require cold storage [9]. Fruit pulps or fruit juice concentrates are generally used for manufacturing fruit leathers. Beside fruit, other ingredients such as sugar, acid, pectin, or starch, can be used to improve the texture, physicochemical and sensory characteristics of fruit leather products [10]. In fruit leather production, sun drying or industrial dryers such as oven or cabinet dryers can be used, which helps in faster drying and obtaining a standard product [8, 11].

Color change is one of the important changes that may occur during drying in dried foods like fruit leather. The changes in color occur mainly through enzymatic and non-enzymatic browning reactions. Browning reactions not only cause color change in foods but also lead to decreased nutritional value, undesired taste and aroma, and textural changes [12, 13].

In this study, fruit leathers were produced from cape gooseberry using different drying methods and temperature. Then, the color properties of the products, important quality criteria for marketing dried fruit products, and some of their bioactive properties were monitored during storage. The effect of different drying conditions on the quality and nutritional properties of the fruit leather products during storage were determined.

MATERIALS and METHODS

Materials

Cape gooseberry fruits were obtained from local producers in Antalya (Türkiye). Low density polyethylene (LDPE) packaging materials, wheat starch, sugar and lemon were purchased from local suppliers or markets in Isparta (Türkiye). Fruits were stored at 4°C until they were used in production. All fruit leather products were kept in sealed vacuum bags at room temperature during storage.

Methods

Production of Cape Gooseberry Leather

First, the calyx was removed from the fruits. The fruits were then washed thoroughly to eliminate any dirt or foreign matter. Stems were detached, and the fruits were blended into a puree. Wheat starch was added at 8% of the puree weight, and water was incorporated at 25% of the puree weight. Sugar and lemon juice were prepared to correspond to 15% and 2% of the puree weight, respectively (see Table 1). The puree was then heated, and sugar was gradually mixed in until fully dissolved. The starch slurry was added, followed by continuous heating and stirring, after which lemon juice was incorporated. Heating was stopped when the mixture reached 63°C. The hot mixture was poured into trays and spread evenly to a thickness of 4 mm. Drying was conducted in a cabinet dryer at 0.8 m/s air velocity with temperatures of 60°C, 70°C, and 80°C, as well as by sun drying. Drying continued until the final product reached a dry matter content of 87%. This process mirrors the traditional pestil-making method used in Turkey for fruit leathers. After drying, the cape gooseberry leathers were vacuum-packed in low-density polyethylene (LDPE) packaging and stored at room temperature.

Table 1. Raw materials used in cape gooseberry leather production

Raw materials	Amounts
Cape gooseberry puree	1000 g
Wheat Starch	80 g
Water	250 mL
Sugar	150 g
Lemon juice	20 mL

Chemical Analyses

The pH of the fruit leather products was determined by a pH-meter (Hanna HI 2211-02, Romania) and their acidity values were determined as citric acid by titrimetric method [14]**.** Moisture and ash values were determined according to Pestil Standard Methods for cape gooseberry leathers [15]. Results calculated in g/100 g. Total phenolic contents were determined using the Folin-Ciocalteu colorimetric method by extracting the samples with methanol [16]. Absorbance values were measured at 720 nm in a spectrophotometer. Results were expressed as mg gallic acid equivalent (GAE) per g fresh weight or g dry weight using the calibration curve obtained from gallic acid solutions.

$$\% \text{ İnhibition (DPPH)} = [(\text{Abs Control} - \text{Abs Sample}) / \text{Abs Control}]*100 \quad (1)$$

In total carotenoid analysis, the fruit leather products were extracted with methanol and the absorbance values of the extract were measured at 666, 653 and 470 nm in a spectrophotometer (Unicam, UK) and the total carotenoid content was calculated with the help of the equations below (Equations 2-4) [18].

$$Ca=15.65 \times A666 - 7.340 \times A653 \quad (2)$$

$$Cb=27.05 \times A653 - 11.21 \times A666 \quad (3)$$

$$Cx+c=1000 \times A470 - 2.860 \times Ca - 129.2 \times Cb/245 \quad (4)$$

where Ca: Chlorophyll a, Cb: Chlorophyll b, and Cx+c: Total carotenoid content.

Ascorbic acid was determined by a spectrophotometric method of 2.6-dichlorophenoldophenol-xylene extraction procedure [14]. Absorbance values were measured at 500 nm. Results were calculated using the calibration curve obtained from the solutions prepared with dilute ascorbic acid solution. Soluble solid contents of samples measured according to [14], by refractometer. The water activity (aw) values of fruit leather samples were determined with a digital Aqualab CX-2 water activity meter (Decagon Devices Inc. Pulman, Washington, USA) [19].

Color Measurements

The color changes in fruit leather products were determined by two different methods. Measurements were made on the color measuring device (PCE-TCR 200) using Hunter (L, a, b) and (L, C, H) color scales. The device was calibrated before each analysis using the device's standard black and white calibration table. Also, the browning index values of samples were determined [14]. For this purpose, 50 mL of 2% acetic acid solution was added to 5 grams of sample cut into small pieces. Samples were kept in the refrigerator overnight for rehydration and then homogenized for 3 min. Each homogenate was transferred to a centrifuge tube and centrifuged at 8000 g for 10 min. Then, the supernatant was transferred to a 200 mL measuring flask. The remaining part at the bottom of the tube was added with 25 mL of 2% acetic acid, mixed with a glass rod and centrifuged again. The supernatant in the centrifuge tube was added to the measuring flask again. Then, 10 mL of 10% lead acetate solution was added to the flask containing the extract, and the flask was filled to its volume with 2% acetic acid. The contents of the flask were centrifuged again at 8000 g for 5 minutes. Then, an equal volume of ethyl alcohol was added to the upper clear part in the centrifuge tube and centrifugation was performed once more. The absorbance of the obtained extract was measured against the blank consisting of equal volumes of 2% acetic acid and ethyl alcohol in a UV-VIS spectrophotometer (Unicam, England) at two different wavelengths, 420 and 600 nm, and the browning index was determined using the difference between them. The browning value is expressed in units of $A_{420/g}$ sample. In the examples, $A_{420/g}$ values were calculated with the help of the formula below (Equation 5) [14]. These values were determined after the production of the fruit leather and during storage.

$$A_{420}/g_{sample}= (A_{420}-A_{600})xS_f \quad (5)$$

where S_f indicates a dilution factor.

Determination of Phenolic Composition

The composition of phenolic substances in samples were determined by HPLC (high performance liquid chromatography) [20]. First of all, 10 g from each sample were weighed and homogenized with 20 mL of methanol:water (4:1, v/v) solution. UltraTorax (IKA T18, Staufen, Germany) was used for the homogenization process. Afterwards, samples were taken into centrifuge tubes and centrifuged in a centrifuge device (Sigma 2-6E, Osterode am Harz, Germany), at 4000 rpm, 4°C, for 20 min, and the supernatant was taken. Before analysis, these fractions were passed through membrane filters (Millex, HV) with a pore diameter of 0.45 µm and 20 µL of the filtrates were injected into the device, and the phenolic composition was determined. All analyses were performed with an HPLC instrument (Shimadzu, Kyoto, Japan). Measurements were made with a DAD detector at a wavelength of 278 nm. The column temperature was set to 30°C. Agilent Eclipse XDB-C18 (250 × 4.60 mm, particle size 5 µm) column was used as the column. Gradient program was used in the analysis. The mobile phases used are solvent A: water:acetic acid (3:97, v/v), solvent B: methanol. The flow rate was set to 0.8 mL/min.

Determination of Carotenoid Composition

Carotenoid composition determination was carried out by an HPLC unit (Shimadzu, Kyoto, Japan), and α- and β-carotene contents were determined in each sample according to Olives Barba et al. [21]. First, the extraction solution was obtained by mixing hexane, acetone and ethanol (50/25/25, v/v/v). Then, 50 mL of extraction solution was added to 5 g of sample. The solution was stirred on a magnetic stirrer for 30 minutes. The upper phase was transferred to a balloon by adding 10 mL of distilled water. Drying was carried out in the evaporator (IKA Rv10, Staufen, Germany) and then the flask was washed with 1 mL of chloroform. Dilutions were made with methanol/acetonitrile/tetrahydrofuran (55/30/15, v/v/v) and 20 µL of the samples were injected into the device for analysis. Measurements were made with a DAD detector at 450 nm. The column temperature was set to 30°C. Luna C18 (250×4.60 mm, particle size 5 µm) column was used as the column. The mobile phase included methanol/acetonitrile (90/10, v/v) and 9 µM triethyl amine. The flow rate was set to 1.2 mL/min.

Determination of Sugar Composition

The sugar composition of fruit leather samples were determined chromatographically [22]. Extraction was carried out with water. 40 mL of water was added to 10 g sample and homogenized by a homogenizer (IKA T18, Staufen, Germany). Then, centrifugation was performed at 6000 rpm for 30 min in a centrifuge (Sigma 2-6E, Osterode am Harz, Germany). The upper clear part was passed through membrane filters (Millex, HV) with a pore diameter of 0.45 µm, 20 µL of which was injected into HPLC. A Shimadzu brand HPLC unit (Kyoto, Japan) was used for analyses. Measurements were made with a RID 10A detector. The column temperature was set to

80°C. Aminex HPX-87C (300×7.8 mm) carbohydrate column was used as the column. The mobile phase used was ultrapure water. The flow rate was set to 0.6 mL/min.

Statistical Analysis

In statistical analysis, SPSS program (version 19, IBM, 2010) was used. Analyses were repeated three times and averaged. One-way analysis of variance with the Duncan multiple comparison test was used to compare means.

RESULTS and DISCUSSION

The physicochemical properties of cape gooseberry leathers are shown that in Table 2. The effects of different temperature applications and sun drying method on the physicochemical properties of the products can be seen in this table. The table shows that the products have high acidity and low water activity. Cape gooseberry leathers pH values between 3.56-3.61. These values below limit pH value (4.0) for bacterial growth. Below this limit only molds and yeast can grow [23]. Cape gooseberry leathers a_w values between 0.53-0.56, with no differences among treatments. Microbial growth doesn't occur below the 0.6 a_w [24]. Also, different preservation factors such as pH and water activity showed synergetic effects on inhibition of microbial growth [23]. Therefore, the high acidity and low water activity of the products increases their microbial stability. In addition, it is seen from the table that temperature change has a similar effect on the phenolic substance contents and antioxidant activities of the products. It was determined that as the temperature applied at drying increased, the amounts of phenolic compounds and antioxidants were found to be higher. This is probably due to the fact that the antioxidant activity in foods is mainly due to phenolic compounds. It was reported that phenolic compounds are mainly responsible for antioxidant activity of fruit juice [4]. In terms of total carotenoid and vitamin C amounts which are other antioxidant compounds in the products, the best preservation among fruit leather production methods was provided by the sun drying method.

Table 2. The physicochemical properties of cape gooseberry leathers dried either in a cabinet at three different temperatures or sun-dried (means ± standard deviation)

Properties	Drying at 60°C	Drying at 70°C	Drying at 80°C	Sun Drying
pH	3.60^{a}±0.01[1]	3.61^{a}±0.01	3.61^{a}±0.01	3.56^{b}±0.02
Titratable acidity (%)	3.43^{a}±0.12	$3.53^{a,b}$±0.04	$3.52^{a,b}$±0.02	3.63^{b}±0.05
a_w	0.56^{a}±0.01	0.53^{a}±0.01	0.55^{a}±0.008	0.55^{a}±0.008
Ash (% dw[2])	2.44^{a}±0.02	2.40^{a}±0.01	2.44^{a}±0.002	2.57^{a}±0.13
Soluble solid	65.33^{a}±0.86	65.66^{a}±0.58	66.09^{a}±0.32	64.37^{a}±2.46
Dry matter (%)	87.12^{a}±0.08	87.87^{a}±0.20	87.55^{a}±0.31	87.52^{a}±0.53
Total phenolic content (mg/g dw)	0.57^{a}±0.02	0.71^{b}±0.029	0.88^{c}±0.053	$0.62^{a,b}$±0.07
Total carotenoid content (mg/100 g dw)	3.19^{a}±0.01	3.15^{a}±0.040	2.97^{b}±0.098	4.19^{c}±0.07
Vitamin C (mg/100 g dw)	$26.22^{a,b}$±0.80	$19.26^{b,c}$±0.77	11.62^{c}±0.35	31.46^{a}±2.51
Antioxidant activity (%)	59.10^{a}±1.38	66.68^{b}±0.42	70.25^{c}±0.71	71.50^{c}±1.09

[1]Means in a same row with different letters are significantly different ($p<0.05$). [2]dw: dry weight

Browning values were determined in the products after production and during storage. Analysis of browning of the products were performed during storage at the end of the second, fourth and sixth months of storage. Browning absorbance reading values are shown in the Table 3, and A_{420}/g dry matter values are shown in the Figure 3. In the samples, browning values after production were found to be quite low. The browning indices of cape gooseberry leather samples were between 1.25 and 1.73 A_{420}/g dw. This was expected since the acidity values of the products were very high, as shown in Table 3. High acidity is one of the restricted factors for nonenzymatic browning [13]. Therefore, high acidity in products is probably one of the main reasons for low browning values. For cabinet dried leathers there were slightly increase browning at higher degree. Che man et al. [25] in a study on the durian leather showed that an increase in temperature also increased nonenzymatic browning index values. Miranda and Berna, [26] also reported that temperature was the determining factor on browning in dried apricots and raisins. As for sun-dried samples in our study, their browning index values were found near to cabinet dried samples values. Also, results showed that browning development for first months occur very slowly. However for the last two months of storage, the browning indices of all samples increased. The browning indices of cape gooseberry leathers at end of the storage were found between 2.762 and 3.278 A_{420}/g dw. The samples that showed the highest browning values after six months of storage were those dried at 80°C, which was the highest temperature in cabinet drying.

Table 3. Browning index absorbance values at 420nm of fruit leathers dried at different conditions during storage

Drying Temperature/Method	After production	2. month	4.month	6. month
60°C	0.014±0.001	0.015±0.001	0.018±0.002	0.031±0.001
70°C	0.018±0.001	0.019±0.001	0.020±0.001	0.030±0.001
80°C	0.019±0.001	0.020±0.001	0.022±0.002	0.036±0.002
Sun Drying	0.018±0.002	0.020±0.003	0.020±0.002	0.030±0.002

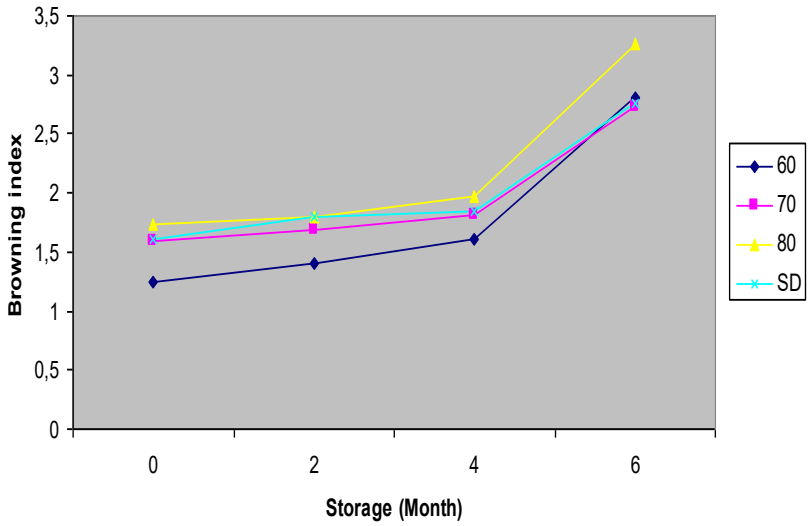

Figure 3. Change of the browning index (A_{420}/g dw) values of samples during storage

Color is an important quality criterion for fruit leather samples. The color values of L (lightness), a (redness), b (yellowness), C (chroma) and h (hue angle) obtained from the measurements made after production and in the 2nd, 4th and 6th months of storage are shown in the Tables 4, 5, 6 and 7 below. Color measurements performed after production are shown in Table 4. The lightness and yellowness of the samples dried in the sun after production were statistically significantly ($p<0.05$) higher from samples dried in the cabin. However, this difference was not observed in the results of browning analyses. In addition, during six months of storage, the highest decrease in lightness values occurred in the samples dried at the highest temperature (80°C), while the highest increase in browning values was observed in the same samples dried at 80°C. This indicated the effect of the increase in browning on the decrease in lightness values. It was reported that a decrease in L (lightness) values was in parallel with an increase in the browning indices of foods [8]. This was also observed in present study for samples dried at 80°C. Reasons for this color change in foods were reported as heat treatment, moisture content, water activity, sugar content, storage time and storage temperature [27]. Enzymes were inactivated by applying heat treatment to the samples; however, the formation of darker pigments is possible by non-enzymatic browning or ascorbic acid oxidation [9].

The phenolic composition of fruit leather samples is shown in Table 8. Rutin, gallic acid, vanillic acid, *p*-coumaric acid, ferulic acid and cinnamic acid were determined in fruit leather samples. Rutin was determined as the dominant phenolic compound in the samples. The second highest phenolic compound was gallic acid. Licodiedoff et al. [28] studied the flavonols in cape gooseberry fruit. They examined for rutin, myricetin and quercetin. They reported that quercetin was present in the fruit. They found mainly rutin and also myricetin in the fruit. After six months of storage, the phenolic compound contents were determined again in all samples. After storage, some phenolic compounds significantly decreased (Table 8).

Table 4. Color values of fruit leather products dried either in a cabinet at three different temperatures or sun-dried after production (means ± standard deviation)

Parameter	Drying at 60°C	Drying at 70°C	Drying at 80°C	Sun Drying
L	$41.01^{a}\pm0.61$[1]	$39.78^{a}\pm0.31$	$39.90^{a}\pm0.13$	$44.33^{b}\pm0.96$
a	$9.81^{a}\pm0.64$	$12.11^{b}\pm0.63$	$13.36^{b,c}\pm0.05$	$14.05^{c}\pm0.76$
b	$21.53^{a}\pm0.69$	$17.09^{b}\pm1.05$	$19.86^{a}\pm0.70$	$23.76^{c}\pm0.81$
C	$23.66^{a}\pm0.85$	$20.95^{b}\pm1.12$	$23.94^{a}\pm0.61$	$27.60^{c}\pm1.09$
h	$65.51^{a}\pm0.95$	$54.66^{b}\pm1.36$	$56.06^{b}\pm0.83$	$59.42^{c}\pm0.50$

[1]Means in a same row with different letters are significantly different ($p<0.05$).

Table 5. Color values of fruit leather products dried either in a cabinet at three different temperatures or sun-dried after two months of storage (means ± standard deviation)

Parameter	Drying at 60°C	Drying at 70°C	Drying at 80°C	Sun Drying
L	$43.54^{a}\pm0.54$[1]	$46.37^{b}\pm0.78$	$42.61^{a}\pm0.49$	$47.22^{b}\pm0.25$
a	$15.12^{a}\pm0.32$	$12.66^{b}\pm0.38$	$15.21^{a}\pm0.97$	$16.15^{a}\pm0.39$
b	$23.88^{a}\pm0.55$	$25.46^{b}\pm0.25$	$25.20^{b}\pm0.19$	$27.43^{c}\pm0.77$
C	$28.27^{a}\pm0.30$	$28.44^{a}\pm0.39$	$29.45^{b}\pm0.54$	$31.83^{c}\pm0.49$
h	$57.65^{a}\pm1.14$	$63.56^{b}\pm0.49$	$58.91^{a}\pm1.62$	$59.50^{a}\pm1.29$

[1]Means in a same row with different letters are significantly different ($p<0.05$).

Table 6. Color values of fruit leather products dried either in a cabinet at three different temperatures or sun-dried after four months of storage (means ± standard deviation)

Parameter	Drying at 60°C	Drying at 70°C	Drying at 80°C	Sun Drying
L	44.73^{a}±1.14[1]	43.56^{a}±0.74	38.75^{b}±0.25	48.16^{c}±0.66
a	12.44^{a}±0.64	14.99^{b}±0.38	14.86^{b}±0.38	13.27^{a}±0.02
b	24.33^{a}±0.76	24.73^{a}±0.93	17.52^{b}±0.27	26.93^{c}±0.21
C	27.33^{a}±0.45	28.92^{b}±0.99	22.97^{c}±0.45	30.02^{b}±0.19
h	62.91^{a}±1.86	58.77^{b}±0.31	49.70^{c}±0.31	63.77^{a}±0.16

[1]Means in a same row with different letters are significantly different (p<0.05).

Table 7. Color values of fruit leather products dried either in a cabinet at three different temperatures or sun-dried after six months of storage (means ± standard deviation)

Parameter	Drying at 60°C	Drying at 70°C	Drying at 80°C	Sun Drying
L	46.96^{a}±1.77[1]	45.03^{a}±0.59	31.91^{b}±3.56	45.61^{a}±2.60
a	12.05^{a,b}±0.13	12.50^{a}±1.02	10.28^{b}±0.32	13.67^{a}±1.16
b	24.51^{a}±0.64	27.38^{a}±1.26	17.10^{b}±2.52	25.01^{a}±0.26
C	27.32^{a}±0.54	30.10^{a}±1.56	19.98^{b}±2.16	28.51^{a}±0.78
h	63.81^{a}±0.77	65.49^{a,b}±0.83	58.71^{a,c}±3.93	61.38^{a}±1.82

[1]Means in a same row with different letters are significantly different (p<0.05).

Table 8. Phenolic composition of samples (mg /100 g dry weight) initial and after six months storage (means ± standard deviation)

Sample	Storage Time	Gallic Acid	Vanillic Acid	p-Coumaric Acid	Ferulic Acid	Rutin	Cinnamic Acid
60°C	0	0.92^{a}±0.20[1]	0.30^{a}±0.40	0.11^{a}±0.20	0.18^{a}±0.00	0.82^{a}±0.40	0.05^{bc}±0.00
70°C	0	0.87^{a}±0.20	0.32^{a}±0.20	0.11^{a}±0.25	0.21^{a}±0.25	0.57^{b}±0.20	0.05^{bc}±0.00
80°C	0	0.89^{a}±0.20	0.32^{a}±0.00	0.11^{a}±0.25	0.18^{a}±0.20	0.44^{bc}±0.25	0.09^{a}±0.20
Sun dried	0	0.64^{a}±0.40	0.18^{a}±0.25	0.09^{a}±0.00	0.21^{a}±0.25	0.57^{b}±0.25	0.02^{d}±0.00
60°C	6 months	0.80^{a}±0.11	0.10^{a}±0.11	0.06^{a}±0.11	0.0013^{b}±0.11	0.29^{cd}±0.23	0.03^{cd}±0.11
70°C	6 months	0.72^{a}±0.11	0.08^{a}±0.11	0.08^{a}±0.11	0.0013^{b}±0.11	0.30^{cd}±0.20	0.04^{bcd}±0.11
80°C	6 months	0.80^{a}±0.20	0.15^{a}±0.11	0.08^{a}±0.23	0.0013^{b}±0.11	0.27^{d}±0.11	0.06^{b}±0.11
Sun dried	6 months	0.53^{a}±0.20	0.08^{a}±0.11	0.06^{a}±0.11	0.0015^{b}±0.23	0.29^{cd}±0.11	0.02^{d}±0.00

[1]Means in a same column with different letters are significantly different (p<0.05).

The β-carotene contents of the samples were significantly higher than the α-carotene contents (Table 9). After six months of storage, the amounts of both α-carotene and β-carotene significantly decreased in all samples. However, the amounts of β-carotene decreased the most in sun-dried samples (Table 9). In the literature, Korese et al. [27], in their study examining the effects of different packaging materials on the storage stability of *Gardenia erubescens* Stapf. and Hutch. dried fruits, found that the β-carotene amounts decreased significantly during three months of storage.

Table 9. α- and β-carotene contents of samples (mg/100 g dry weight) initial and after six months storage (means ± standard deviation)

Sample	Storage Time	α-Carotene	β-Carotene
60°C	0	0.751^{c}±0.02[1]	1.227^{d}±0.01
70°C	0	0.873^{a}±0.01	1.500^{a}±0.01
80°C	0	0.767^{b}±0.02	1.378^{c}±0.02
Sun dried	0	0.722^{d}±0.02	1.480^{b}±0.02
60°C	6 months	0.009^{e}±0.02	0.435^{e}±0.01
70°C	6 months	0.008^{e}±0.01	0.394^{f}±0.01
80°C	6 months	0.007^{e}±0.02	0.377^{g}±0.02
Sun dried	6 months	0.007^{e}±0.02	0.346^{h}±0.02

[1]Means in a same column with different letters are significantly different (p<0.05).

In our study, the primary sugars identified in cape gooseberry fruit leathers were sucrose, glucose, and fructose. Because sugar was added during production, the sucrose content in the leathers was significantly higher than that of the other sugars (see Table 10). According to previous research on cape gooseberry's sugar composition, the main sugars found in the fruit are glucose, fructose, and sucrose, with sucrose levels exceeding those of glucose and fructose [29]. Although there were some changes in the amounts of sucrose, glucose, and fructose after storage, these sugars did not experience notable losses, unlike the phenolic compounds or carotene levels (Table 10).

Table 10. Sugar composition of samples (g/100 g dry weight) initial and after six months storage (means ± standard deviation)

Sample	Storage Time	Sucrose	Glucose	Fructose
60°C	0	27.47^{ab}±1.18[1]	4.62^{cd}±0.30	5.07^{cd}±0.57
70°C	0	26.97^{ab}±1.61	5.67^{bc}±0.09	5.84^{bc}±0.08
80°C	0	25.31^{b}±1.78	7.64^{a}±0.35	7.76^{a}±0.37
Sun dried	0	33.78^{a}±4.84	4.74^{cd}±0.35	5.19^{cd}±0.13
60°C	6 months	26.23^{ab}±1.42	4.97^{c}±0.12	5.27^{cd}±0.11
70°C	6 months	26.93^{ab}±1.12	5.06^{c}±0.38	5.51^{cd}±0.32
80°C	6 months	23.89^{b}±1.33	6.61^{b}±0.22	6.83^{ab}±0.25
Sun dried	6 months	28.59^{ab}±2.84	3.77^{d}±0.45	4.57^{d}±0.51

[1]Means in a same column with different letters are significantly different ($p<0.05$).

CONCLUSION

Fruit leathers made from various fruits serve as healthy snack alternatives to traditional confectionery. In this study, cape gooseberry leathers were prepared using both hot air and sun drying techniques. After production, changes in color metrics, browning index, phenolic content, sugar composition, and levels of α- and β-carotene in the fruit leathers were monitored over a six-month storage period. Color is a key quality factor influencing consumer preference and reflecting the physical and structural characteristics of food products. The browning index was initially low after production but increased notably after the fourth month, reaching much higher levels by the end of six months.

Among the samples, those dried at 80°C showed the highest browning after six months. It was also found that β-carotene levels were significantly higher than α-carotene levels. Over the six months, both α- and β-carotene contents decreased significantly in all samples. The phenolic profile analysis revealed the presence of rutin, gallic acid, vanillic acid, *p*-coumaric acid, ferulic acid, and cinnamic acid, with some phenolic compounds exhibiting significant declines after storage.

Cape gooseberry is a tropical fruit rich in bioactive compounds, including phenolic substances, vitamin C, and carotenoids, making it valuable for healthy diets. Turning cape gooseberry into fruit leather is an effective way to add value, increase demand, and encourage consumption of fruit-based products. This research showed that cape gooseberry leathers retain essential bioactive and functional nutrients and can preserve these qualities for an extended period without added preservatives. Furthermore, the use of hot air and sun drying, common fruit and vegetable drying methods in the food industry, proved suitable for producing this product.

REFERENCES

[1] Petkova, N.T., Popova, V.T., Ivanova, T.A., Mozova, N.N., Parayotov, N.D., Stoyanova, A. (2021). Nutritional composition of different cape gooseberry genotypes (*Physalis peruviana L.*)- a comporative study. *Food Research*, 5(4), 191-202.

[2] Rodrigues, E., Rockenbach, I.I., Cataneo, C., Gonzaga, L.V., Chaves E.S, Fett, R. (2009). Minerals and essential fatty acids of the exotic fruit *Physalis peruviana L. Ciencia e Tecnologia de Alimentos*, Campinas, 29(3), 642-645.

[3] Aamer, R. (2018). Evaluation of new non-traditional products processed from cape gooseberry (*Physalis peruviana L.*). *Egyptian Journal Agricultural Research*, 96(4), 1493-1511.

[4] Valdenegro M, Almonacid S, Henriquez C, Lutz M, Fuentes L., Simpson, R. (2013). The effects of drying processes on organoleptic characteristics and the health quality of food ingredients obtained from goldenberry fruits (*Physalis peruviana*). *Open Access Scientific Reports*, 2(2), 642.

[5] Kara, O. O., Karacabey, E., & Küçüköner, E. (2021). Altınçilek (Physalis peruviana L.) Meyvesinin Bazı Biyoaktif Özellikleri. *Akademik Gıda*, 19(2), 169-176.

[6] Raj, GVS.B., Dash, K.K. (2022). Development of hydrocolloids incorporated dragon fruit leather by conductive hydro drying: characterization and sensory evaluation. *Food Hydrocolloids for Health*, 2, 100086.

[7] Che Man, Y.B., Sin, K.K. (1997). Processing and consumer acceptance of fruit leather from the unfertilised floral parts of jackfruit. *Journal of The Science of Food and Agriculture*, 75, 102-108.

[8] Huang, X., Hsieh, F. (2005). Physical properties, sensory attributes and consumer preference of pear fruit leather. *Journal of Food Science*, 70(3), 177-186.

[9] Ruiz, N.A.Q., Demarchi, S.M., Massolo, J.F., Rodoni, L.M., Giner, S.A. (2012). Evaluation of quality during storage of apple leather. *Food Science and Technology*, 47, 485-492.

[10] Phimpharian C, Jangchud A, Jangchud K., Therdther N, Prinyawiwatkul, W., No, H.K. (2011). Physicochemical characteristics and sensory optimization of pineapple leather snack as affected by glucose syrup and pectin concentrations. *International Journal of Food Science and Technology*, 46, 972-981.

[11] Chen, Y., Martynenko, A. (2018). Combination of hydrothermodynamic (HTD) processing and different drying methods for natural blueberry leather. *Food Science and Technology*, 87, 470-477.

[12] Bonazzi, C., Dumoulin, E. (2011). Quality Changes in Food Materials as Influenced by Drying Processes. In Modern Drying Technology, 3: Product Quality and Formulation, First Edition. Edited by Tsotsas, E., Mujumdar, A.S., Wiley-VCH.

[13] Sablani, S.S., Rahman, M.S. (2008). Fundemantals of Food Dehydration. In Food Drying Science and Technology. Edited by Hui, Y.H., Clary, C., Farid, M.M., Fasina, O.O., Noomhorm, A., Welti-Chanes, J. DEStech Publiciations, Inc. 439 North Duke St., 1st Flor Lancaster, Pennsylvania 17601 U.S.A., 792 p.

[14] Cemeroğlu, B., (2010). Gıda Analizleri, 2. baskı. Gıda Teknolojisi Derneği Yayınları No: 34, 657 s, Ankara.

[15] Anonymous, (1992). *Pestil Standardı*, TS 9776/Ocak 1992, Necatibey Cad., No: 112,Bakanlıklar, Ankara.

[16] Singleton, V.L., Rossi, J.R. (1965). Colorimetry of total phenolics with phosphomolibdic-phosphothungstic acid. *American Journal of Enology and Viticulture*, 16, 144-158.

[17] Dorman, H.J.D., Peltoketo, A., Hiltunen, R., Tikkanen, M.J. (2003). Characterization of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs, *Food Chemistry*, 83(2), 255-262.

[18] Dere, Ş., Güneş, T., Sıvacı, R. (1997). Spectrophotometric determination of chlorophyll-A, B and total carotenoid contents of some algae species using different solvents. *Turkish Journal of Botany*, 22, 13-17.

[19] AOAC (2011). *Official methods of analysis*, 18th ed. (2005) Revision 4. Gaithersburg, MD.

[20] Tomas Barberan, F.A., Gil, M.I, Cremin, Waterhouse, A., Hess Pierce, B., Adel, K. (2001). HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches and plums. *Journal of Agricultural and Food Chemistry*, 49, 4748-4760.

[21] Olives Barba, A.I., Camara Hurtado, M., Sanchez Mata, M.C., Fernandez Ruiz, V., Lopez Saenz de Tejada, M. (2006). Application of a UV-vis detection-HPLC method for a rapid determination of lycopene and β-carotene in vegetables. *Food Chemistry*, 95, 328-336.

[22] Karkacier, M., Erbaş M, Uslu, M.K., Aksu, M. (2003). Comparison of different extraction and detection methods for sugars using amino-bonded phase HPLC. *Journal of Chromatographic Science*, 41, 331-333.

[23] Azeredo, H.M.C., de Brito, E.S., Farias, V., (2006). Effect of drying and storage time on the physico-chemical properties of mango leathers. *International Journal of Food Science & Technology*, 41(6), 635-638

[24] Fontana, A.J. (2007). Appendix D: Minimum Water Activity Limits for Growth of Microorganisms. In Water activity in foods: fundamentals and applications. Blackwell Publishing and the Institute of Food Technologists, 640 p.

[25] Che Man, Y.B., Jaswir, I., Yusof, S., Selamat, J., Sugisawa, H. (1997). Effect of different dryers and drying conditions on acceptability and physicochemical characteristics of durian leather. *Journal of Food Processing and Preservation*, 21, 425-441.

[26] Miranda, G., Berna, A. (2021). Evolution of colour in dried fruit storage: relation between colour and SO_2 content. *International Journal of Innovative Science and Research Technology*, 6(12), 682-697.

[27] Korese, J.K., Achaglinkame, M., Adzitey, F. (2022). Effect of different packaging materials on storage stability of *Gardenia erubescens* stapf. & Hutch. Dried fruits and powder. *Applied Food Research,* 2(2), 100143.

[28] Licodiedoff, S., Koslowski, L.A.D., Ribani, R.H. (2013). Flavonols and antioxidant activity of Physalis peruviana L. fruit at two maturity stages. *Acta Scientiarum. Technology*, 35(2), 393-399.

[29] Fischer, G., Lüdders, P. (1997). Developmental changes of carbohydrates in cape gooseberry (*Physalis peruviana L.*) fruits in relation to the calyx and the leaves. *Agronomia Colombiana*, XIV(2), 95-107.

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Research Paper / Araştırma Makalesi

Convective Drying Behavior and Mass Diffusivity Modeling of Persimmon Fruit Leather with Comparative Computer Methodologies

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ABSTRACT

An engineering perspective using computer-aided comparison were introduced in this research to investigate the effects of starch concentration, product thickness and drying temperature on the drying time, effective moisture diffusivity and drying kinetics of fifteen differently treated persimmon fruit leather samples. The best descriptive mathematical model of the drying behaviors of the leather products was found by testing the equations of Page, Midilli and others, Two-term and Logarithmic models. Fick's second law model was used to calculate the water diffusion coefficient and the diffusivity mechanism was modeled with feed forward backpropagation artificial neural network and response surface methodologies. The product thickness and drying temperature were the most effective operating parameters. The drying process for persimmon leather production took place in a falling rate period over a minimum of 120 minutes. The values of effective moisture diffusivity varied from $1.1^{\times}10^{-7}$ to $13.8^{\times}10^{-7}$ m^2/s. Semi-theoretical Midilli and others model presented a better fit depending on the statistical analyses. The artificial network produced a more powerful prediction than the response surface methodology with higher R-squared value (0.9846), lower mean absolute error (0.0567) and root mean square error (0.0715).

Keywords: Artificial neural network, Drying kinetics, Effective moisture diffusivity, Feed forward backpropagation, Persimmon fruit leather

Trabzon Hurması Pestilinin Konvektif Kuruma Davranışı ve Kütle Difüzivitesinin Karşılaştırmalı Bilgisayar Metodolojileri ile Modellenmesi

ÖZ

Bu araştırmada, nişasta konsantrasyonu, ürün kalınlığı ve kurutma sıcaklığının on beş farklı işlem görmüş Trabzon hurması pestil örneğinin kuruma süresi, etkin nem difüzivitesi ve kuruma kinetiği üzerindeki etkilerini araştırmak için bilgisayar destekli karşılaştırma kullanan bir mühendislik perspektifi sunulmuştur. Pestil ürünlerinin kuruma davranışlarının en iyi tanımlayıcı matematiksel modeli Page, Midilli ve diğerleri, İki-terimli ve Logaritmik modeller test edilerek bulunmuştur. Su difüzyon katsayısını hesaplamak için Fick'in ikinci yasa modeli kullanılmış ve difüzivite mekanizması ileri beslemeli geri yayılımlı yapay sinir ağı ve yanıt yüzeyi metodolojileri ile modellenmiştir. Ürün kalınlığı ve kurutma sıcaklığı en etkili çalışma parametreleri olmuştur. Trabzon hurması pestili üretimi için kurutma işlemi, minimum 120 dakika boyunca düşen bir hız periyodunda gerçekleşmiştir. Etkin nem difüzivitesi değerleri 1,1×10-7 ile 13,8×10-7 m^2/s arasında değişmiştir. Yarı teorik Midilli ve diğerleri modeli istatistiksel analizlere bağlı olarak daha iyi bir uyum göstermiştir. Yapay ağ, daha yüksek R-kare değeri (0,9846), daha düşük ortalama mutlak hata (0,0567) ve kök ortalama kare hatası (0,0715) ile yanıt yüzeyi metodolojisinden daha güçlü bir tahmin üretmiştir.

Anahtar Kelimeler: Yapay sinir ağı, Kurutma kinetiği, Etkin nem difüzivitesi, İleri beslemeli geri yayılım, Trabzon hurması meyve pestili

INTRODUCTION

The drying process is defined by the use of a heating source for moisture removal by which allows fruits to be consumed regardless of the time, season and environmental conditions. The amount of moisture determines the drying behaviors and the properties of the product being dried. Mass and heat transfers take place between the product and the drying system during the moisture removal. The mass diffusivity and activation energy changes in the material are calculated to observe the transfers. A mathematical description of the transfers is also carried out to reveal the drying kinetics using several models [1-3]. Mass diffusivity is the fundamental phenomenon of the drying behaviors and is so important that the drying time, the final product properties, the energy consumption and the efficiency of the drying system may be greatly affected [4]. Therefore, there should be an accurate explanation of the modeling construction used. Artificial neural networks (ANNs) and response surface methodology (RSM) have been used to express the drying behaviors of different materials such as fruits and fruit products [5-8]. Both methodologies yield good fittings; however, RSM has limitations on the modeling of non-linear processes and engineering applications, although it is one of the most popular, powerful and widely used methods for the design of experiments, modeling and optimization [8, 9]. ANN is derived from the human brain system and is very good at predicting the response output(s) using transfer and training functions with a topology map that includes nodes, layers and connections. Choosing an appropriate network is important to set up an accurate relationship between input and output variables that is simple to understand. Feed forward back-propagation (FF-BP) is a commonly used network in artificial neural systems. It uses a multi-layer structure consisting of inputs (process variables), hidden neurons and output layers, so that it forms many ways to reach the most fitting result for the output [9, 10].

It is important to determine the factors affecting the drying kinetics, and accordingly, to realize the appropriate drying model for the dried fruit products such as fruit leathers. The healthy fruit leather, soft food snacks, are produced by drying fruit puree, concentrate or juice [11-13]. It has been reported that the most effective parameters in the drying kinetics of fruit and fruit products are temperature and thickness −by taking into account the properties of the drying system used− whereas air velocity and relative humidity are fewer effective ones [3]. It is known that the effect of heat on drying time of fruit leather is the most fundamental [12]. Additionally, it has been shown that the effect of the water holding capacities of the gelling or thickening agents (vegetable oil, starch, pectin, and other hydrocolloids) used during the fruit leather production on the moisture content of the final product is substantial and the physical properties are significantly affected [11].

The Asian fruit persimmon (*Diospyros kaki* L.) is widely consumed in Mediterranean nations, mostly in its fresh form during a brief but distinct season (autumn) [14-16]. Several studies into the drying characteristics of persimmon fruit in various forms such as whole or in slices have been conducted [15-22]. For persimmon fruit leather as a novel product, no drying kinetic study has been done, though.

Given the foregoing information, the manufacturing of persimmon leather was carried out in an experimental design matrix, with the parameters of thickness, temperature, and starch content being examined. At the beginning and completion of the drying process, the moisture content of the persimmon leather was measured. Moisture ratios that were determined at any point throughout the drying process were used to establish the drying kinetics. A total of four drying curve models were employed in the study since it is impossible to determine a general drying equation because the models can differ based on *(i)fruit features, (ii)drying conditions, (iii)drying techniques, and (iv)fruit leathering processes*. The semi-theoretical models of Page, Midilli and others, Two-term and Logarithmic were selected because they fit the drying behaviors of fruits well and are commonly employed for natural products [3, 6].

A comparative analysis of the models' coefficients was conducted using statistical methods to determine which the best is. The moisture weight loss during drying was used to calculate the mass diffusivity and drying rate. The mass diffusivity was modeled using the RSM and FF-BPANN approaches, and their prediction accuracy was statistically evaluated.

MATERIALS and METHODS

Experimental Design

The aim of generating this experimental design was to produce different characterizations of persimmon leather products; to investigate the effect of the production variables on drying time and moisture diffusion; and to model the drying kinetics for each product. The experimental study was designed using the independent variables of starch concentration, product thickness, and drying temperature using a Box-Behnken Design (BBD) in Design Expert Version 7.1.6 (Minneapolis, USA). Fifteen leather products (three in the center point) were prepared according to the starch concentration and product thickness and then dried at the discussed drying temperature levels.

Preparation of Fruit Leather

Persimmon fruit was purchased from a local market in Gaziantep, Turkey. After the fruit had been cleaned, peeled, and shredded, a hand blender was used to homogenize it. A 1:2 (w/v) starch solution made of commercial wheat starch and distilled water was combined with 100 g of homogenized fruit. Following thorough mixing, the mixture was heated using a magnetic stirrer until the final brix value was 30.0±1.0. The cooked material was spread on a clean fabric by

using circular shaped molding apparatus of a certain thickness. The fabric with the sample was placed in tray drier (UOP 8 Tray Drier, Armfield, UK) with the velocity of hot air maintained constant (0.5±0.05 m/s) for all drying temperature levels [23]. A balance installed on the tray drier was used to measure the material's weight reduction every 20 minutes. As a result, the products' free moisture content could be determined at any moment. When the difference between the two most recent weight reductions was measured to be less than one gram, the drying process was halted, and the drying duration for each persimmon leather product was established [19]. The ultimate moisture content of the products was determined using the oven method [24].

Mathematical Modeling of Drying Kinetics and Statistical Analysis

The weight reduction of the leather products was measured every twenty minutes so that the remaining moisture content could be calculated in the products. The moisture ratio (*MR*) of each product was calculated by Equation 1 [25]. The equilibrium moisture content was determined as the moisture content calculated at the end of drying time.

$$MR = \frac{(M - M_e)}{(M_o - M_e)} \quad (1)$$

Where the definitions are *MR,* moisture ratio; *M,* moisture content of the product at any time; M_o, the initial moisture content of the product; M_e, the equilibrium moisture content of the product.

Four drying curve models were selected and performed for each experimental run based on *MR* values. The equations of the models are given below for Page (Equation 2) [18], Two-term (Equation 3) [26], Logarithmic (Equation 4) [27], and Midilli and others (Equation 5) [30]. The models were fitted to the experimental data by SigmaPlot software (Version 11, Erkrath, Germany).

Page Equation: $MR = \exp\left(-k_0 t^n\right)$ (2)

Two-term Equation: $MR = a\exp\left(-k_0 t\right) + b\exp\left(-k_1 t\right)$ (3)

Logarithmic Equation: $MR = a\exp\left(-k_0 t\right) + c$ (4)

Midilli and others Equation: $MR = a\exp\left(-k_0 t^n\right) + bt$ (5)

Statistical evaluation of the predicted data for goodness of fitting was performed by root mean square error (*RMSE*; Equation 6), chi-square (χ^2 ; Equation 7), coefficient of determination (R^2; obtained from the software) and the correlation coefficient (*CC*; Equation 8) as the primary criteria for the best model selection [26]. The goodness of fit of the experimental and predicted data was revealed by high R^2 and *CC* values, and by low chi-square and *RMSE* values. Consequently, the best drying curve model was determined.

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(MR_{pre,i} - MR_{\exp,i})^2} \quad (6)$$

$$\chi^2 = \frac{\sum_{i=t}^{N}(MR_{\exp,i} - MR_{pre,i})^2}{N - z} \quad (7)$$

$$CC = \frac{\sum_{i=1}^{N}((MR_{\exp,i} - \overline{MR_{\exp,i}}) \times (MR_{pre,i} - \overline{MR_{pre,i}}))}{\sqrt{\sum_{i=1}^{N}(MR_{\exp,i} - \overline{MR_{\exp,i}})^2} \times \sqrt{\sum_{i=1}^{N}(MR_{pre,i} - \overline{MR_{pre,i}})^2}} \quad (8)$$

Where the definitions are *N,* number of data; $MR_{pre,i}$, predicted model value; $MR_{exp,i}$, experimental value; $\overline{MR_{pre,i}}$, mean of predicted model value; $\overline{MR_{\exp,i}}$, mean of experimental value; and *z* number of constants in the Equations of 6, 7 and 8.

Experimental free moisture content values of the products at any drying time were predicted using the best drying curve model equation which was discussed at the drying curve stage. This predicted data were utilized to obtain the rates of the drying curve for each run in Equation 9 [29] where *R* is the drying rate (kg water/h× m), *Ls* dry solid (kg), *A* heat exposed area (m^2), *dx/dt* (kg water/kg dry solid×h) water loss at *t* time. Falling and constant rate periods of the drying procedure were determined by plotting the rates versus the predicted free moisture content values.

$$R = -\frac{Ls}{A}\frac{dx}{dt} \quad (9)$$

Effective Mass Diffusivity Calculation and Modeling

The effective mass diffusivity coefficient of the leather products during the drying period was estimated by using Fick's second law of diffusion [30]. The leather products were assumed to be in slab form. According to the diffusion law, the drying behavior of the products can be described in Equation 10 where *MR'* is the derived moisture ratio from the best drying curve model equation; *n,* the number of terms of the series; $D_{eff,}$ the effective diffusivity coefficient (m^2/s); *t,* drying time and *L,* the half thickness of the leather product. Since the drying of leather products takes a long time, Equation 10 can be simplified to Equation 11 using only the first term of the series [31].

$$MR' = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left(-\frac{(2n+1)D_{eff}\pi^2 t}{4L^2}\right) \quad (10)$$

$$In(MR') = In(\frac{8}{\pi^2}) - (\frac{D_{eff}\pi^2 t}{4L^2}) \quad (11)$$

The derived D_{eff} values were predicted by the equations for the response surface and artificial neural network methodologies. A second order polynomial equation (Equation 12) of the RSM (Design Expert Version 7.1.6, Minneapolis, USA) shows that y is the response, β_0 is constant, β_j, β_{ij} and β_{jj} are the regression coefficients, and x_j and x_i are the levels of the independent variables. FF-BPANN (MATLAB Version 7.10, USA) was trained under supervision with the Levenberg-Marquardt (LM) algorithm due to its speed and lower training requirement. Three input layers were transferred by the tan-sing function with 1 hidden layer, 10 neurons (nodes) to 1 output layer. The prediction competencies of the methodologies were compared by *RMSE,* mean absolute error (*MAE*; Equation 13) and R^2. The coefficient of determination values for RSM and FF-BPANN were computed with the regression tool of the MS Excel program.

$$y = \beta_0 + \sum_{j=1}^{k}\beta_j x_j + \sum_{j=1}^{k}\beta_{jj}x^2{}_j + \sum_{i=1}^{j-1}\sum_{j=2}^{k}\beta_{ij}x_i x_j \quad (12)$$

$$MAE = \frac{1}{N}\sum_{i=1}^{N}(MR_{pre,i} - MR_{\exp,i}) \quad (13)$$

RESULTS and DISCUSSION

The drying process provides a long shelf life for fruits by reducing water content. The mathematical modeling approach is used for drying kinetics based on the efficiency and calculations of water removal to control and consider an accurate design of the drying process. Dried fruits are exposed to changing conditions and nutritional loss over a period of time. To avoid such problems and operate the drying successfully, the process parameters needed to be modeled and optimized [3, 32].

The time and moisture diffusivity, which are important factors in drying [3], were discussed for the differently treated persimmon leathers within the scope of the BBD matrix depending on the moisture ratio changes (Table 1). The graphics (Figure 1) of MR and time show the effects of the production parameters on the drying time. The drying time extends when the leather thickness increases at any concentration of starch and drying temperature, as seen in Figure 1a and 1b. As Figures 1a and 1c demonstrate, drying times are shorter as temperatures rise. This is a trend that is anticipated. The effect of the starch concentration on the drying time is related to the temperature value because the gelatinization characteristics of the starch hydrocolloid are affected by its type, temperature and water content [33]. The minimum leather production period is 120 minutes, whereas the maximum one is 360 minutes, which shows that drying thickness and temperature are the most effective process parameters for persimmon leather.

Demiray and Tülek [17] applied pretreatments of blanching and osmotic dehydration to whole persimmon fruit. They dried the fruit with a cabinet dryer at 55, 65 and 75°C temperatures and 0.2 m/s air velocity. Specific moisture content (35%) was reached at 75°C 27 h and 22 h respectively for blanched and osmo-dehydrated fruit. Tontul and Topuz [34] produced pomegranate fruit leather at constant product thickness (4 mm) and concentration of wheat starch (10%). They indicated that increasing drying temperature and presence of hydrocolloid caused decreasing drying time. Yılmaz et al. [35] produced pomegranate fruit leather at three leather thickness (1, 2, 3 mm) and drying temperature degrees (50, 60, 70°C), and constant wheat starch concentration (5%). They indicated that the drying conditions had significant effects on drying time and effective mass diffusivity of the leather samples. When the thickness of pomegranate fruit leather increased, drying time significantly decreased. The increasing drying temperature resulted in reverse effect on drying time. The effective mass diffusivity was also significantly affected by the parameters. Increasing levels of the parameters cause a faster moisture removal from the leather samples. They concluded that optimization of drying conditions provides an enhancement for the process efficiency. Azeredo et al. [12] found the same effects for drying temperature and puree load (puree mass per dish area) on the drying time to produce mango fruit leather. Although they designated the drying time as a parameter for leather production, they stated that it could not be minimized, but the minimum temperature could be determined to be the optimum result for mango leather.

Developing a new food product requires design in terms of process tools, operational parameters and outputs. Modeling and optimization achieves the goals of reduction in time and cost, operational minimization, a well-controlled system, statistical evaluation and reporting of the outputs and an understanding of the relationship between the materials to be processed and the system to be used [36]. The phenomenon of water loss from a product during a conventional drying process depends on external and internal conditions, which can cause a challenge for drying of fruits. Therefore, mathematical modeling is a good way of attending to the fruit drying. Additionally, validation of models used is important and it should be considered statistically in terms of both accuracy and precision [37]. This study proposed that drying kinetics for fruits would be described, evaluated and compared by modeling. Page, Midilli and others, Two-term and Logarithmic models are those that provide good predictions and the right perspectives for various fruit drying processes [3]. The equations for these semi-theoretical models (Table 1) were tested to determine the best drying curve model for the mathematical description of persimmon leather products. Initial constant values and coefficients of the models for each product run are presented in Table 2. The Midilli and others model was specified as the best model by its low RMSE and χ^2 values and high R^2 and CC values (Table 3).

Table 1. The design matrix showing the results of drying time and experimental and predicted effective mass diffusivity values

Runs	Starch concentration (%)	Drying thickness (mm)	Drying temperature (°C)	Time (min)	D_{eff} ($m^2/s \times 10^{-7}$)		
					Derived	RSM	FF-BPANN
1	7	3	50	240	8.2932	8.1500	8.2105
2	5	1	60	140	2.2709	2.4250	2.2845
3	3	1	50	120	1.6347	1.7780	1.8158
4	5	3	40	320	5.6617	5.5070	6.4660
5	5	2	50	180	5.6422	5.3770	5.4756
6	5	2	50	180	5.0819	5.3770	5.4756
7	3	2	40	260	3.6471	2.9890	2.4804
8	5	2	50	180	5.4061	5.3770	5.4756
9	3	3	50	220	8.4249	9.2370	8.3644
10	3	2	60	120	9.9218	9.6250	9.9041
11	7	1	50	140	1.6733	8.6140	1.9997
12	7	2	40	160	4.0344	4.3310	3.9469
13	7	2	60	160	5.6220	6.2800	5.6403
14	5	1	40	220	1.1008	1.6160	1.4148
15	5	3	60	180	13.7963	13.2800	13.3260
					RMSE	0.0759	0.0715
			Statistical sources		MAE	0.0635	0.0567
					R^2	0.9815	0.9846

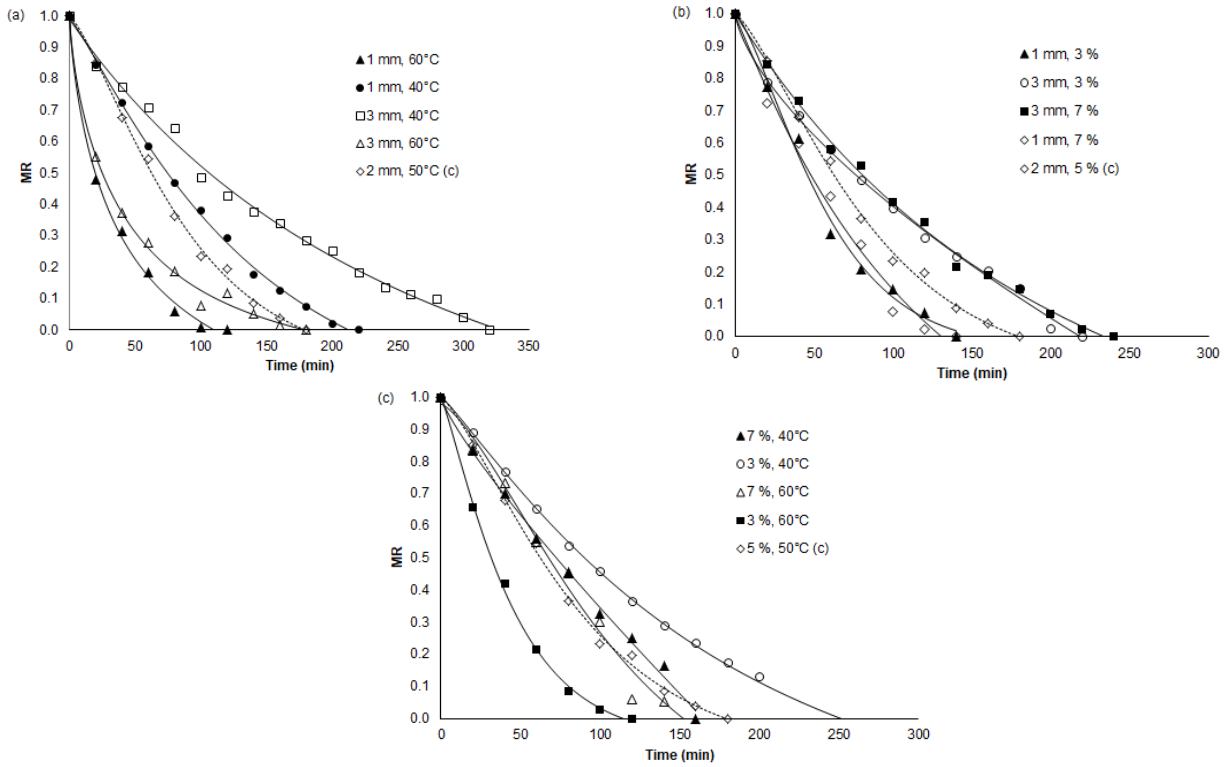

Figure 1. Experimental and estimated (lines) MR values vs time, (a): at constant starch concentration, (b): at constant drying temperature, (c): at constant drying thickness, (c): center point depiction

The model suggested by Hii et al. [38] was selected as the best model for sliced and blanched persimmon fruit drying among twelve thin-layer drying kinetic models in the study of Hanif et al. [19]. Demiray and Tülek [17] determined Page [39] and Modified Page [40] models as the best models for the drying kinetics of whole persimmon fruit. Sampaio et al. [21] and Doymaz [41] reported that Midilli and others model presented the best fitted modeling for drying process of persimmon fruit.

It is known that fruits and vegetables mostly dry in the falling rate period [41, 42, 43]. The leather of persimmon fruit also dried in the falling rate period as seen in Figure 2. Except for Runs 9 and 12, their drying process took place in both the first and second falling rate periods; the others reached their equilibrium moisture content in the first falling-rate period. Doymaz [18] observed the drying process of persimmon fruit occurring in the falling rate period.

Table 2. Equation constants of the drying curve models for each run

Model	Coefficients	Values	Run1	Run 2	Run3	Run4	Run5	Run6	Run7	Run8	Run9	Run10	Run11	Run12	Run13	Run14	Run15
Page	k_0	10^{-3}	0.0025	0.0393	0.0031	0.0026	0.0016	0.001	0.0014	0.0042	0.0048	0.0079	0.0031	0.0021	0.0005	0.0019	0.0464
	n	1	1.2926	0.9466	1.4179	1.2049	1.4735	1.5714	1.3807	1.2672	1.1597	1.2974	1.4062	1.3796	1.7182	1.3772	0.832
Two-term	a	0.5	0.5244	0.4952	0.5276	0.5166	0.5377	0.5482	0.537	0.5231	0.5077	0.5144	0.5201	0.5256	0.5416	0.5313	0.8163
	b	0.5	0.5244	0.4952	0.5276	0.5166	0.5377	0.5482	0.537	0.5231	0.5077	0.5144	0.5201	0.5256	0.5416	0.5313	0.1837
	k_0	10^{-3}	0.0103	0.0319	0.0186	0.0075	0.0141	0.0139	0.0096	0.0145	0.0104	0.0257	0.0179	0.012	0.0138	0.0115	0.0193
	k_1	10^{-3}	0.0103	0.0319	0.0186	0.0075	0.0141	0.0139	0.0096	0.0145	0.0104	0.0257	0.0179	0.012	0.0138	0.0115	16.4741
Logarithmic	a	1	1.365	1.0111	1.2729	1.2665	1.409	1.4593	1.434	1.261	1.3621	1.1476	1.4941	2.4706	2.5811	1.3954	0.9564
	c	-0.1	-0.3704	-0.0264	-0.2504	-0.2822	-0.3817	-0.4121	-0.4198	-0.2554	-0.3982	-0.1395	-0.5022	-1.4827	-1.5671	-0.3866	0.0183
	k_0	10^{-3},k>0	0.0056	0.0296	0.0118	0.0045	0.0076	0.0074	0.0049	0.009	0.0054	0.019	0.0084	0.0031	0.0033	0.0061	0.0243
Midilli & others	a	0.9	0.9964	0.9991	0.9962	0.9901	0.9971	1.0057	1.0018	1.0041	0.9993	0.9972	0.9886	1.0012	0.9779	0.9934	0.9997
	b	-10^{-6}	-0.0008	-0.0009	-0.0002	-0.0005	-0.0004	-0.0002	-0.0006	-0.0008	-0.0019	-0.0004	-0.0016	-0.0039	-0.0011	-0.0006	-0.0003
	k_0	10^{-3}	0.0068	0.0748	0.0037	0.0061	0.0026	0.0014	0.0037	0.0093	0.0259	0.0109	0.0089	0.0122	0.001	0.0041	0.0683
	n	1,n>0	1.0059	0.7326	1.3624	0.975	1.3346	1.4747	1.125	1.0376	0.6499	1.1905	1.0626	0.6995	1.4914	1.1576	0.7137

*Initial values of the constants

Table 3. Statistical evaluation results of the fitted drying curve models

Model	Statistics	Run1	Run 2	Run3	Run4	Run5	Run6	Run7	Run8	Run9	Run10	Run11	Run12	Run13	Run14	Run15
Page	RMSE	0.0347	0.0302	0.0302	0.0320	0.0226	0.0157	0.0287	0.0286	0.0424	0.0189	0.0528	0.0424	0.0520	0.0282	0.0246
	CC	0.9946	0.9976	0.9960	0.9943	0.9979	0.9990	0.9962	0.9962	0.9902	0.9987	0.9914	0.9908	0.9909	0.9965	0.9972
	χ^2	0.0014	0.0013	0.0012	0.0012	0.0006	0.0003	0.0010	0.0010	0.0022	0.0005	0.0037	0.0023	0.0035	0.0010	0.0008
	R^2	0.9877	0.9917	0.9921	0.9882	0.9955	0.9979	0.9919	0.9921	0.9797	0.9970	0.9758	0.9812	0.9773	0.9924	0.9931
Two-term	RMSE	0.0513	0.0308	0.0586	0.0424	0.0603	0.0664	0.0543	0.0449	0.0482	0.0414	0.0733	0.0626	0.0942	0.0545	0.0216
	CC	0.9894	0.9971	0.9865	0.9906	0.9858	0.9834	0.9874	0.9916	0.9879	0.9943	0.9844	0.9807	0.9679	0.9877	0.9977
	χ^2	0.0038	0.0022	0.0069	0.0024	0.0061	0.0074	0.0041	0.0034	0.0035	0.0040	0.0107	0.0071	0.0160	0.0045	0.0008
	R^2	0.9731	0.9913	0.9701	0.9793	0.9676	0.9624	0.9711	0.9805	0.9738	0.9856	0.9534	0.9589	0.9256	0.9715	0.9946
Logarithmic	RMSE	0.0180	0.0290	0.0372	0.0210	0.0248	0.0322	0.0127	0.0155	0.0235	0.0171	0.0364	0.0191	0.0436	0.0138	0.0322
	CC	0.9983	0.9967	0.9940	0.9975	0.9973	0.9956	0.9992	0.9988	0.9969	0.9988	0.9942	0.9981	0.9926	0.9991	0.9947
	χ^2	0.0004	0.0015	0.0022	0.0005	0.0009	0.0015	0.0002	0.0003	0.0007	0.0005	0.0021	0.0005	0.0028	0.0003	0.0015
	R^2	0.9967	0.9924	0.9880	0.9949	0.9945	0.9912	0.9984	0.9977	0.9938	0.9976	0.9885	0.9962	0.9841	0.9982	0.9881
Midilli & others	RMSE	0.0182	0.0185	0.0294	0.0208	0.0157	0.0128	0.0114	0.0152	0.0155	0.0123	0.0370	0.0168	0.0396	0.0121	0.0195
	CC	0.9983	0.9984	0.9962	0.9975	0.9989	0.9993	0.9994	0.9989	0.9986	0.9994	0.9940	0.9985	0.9941	0.9993	0.9979
	χ^2	0.0005	0.0008	0.0017	0.0006	0.0004	0.0003	0.0002	0.0004	0.0004	0.0004	0.0027	0.0005	0.0028	0.0002	0.0006
	R^2	0.9966	0.9969	0.9925	0.9950	0.9978	0.9986	0.9987	0.9978	0.9973	0.9987	0.9881	0.9971	0.9868	0.9986	0.9957

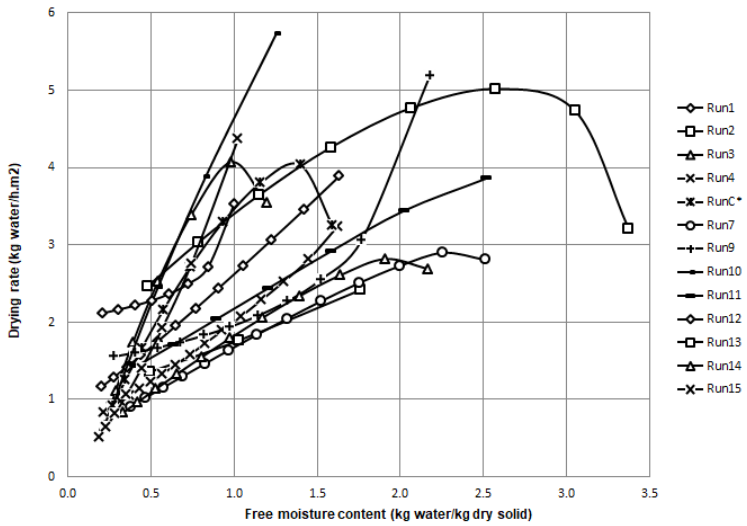

Figure 2. Drying rate curves of persimmon leather at different process conditions. Run C* depicts the drying rate curve of the mean values of the center points runs, Run 5, Run 6, and Run 8

The drying process is controlled by the moisture diffusion mechanism [3]. Hence, its accurate portrayal is important. The effective moisture diffusivity of food products is in the range from 10^{-8} to 10^{-12} m^2/s [2, 17]. We calculated D_{eff} to be from 1.1 to 13.8×10^{-7} m^2/s for the persimmon leather products. The highest D_{eff} value (Run 15) was obtained at a maximum temperature of 60°C and a thickness of 3 mm whereas the minimum temperature of 40°C and thickness of 1 mm gave the lowest value (Run 14). It is understood that the moisture removal is fundamentally affected by the drying temperature and leather thickness. Cárcel et al. [16], Doymaz [18] and Demiray and Tülek [17] obtained D_{eff} values varying from 0.7 to 10×10^{-10} m^2/s for hot air drying of persimmon fruit in different drying conditions. Doymaz [18] and Demiray and Tülek [17], respectively worked with 50 to 70°C and 55 to 75°C temperature intervals, and indicated that the drying temperature increased the D_{eff} values. Lower D_{eff} values were reported for tomato slices (5.11×10^{-6} m^2/s) and date palm fruit (4.73×10^{-6} m^2/s) by Onwude et al. [3].

There are many fruit drying studies where the fundamental point is to explain the moisture removal mechanism by modeling methods [5, 6, 8, 30, 31, 44, 45, 46, 47, 48, 49]. The effective moisture diffusivity was modeled in this study. Statistical comparison of RSM and FF-BPANN methods are presented in Table 1. The topology of FF-BPANN (3-1-10-1) predicted more accurate effective moisture diffusivity values. In other words, it revealed better data fitting and estimation capability since ANN methodology has better abilities of modeling of non-linear processes and adapting to the changes of the process. These properties make ANN powerful and flexible. RSM tries to explain the changes of the process by second order polynomial equation, which creates an important limitation for its representation [36]. However, there is so little difference that both methods can be considered successful. Data distribution on the regression line in Figure 3 showed that FF-BPANN provided better prediction of D_{eff} values.

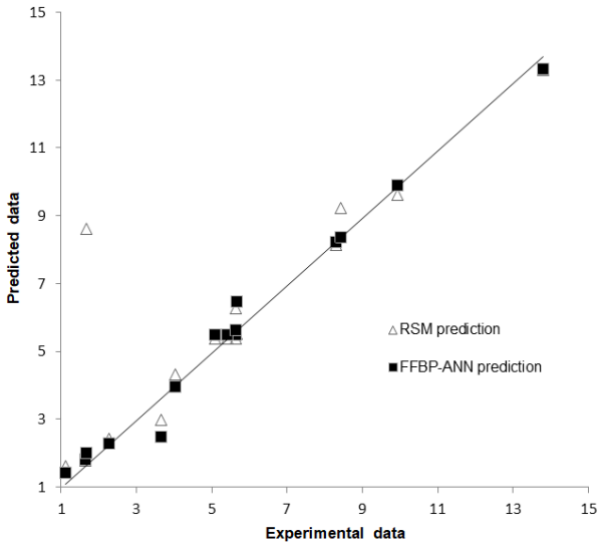

Figure 3. Visual demonstration of the prediction competence of the modeling methods for D_{eff}

CONCLUSION

The drying models based on the experimental studies allows fewer assumptions to be made about the drying processes, which results in more accurate and feasible applications for designers and engineers. In this respect, it is important to study the drying kinetics of persimmon fruit leather as a novel product. The drying time and moisture removal mechanisms were most affected by the leather thickness and drying temperature parameters. The drying rate curves showed that the drying process of the leather products took place during the falling rate period. The fitting quality of Midilli and others model was statistically shown to be the best fit for the persimmon leather drying kinetics. The effective moisture diffusivity (D_{eff}) values for different persimmon leather products ranged from 1.1×10^{-7} to 13.8×10^{-7} m^2/s, and were successfully modeled by RSM and ANN methods. The artificial neural methodology produced slightly better modeling results compared to RSM.

REFERENCES

[1] Azeez, L., Adebisi, S.A., Oyedeji, A.O., Adetoro, R.O., Tijani, K.O. (2017). Bioactive compounds' contents, drying kinetics and mathematical modeling of tomato slices influenced by drying temperatures and time. *Journal of the Saudi Society of Agricultural Sciences,* 18(2), 120−126.

[2] Mahjoorian, A., Mokhtarian, M., Fayyaz, N., Rahmati, F., Sayyadi, S., Ariaii, P. (2017). Modeling of drying kiwi slices and its sensory evaluation. *Food Science and Nutrition,* 5(3), 466−473.

[3] Onwude, D., Hashim, N., Janius, R.B., Nawi, N.M., Abdan, K. (2016). Modeling the thin-layer drying of fruits and vegetables: A review. *Comprehensive Reviews in Food Science and Food Safety,* 15, 599−618.

[4] Zhang, M., Hao, J., Lim, R. (2010). Recent developments in microwave-assisted drying of vegetables, fruits, and aquatic products−Drying kinetics and quality considerations. *Drying Technology,* 28, 1307−1316.

[5] Erentürk, S., Erentürk, K. (2007). Comparison of genetic algorithm and neural network approaches for the drying process of carrot. *Journal of Food Engineering,* 78, 905−912.

[6] Movagharnejad, K., Nikzad, M. (2007). Modeling of tomato drying using artificial neural network. *Computers and Electronics in Agriculture,* 59, 78−85.

[7] Nazghelichi, T., Kianmehr, M.H., Aghbashlo, M. (2011). Prediction of carrot cubes drying kinetics during fluidized bed drying by artificial neural network. *Journal of Food Science and Technology,* 48(5), 542−550.

[8] Youssefi, S., Emam-Djomeh, Z., Mousavi, S.M. (2009). Comparison of artificial neural network (ANN) and response surface methodology (RSM) in the prediction of quality parameters of spray-dried pomegranate juice. *Drying Technology,* 27, 910−917.

[9] Witek-Krowiak, A., Chojnacka, K., Podstawczyk, D., Dawiec, A., Pokomeda, K. (2014). Application of response surface methodology and artificial neural network methods in modeling and optimization of biosorption process. *Bioresource Technology,* 160, 150−160.

[10] Kariyama, I.D. (2014). Temperature-based feed-forward backpropagation artificial neural network for estimating reference crop evapotranspiration in the upper west region. *International Journal of Science and Technology Research,* 3(8), 357−364.

[11] Al-Hinai, K.Z., Guizani, N., Singh, V., Rahman, M.S., Al-Subhi, L. (2013). Instrumental texture profile analysis of date-tamarind fruit leather with different types of hydrocolloids. *Food Science and Technology Research,* 19(4), 531−538.

[12] Azeredo, H.M.C., Brito, E.S., Moreira, G.E.G., Farias, V.L., Bruno, L.M. (2006). Effect of drying and storage time on the physico-chemical properties of mango leathers. *International Journal of Food Science and Technology,* 41, 635−638.

[13] Huang, X., Hsieh, F.H. (2005). Physical properties, sensory attributes, and consumer preference of pear fruit leather. *Journal of Food Science,* 70(3), 177−186.

[14] Baltacıoğlu, H., Artık, N. (2013). Study of postharvest changes in the chemical composition of persimmon by HPLC. *Turkish Journal of Agriculture and Forestry,* 37, 568−574.

[15] Bölek, S., Obuz, E. (2014). Quality characteristics of Trabzon persimmon dried at several temperatures and pretreated by different methods. *Turkish Journal of Agriculture and Forestry,* 38, 242−249.

[16] Cárcel, J.A., García-Pérez, J.V., Riera, E., Mulet, A. (2007). Influence of high-intensity ultrasound on drying kinetics of persimmon. *Drying Technology,* 25, 185−193.

[17] Demiray, E., Tülek, Y. (2017). The effect of pretreatments on air drying characteristics of persimmons*. Heat and Mass Transfer,* 53, 99−106.

[18] Doymaz, İ. (2012). Evaluation of some thin-layer drying models of persimmon slices (*Diospyros kaki* L.). *Energy Conversion and Management,* 56, 199−205.

[19] Hanif, M., Khattak, M.K., Rahma, M., Khan, M.U. (2015). Evaluation of existing thin layer drying models on drying kinetics of persimmon (*Diospyros kaki* L.). *Journal of Agricultural Research,* 53(2), 269−275.

[20] Khaled, A.Y., Kabutey, A., Selvi, K.Ç., Mizera, Č., Hrabe, P., Herák, D. (2020). Application of computational intelligence in describing the drying kinetics of persimmon fruit (*Diospyros kaki*) during vacuum and hot air drying process. *Processes*, 8(5), 544.

[21] Sampaio, R.M., Neto, J.P.M., Perez, V.H., Marcos, S.K., Boizan, M.A., Silva, L.R. (2016). Mathematical modeling of drying kinetics of persimmon fruits (*Diospyros kaki* cv. fuyu). *Journal of Food Processing and Preservation,* 41(1), 1−7.

[22] Zhao, C., Ameer, K., Eun, J. (2021). Effects of various drying conditions and methods on drying kinetics and retention of bioactive compounds in sliced persimmon. *LWT-Food Science and Technology,* 143, 111149.

[23] Dursun, D., Dalgıç, A.C. (2018). Production and preference mapping of persimmon fruit leather: An optimization study by Box–Behnken. *Journal of Food Process Engineering,* 41(8), e12899.
[24] AOAC (1990). Official Methods of Analysis. Association of Analytical Communities, Arlington, VA.
[25] Cakmak, H., Kumcuoglu, S., Tavman, Ş. (2013). Thin layer drying of bay leaves (*Laurus nobilis* L.) in conventional and microwave oven. *Akademik Gıda,* 11(1), 20-26.
[26] Akpınar, E.K. (2006). Determination of suitable thin layer drying curve model for some vegetables and fruits. *Journal of Food Engineering,* 73, 75−84.
[27] Toğrul, İ.T., Pehlivan, D. (2003). Modeling of drying kinetics of single apricot. *Journal of Food Engineering,* 58, 23−32.
[28] Midilli, A., Küçük, H., Yapar, Z.A. (2002). A new model for single-layer drying. *Drying Technology,* 20, 1503−1513.
[29] Geankoplis, C.J. (1993). Transport Processes and Unit Operations. Prentice-Hall International Inc., New Jersey.
[30] Ju, H., Law, C., Fang, X., Xiao, H., Liu, Y., Gao, Z. (2016). Drying kinetics and evolution of the sample's core temperature and moisture distribution of yam slices (*Dioscorea alata* L.) during convective hot-air drying. *Drying Technology,* 34(11), 1297−1306.
[31] Simpson, R., Ramírez, C., Birchmeier, V., Almonacid, A., Moreno, J., Nuñez, H., Jaques, A. (2015). Diffusion mechanisms during the osmotic dehydration of Granny Smith apples subjected to a moderate electric field. *Journal of Food Engineering,* 166, 204−211.
[32] Saavedra, J., Cordova, A., Navarro, R., Díaz-Calderon, P., Fuentealba, C., Astudillo-Castro, C., Toledo, L., Enrione, J., Galvez, L. (2017). Industrial avocado waste: Functional compounds preservation by convective drying process. *Journal of Food Engineering,* 198, 81−90.
[33] Sullivan, J.W., Johnson, J.A. (1964). Measurements of starch gelatinization by enzyme susceptibility. *Cereal Chemistry,* 41, 73−79.
[34] Tontul, İ. and Topuz, A. (2017). Effects of different drying methods on the physicochemical properties of pomegranate leather (pestil). *LWT - Food Science and Technology*, 80, 294−303.
[35] Yılmaz, F.M., Yüksekkaya, S., Vardin, H., Karaaslan, M. (2017). The effects of drying conditions on moisture transfer and quality of pomegranate fruit leather (pestil). *Journal of the Saudi Society of Agricultural Sciences,* 16, 33−40.
[36] Baş, D., Boyacı, I.H. (2007). Modeling and optimization I: Usability of response surface methodology. Journal of *Food Engineering,* 78, 836−845.
[37] Castro, A.M., Mayorga, E.Y., Moreno, F.L. (2018). Mathematical modeling of convective drying of fruits: A review. *Journal of Food Engineering,* 223, 152−167.
[38] Hii, C.L., Law, C.L., Clocke, M. (2008). Modeling of thin layer drying kinetics of cocoa beans during artificial and natural drying. *Journal of Engineering Science and Technology,* 3(2), 1−10.
[39] Roberts, J.S., Kidd, D.R., Padilla-Zakour, O. (2008). Drying kinetics of grape seeds. *Journal of Food Engineering,* 89(4), 460−465.
[40] Toğrul, H. (2006). Suitable drying model for infrared drying of carrot. *Journal of Food Engineering,* 77(3), 610−619.
[41] Darıcı, S., Şen, S. (2015). Experimental investigation of convective drying kinetics of kiwi under different conditions. *Heat and Mass Transfer,* 51(8), 1167−1176.
[42] Darvishi, H., Khoshtaghaza, M.H., Minaei, S. (2014). Drying kinetics and color change of lemon slices. *International Agrophysics,* 28(1), 1−6.
[43] Rabha, K., Muthukumar, P., Somayaji, C. (2017). Experimental investigation of thin layer drying kinetics of ghost chilli pepper (*Capsicum Chinense* Jacq.) dried in a forced convection solar tunnel dryer. *Renewable Energy,* 105, 583−589.
[44] Bala, B.K., Ashraf, M.A., Uddin, M.A., Janjai, S. (2005). Experimental and neural network prediction of the performance of a solar tunnel drier for drying jackfruit bulbs and leather. *Journal of Food Process Engineering*, 28, 552−566.
[45] Doymaz, İ., Karasu, S., Başlar, M. (2016). Effects of infrared heating on drying kinetics, antioxidant activity, phenolic content, and color of jujube fruit. *Journal of Food Measurement and Characterization,* 10(2), 283−291.
[46] Fathi, M., Mohebbi, M., Razavi, S.M.A. (2011). Application of image analysis and artificial neural network to predict mass transfer kinetics and color changes of osmotically dehydrated kiwifruit. *Food and Bioprocess Technology,* 4(8), 1357−1366.
[47] Guiné, R.P.F., Cruz, A.C., Mendes, M. (2014). Convective drying of apples: kinetic study, evaluation of mass transfer properties and data analysis using artificial neural networks. *International Journal of Food Engineering,* 10(2), 281−299.
[48] Maran, J.P., Sivakumar, V., Thirugnanasambandham, K., Sridhar, R. (2013). Artificial neural network and response surface methodology modeling in mass transfer parameters predictions during osmotic dehydration of *Carica papaya* L. *Alexandria Engineering Journal,* 52(3), 507−516.
[49] Singh, V., Guizani, N., Al-Alawi, A., Claereboudt, M., Rahman, M.S. (2013). Instrumental texture profile analysis (TPA) of date fruits as a function of its physico-chemical properties. *Industrial Crops and Products,* 50, 866–873.

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Araştırma Makalesi / Research Paper

Organik Atıklardan Elde Edilen Besiyerinde Üreyen *Aspergillus niger*'den Proteaz Enzimi Üretimi ve Karakterizasyonu

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0 264 295 59 50 0 264 295 59 20

ÖZ

Ticari enzim pazarında önemli bir yeri olan proteolitik enzim grubundan proteazlar, geniş ve farklı biyoteknolojik uygulamaları nedeniyle yüksek talep gören bir enzim grubudur. Bu enzimlerin küfler kullanılarak fermantasyon yoluyla üretilmesi tercih edilen bir yöntemdir. Çünkü, enzimlerin konsantre halde ekstraselüler olarak üretilmesiyle geri kazanımı daha kolay ve maliyet açısından daha elverişli bir üretim yöntemi olmaktadır. Bu nedenle çalışmada, organik çöpler substrat olarak kullanılarak *Aspergillus niger*'den proteazların üretimine alternatif oluşturmak ve enzimin saflaştırılmasına bir temel oluşturmak amaçlanmıştır. Araştırmada, *A. niger* inoküle edilen %50 protein oranında organik atıklardan hazırlanan besiyerinin, 30°C'de 24 saat inkübasyon süresinde, pH 5.5'te enzim üretimi için optimum besiyeri olduğu tespit edilmiştir. *A. niger*'den üretilen proteaz enzimi geniş bir uygulama alanına sahip, çok yönlü bir biyo-ayırma aracı haline gelen, yüksek geri kazanım ve saflık seviyelerine sahip bir dizi enzim ve proteini saflaştırmak için kullanılan üçlü faz ayırma (TPP) sistemi ile kısmen saflaştırılmış ve biyokimyasal karakterizasyonları incelenmiştir. TPP sistemi ile proteaz enzimi 1.0:1.5 (ham enzim çözeltisi: t-bütanol oranı) ve %80 doygun amonyum sülfat konsantrasyonu ile %235.7 verimle 13.15 kat saflaştırılmıştır. Enzimin molekül ağırlığı MALDI-TOF yöntemiyle 15.56 kDa olarak belirlenmiştir. *A. niger*'den saflaştırılan proteaz enziminin optimum ve stabil pH'ları sırasıyla 10.0 ve 9.0 iken optimum ve stabil sıcaklığı ise 60°C olmuştur. Okside edici ajanlar, yüzey aktif maddeleri, inhibitör ve substrat varlığı proteaz enziminin aktivite değerlerinde azalmaya neden olmuştur.

Anahtar Kelimeler: Proteaz, *Aspergillus niger*, Üçlü faz ayırma sistemi (TPP), Taguchi

Protease Enzyme Production and Characterization from *Aspergillus Niger* Grown in Medium Obtained from Organic Waste

ABSTRACT

Proteases, a subgroup of proteolytic enzymes with a significant role in the commercial enzyme market, are in high demand due to their broad and diverse biotechnological applications. Fermentation using molds is a preferred method for producing these enzymes because their extracellular production in a concentrated form facilitates easier recovery, making the process more cost-effective. Therefore, this study aims to provide an alternative approach for the production of proteases from *Aspergillus niger* using organic waste as a substrate and to establish a basis for enzyme purification. In this study, the optimal medium for enzyme production was determined as a culture medium prepared from organic waste with a 50% protein content, inoculated with *A. niger*, and incubated at 30°C for 24 h at pH 5.5. The protease enzyme produced from *A. niger* was partially purified using the three-phase partitioning (TPP) system, which has become a versatile bio-separation tool with broad applications, high recovery rates, and high purity levels. Using the TPP system, the protease enzyme was purified 13.15-fold with a yield of 235.7% under conditions of a 1.0:1.5

ratio (crude enzyme solution: t-butanol) and 80% ammonium sulfate saturation. The molecular weight of the enzyme was found to be 15.56 kDa using the MALDI-TOF method. The optimum and stable pH values of the purified protease enzyme from *A. niger* were determined as 10.0 and 9.0, respectively, while the optimum and stable temperature was 60°C. The presence of oxidizing agents, surfactants, inhibitors, and substrates caused a decrease in the enzyme activity values.

Keywords: Protease, *Aspergillus niger*, Triple phase separation system (TPP), Taguchi

GİRİŞ

Enzimler, biyokimyasal katalizörler olarak çalışan ve reaksiyonları yüksek verimle gerçekleştiren biyomoleküllerdir. Canlı organizmalar tarafından spesifik biyokimyasal reaksiyonları gerçekleştirmek amacıyla üretilirler [1]. Enzimlerin büyük kısmı, enzimatik aktivite için gerekli olan ve protein yapısında bulunmayan kofaktörlere bağımlı iken, bazıları yalnızca protein yapısıyla fonksiyon gösterir. Endüstrinin hemen hemen her alanında enzimlere ihtiyaç duyulmakta olup, bu enzimler genellikle mikrobiyal kökenlidir. Mikrobiyal kökenli enzimlerin tercih edilmesinin sebebi, hayvansal ve bitkisel enzimlere kıyasla daha yüksek kalitatif aktivite göstermeleridir. Ayrıca mikrobiyal enzimler diğerlerine göre daha dayanıklı, ekonomik ve daha büyük çapta üretilebilir [2]. Enzimler, tekstil, kâğıt, içecek ve yiyecek, deterjan, deri, hayvan yemi, yakıt, ilaç, kozmetik gibi çeşitli endüstrilerde yaygın olarak kullanılmaktadır.

Mikrobiyal enzim grubu içinde olan proteaz, aminoasitlerin oluşumu ve protein katabolizması için gereklidir. Proteinlerin peptit bağlarının hidrolizini katalize eden bu enzim, proteolitik enzimler veya peptidazlar olarak adlandırılır. Peptitleri ve amino asitleri serbest bırakır ve gıdanın bileşimi, süreci ve bozulması üzerindeki etkileri nedeniyle gıda endüstrisinde önemli bir rol oynar. Fonksiyonel ve aktif gruplarına göre altı ana başlık altında sınıflandırılır: serin proteazlar, sistein proteazlar, metalloproteazlar, aspartilproteazlar, treonin proteazlar ve glutamik asit proteazlar [3].

Biyoteknoloji alanındaki endüstriyel enzim araştırmaları giderek daha fazla önem kazanmaktadır. Bunun başlıca sebepleri arasında enzim teknolojisinin gelişmesi, ürün kullanım alanlarının genişlemesi ve ekonomik değerinin artması yer almaktadır. Son yıllarda, bu çalışmaların bir kısmı atıklardan enzim üretimi üzerine yoğunlaşmıştır. Dünya genelinde nüfus artışına paralel olarak atık miktarı da hızla yükselmekte ve bu atıkların değerlendirilmesi çevresel açıdan büyük önem taşımaktadır. Özellikle meyve ve sebzelerden kaynaklanan biyolojik olarak parçalanabilir atıkların yılda yaklaşık 50 milyon ton olduğu tahmin edilmektedir ve bunun sadece %0.5'i endüstriyel işlemlerde kullanılmaktadır. Bu nedenle, endüstriyel süreçlerin yan ürünü olarak oluşan atıkların değerlendirilmesi çevresel ve ekonomik açıdan önemlidir. Bu kapsamda, Madhumithah ve ark. [4] bitkisel atıklar, Oyeleke ve ark. [5], bakliyat atıkları; Lanka ve ark. [6] hayvansal protein kaynaklı süt ürünleri atıklarını kullanarak *Aspergillus niger'*den proteaz üretimini araştırmıştır. Benzer şekilde bu çalışma da organik atıkların *A. niger* tarafından proteaz üretimi için kullanılabilirliğini değerlendirilmeyi ve enzim üretimini ekonomik olarak teşvik etmeyi amaçlamaktadır.

Bu çalışmada proteaz enzimini saflaştırmak için üçlü faz ayırma (TPP) yöntemi kullanılmıştır. TPP, geniş bir uygulama alanına sahip, protein çökelmesinin pek çok basamağını kapsayan çok yönlü bir biyo-ayırma aracı olarak yüksek geri kazanım ve saflık seviyelerine sahip bir dizi enzim ve proteini saflaştırmak için kullanılmaktadır. Proteaz, invertaz, pektinaz, a-galaktosidaz, tripsin inhibitörü, lakkaz, katalaz gibi enzimlerin saflaştırılmasında yaygın olarak kullanılan TPP yönteminin mekanizması tam olarak literatürde tanımlanmamış olsa da, salting out, ozmolitik elektrostatik kuvvetler, kozmotropik çökeltme, izo-iyonik çökeltme, konformasyon sıkılaşması ve protein hidrasyon kaymaları gibi çeşitli ayırma işlemlerinin kombinasyonlarından oluştuğu belirtilmektedir [8].

Bu çalışmanın amaçlarından biri de Taguchi test metodu kullanılarak organik atıklardan elde edilen besiyerlerinde *A. niger* fermentasyonu ile üretilen proteaz enzimlerinin en verimli koşulları belirlemek ve geleneksel yöntemlere göre daha basit, verimli, ekonomik ve tek adımda yüksek geri kazanımla saflaştırılabilen TPP yöntemi ile proteaz enziminin saflaştırılmasının karakterizasyonunu yapmaktır.

MATERYAL ve METOT

Kullanılan Mikroorganizma

A. niger MRC 200806 saf kültürleri TÜBİTAK Marmara Araştırma Merkezi Küf Kültür Koleksiyonu'ndan temin edilmiştir. Saf kültürler 650 µL gliserol, 350 µL Tryptic Soy Broth (TSB) içinde -18°C'de muhafaza edilmiştir.

Küfün Çoğaltılması

Sporulasyon ortamı olarak TSA (Tryptic Soy Agar) besiyeri kullanılmıştır. Seyreltilmiş örnekler, steril öze kullanılarak TSA içeren petri kaplarına çizgi ekim yöntemiyle inoküle edilmiştir. Kültürler, 30°C'de 72 saat inkübasyona bırakılmıştır. İnkübasyon süresi sonunda, gelişen kolonilerden 100 µL alınarak 7 mL TSB (Tryptic Soy Broth) besiyerine inoküle edilmiş ve 30°C'de 72 saat boyunca inkübe edilerek çoğaltılmıştır. Elde edilen kültür, enzim üretiminde inokulum kaynağı olarak kullanılmıştır.

Uygun Besiyeri İçeriğinin Belirlenmesi

Proteaz üretimi için en uygun besiyeri koşullarını belirlemek amacıyla Taguchi (3x3) deney tasarım

yöntemi uygulanmıştır. Bu yöntemin temel adımları; faktörlerin ve etkileşimlerin belirlenmesi, her bir faktörün farklı seviyelerinin tanımlanması, uygun ortogonal matrisin seçilmesi, etkileşimlerin ve faktörlerin matrise aktarılması, deneylerin gerçekleştirilmesi, elde edilen verilerin analizi, optimal koşulların belirlenmesi ve doğrulama deneylerinin yapılması şeklinde özetlenebilir [9]. *A. niger* kültürü, Taguchi yöntemine göre tasarlanan deney düzeninde, Tablo 1'de belirtilen faktörler doğrultusunda üç paralel analiz ile proteaz aktivitesi açısından değerlendirilmiştir.

Tablo 1. Proteaz üretimi için kullanılan fermentasyon koşulları
Table 1. Fermentation conditions used for protease production

Hayvansal Protein (%)-Bitkisel Protein (%)	pH	İnkübasyon Süresi (saat)
50-50	5.0	24
50-50	5.5	48
50-50	6.0	72
60-40	5.0	48
60-40	5.5	72
60-40	6.0	24
70-30	5.0	72
70-30	5.5	24
70-30	6.0	48

Sakarya YURTKUR Kız Öğrenci Yurdu Yemekhanesi'nden toplanan gıda artıkları (Tablo 2), hayvansal-bitkisel protein, meyve-sebze ve polisakkarit ağırlıklı olarak sınıflandırılmış ve blender yardımıyla püre haline getirilerek toplam 50 g olacak şekilde karışım hazırlanmıştır. Karışıma 100 mL saf su eklenmiş ve homojen bir kıvam elde etmek amacıyla tekrar blender ile işlem görmüştür. Elde edilen besiyeri, gıda partiküllerinden arındırılarak pürüzsüz bir yapı elde etmek için No:1 Whatman™ filtre kâğıdı ile süzülmüştür.

Tablo 2. Besiyeri kompozisyonunda yer alan biyolojik atıkların içeriği
Table 2. Composition of biological waste in the growth medium

1. Sebze ve meyve ağırlıklı atıklar	Kabak, havuç, lahana (çiğ ve turşu), domates (çiğ ve konserve), biber (kapya, çarliston), patlıcan, soğan, patates, karnabahar, maydanoz, marul, dereotu, roka, nane, pırasa biberiye, kereviz, mandalina, portakal, limon, karpuz, muz, ananas, iğde, elma, ayva, Trabzon hurması, armut, greyfurt, kivi
2. Hayvansal Protein ağırlıklı atıklar	Mantar, yumurta (çiğ ve haşlanmış), peynir (beyaz peynir, kaşar peyniri), kırmızı et, tavuk eti
3. Bitkisel Protein ağırlıklı atıklar	Kuru fasulye yemeyi, nohut yemeyi, yeşil mercimek, bezelye, börülce
4. Polisakkarit ağırlıklı atıklar	Ekmek (kepekli, çavdar, beyaz, tam buğday), kek, pankek, simit, poğaça (dereotlu, kaşarlı, domatesli), açma, üzümlü çörek, kruvasan (çikolatalı ve sade), şekerli çörek, ay çöreği.

Besiyerinin pH değeri deney düzenine uygun olarak 5.0, 5.5 ve 6.0 seviyelerine ayarlanmış, ardından her biri 30 mL fermantasyon ortamı içerecek şekilde 100 mL'lik Eppendorf® tüplerine aktarılmıştır. Sterilizasyon amacıyla 121°C'de 15 dakika otoklavlanmıştır. Soğutulan besiyerleri, 8 mm çapında *A. niger* kültürü ile inoküle edilerek vortekslenmiştir. İnoküle edilen tüm besiyerleri, 30°C'de 24, 48 ve 72 saat boyunca inkübe edilmiştir. Fermantasyon süresi sonunda, biyolojik kabin içerisinde Buhner hunisi kullanılarak No:1 Whatman™ filtre kağıdı yardımıyla süzme işlemi gerçekleştirilmiş ve elde edilen süzüntü ham enzim kaynağı olarak kullanılmıştır. Her bir süzüntünün proteaz aktivitesi ölçülmüş ve tüm analizler üç paralel tekrar ile gerçekleştirilmiştir.

Proteaz Enziminin Üçlü Faz Ayırma Yöntemi Kullanılarak Saflaştırılması

Optimum koşullarda enzim üretimi gerçekleştirildikten sonra, fermantasyon ortamındaki küf miselleri filtre kağıdı kullanılarak uzaklaştırılmıştır. Homojenattaki hücre kalıntılarının giderilmesi amacıyla, süzüntü 5000 rpm'de, 24°C'de 25 dakika santrifüj edilmiştir. Enzim saflaştırma sürecinde, optimum amonyum sülfat/t-bütanol oranını belirlemek için homojenattan her bir tüpe 2 mL aktarılmış ve farklı amonyum sülfat doygunluk seviyelerine (%30, %40, %50, %60, %70, %80 ve %90) ayarlanmıştır. Ardından, her tuz konsantrasyonu için farklı homojenat/t-bütanol oranları (1.0:0.5, 1.0:1.0, 1.0:1.5 ve 1.0:2.0) hesaplanarak uygun hacimde t-bütanol eklenmiş, her ekleme sonrası vortekslenme işlemi uygulanmıştır. Numuneler oda sıcaklığında 1 saat inkübe edilmiş ve üç fazın oluştuğu gözlemlenmiştir. Bunu takiben, çözeltiler 5000 rpm'de, 24°C'de 10 dakika santrifüj edilmiştir. Santrifüj sonrası üst faz pastör pipeti ile ayrılmış, kalan orta ve alt fazlar ayrı tüplere aktarılmıştır. Son olarak, bu fazların protein tayini ve enzim aktivitesi analizleri gerçekleştirilmiştir.

Proteazların Enzim Aktivitesi

Proteaz enziminin aktivitesi, substrat olarak kazein kullanılan modifiye bir yöntemle belirlenmiştir [2].

Yöntemde, %0.65 konsantrasyonda hazırlanan kazeinden 2.5 mL alınarak üzerine 0.5 mL enzim çözeltisi eklenmiştir. Reaksiyon karışımı, 37°C'de su banyosunda 10 dakika inkübe edilmiştir. Kontrol (kör) için enzim çözeltisi yerine Tris-HCl tampon (100 mM, pH 8.5) kullanılmıştır. İnkübasyonun ardından reaksiyonu durdurmak için 2.5 mL 110 mM TCA çözeltisi eklenmiş ve karışım tekrar 37°C su banyosunda 30 dakika bekletilmiştir. Bu işlemi takiben karışım 5000 rpm'de 10 dakika santrifüj edilmiş, oluşan süpernatanttan 1 mL alınmıştır. Süpernatanta 2.5 mL Na_2CO_3 (0.5 M) çözeltisi ve 0.5 mL Folin-Ciocalteu reaktifi (0.5 M) eklenerek karışım bir kez daha 37°C'de 30 dakika inkübe edilmiştir. İnkübasyonun sonunda, reaksiyon karışımının absorbans değerleri 660 nm'de spektrofotometrede ölçülmüştür. 37°C ve pH 8.5 koşullarında gerçekleşen hidroliz sırasında, %0.65 kazeinden dakikada 1 µg tirozin açığa çıkması için gereken enzim miktarı, bir ünite proteaz aktivitesi (U/mL) olarak tanımlanmıştır.

Bradford Yöntemiyle Protein Tayini

Seyreltilmiş BSA örneklerinin üzerine hazırlanan Coomassie Brillant Blue G-250 çözeltisinden 2 mL eklenmiş ve vortekslenmiştir [2]. On dakika boyunda oda sıcaklığında bekletildikten sonra standartlar 595 nm dalga boyunda köre karşı okunmuş ve elde edilen değerlerle protein standart grafiği çizilmiştir. Numunelerdeki bilinmeyen protein konsantrasyonları, bu grafik yardımıyla hesaplanmıştır.

Moleküler Ağırlığı

Saflaştırılan proteaz enziminin moleküler ağırlığı MALDI-TOF cihazı kullanarak hesaplanmıştır. Saflaştırılmış numunenin analizi için numune 500 µL (1/1/1 asetonitril/ultra saf su/ethanol) karışım kullanılarak seyreltilmiştir. Matriks için ise iki farklı şekilde; 5 mg CHCA (α-siyano-4-hidroksisinnamik asit), 10 mg sinapinik asit (3,5-dimetoksi-4-hidroksisinnamik asit) kullanılmıştır. Matriksler 1000 µL (50.0/50.0/0.1 asetonitril/ ultra saf su/ trifloroasetik asit) karışım kullanılarak seyreltilmiştir. Analiz işlemi 0.5 mL numune ve 0.5 mL matriks karıştırılarak gerçekleştirilmiştir. Analizde kullanılan aralıklar: 'Mass Range' 1000-4000 Da, 'Max Laser Repetition Rate' 50, 'Power' 95, Profile 100, 'Shots' 20, 'Pulsed Extraction Optised at' (Da) 2300 olarak bildirilmiştir.

Saflaştırılan Enzimin Özellikleri

Saflaştırılan enzimin optimum sıcaklık değerini belirlemek amacıyla, 100 mM Tris-HCl tamponu (pH 8.5) içerisinde %0.65 konsantrasyonunda hazırlanan kazein çözeltisinden 2.5 mL alınarak üzerine 0.5 mL enzim çözeltisi eklenmiştir. Enzim aktivitesinin belirlenmesi için reaksiyon karışımları 20°C'den 80°C'ye kadar farklı sıcaklıklarda 10 dakika inkübe edilmiştir. Ardından, enzim aktivite değerleri hesaplanarak optimum sıcaklık belirlenmiştir. Optimum pH değerinin tespiti için, %0.65 kazein içeren farklı tampon çözeltileri hazırlanmıştır: Sodyum fosfat tamponu (pH 6.0-8.0), Tris-HCl tamponu (pH 7.0-9.0) ve glisin-NaOH tamponu (pH 9.0-11.0). Bu çözeltilere 0.5 mL enzim eklenerek, 37°C'de 10 dakika inkübe edilmiştir. Ardından, proteaz aktivite tayini gerçekleştirilmiştir. Proteaz enziminin aktif bölgesindeki katalizden sorumlu amino asitlerin varlığını tespit etmek için, enzim %1 ve %8 oranında üre, %1 ve %5 oranında inhibitörler (beta-merkaptoetanol, serin proteaz inhibitörü PMSF ve metaloproteaz inhibitörü EDTA), %1 ve %5 oranında yüzey aktif maddeler (Tween 80, Tween 20, SDS, Triton X-100) ve oksitleyici madde H2O2 ile muamele edilmiştir. Enzim çözeltileri, ilgili kimyasallarla 30 dakika inkübe edildikten sonra aktivite ölçümleri yapılmıştır. Enzimin doğal substratlara karşı aktivitesini incelemek amacıyla, 100 mM Tris-HCl tamponu (pH 8.5) içerisinde hazırlanan %0.65 konsantrasyonundaki hemoglobin, kazein, BSA, azokazein ve jelatin substratları kullanılmıştır. Farklı substratlar varlığında enzim aktivite değişimleri analiz edilerek, enzimin substrat spesifikliği değerlendirilmiştir.

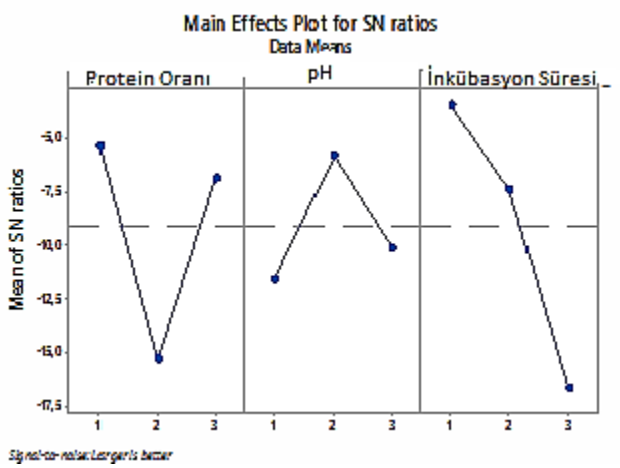
İstatistiksel Analiz

Analizlerden alınan değerler, 3 tekrarın ortalamasıdır. Çalışmada istatistiksel analizler Minitab (18.0 versiyon, State College, PA, ABD) paket programı kullanılarak gerçekleştirilmiştir. Veriler varyans analizi (tek-yönlü ANOVA) ile analiz edilmiş, istatistiksel farklılık Tukey çoklu karşılaştırma testi yardımıyla $p < 0.05$ düzeyinde belirlenmiştir.

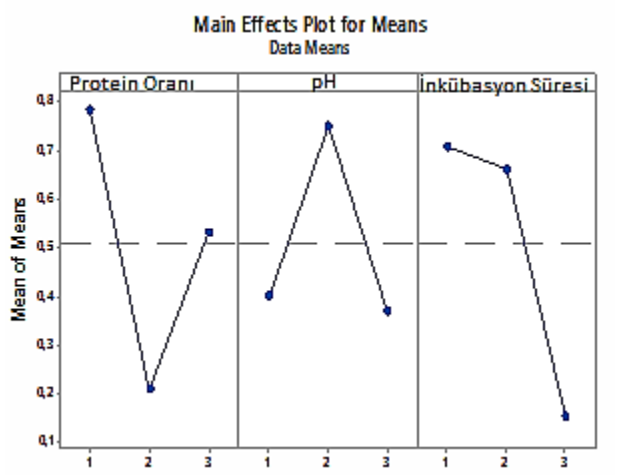
BULGULAR ve TARTIŞMA

Proteaz Enziminin Üretimi

Taguchi yöntemi kullanılarak yapılan hesaplamalara göre parametrelerin optimum seviyeleri çoğunlukla S/N oranının en büyük olduğu seviyelerde meydana gelmektedir ve grafikler incelendiğinde protein oranı parametresinde en yüksek piki birinci seviyenin verdiği görülmektedir (Şekil 1 ve 2). Grafikler incelendiğinde %50-50 protein oranının aktivite tayininde en yüksek değeri verdiği gözlenmiştir. pH parametresinde proteaz enzimi üretimi için optimum değerin 5.5 olduğu tespit edilmiştir. *A. niger*'in proteaz enzimi üretimi için optimum inkübasyon süresi ise 24 saat olarak bulunmuştur. Çalışmada inkübasyon süresi arttıkça enzim üretiminin düşmesi kullanılan katı substrat yapısının zamana bağlı olarak bozulması ve ortamdaki diğer bileşiklerden etkilenmesine bağlanmaktadır. Mikroorganizma tarafından kısa sürede enzim üretiminin gerçekleştirilmesi önemli avantaj sağlamaktadır. Bu parametrelerle üretilen besiyerinde proteaz aktivitesi 60.79 U/mL olarak hesaplanmıştır. Literatürdeki benzer çalışmalara bakıldığında Paranthaman ve ark. [10], *A.niger* bakterisi kullanarak pirinç atıklarından proteaz enzimi üretiminde aktiviteyi 67.7 U/g bulurken, Devi ve ark. [11] da *A. niger* küfü ile yaptıkları optimizasyon çalışmalarında proteaz aktivitesini 89.1 U/mL olarak belirtmişlerdir. Çalışmamızdan elde edilen değer ile yakın sonuçlar elde edilmiştir.



Şekil 1. Sinyal gürültü oranları için tepki tablosu (daha büyük olan daha iyidir)
Figure 1. Response table for signal-to-noise ratios (the larger, the better)



Şekil 2. Taguchi deney tasarımında enzim üretiminin sinyal/gürültü oranı sonuçları
Figure 2. Results of the signal-to-noise ratio for enzyme production in the Taguchi experimental design

Çalışmada proteaz üretimini etkileyen faktörlerden biri besiyerinde bulunan protein çeşidi olmuştur. Proteinler aminoasitlerin polimerleşmesi sonucu oluşan polimerlerdir ve her aminoasidin miktarı ve türü protein kaynağına göre farklılık gösterir. Örneğin hayvansal ve bitkisel proteinlerin de içerdikleri aminoasitler farklıdır ve bu aminoasit farklılıkları proteaz çeşidini de etkiler. Proteazlar aktif bölgelerindeki fonksiyonel aminoasit köküne göre serin proteaz, sistein proteaz, asidik proteaz ve metalloproteazlar olarak sınıflandırılmaktadır Çalışmamızda hazırlanan besiyerinin bileşiminde bulunan bitkisel proteinlerden bakliyatlar, asidik asit, lisin, glutamik asit ve arjinin gibi aminoasitleri içerir. Hayvansal proteinler proteazın proteini parçalamak için kullandığı enzimlerden sistein ve serin dahil 20 aminoasidin tamamını içerir [12]. Proteaz üretimi için hayvansal proteinler bitkisel proteinlere kıyasla aminoasit açısından daha zengin olmasına rağmen, bitkisel ve hayvansal proteinin eşit miktarda olduğu besiyeri proteaz üretimi için en uygun besiyeri olarak belirlenmiştir. Literatürdeki protein farklılığı üzerine yapılan bir çalışmada Pinar ve ark. [13], pirinç çeşitlerinin farklı coğrafi bölgelerde yetiştirildiği için numunelerdeki protein seviyesinin oldukça farklı olduğunu raporlamıştır. Çeşitli pirinç atığı örneklerinden ekstrakte edilen proteazların aktivitelerini karşılaştırmış ve farklı pirinç numunelerindeki proteaz üretim seviyesindeki farkın, numunelerin protein içeriğindeki farktan kaynaklandığını belirtmiştir. İnkübasyon süresinin etkisi yapılan çalışmalarda incelendiğinde; Upgade ve ark. [14], Fransız fasulyesi kullanarak gerçekleştirdiği fermantasyonda; Qazı ve ark. [15] ise buğday kepeği ve soya fasulyesinden proteaz üretimini, çalışmamızdan farklı olarak optimum 48. saatte elde etmiştir ve bunun yanı sıra Shivakumar [16] yaptığı benzer çalışmada optimal inkübasyon süresinin 120. saatte 280 U/g enzim aktivitesi ile elde ettiğini belirtmiştir. Fakat tüm çalışmalar verimin artan inkübasyon süresince düştüğü yönünde ortak bir karara varmıştır.

Çalışmada optimum enzim üretimi besiyerinin pH'sı 5.5 olduğunda gözlenmiştir. Bunun yanı sıra benzer çalışmalarda da farklı sonuçlar alınmıştır. Örneğin, Oyeleke ve ark. [5] bitkisel proteinlerden fasulyeyi kullanarak hazırladıkları besiyerinde *A. niger*'den proteaz üretimi için optimum pH'ın 6 olduğunu belirtirken; Shivakumar [16], *Aspergillus sp*. tarafından asit proteaz üretimi için optimum pH'nın 5.0 olduğunu ve 5.0'ın altındaki ve üzerindeki pH'nın enzim üretimini

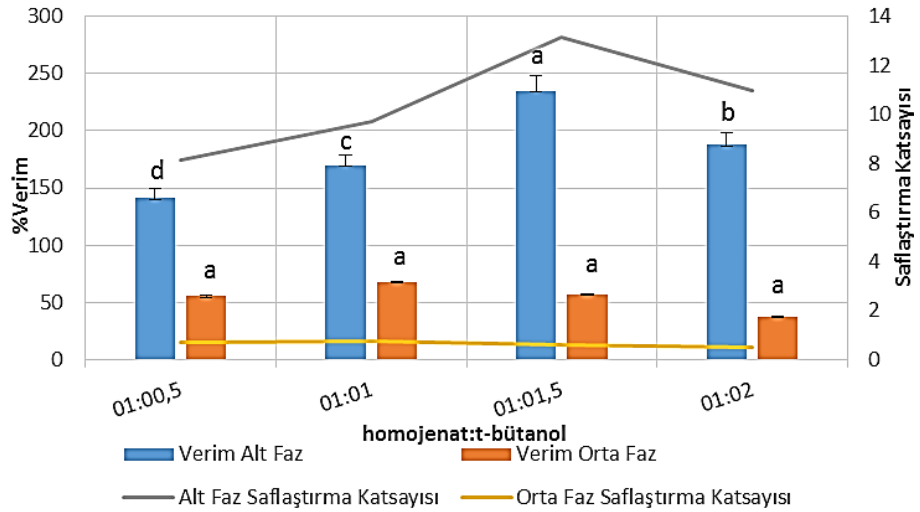
olumsuz etkilediğini belirtmiştir. Benzer şekilde Oyewole ve ark. [17], mezbaha atıklarından elde ettikleri proteaz enzim aktivitesi için sırasıyla *A. niger* ve *P. frequesans* için 5.0 ve 6.0 pH değerlerinin optimum olduğunu kaydetmiştir. Optimum enzim üretimi için pH'nın mikroorganizma türüne, besiyeri bileşimine ve çalışma koşullarına bağlı olarak 5 ile 6 arasında değiştiği söylenebilir.

Proteaz Enziminin TPP Sistemiyle Organik Çözgen ve Amonyum Sülfat Oranının İncelenmesi

A. niger'den üretilen proteaz enziminin sırasıyla %30, 40, 50, 60, 70, 80 ve 90 konsantrasyonlardaki amonyum sülfat oranları ve 1.0:0.5, 1.0:1.0, 1.0:1.5 ve 1.0:1.2 gibi değişik homojenat:t-bütanol oranları ile yapılan analizler sonrası bulunan verilerle, yüzde verim ve saflaştırma katsayıları hesaplanmıştır. Saflaştırılan proteaz enziminin, %80 (w/v) amonyum sülfat ve 1.0:1.5 (v/v) homojenat: t-bütanol oranında, alt fazda en iyi % verim ve saflaştırma katsayısını elde ettiği tespit edilmiştir ($p<0.05$) (Tablo 3, Şekil 3). En yüksek enzim verimi ve saflaştırma katsayısı sırasıyla %235.2 ve 13.15 olarak raporlanmıştır.

Tablo 3. *A. niger*'den proteaz enzimi saflaştırma süreci ve aktivite analizi değerleri (n=3, ortalama ± SS)
Table 3. The protease enzyme purification process from A. niger and enzyme activity values (n=3, mean ± SD)

Faz	Toplam Hacim (mL)	Aktivite (EU/mL)	Protein (mg/mL)	Toplam Aktivite (EU)	Toplam Protein (mg)	Spesifik Aktivite (EU/mg)	%Verim	Saflaştırma Katsayısı
Homojenat	2.00	25.85±3.2	5.20±1.4	51.70±4.5	10.40±2.3	4.97±2.1	100.00±0	100.00±0
Alt faz	2.00	60.79±7.8	0.93±0.1	121.58±9.6	1.86±0.4	65.37±6.9	235.17±12.5	13.15±5.6
Ara faz	2.00	14.70±2.3	4.90±1.3	29.40±6.5	9.80±1.7	3.00±0.8	56.86±6.9	0.12±0.1



Şekil 3. Homojenat:t-Bütanol oranlarının proteaz enziminin verim ve saflaştırma katsayısına etkisi. Aynı harfle belirtilen bloklardaki ortalamalar arasındaki fark önemsizdir ($p\geq0.05$).
Figure 3. The effect of homogenate-to-t-butanol ratios on the yield and purification factor of the protease enzyme. The differences between the means in bars marked with the same letter are insignificant ($p\geq0.05$).

Literatürdeki saflaştırma çalışmalarında çoğunlukla çok aşamalı kromatografik yöntemler tercih edilir fakat TPP yöntemiyle saflaştırmaya ait az sayıda çalışma vardır. TPP yöntemi kullanılarak yapılan saflaştırmalarda genellikle %25-363 arası verim ve 2.7-95 arası saflaştırma katsayısına ait sonuçların bildirildiği gözlenmiştir [1]. *Calotropis procera* ve *Cucumis melo (Cucumisin)* 'nun TPP yöntemiyle proteaz saflaştırması üzerine yapmış olduğu çalışmalarda %60-65 amonyum sülfat konsantrasyonunda, yüksek oranda enzim geri kazanımı ve saflaştırma tespit edilirken, t-bütanol hacmindeki artışın, enzim aktivitesinde bir azalmaya sebep olduğu belirtilmiştir [2]. Pinjara ve ark. [13] *A.niger* ve *A.flavus* proteazlarını %70 doygunlukta amonyum sülfat çöktürme ve diyaliz ile sırasıyla 11 ve 9 kat saflaştırmıştır. Chimbekujwo ve ark. [18] *A. brasiliensis* BCW2 tarafından üretilen ham proteazı, amonyum sülfat (%80) kullanılarak kısmen saflaştırılmış ve %28 proteaz geri kazanımı ile 13.3 kat saflık vermiştir. Rüzgar'ın *B. licheniformis* OSB6'dan proteaz enzimlerinin TPP ile saflaştırılması üzerine yaptığı çalışmada %70 amonyum sülfat ve 1.0:1.5 t-bütanol oranıyla saflaştırmayı gerçekleştirdiği bildirilmiştir [2]. Başka bir çalışmada ipek otundan proteaz enzimini saflaştırmak için ilk olarak 1.0:0.5 homojenat:t-bütanol ve %50 amoyum sülfat oranında ilk TPP yapılmış ve sonrasında alt faz alınarak 1.0:0.5 homojenat:t-bütanol oranında %65 amonyum sülfat ile ikinci bir TPP

yapılmıştır. Analiz sonucunda enzimin %132 verim ve 6.92 kat saflaştırıldığı ve enzimin alt fazdan elde edildiği tespit edilmiştir. Bir diğer çalışmada balık iç organlarında alkalin proteazı saflaştırmak amacıyla 1.0:0.5 oranında t-bütanol, %50 amonyum sülfat kullanılarak pH 8.0' de TPP yöntemi uygulanmış ve enzimin %154 verimle saflşatırıldığı bildirilmiştir. Sonuç olarak t-bütanol miktarı daha düşük olduğunda amonyum sülfat ile yeterli sinerji oluşturamaz ama daha yüksek ise protein denatürasyonuna neden olması kaçınılmazdır. Ayrıca yapılan çalışmalarda TPP prosedürleri sırasında amonyum sülfat konsantrasyonundaki artışın enzimlerin aktivitesini arttırdığı rapor edilmiştir [2].

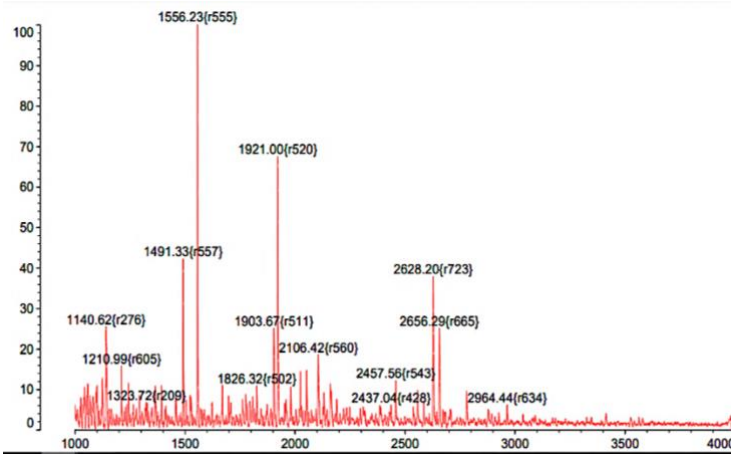
Moleküler Ağırlığın Belirlenmesi

Bu çalışmada, *A. niger* tarafından üretilen proteaz enziminin moleküler ağırlığı, MALDI-TOF kütle spektrometresi kullanılarak belirlenmiştir. Spektrum analizi sonucunda, en belirgin pik 1556.23 Da bölgesinde gözlemlenmiştir. Bu sonuç, proteaz enziminin yaklaşık 15.56 kDa moleküler ağırlığa sahip olduğunu göstermektedir.

Elde edilen moleküler ağırlık, literatürde yer alan diğer *Aspergillus* türlerinden izole edilen alkali proteazlarla kıyaslandığında benzerlik göstermektedir. Örneğin, farklı mikroorganizmalardan üretilen proteaz enzimlerinin moleküler ağırlıklarının genellikle 10-45 kDa aralığında olduğu bilinmektedir [19]. Bu bağlamda, çalışmamızdaki enzim, düşük moleküler ağırlıklı proteazlar sınıfına girmektedir.

Bununla birlikte, grafikte gözlemlenen diğer pikler, enzimdeki olası post-translasyonel modifikasyonlar, fragmentasyon veya saflaştırma yöntemine bağlı olarak küçük peptit kontaminasyonlarıyla ilişkilendirilebilir. Ayrıca, 1921.00 Da ve 2106.42 Da bölgelerinde görülen sinyaller, enzim yapısında bulunan olası alt birimlere veya parçalanmış protein formlarına işaret edebilir.

Sonuç olarak, MALDI-TOF analizi ile belirlenen 15.56 kDa moleküler ağırlık, proteaz enziminin karakterizasyonuna önemli bir katkı sağlamaktadır. Bu enzim, özellikle deterjan, deri işleme ve gıda endüstrisinde kullanım potansiyeli olan alkali proteazlarla benzer özellikler göstermektedir [11]. Gelecekte, daha ileri saflaştırma teknikleri ve kristalografi çalışmaları ile enzimin yapısal ve fonksiyonel özellikleri daha ayrıntılı olarak incelenebilir.



Şekil 4. Saflaştırılmış enzimin moleküler ağırlığı

Figure 4. The molecular weight of the purified enzyme

Proteaz Enzimi Üzerine Optimum Şartlarının Tespitine Yönelik Sonuçlar

Saflaştırılan proteaz enziminin pH 10.0 glisin-NaOH (pH 9.0-11.0) tamponunda optimum aktiviteye sahip olduğu tespit edilmiştir (Tablo 4). Benzer şekilde Devi ve ark.'nın [11], *A. niger* ile yaptıkları optimizasyon çalışmalarında ve Lanka ve ark.'nın [6] süt ürünleri atık suyunda alkalin proteaz üretiminde de optimum pH'ın 10.0 olduğunu bulmuştur. Bununla birlikte, Coral ve ark.'nın [20], yanı sıra Oyeleke ve ark. da [5] *A. niger*' den proteaz aktivitesi için optimum pH'nın 9.0 olduğunu belirtmişlerdir. Bu çalışmaların aksine Olajuyigbe ve Ajele [21] *A. niger*'den asit proteaz üretiminde pH kararlılığının pH 3.5'te en iyi olduğunu ve 4.0'dan sonra hızla düştüğünü belirtmiştir fakat *A. niger* ile yapılan çalışmaların çoğunda optimum pH 10.0 olarak bulunmuştur. Çalışmalar karşılaştırıldığında elde edilen enzimin pH kararlılığının oldukça iyi olduğu ve literatürdeki çalışmalar ile uyumlu olduğu görülmüştür. Bununla birlikte, optimum pH'nın 10.0 olarak bulunması, enzimin alkali şartlarda da aktif olduğunu göstermektedir.

Sıcaklık çalışmaları sonucunda proteazın en iyi aktiviteyi 60°C'de 1 saatlik inkübasyon süresinin ardından %98.91'lik değer ile sağladığı görülmüş ve diğer sıcaklık değerleriyle karşılaştırıldığında değişen sürelerde aktivitesini en iyi koruyan sıcaklık olduğu gözlenmiştir. Qazı ve ark.'nın [15] yaptığı benzer bir çalışmada da buğday kepeği ve soya fasulyesini kullanarak *A. niger*'den proteaz üretimi için optimum sıcaklığın 60°C olduğunu buldukları gibi başka çalışmalarda yine *A. niger* kullanılarak üretilen proteazın 40 ile 50°C'de optimum aktivite sergilediği gösterilmiştir. Literatürde çoğunlukla sıcaklık ve inkübasyon süresi arttıkça

aktivitenin düştüğü görülür fakat termofilik enzimlerin genellikle sıcaklık sonucu aktivitesinin arttığı belirlenmiştir. Endüstriyel çalışmalarda sıcaklığa dayanıklılık ve yüksek alkalilik olması istenen bir durumdur ve enzimin tercih edilme sebepleri arasında yer alır. Biyoteknolojik uygulamalar ve biyomühendislik alanlarında da yüksek pH ve yüksek sıcaklık aralığında aktivitesini ve stabilitesini yüksek tutan proteazlar ve daha fazla kullanılır [2]. Dolayısıyla bu yönüyle *A.niger*'den üretilen proteaz enziminin oldukça avantajlı olduğu söylenebilir.

Tablo 4. pH değerinin ve sıcaklığın proteaz enziminin yüzde bağıl aktivitesine etkisi (n=3, ortalama ±SS)
Table 4. The effect of pH and temperature on the percentage *relative activity of the protease enzyme (n=3, mean ±SD)*

pH							
6	7	8	9	10	11		
85.2±5.6	82.2±7.3	78.2±4.2	75.2±2.5	100.0±0.0	54.5±6.3		
Sıcaklık (°C)							
20	30	40	50	60	70	80	90
80.7±4.5	89.3±3.9	88.0±9.2	91.6±4.3	100.0±0.0	96.0±3.4	81.47±3.9	71.5±5.7

Saflaştırılan proteaz enzimi üzerine okside edici ajan olan H_2O_2'nin ve yüzey aktif maddelerin etkisi incelendiğinde; %1 konsantrasyondaki SDS enzim aktivitesinde ciddi bir aktivite kaybına sebep olurken, %5'lik SDS' in enzimin aktivitesini tamamen inhibe ettiği tespit edilmiştir (Tablo 5). Yüzde 1'lik H_2O_2'nin ise enzimin aktivitesini %87'ye kadar koruduğu görülmüştür. Sonuç olarak yüzey maddelerinin konsantrasyonunun artmasının enzim aktivitesine olan etkinin de artmasına neden olduğu belirlenmiştir. Literatürdeki çalışmalara bakıldığında; Sattar ve ark. [22] *A. niger* kaynaklı proteaz aktivitesi üzerine yüzey aktif maddelerin, metal iyonlarının ve çözücülerin etkisini incelediği çalışmada 30 dakikalık inkübasyon sonrasında Tween 80 ve Trion X-100' ün yaklaşık %25 inhibisyona sebep olduğu, SDS varlığında enzimin %50 oranında aktivitesini kaybettiğini bildirmiştir. Benzer şekilde Pham ve ark. [23] *A. niger* VTCC-F021'den ürettikleri proteaz aktivitesinin %0.5−%2 (w/v) konsantrasyonlarda Tween 80 ve Tween 20' ye karşı yüksek direnç gösterdiğini ve başlangıç aktivitesinin %80'inden fazlasını koruduğunu tespit etmiştir. SDS ve Triton X-100 ise enzim aktivitesini üçte iki oranında azaltmıştır.

Tablo 5. Yüzey aktif maddelerin ve okside edici ajanın saflaştırılan proteaz enzimi aktivitesi (n=3, ortalama ±SS) üzerine etkisi
Table 5. The effect of surfactants and oxidizing agents on the activity of the purified protease enzyme (n=3, mean ±SD)

Yüzey Aktif Maddeler	%1	%5
Kontrol	100.00±0.00	100.00±0.00
SDS	9.23±2.40	0.00±0.00
Tween-20	43.24±2.50	28.12±3.70
Tween-80	57.32±4.50	37.51±4.30
TritonX-100	50.32±3.40	25.17±2.80
H2O2	87.32±5.40	63.77±6.40

Proteaz enzimine inhibitörlerin etkisini incelendiğinde %1 PMSF varlığında aktivitesinin yaklaşık %95'ini inhibe ettiği, %5 konsantrasyonda ise neredeyse tamamını inhibe ettiği görülmektedir (Tablo 6). Serin spesifik inhibitörü olan PMSF'nin enzimin üzerindeki inhibe etkisi aktif bölgesinde bir serin proteaz olduğunu ve enzimin alkalin serin proteaz olduğunu göstermektedir. Enzim aktivitesinin EDTA varlığında da azalması enzimin metal bağlama bölgesine sahip olduğunu göstermektedir. Basu ve ark.'nın [24] yaptığı benzer karakterizasyon çalışmalarında, *A. niger* tarafından ürettirilen proteazın EDTA varlığında %90 inhibe olduğu ve PMSF tarafından aktif bölgedeki temel serin kalıntısının ve aktivitenin tamamen kaybolmasına neden olduğu görülmüştür.

Tablo 6. İnhibisyon çözeltilerinin proteaz aktivitesi üzerine etkisi (n=3, ortalama ± SS)
Table 6. The effect of inhibition solutions on protease activity (n=3, mean ± SD)

İnhibisyon çözeltileri	Konsantrasyon (mM)	Kalan aktivite (%)
Kontrol	0	100.00±0.00
β-merkaptoetanol	1	41.32±3.20
	5	23.45±1.60
EDTA	1	58.12±5.40
	5	29.68±2.30
PMSF	1	4.85±0.90
	5	1.23±0.20
DTNB	1	75.11±5.30
	5	60.23±3.80
Üre	1	48.41±3.10
	8	31.57±2.70

Proteaz enziminin farklı substratlardaki aktivite miktarı incelendiğinde enzimin en iyi kazein varlığında aktivite gösterdiği ve kazeini sırasıyla azokazein, hemoglobin, jelaitin ve en son olarak da BSA'nın takip ettiği görülmektedir (Tablo 7). Muazu'nun [25] *A. niger* üzerine yaptığı çalışmasında da kazeinin substrat olarak etkisi çalışılmış ve 0.44 µg/mL ile en yüksek proteaz aktivitesi kazeinde elde edilmiştir. Benzer olarak Leng ve ark. [26] *A. oryzae* CH93'den saflaştırılan proteaz enziminin BSA, jelatin, kazein ve azokazein subsratları varlığında aktivitesi incelendiğinde kazeinin en iyi aktiviteyi gösterdiği tespit edilmiştir. Çeşitli substratlara enzimin ilgisinin olması kullanım alanlarının genişlemesine olanak sağlar. Bu nedenle bazı belli koşullar altında stabil kalabilen enzimler, stabilitenin moleküler temelini anlamak adına bir model sistem oluşturur ve bu durum endüstriyel uygulamalar için proteazların tasarlanmasına öncülük edebilir [27-28].

Tablo 7. Proteaz enziminin doğal substratlara (%0.6, w/v) karşı yüzde bağıl aktivitesi (n=3, ortalama ± SS)

Table 7. Percent relative activity of the protease enzyme against natural substrates (0.6%, w/v) (n=3, mean ± SD)

Kazein	Azokazein	Hemoglobin	Jelatin	BSA
100	83.6±4.6	47.3±3.2	35.4±3.8	21.2±1.9

SONUÇ

Bu çalışma, organik atıklardan elde edilen besiyerlerinde *A. niger* kullanılarak proteaz enzimi üretimini, saflaştırılmasını ve karakterizasyonunu kapsamlı bir şekilde incelemiştir. Elde edilen sonuçlar, organik atıkların enzim üretimi için uygun ve ekonomik bir substrat olabileceğini göstermektedir. Taguchi deney tasarımına göre optimize edilen besiyeri koşullarında (%50 hayvansal-%50 bitkisel protein, pH 5.5, 24 saat inkübasyon süresi), en yüksek proteaz üretimi gözlemlenmiştir.

Üçlü faz ayırma yöntemi kullanılarak gerçekleştirilen saflaştırma sürecinde, %80 amonyum sülfat ve 1.0:1.5 (v/v) homojenat:t-bütanol oranında %235.7 verimle 13.15 kat saflaştırma sağlanmıştır. Saflaştırılan proteaz enziminin moleküler ağırlığı 15.56 kDa olarak belirlenmiştir. Optimum pH değeri 10.0, optimum sıcaklık değeri ise 60°C olarak tespit edilmiş olup, enzim yüksek sıcaklık ve alkalin koşullara dayanıklılık göstermiştir.

Bununla birlikte, enzim aktivitesi okside edici ajanlar, yüzey aktif maddeler ve inhibitörlerin varlığında değişkenlik göstermiştir. Özellikle SDS ve PMSF gibi inhibitörlerin enzim aktivitesini önemli ölçüde azalttığı tespit edilmiştir. Substrat spesifikliği açısından ise en yüksek aktivitenin kazein varlığında gerçekleştiği belirlenmiştir.

Sonuç olarak, çalışmamız, atık değerlendirme ve biyoteknolojik enzim üretimi alanlarında sürdürülebilir ve ekonomik bir yaklaşım sunmaktadır. Endüstride proteaz enzimlerinin kullanım alanlarını genişletmek ve üretim süreçlerini daha verimli hale getirmek amacıyla, organik atık bazlı üretim süreçlerinin geliştirilmesi önemli bir adım olacaktır.

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Yazarlar herhangi bir ortak çıkar beyanı ya da çıkar çatışması beyan etmemiştir.

YAZARLARIN KATKISI

Çalışmaya yazarların eşit oranda katkısı olmuştur.

KAYNAKLAR

[1] Tolan, M. (2015). Mikrobiyal Kökenli Proteaz Üretimi, Saflaştırılması ve Karakterizasyonu. Gaziosmanpaşa Üniversitesi, Fen Bilimleri Enstitüsü, Biyomühendislik Anabilim Dalı, Tokat.

[2] Rüzgar, D. (2019*). Bacillus licheniformis* OSBS 6'dan Amilaz ve Proteaz Enzimlerinin Üçlü Faz Ayırma Sistemi (TPP) ile Saflaştırılması ve Karakterizasyonu. Atatürk Üniversitesi, Fen Bilimleri Enstitüsü, Moleküler Biyoloji ve Genetik Anabilim Dalı, Erzurum.

[3] Duman, Y. (2016). Üçlü faz ayrımı (ÜFA) ile geleneksel enzim saflaştırma tekniğinin karşılaştırılması; ÜFA ile saflaştırılan β-galaktosidazın termodinamik özellikleri. *Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi,* 6(3), 107-117.

[4] Madhumithah, C.G., Krithiga, R., Sundaram, S, Sasikumar, C.S., Guhathakurta, S., K.M. Cherian, K.M. (2011). Utilization of vegetable wastes for production of protease by solid state fermentation using *Aspergillus niger. World Journal of Agricultural Science,* 7(5), 550-555.

[5] Oyeleke, S.B., Oyewole, O.A., Egwim, E.C. (2011). Production of protease and amylase from bacillus subtilis and *Aspergillus niger* using *Parkia biglobossa* (Africa Locust Beans) as substrate in solid state fermentation. *Advances in Life Science*, 1(2), 49-53.

[6] Lanka, S. Anjali, C.H., Pydipalli, M. (2017). Enhanced production of alkaline protease by *Aspergillus niger* DEF 1 isolated from dairy form effluent and determination of its fibrinolytic ability. *African Journal of Microbiology Research,* 11(11), 440-449.

[7] Gagaoua, M., Equipe, K.H. (2013). Three phase partitioning system, an emerging non-chromatographic tool for proteolytic enzymes recovery and purification. *Biosensors Journal,* 5(1), 1-4.

[8] Gurung, N., Ray, S., Bose, S., Rai, V. (2013). A broader view: microbial enzymes and their relevance in industries, medicine, and beyond. *BioMed Research İnternational*, 18(1), 1-19.

[9] Gupta, R., Beg, Q., Lorenz, P. (2002). Bacterial alkaline proteases: molecular approaches and industrial applications. *Applied Microbiology and Biotechnology*, 59(1), 15- 32.

[10] Paranthaman, R., Alagusundaram, K., Indhumathi, J. (2009). Production of protease from rice mill wastes by *Aspergillus niger* in solid state fermentation. *World Journal of. Agricultural Science*, 5(3), 308-312.

[11] Devi, M.K., Banu, A.R., Gnanaprabhal, G.R., Pradeep, B.V., Palaniswamy, M. (2008). Purification, characterization of alkaline protease enzyme from native isolate *Aspergillus niger* and its compatibility with commercial detergents. *Indian Journal of Science and Technology*, 1(7), 1-6.

[12] Çetiner, M.E., Bilek, S. (2018). Protein kaynakları. *Çukurova Tarım Gıda Bilim Dergisi,* 33(2), 111-126.

[13] Sankeerthana, C., Pinjar, S., Jambagi, S., Bhavimani, R.T., Anupama, S., Sarovar, B.S., Inamdar, S.R. (2013) Production and partial characterization of protease from aspergillus flavus using rice mill waste as a substrate and its comparison with *Aspergillus niger* protease. *International Journal of Current Engineering Technology,* 1, 143-147.

[14] Upgade, A., Nandeshwar, A., Samant, L. (2011). Assessment of fungal protease enzyme from French bean using *A. niger* by solid state fermentation. *Journal of Microbiology and Biotechnology Research*, 1(4), 45-51. A. Upgade,

[15] Qazi, J.I., Jamshaid, H., Nadeem, M., Ali S.S. (2008). Production of proteases by *Aspergillus niger* through solid state fermentation. *Punjab University Journal of Zoology*, 23(1-2), 037-046.

[16] Shivakumar, S. (2012). Production and characterization of an acid protease from a local Aspergillus sp. by Solid substrate fermentation. *Archives of Applied Science Research*, 4(1), 188-199.

[17] Oyewole, O.A., Oyeleke, S.B., Dauda, B.E.N., Emiade, S. (2011). Production of amylase and protease enzymes by *Aspergillus niger* and *Penicillium frequestans* isolated from abattoir effluent. *Microbiology Journal*, 1(5), 174-180.

[18] Chimbekujwo, K.I., Ja'afaru, M.I., Adeyemo, O.M. (2020). Purification, characterization and optimization conditions of protease produced by *Aspergillus brasiliensis* strain BCW2. *Scientific African*, 8(1), 2-9.

[19] Rao, M.B., Tanksale, A.M., Ghatge, M.S., Deshpande, V.V. (1998). Molecular and biotechnological aspects of microbial proteases. *Microbiology and molecular biology reviews*, 62(3), 597-635.

[20] Coral, G., Arikan, B., Unaldi, M.N., Guvenmez, H. (2003). Thermostable alkaline protease produced by an *Aspergillus nig*er strain. *Annals of Microbiology*, 53(4), 491-498.

[21] Olajuyigbe, F.M. (2006). Influence of media composition on acid protease production by *Aspergillus niger* (NRRL 1785). *Global Journal of Pure and Applied Sciences*, 12(1), 85-88.

[22] Sattar, H., Aman, A., Qader, S.A.U. (2017). Effect of metal ions, solvents and surfactants on the activity of protease from *Aspergillus niger* KIBGE-IB36. *Journal of Basic & Applied Sciences*, 13, 491-495.

[23] Pham, T. H., Quyen, D.T., Nghiem, N.M. (2012). Purification and properties of an endoglucanase from *Aspergillus niger* VTCC-F021. *Turkish Journal of Biology*, *36*(6), 694-701.

[24] Basu, B. R., Banik, A.K., Das, M. (2008). Production and characterization of extracellular protease of mutant *Aspergillus niger* AB 100 grown on fish scale. *World Journal of Microbiology and Biotechnology,* 24, 449-455.

[25] Muazu, A. (2015). Production and characterization of extracellular protease enzyme from *Aspergillus niger* using different agro-industrial residues. *International Journal of Science and Technology*, 4(12), 535-541.

[26] Leng, Y.W., Xu, Y. (2011). Improvement of acid protease production by a mixed culture of *Aspergillus niger* and *Aspergillus oryzae* using solid-state fermentation technique. *African Journal of Biotechnology*, 10(35), 6824-6829.

[27] Saxena, L., Iyer, B.K., Ananthanarayan, L. (2007). Three phase partitioning as a novel method for purification of ragi (*Eleusine coracana*) bifunctional amylase/protease inhibitor. *Process Biochemistry*, 42(3), 491-495.

[28] Konak, Ü. İ., Karakaş, B., Certel, M., Erem, F. (2012). Katı faz fermentasyon yöntemi ile enzim üretimi. *Akademik Gıda*, 10(3), 84-92.

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Araştırma Makalesi / Research Paper

Nar Kabuğundan Ultrason Destekli Ekstraksiyon Uygulamasıyla Fenolik Bileşen Ekstraksiyonu ve Ekstraktların Antioksidan Aktiviteleri

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ÖZ

Bu çalışmanın amacı, sıfır atık yaklaşımıyla nar kabuklarından (Punica granatum L.) biyoaktif bileşenlerin ultrason destekli ekstraksiyon (UDE) yöntemi kullanılarak elde edilmesi ve farklı ekstraksiyon koşullarının fenolik madde içeriği ile antioksidan kapasite üzerindeki etkilerinin belirlenmesidir. Taze nar kabuklarının iç ve dış kısımları ayrı ayrı değerlendirilmiş, %80'lik metanol çözgeni ile farklı ultrasonik güç (%50 ve %100) ve süre (5, 10 ve 20 dakika) kombinasyonlarında ekstraksiyon gerçekleştirilmiştir. Elde edilen sonuçlara göre, dış kabuğun toplam fenolik madde içeriği %100 güç ve 10 dakika ekstraksiyon süresinde maksimum 58.73 ± 0.79 mg GAE/g KM değerine ulaşmıştır. En yüksek antioksidan kapasite ise dış kabuk örneklerinde %100 güç ve 20 dakikada 3177.75 ± 6.06 ppm TEAC/g KM olarak ölçülmüştür. İç zar örneklerinde ise en yüksek fenolik madde miktarı 7.31 ± 0.03 mg GAE/g KM değerinde bulunmuştur. FTIR ve SEM analizleri, nar kabuğunun yapısal bütünlüğünü koruyarak aktif bileşenlerin salımını kolaylaştırdığını göstermiştir. Bu bulgular, nar kabuklarının çevre dostu yaklaşımlarla değerlendirilerek gıda ve ilaç sanayilerinde katma değerli ürünler elde edilmesi için önemli bir potansiyele sahip olduğunu ortaya koymaktadır.

Anahtar Kelimeler: Nar kabuğu, Antioksidan kapasite, Fenolik bileşenler, Ultrasonik ekstraksiyon

Extraction of Phenolic Compounds from Pomegranate Peels by Ultrasound Assisted Extraction and Determination of Antioxidant Activities

ABSTRACT

The aim of this study was to extract bioactive compounds from pomegranate peels (Punica granatum L.) using the ultrasound-assisted extraction (UDE) method within a zero-waste approach and to investigate the effects of different extraction conditions on total phenolic content and antioxidant capacity. Fresh pomegranate peels were separated into inner and outer layers, and extractions were performed using 80% methanol as a solvent under different ultrasound power levels (50% and 100%) and durations (5, 10, and 20 minutes). The results showed that the outer peel exhibited the highest total phenolic content, reaching 58.73 ± 0.79 mg GAE/g DM under 100% power and 10 minutes of extraction. The maximum antioxidant capacity was observed in outer peel extracts at 3177.75 ± 6.06 ppm TEAC/g DM with 100% power and 20 minutes. In the inner peel, the highest total phenolic content was found as 7.31 ± 0.03 mg GAE/g DM. FTIR and SEM analyses demonstrated that the structural integrity of the pomegranate peel was preserved while facilitating the release of active compounds. These findings suggest that pomegranate peels possess significant potential for valorization through environmentally friendly processes, offering opportunities for the development of value-added products in the food and pharmaceutical industries.

Keywords: Pomegranate peel, Antioxidant capacity, Phenolic compounds, Ultrasonic extraction

GİRİŞ

Nar (*Punica granatum L.*) kabuğu, meyve işleme endüstrisinin en önemli yan ürünlerinden biri olup, meyvenin ağırlığının %26-30'unu oluşturmaktadır [1]. Geleneksel olarak hayvan yemi olarak kullanılan ya da atık olarak çevreye bırakılan nar kabukları, içerdiği biyoaktif bileşenler sayesinde son yıllarda büyük ilgi görmektedir. Punicalagin, ellajik asit, gallik asit ve kuersetin gibi fenolik bileşikler ile antosiyaninler ve ellajitanenler açısından zengin olan nar kabukları, güçlü antioksidan, antimikrobiyal ve sağlığı destekleyici özellikler sergilemektedir [2, 3]. Bu özellikler, nar kabuklarının doğal koruyucu ve fonksiyonel gıda bileşeni olarak kullanımına yönelik önemli bir potansiyel sunduğunu göstermektedir [4, 5]. Nar kabuğu, meyvenin yenilebilir kısmına kıyasla çok daha yüksek miktarda fenolik bileşik içermekte ve güçlü antioksidan özellikler sergilemektedir [9].

Biyoaktif bileşiklerin bitkisel materyallerden eldesinde kullanılan geleneksel yöntemlerden Soxhlet ekstraksiyonu, maserasyon ve hidrodistilasyon, uzun süredir yaygın olarak kullanılan yöntemlerdir [6]. Ancak bu yöntemler, uzun ekstraksiyon süreleri, pahalı oluşları, saf çözücü ihtiyacı ve ısıya hassas bileşenlerin zarar görmesi gibi dezavantajlara sahiptir [1, 7, 8]. Bitkisel materyallerden biyoaktif bileşenlerin eldesi için yaygın olarak kullanılan geleneksel ekstraksiyon yöntemleri, uzun işlem süreleri, yüksek çözücü tüketimi ve ısıya duyarlı bileşiklerin bozulması gibi sınırlamalara sahiptir [6]. Bu durum, daha verimli ekstraksiyon yöntemlerine olan ihtiyacı artırmıştır. Ultrason destekli ekstraksiyon (UDE), bu alandaki en etkili yöntemlerden biri olarak öne çıkmaktadır. UDE, yüksek frekanslı ses dalgalarının bir sıvı ortamda yayılmasıyla oluşan kavitasyon etkisini kullanarak bitki materyalinden biyoaktif bileşiklerin çözgen içine salımını kolaylaştıran bir yöntemdir. UDE, kavitasyon etkisiyle bitki hücre duvarlarını parçalayarak biyoaktif bileşenlerin çözgen içine daha hızlı ve etkin bir şekilde geçmesini sağlamaktadır [9]. Literatürde UDE'nin geleneksel yöntemlere kıyasla ekstraksiyon verimini %20-30 oranında artırdığı ve işlem süresini %80-90 oranında azalttığı görülmüştür [10, 11]. Ayrıca, düşük sıcaklıkta çalışması sayesinde ısıya hassas bileşiklerin korunmasını ve enerji tasarrufu sağlamaktadır [12].

Bu çalışmanın amacı, nar kabuğundan ultrason destekli ekstraksiyon (UDE) yöntemi kullanılarak fenolik maddeler gibi biyoaktif bileşenlerin eldesi için uygun ekstraksiyon koşullarının belirlenmesidir. Çalışmada, fenolik bileşiklerin çözünürlüğünün yüksek olması nedeniyle ekstraksiyon çözgeni olarak %80'lik metanol tercih edilmiştir. Farklı süreler ve ultrasonik güç seviyeleri kullanılarak gerçekleştirilen ekstraksiyon işlemleriyle, fenolik madde ve antioksidan kazanımını etkileyen koşullar araştırılmıştır. Bu kapsamda, UDE yönteminin geleneksel ekstraksiyon yöntemlerine kıyasla solvent kullanımını azaltma, ekstraksiyon süresini kısaltma ve işlem verimini artırma potansiyeli değerlendirilmiştir. Çalışmanın özgün yönü, nar kabuklarının sıfır atık yaklaşımıyla katma değerli ürünlere dönüştürülmesi ve yenilikçi ekstraksiyon tekniklerinin gıda ve ilaç endüstrilerinde sürdürülebilir kullanımına yönelik literatüre katkı sağlamasıdır.

MATERYAL ve METOT

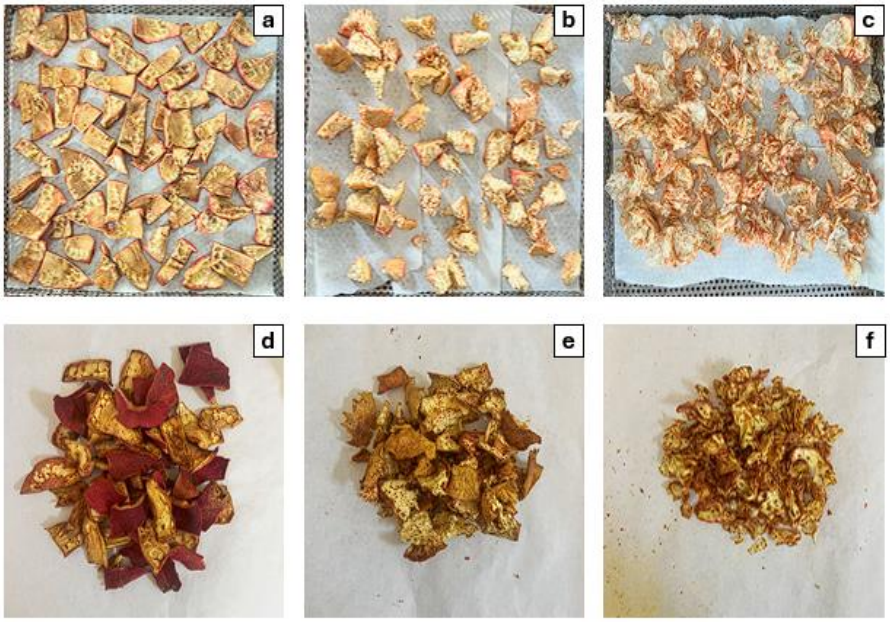
Materyal

Çalışmada kullanılan taze nar (*Punica granatum L.*) örnekleri, 2024 yılı Eylül ayında bir yerel marketten (Bornova, İzmir, Türkiye) sağlanmış ve ekstraksiyon işlemlerine kadar 4 °C'de muhafaza edilmiştir. Narlar yıkandıktan sonra kurulandı ve nar dış kabukları (perikarp), narların tutunduğu bölüm (mezokarp) ve iç zarlar, nar tanelerinden el yardımıyla ayıklanarak elde edilmiştir. Örnekler polietilen torbalarda paketlenmiştir ve 4°C sıcaklıkta, buzdolabında, kurutma işlemi yapılana kadar bekletilmiştir. Çalışmada kullanılan narların genel özelliklerini temsil etmek amacıyla seçilmiş örneklerin görselleri Şekil 1'de verilmiştir.

Kurutma Yöntemi

Kurutma işleminde Mphahlele vd. [13] tarafından yapılan çalışmada belirtilen yöntem kullanılmıştır. Kurutma işleminden önce 4°C sıcaklıktaki örnekler oda sıcaklığına ulaşılıncaya kadar bekletilmiştir. Çalışma kapsamında kurutma işlemi için pilot ölçekli tepsili kurutucu (Armfield Lim., Ringwood, Hampshire, Birleşik Krallık) kullanılmıştır. Oda sıcaklığındaki örnekler, cihazın tepsilerine yerleştirilip kurutma işlemine tabi tutulmuştur. Kurutma 40°C hava sıcaklığı ve 1.0 m/s hava hızında gerçekleştirilmiştir. Örnekler sabit tartıma gelene kadar kurutma işlemine devam edilmiştir. Nar dış kabuğu, mezokarp ve iç zarının kurutma öncesi ve sonrasındaki görünümü Şekil 2'deki gibidir.

Şekil 1. Satın alınan narlar (a), yıkanmış ve temizlenmiş nar (b), nar dış kabuğu (c), narların tutunduğu bölüm (d) ve iç zarlar (e)
Figure 1. Purchased pomegranates (a), washed and cleaned pomegranates (b), pomegranate outer peel (c), the part where the pomegranates are attached (d) and inner membranes (e)



Şekil 2. Kurutmadan önce nar dış kabuğu (a), mezokarp (b) ve iç zar (c); kurutulduktan sonra nar dış kabuğu (d), mezokarp (e) ve iç zarı (f)
Figure 2. Pomegranate outer peel (a), mesocarp (b) and inner membrane (c) before drying; pomegranate outer peel (d), mesocarp (e) and inner membrane (f) after drying

Kurutmadan sonra örnekler oda sıcaklığına getirilmiş ve öğütücü (Beehive Mechanical Grinder, ABD) yardımıyla öğütülerek 250 µm elekten geçirilmiştir. Mezokarp ve iç zar karıştırılarak örnek nar kabuğu iç zarı olarak adlandırılmış ve örnekler polietilen torbalarda analizler gerçekleşene kadar saklanmıştır. Şekil 3'de, çalışmada kullanılan nar kabuğunun dış kabuk, iç zar ve orta kısımlarına ait kurutulmuş ve öğütülmüş örneklerin görünümü verilmiştir.

Şekil 3. Öğütülmüş nar kabuğu dış kabuk (a), mezokarp (b) ve iç zar (c)
Figure 3. Ground pomegranate peel outer shell (a), mesocarp (b) and inner membrane (c)

Nem ve Su Aktivitesi Tayini

Yaş örneklerin nem içerikleri, AOAC (2005), Official Method 934.06 [14] yöntemine bazı küçük değişiklikler yapılarak belirlenmiştir. Örnekler 105±0.5 °C sıcaklıktaki etüvde kurutulmuş ve kütle kaybı yöntemi esas alınarak nem oranları hesaplanmıştır. Kuru örneklerin nem içerikleri ise nem tayini cihazı (Radwag MA 50.R, Polonya) kullanılarak, üretici firmanın standart protokolüne uygun şekilde ölçülmüştür.
Kurutulmuş nar kabuğu ve zar kısmının su aktivitesi değerlerinin ölçümünde su aktivitesi ölçüm cihazı (Testo AG400, Lenzkirch, Almanya) kullanılmıştır. Numuneler sızdırmaz bir çelik hazneye yerleştirilmiş, sabit sıcaklıkta (25 °C) tutulmuş ve ürün-hava arasındaki nem alışverişinin durmasıyla denge sağlanıncaya kadar beklenmiştir. Dengeye ulaşıldığında hazne içindeki havanın bağıl nemi cihazın probu ile ölçülmüş ve bu değer doğrudan su aktivitesi (aw) olarak kaydedilmiştir.

Toplam Fenolik Madde Miktarı Tayini

Toplam fenolik madde miktarının belirlenmesi amacıyla ekstraktların hazırlanmasında 5 g toz haline getirilmiş örneğe 75 mL %80'lik metanol eklenmiş ve bu karışım oda sıcaklığında 250 rpm hızla çalışan manyetik karıştırıcıda 21 saat boyunca karıştırılarak ön ekstraksiyon gerçekleştirilmiştir. Ardından, homojenize edilen karışıma oda sıcaklığında %50 ve %100 güç seviyelerinde, 5, 10 ve 20 dakika süreyle ultrason destekli ekstraksiyon (UDE) uygulanmıştır. Bu yöntemle, fenolik bileşiklerin ekstraksiyon veriminin artırılması ve ekstraksiyon süresinin azaltılması amaçlanmıştır. Ultrason destekli ekstraksiyon sırasında özellikle yüksek güç seviyelerinde (özellikle %100 güçte) oluşabilecek ısınmayı önlemek amacıyla, çift cidarlı örnek haznesi kullanılmıştır. Haznenin cidarları arasında dolaştırılan antifrizli su sayesinde, işlem süresince örnek ve çözücü sıcaklığı stabil tutulmuş ve sıcaklığa bağlı olası fenolik madde bozulmaları engellenmiştir. Elde edilen ekstraktlardan toplam fenolik madde miktarı tayini Singleton vd. (1999) tarafından verilen yönteme göre yapılmıştır [15]. Bunun için ekstraktlardan 200 µL alındıktan sonra sonra 1 mL saf su ve 1 mL 0.2 N Folin reaktifi ile karıştırılıp 5 dakika süreyle beklenmiştir. Reaksiyon karışımına 800 µL %20'lik Na_2CO_3 ilave edilmiş ve oda sıcaklığında 1 saat bekletilmiştir. İnkübasyon sonrası karışımın 760 nm dalga boyunda Cary 60 UV–vis spektrofotometrede absorbans değerleri belirlenmiş, sonuçlar 'mg/g KM gallik asit eşdeğeri (GAE)' olarak verilmiştir.

Antioksidan Aktivitesinin Belirlenmesi

Antioksidan aktivitenin belirlenmesi amacıyla, fenolik madde analizi için hazırlanmış ekstraktlar kullanılmıştır. Ekstraktlardaki antioksidan aktivitenin kapsamlı değerlendirmesinde Re vd. (1999) tarafından yapılmış olan çalışmadaki yöntemden yararlanılmıştır [16]. Bu amaçla kullanılacak ABTS çözeltisi; 1.92 mg ABTS reaktifinin 0.5 mL saf su içerisinde çözündürülmesiyle, potasyum persülfat çözeltisi; 0.33125 mg Potasyum persülfatın 0.5 mL saf su içerisinde çözündürülmesiyle hazırlanmıştır. İki çözelti birleştirilip 16 saat oda sıcaklığında karanlıkta reaksiyona bırakılmıştır. Sonuçlar Trolox® eşdeğer antioksidan kapasitesi (TEAC) açısından niceliksel olarak belirlenmiştir.

Hem Trolox® çözeltileri hem de örnekler için inhibisyon değerlerinin yüzdesi aşağıdaki Denklem 1 kullanılarak belirlenmiştir.

$$inhibisyon(\%) = \frac{(A_{ABTS^{\bullet+}} - A_{örnek})}{A_{ABTS^{\bullet+}}} \times 100 \quad (1)$$

$A_{ABTS^{\bullet+}}$, $ABTS^{\bullet+}$'nin 734 nm'deki absorbansı, $A_{örnek}$ örneğin 734 nm'deki absorbansıdır.

Örneklerin antioksidan kapasiteleri, 734 nm'ye ayarlanmış bir Cary 60 UV-vis spektrofotometresi kullanılarak, reaksiyon başlangıcından 6 dakika sonra ölçülmüştür. Antioksidan kapasiteleri, Trolox® standart çözeltileri kullanılarak oluşturulan kalibrasyon eğrisi üzerinden hesaplanmıştır. Kalibrasyon eğrisi, 5 ppm ile 500 ppm arasında değişen Trolox® konsantrasyonları kullanılarak hazırlanmış ve sonuçlar mM TEAC (Trolox® Equivalent Antioxidant Capacity) cinsinden ifade edilmiştir. Tüm analizler her örnek için üç paralel ölçüm yapılarak gerçekleştirilmiş ve çalışma iki bağımsız tekerrür ile yürütülmüştür.

Fourier Dönüşümlü Kızılötesi Spektroskopisi (FT-IR) Analizi

Dondurularak kurutulmuş ekstrakt örneklerinin spektrum analizleri, 400–4000 cm^{-1} dalga boyu aralığında çalışan bir FT-IR spektrofotometre (Thermo Scientific NICOLET iS10, ABD) kullanılarak gerçekleştirilmiştir.

Taramalı Elektron Mikroskop (SEM) Analizi

Kurutulmuş nar kabuğu iç zarının ve dış kabuğunun morfolojik görüntüleri, 5.0 kV ivme voltajı kullanılarak bir taramalı elektron mikroskobu (SEM, Thermo Fisher Scientific QUANTA FEG 250, Waltham, MA, USA) ile elde edilmiştir. Örnekler Au-Pd alaşım ile kaplanarak 250-5000 kat büyütülerek örneklerin görüntülerinin alınması sağlanılmıştır. Mikro gözenekli yapılar (Şekil 8 ve Şekil 9) 250x ve 5000x çözünürlükte gözlenirken, görüntüler sırasıyla 400 µm ve 20µm parçacık boyutuyla görüntüleme yapılmıştır.

İstatistiksel Analiz

Tüm veriler ortalama değerler (±standart sapma) olarak sunulmuştur. Tek yönlü varyans analizi (ANOVA) SPSS (SPSS Inc., Chicago, ABD) kullanılarak gerçekleştirilmiştir. İstatistiksel anlamlılık ($p<0.005$), ortalamalar Duncan Çoklu Karşılaştırma Testi kullanılarak verilmiştir.

BULGULAR ve TARTIŞMA

Nem ve Su Aktivitesi Tayini

Kurutulmuş örneklerin nem (%) içerikleri ve su aktiviteleri belirlenmiştir. Kurutulmuş nar kabuğu iç zarının nem oranı %77.83'ten %9.05'e, dış kabuğunun nem oranı ise %73.65'ten %8.38'e düşmüştür. Literatürde nar kabuklarının farklı kurutma teknikleri (konveksiyonel kurutma ve dondurarak kurutma) kullanılarak kurutulmasına yönelik gerçekleştirilen bir çalışmada [17] da benzer sonuçlar rapor edilmiştir. Söz konusu çalışmada dondurarak kurutulan nar kabuklarının başlangıç ve son nem içeriklerinin sırasıyla %73.6 ve %7.7 olduğu belirtilmiş olup, bu sonuçların mevcut çalışma bulgularıyla paralellik gösterdiği görülmektedir.
Kurutma işleminden sonra ürünlerin su aktivitesi değerleri ise 0.300 ve 0.381 olarak ölçülmüştür. Düşük su aktivitesi ürünlerdeki gıda bozulmasına ve mikroorganizma büyümesine yol açan enzimatik ve oksidatif reaksiyonların gerçekleşmesini azaltmakta ya da önleyebilmektedir [18, 19]. Kurutma işlemi sonrasında elde edilen nar kabuğu örneklerinde su aktivitesinin düşmesi, mikrobiyal gelişim riskini azaltarak depolama stabilitesine katkı sağlayabilecek potansiyel göstermiştir.

Toplam Fenolik Madde İçeriği Tayini

Nar kabuğu iç zarı örneklerindeki toplam fenolik madde miktarları Tablo 1'de görüldüğü gibidir. Çalışmada nar kabuğu iç zarı için fenolik madde miktarı, 7.30±0.02 mg GAE/g KM olarak ölçülmüştür. Dış kabuk için fenolik madde miktarı ise en çok 58.73±0.79 mg GAE/g KM olarak ölçülmüştür. Bu bulgu, literatürde bildirilen bazı değerlerden düşük, bazılarıyla ise benzerlik göstermektedir. Örneğin, Derakhshan vd. [20] çalışmasında 100 gram toz nar kabuğu örneğinin 48 saat boyunca oda sıcaklığında etanol (%80) kullanılarak yapılan ekstraksiyonunda fenolik madde miktarı 276-413 mg GAE/g KM olarak belirlenmiştir. El-Hamamsy ve El-Khamissi [21] tarafından yapılan çalışmada ise, etanol çözgeni ile elde edilen fenolik madde miktarı 161.5±0.5 mg GAE/g KM olarak rapor edilmiştir [22]. Bu farklar, kullanılan çözgen, ekstraksiyon yöntemi, sıcaklık ve süre gibi parametrelerin yanında nar türü, yetiştirilme koşulu gibi parametrelerin de etkisinden kaynaklanabilir. etkisinden kaynaklanabilir.

Ek olarak, Campos vd. (2022) nar kabuğu üzerine yaptığı çalışmasında etanol/su karışımı (%0, %25, %50 ve %75 EtOH, v/v) kullanılarak yapılan ekstraksiyonlarda fenolik içerik, çözgen konsantrasyonuna bağlı olarak değişkenlik göstermiş ve en yüksek fenolik miktar 0.86 mg GAE/mg (75% EtOH) olarak bildirilmiştir [23]. Elde edilen sonuçlar, kullanılan ekstraksiyon koşulları altında metanolün toplam fenolik madde ekstraksiyonunda etkili bir çözücü olabileceğini göstermektedir. Ayrıca, Suleria vd. [24] nar kabuğu için etanol kullanılarak yapılan ekstraksiyonda toplam fenolik içerik miktarını 3.34 mg GAE/g KM olarak bildirmiştir, bu da çalışmamızdaki verilerin daha yüksek fenolik içeriği işaret ettiğini göstermektedir.

Yapılan bu çalışmada nar kabuğu iç zarı için toplam fenolik madde miktarına bakıldığında, hem ekstraksiyon süresinin hem de ultrasonik gücün etkili olduğu gözlenmiştir. Kontrol grubuna (0 güç) kıyasla %50 ve %100 güç seviyelerinde toplam fenolik madde miktarı artış göstermiştir. Özellikle 10 dakika ve %100 güç kombinasyonu (7.3082±0.02698 mg GAE/g KM), en yüksek fenolik madde miktarını sağlamıştır. Süre açısından, 20 dakikalık ekstraksiyon (hem %50 hem de %100 güçte) benzer fenolik madde miktarları üretmiştir. Bu durum, iç zar için belirli bir güç ve süreden sonra fenolik madde ekstraksiyonunun doyuma ulaştığını düşündürmektedir.

Fenolik içerik değerleri için kolonlardaki küçük harf farklılıkları Duncan testine göre süre için istatistiksel grup farklılıklarını ifade ederken, büyük harfler Duncan testine göre ultrasonik güç için istatistiksel grup farklılıklarını ifade etmektedir.

Tablo 1. Nar kabuğu iç zarı örneklerindeki toplam fenolik madde miktarları
Table 1. Total phenolic content in pomegranate peel inner membrane samples

Örnek	Güç (Watt)	Süre (dakika)	mg GAE/g KM
Nar kabuğu iç zarı	0	0	6.44 ± 0.01^{aA}
	%50	5	6.51 ± 0.21^{bB}
		10	7.26 ± 0.02^{dB}
		20	7.01 ± 0.20^{cB}
	%100	5	6.78 ± 0.39^{bC}
		10	7.31 ± 0.03^{dC}
		20	7.19 ± 0.05^{cC}

Nar kabuğu dış kabuğunda ise toplam fenolik madde miktarı, güç ve süre değişimlerine karşın daha sınırlı bir değişim göstermiştir. Kontrol grubunda (0 güç) elde edilen fenolik madde miktarı (58.5426±0.41682 mg GAE/g KM), diğer gruplarla istatistiksel olarak benzer ya da daha yüksektir. %50 ve %100 güç seviyelerinde 10 dakika ekstraksiyon süresi ile fenolik madde miktarı bir miktar artış göstersede, bu artış istatistiksel olarak anlamlı değildir. 20 dakikalık ekstraksiyonlarda (hem %50 hem de %100 güç) fenolik madde miktarında hafif bir azalma görülmüştür. Bu durum, dış kabuk için daha kısa süreli ve kontrollü bir ekstraksiyonun fenolik bileşenlerin korunması açısından daha uygun olduğunu göstermektedir.

Tablo 2. Nar kabuğu dış kabuk örneklerindeki toplam fenolik madde miktarları
Table 2. Total phenolic content in pomegranate peel samples

Örnek	Güç (Watt)	Süre (dakika)	mg GAE/g KM
Nar kabuğu dış kabuk	0	0	58.54 ± 0.42^{bB}
	%50	5	56.24 ± 0.98^{aA}
		10	57.72 ± 0.53^{bA}
		20	57.01 ± 0.31^{aA}
	%100	5	57.14 ± 0.33^{aA}
		10	58.73 ± 0.79^{bA}
		20	55.89 ± 0.76^{aA}

Fenolik içerik değerleri için kolonlardaki küçük harf farklılıkları Duncan testine göre süre için istatistiksel grup farklılıklarını ifade ederken, büyük harfler Duncan testine göre ultrasonik güç için istatistiksel grup farklılıklarını ifade etmektedir.

Nar kabuğu dış kabuk örneklerindeki toplam fenolik madde miktarları Tablo 2'de görülmektedir. Genel olarak, nar kabuğunun dış kısmı iç zarına kıyasla daha yüksek fenolik madde içeriğine sahiptir. Bu durum, dış kabuğun fenolik bileşiklerin birincil depolama alanı olması ve antioksidan kapasitesinin daha yoğun olmasından kaynaklanabilir. Literatürde de sıkça vurgulandığı üzere, dış kabuktaki fenolik bileşiklerin yüksekliği, bu kısmın doğal bir antioksidan kaynağı olarak daha fazla potansiyele sahip olduğunu göstermektedir [22, 25].
Sonuç olarak, çalışmada bulunan değerler literatürdeki diğer çalışmalarla kıyaslandığında, kullanılan yöntemlerin fenolik içerik miktarını etkilediğini doğrulamaktadır. Elde edilen bulgular, UDE yönteminde uygulanan ekstraksiyon süresi ve ultrasonik güç seviyelerinin, nar kabuğu fenolik bileşenlerinin verimli bir şekilde elde edilmesinde etkili bir yöntem olduğunu göstermektedir.

Antioksidan Aktivite Analizi

Nar kabuğu iç zarı için yapılan analizlerde, ultrasonik güç seviyesinin (%50 ve %100) TEAC değerlerinde anlamlı bir artış sağlamadığı, bu nedenle ultrasonik gücün iç zar üzerindeki etkisinin sınırlı olduğu görülmektedir. Tablo 3, nar kabuğu iç zarı örneklerindeki antioksidan aktivite miktarlarını göstermektedir. Ekstraksiyon süresi açısından, 10 dakika süre ile elde edilen sonuçlar benzer olsa da, %50 güçte 20 dakikalık süre ile TEAC değerinde hafif bir artış gözlenmiştir. Bu durum, iç zarın antioksidan kapasitesini artırmada ekstraksiyon süresinin daha belirleyici olduğunu göstermektedir.

Antioksidan aktivite değerleri için kolonlardaki küçük harf farklılıkları Duncan testine göre süre için istatistiksel grup farklılıklarını ifade ederken, büyük harfler Duncan testine göre ultrasonik güç için istatistiksel grup farklılıklarını ifade etmektedir.

Nar kabuğu dış kabuğunda ise hem ultrasonik güç hem de ekstraksiyon süresinin antioksidan aktivite üzerinde belirgin bir etkisi olduğu görülmektedir. Özellikle %100 güç ve 20 dakika ekstraksiyon süresiyle en yüksek TEAC değerine (3177.75±6.06) ulaşılmış, bu da hem uzun sürelerin hem de yüksek ultrasonik güç seviyelerinin dış kabuğun antioksidan çıkışını artırmada etkili olduğunu göstermektedir. Ayrıca, dış kabuk genel olarak iç zardan daha yüksek TEAC değerlerine sahiptir ve bu durum dış kabuğun daha zengin fenolik bileşen içeriğine sahip olduğunu düşündürmektedir.

Tablo 3. Nar kabuğu iç zarı örneklerindeki antioksidan aktivite değerleri
Table 3. Amounts of antioxidant activity in pomegranate peel inner membrane samples

Örnek	Güç (Watt)	Süre (dakika)	TEAC ppm/g KM
Nar kabuğu iç zarı	0	0	319.50 ± 1.38^{aA}
	%50	5	320.47 ± 0.12^{bB}
		10	321.33 ± 0.57^{bB}
		20	319.54 ± 0.04^{aB}
	%100	5	320.56 ± 0.04^{bB}
		10	320.46 ± 0.02^{bB}
		20	319.02 ± 0.31^{aB}

Tablo 4. Nar kabuğu dış kabuk örneklerindeki antioksidan aktivite miktarları
Table 4. Amounts of antioxidant activity in pomegranate peel outer shell samples

Örnek	Güç (Watt)	Süre (dakika)	TEAC ppm/g KM
Nar kabuğu dış kabuk	0	0	3167.11 ± 5.46^{cB}
	%50	5	3167.96 ± 2.40^{abA}
		10	3167.47 ± 1.84^{bcA}
		20	3111.78 ± 21.26^{aA}
	%100	5	3136.92 ± 23.05^{abAB}
		10	3155.40 ± 3.39^{bcAB}
		20	3177.75 ± 6.06^{aAB}

Antioksidan aktivite değerleri için kolonlardaki küçük harf farklılıkları Duncan testine göre süre için istatistiksel grup farklılıklarını ifade ederken, büyük harfler Duncan testine göre ultrasonik güç için istatistiksel grup farklılıklarını ifade etmektedir.

Tablo 4, nar kabuğu dış kabuk örneklerindeki antioksidan aktivite değerlerini vermektedir. Çalışmada nar kabuğu iç zarı için antioksidan aktivite miktarı en çok 321.33±0.57 TEAC ppm/g KM olarak, dış kabuk için ise antioksidan aktivite miktarı en çok 3177.75±6.06 TEAC ppm/g KM olarak ölçülmüştür. Bu sonuçlar literatürde bildirilen değerler ile karşılaştırıldığında bazı çalışmalardan düşük, bazılarıyla ise benzerlik göstermektedir. Ancak, literatürdeki çalışmaların ekstraksiyon yöntemi, çözgen tipi, analiz koşulları ve kullanılan standart birimleri farklılık gösterebilmektedir. Örneğin Suleria vd.[24] 2.0±0.5 g nar kabuğu örneklerini 20 mL %70 etanol ile ekstrakte ederek ABTS testi uygulamış ve antioksidan aktiviteyi 3.34±0.09 mg AAE/g olarak bulunmuştur.

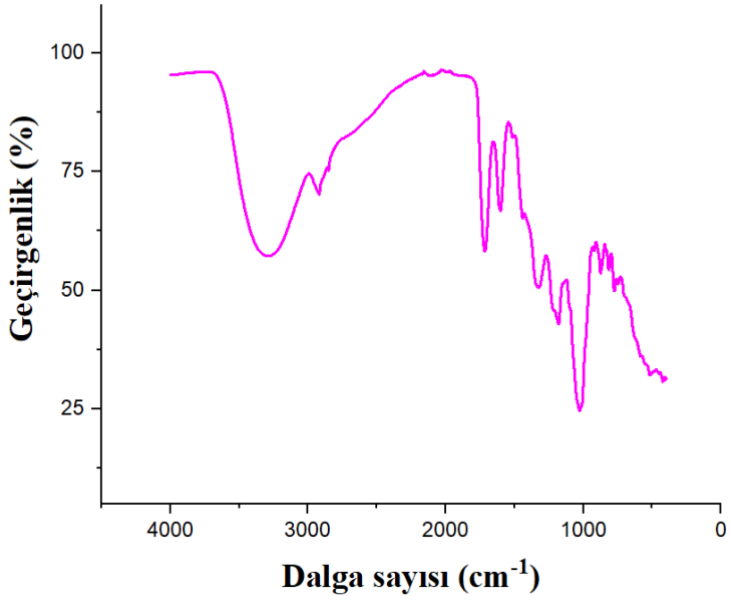
Yan vd. (2017) yapmış olduğu çalışmada ise altı farklı nar çeşidi (Bolicui (SD-BLC), 00Damaya (SD-DMY), Qingpi (SD-QP, Kashgar Suan (XJ-KS), Hotan CeLe1# (XJ-HT), and Piyaman (XJ-PYM)) %80 metanol-su çözeltisi ile ekstrakte edilerek ABTS analizi için antioksidan değerlerine bakılmıştır [26]. Belirtilen farklı nar örneklerinde sırasıyla 422.8±0.7 mg TEAC g^{-1}, 805.9±32.5 mg TEAC g^{-1}, 1105.1±27.6 mg TEAC g^{-1}, 760.8±10.7 mg TEAC g^{-1}, 720.8±30.2 mg TEAC g^{-1}, 579.1±37.6 mg TEAC g^{-1} antioksidan aktivite değerleri bulunmuştur.

Elfalleh vd. (2012) tarafından gerçekleştirilen bir çalışmada, hasat edilen nar örneklerinin kabukları ayrılmış, bu kabuklar güneşte kurutulduktan sonra toz haline getirilmiştir [27]. Daha sonra 10 g kabuk örneği, 30°C'de bir gece boyunca 100 mL metanol içinde ekstrakte edilmiştir. Elde edilen ekstrakt, partiküllerin uzaklaştırılması amacıyla Whatman No.1 filtre kağıdından süzülmüş ve ABTS radikal süpürme aktivitesi analiz edilmiştir. Metanol ekstraktında kabukların ABTS aktivitesi 7.50±0.83 TEAC mmol/100 g kuru ağırlık olarak tespit edilmiştir.

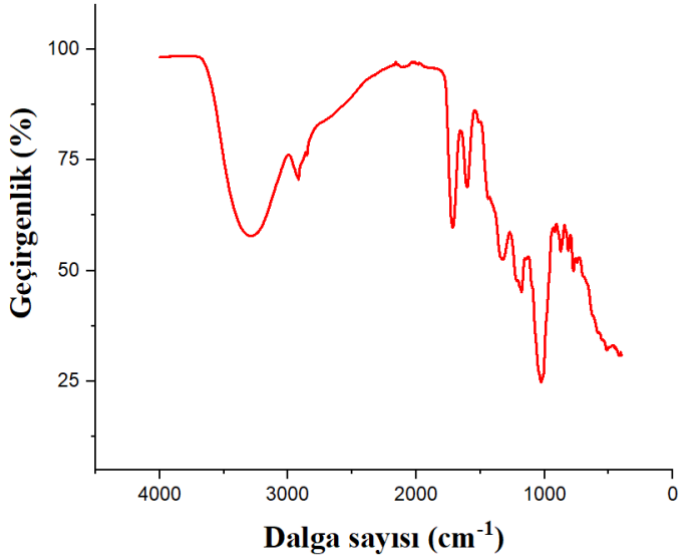
Tüm bu sonuçlara göre nar kabuğundaki antioksidan miktarının farklılığı çözgen madde, ekstraksiyon yöntemi ve nar çeşidi gibi parametrelere göre değişiklik gösterebilmektedir. Ayrıca nar kabuğunun antioksidan içeriğinin, çiçek, yaprak ve çekirdek gibi diğer nar bileşenlerine kıyasla daha yüksek olduğu rapor edilmiştir.

Fourier Dönüşümlü Kızılötesi Spektroskopisi (FT-IR) Analizi

Nar kabuğunun kontrol FTIR spektrumu (kırmızı kabuk) ile %100 gücünde ve 10 dakika UDE işlemiyle elde edilen ekstraktın FTIR spektrumu karşılaştırıldığında, her iki spektrumda da karakteristik bantların yer aldığı gözlemlenmiştir. FTIR spektrumuna ait şekiller, Şekil 4, Şekil 5, Şekil 6 ve Şekil 7'de görüldüğü gibidir. Kontrollere kıyasla ekstrakt spektrumunda belirli piklerin (örneğin 3293 cm^{-1} ve 2917 cm^{-1} gibi) yoğunluklarının arttığı veya kaymalar gösterdiği görülmektedir. Bu değişimler, UDE'nin yapısal değişimlere neden olduğunu ve bazı bileşiklerin (fenolikler veya diğer aktif bileşenler gibi) ekstrakte edildiğini gösterebilir. Özellikle hidroksil grupları ve karbonil grupları ile ilişkili bantlardaki (örneğin 1700-3300 cm^{-1} aralığında) değişiklikler, ekstraktın biyolojik veya kimyasal özelliklerinde farklılıklar olduğuna işaret etmektedir. Bu sonuçlar, ekstraksiyon yönteminin nar kabuğu biyokimyasal yapısına etkisini vurgulamaktadır.

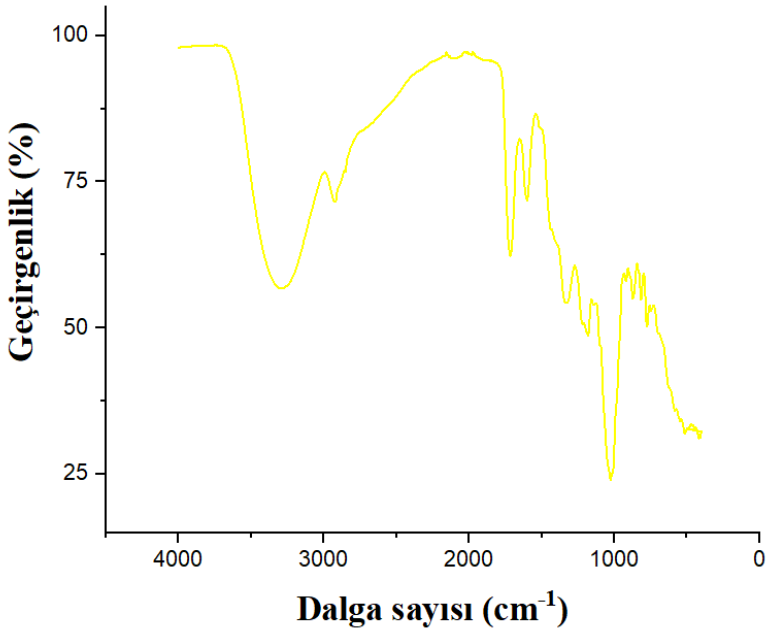


Şekil 4. Nar kabuğu dış kabuk kurutulmuş tozunda FTIR
Figure 4. FTIR on dried powder of pomegranate peel outer shell

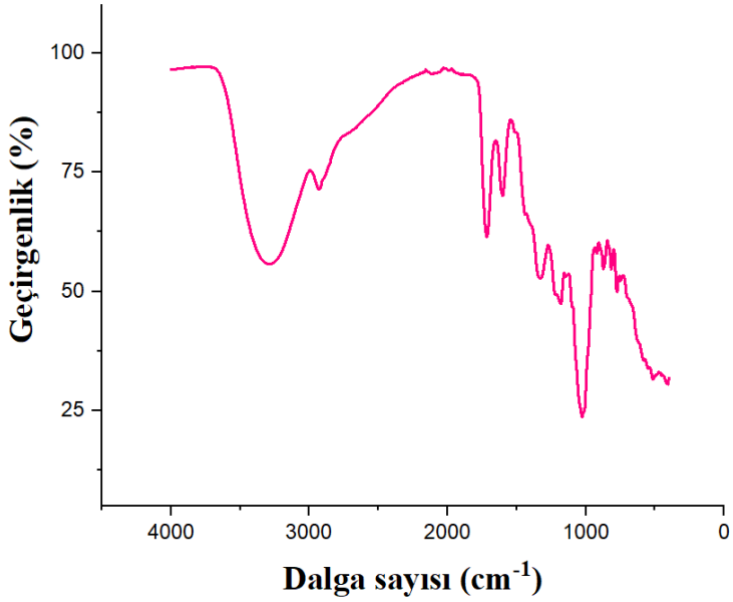


Şekil 5. Nar kabuğu dış kabuğunun kurutulmuş tozu, %100 güçte 10 dakika UDE sonucu elde edilen ekstraktta FTIR
Figure 5. FTIR of dried powder of pomegranate peel outer peel, extract obtained by UDE at 100% power for 10 minutes

UDE ve kontrol örneklerinin FTIR spektrumları karşılaştırıldığında, her iki örnekte de 3293 cm^{-1} (kontrol) ve 3291 cm^{-1} (%100) civarında hidroksil gruplarına (O–H) ait geniş bir pik gözlemlenmiştir. Bu pikler, genellikle fenolik yapıların varlığını göstermektedir [28] . 2920–2932 cm^{-1} aralığında gözlemlenen bantlar, alifatik C–H gruplarının asimetrik gerilme titreşimlerine; 1715 cm^{-1} civarındaki bant ise karbonil (C=O) grubu titreşimlerine karşılık gelmekte ve ester, aldehit veya keton gruplarının varlığına işaret etmektedir [29]. UDE sonrası hidroksil grupları ve 1328 cm^{-1} bölgesindeki polisakkaritlere işaret eden bantların yoğunluğunda azalma gözlemlenmiş, ancak pik konumlarında büyük kaymalar olmamıştır. Bu durum, fenolik bileşikler ve bazı polisakkaritlerin ekstrakte edildiğini, ultrasonik işlemin ise yapısal bütünlükte önemli bir değişiklik yaratmadan aktif bileşenlerin ayrışmasına katkıda bulunduğunu göstermektedir [30, 31]. Bu bulgular, UDE'nin fenolik ve polisakkarit yapıların çözünmesinde etkili bir yöntem olduğunu ortaya koymaktadır.



Şekil 6. Nar kabuğu iç zarı kurutulmuş tozunda FTIR görüntüleri
Figure 6. FTIR images of dried powder of pomegranate peel inner membrane



Şekil 7. Nar kabuğu iç zarı kurutulmuş tozu, %100 güçte 10 dakika ultrasonik ekstraksiyon sonucu elde edilen ekstraktta FTIR
Figure 7. FTIR of the dried powder of pomegranate peel inner membrane, extract obtained by ultrasonic extraction at 100% power for 10 minutes

Nar kabuğunun iç zarı ve dış kabuğunun FTIR spektrumları karşılaştırıldığında, her iki örnekte de benzer fonksiyonel gruplar tespit edilmiştir, ancak pik yoğunluklarında ve bazı bölgelerdeki belirginlikte farklılıklar gözlemlenmiştir. Nar kabuğunun iç zarı ve dış kabuğunun FTIR spektrumları karşılaştırıldığında, her iki örnekte de benzer fonksiyonel gruplar tespit edilmiştir. Ancak, 3293 cm^{-1} (iç zar) ve 3292 cm^{-1} (dış kabuk) bölgelerinde hidroksil gruplarını temsil eden geniş pikler gözlemlenmiş, dış kabuğun bu pikinin daha yoğun ve belirgin olması, fenolik içerik veya nem açısından dış kabuğun daha zengin bir yapıya sahip olabileceğini göstermektedir [32]. Bununla birlikte, 11328 cm^{-1} ve 1026 cm^{-1} bölgelerinde gözlemlenen polisakkarit piklerinin iç zarda daha baskın olması, iç zarın selüloz veya diğer polisakkarit yapıların daha yoğun olduğu bir bölge olduğunu düşündürmektedir [33]. Genel olarak, dış kabuk fenolik içerik açısından daha zengin bir yapı sergilerken,

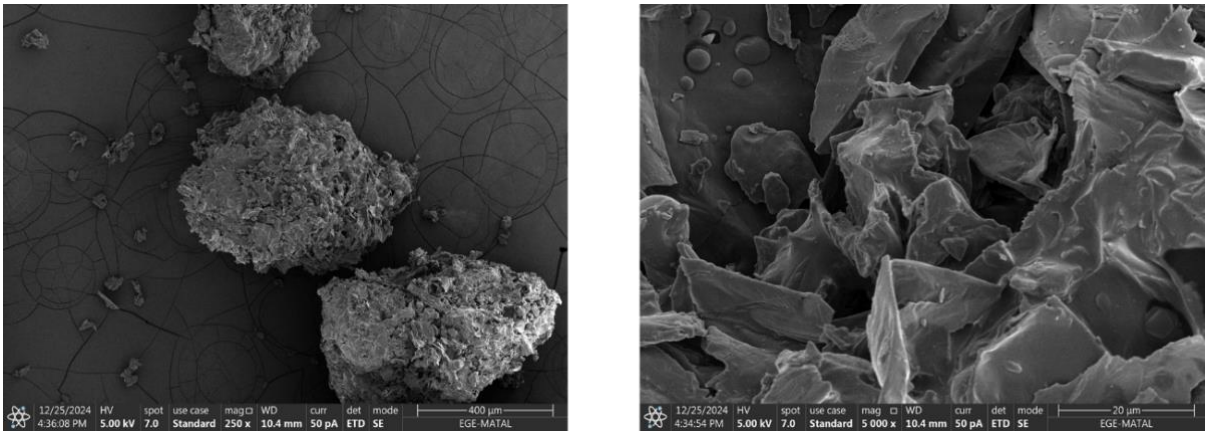
iç zarın daha yüksek polisakkarit içeriğine sahip olduğu söylenebilir. Bu farklılıklar, her iki bölgenin kimyasal ve biyolojik özelliklerinin farklı kullanım alanlarına uygun olabileceğini göstermektedir.

Safdar vd. [34] nar kabuğu FTIR spektrumlarında hidroksil (O-H), karbonil (C=O) ve alifatik C-H gruplarına ait piklerin 3300, 1700 ve 2900 cm^{-1} bölgelerinde yer aldığı belirtilmiştir. Bu çalışmada da bu piklerin benzer şekilde tespit edilmesi, literatürdeki bulgularla uyum göstermektedir. Ayrıca, ekstrakt spektrumunda gözlenen pik yoğunluğu artışları ve kaymalar, literatürde bildirilen UDE'nin fenolik bileşen salınımını artırdığına dair sonuçları desteklemektedir. Dias [35] tarafından yapılan FTIR analizleri, nar kabuğunda farklı piklerde H-bağlı alkolik ve H-X grupları, eter, alkan, fenolik ve eter bileşiklerinin varlığını da kanıtlamıştır. Böylece nar kabuğunun kimyasal yapısının zengin bir fitokimyasal içerik barındırdığı ve çeşitli fonksiyonel grupların belirli dalga boylarında FTIR spektrumunda tespit edilebildiği görülmektedir. Ayrıca, bu bulgular, nar kabuğu ekstraktlarının fenolik bileşikler gibi aktif içerikleri izole etmek ve karakterize etmek için kullanılabilecek uygun bir kaynak olduğunu işaret etmektedir.

Kontrol ve ekstrakt örneklerinin FTIR spektrum sonuçları, Ben-Ali vd. [36] tarafından nar kabuğu ekstraktları üzerine raporlanan hidroksil, karbonil ve alifatik C–H gruplarına ait bantlarla uyumludur. Literatürde nar kabuğu ekstraktında tanen ve gallik asit türevlerine ait yeni bantların ortaya çıktığı belirtilmiştir. Benzer şekilde, UDE ekstraktımızda bu bölgelerdeki yeni pikler, fenolik bileşiklerin ekstraksiyon sırasında salındığını göstermektedir. Bu sonuçlar, ekstraksiyon yöntemimizin biyoaktif bileşiklerin zenginleştirilmesindeki etkinliğini doğrulamaktadır.

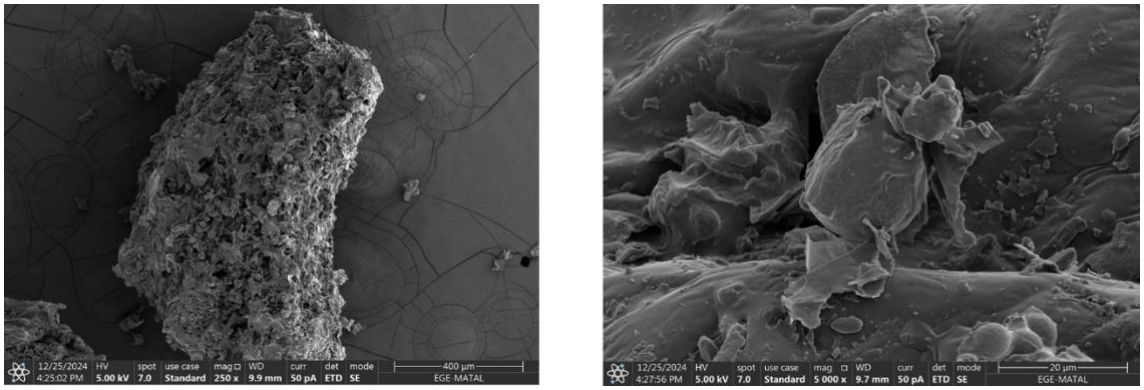
Taramalı Elektron Mikroskop (SEM) Analizi

Kurutulmuş ve öğütülmüş nar kabuklarının yüzey morfolojisi, taramalı elektron mikroskobu (SEM) kullanılarak incelenmiştir. Narın dış kabuğuna ait SEM görüntüleri Şekil 8'de sunulmuştur. Örneklerin sert ve dayanıklı yapısı belirgin şekilde gözlemlenmiş; yüzeylerinin oldukça pürüzlü, heterojen ve çok katmanlı bir morfolojiye sahip olduğu tespit edilmiştir. Bu morfolojik özellikler, dış kabuğun lignoselülozik bileşenlerce zengin olabileceğini ve mekanik işlem sırasında daha büyük, düzensiz parçacıklara ayrılma eğilimi gösterdiğini düşündürmektedir. Benzer şekilde, Siddiqui ve ark. [37] çalışmalarında da nar kabuğunun selüloz, hemiselüloz ve lignin gibi lignoselülozik bileşenler içerdiğini bildirmişlerdir. Nar kabuğunun dış yüzeyindeki pürüzlü ve heterojen yapısı, lignoselülozik bileşenlerin yoğunluğu ve mekanik işlem sırasında oluşan düzensiz parçalanma ile uyumlu olarak gözlemlenmiştir. Benzer şekilde, yapılan bir çalışmada nar kabuğu ekstraktı eklenen filmlerde de pürüzlü bir yüzey ve süngerimsi bir yapı oluştuğu rapor edilmiştir [38]. Başka bir çalışmada da SEM altında nar kabuğu partiküllerinin düzensiz ve lifli bir görünüme sahip olduğu görülmüştür [39].



Şekil 8. Nar kabuğu dış kabuk kurutulmuş tozunda SEM görüntüleri
Figure 8. SEM images of dried powder of pomegranate peel outer Shell

Nar kabuğunun iç zarının SEM görüntüleri Şekil 9'da verilmiştir. Örneğin daha ince, katmanlı ve fibröz bir yapıya sahip olduğunu görülmektedir. Örneğin asimetrik formda ve çok sayıda gözeneğe sahip olduğu gözlemlenmiştir. Bu durum öğütme ile örnekte mekanik hasarın ve moleküller arası bağların kırılmasıyla düzenli bir yapıdan düzensiz bir yapıya dönüşmesinden kaynaklanabilir [40]. Nar kabuğunun vakum altında mikrodalga kullanılarak kurutulan bir çalışmada farklı mikrodalga güçleri ve vakum basınçlarının örneklerin morfolojik özelliklerine etkileri incelenmiş ve elde edilen görüntülerde kurutulan nar kabuklarının çok sayıda asimetrik gözeneklere sahip olduğu bulunmuştur [41]. Yüzeyi nispeten homojen olup, düşük pürüzlülük sergilemektedir. Nar kabuğu iç zarının lifli ve pürüzlü yüzey morfolojisi, taşıyıcı ve emici bir materyal olarak kullanım potansiyelini desteklemektedir [42]. Jerome ve Dwivedi [43], nar kabuğunun düşük nemde kurutularak toz haline getirilebilmesinin, yüksek işlenebilirlik potansiyelini ortaya koyduğunu belirtmiştir.



Şekil 9. Nar kabuğu iç zarı kurutulmuş tozunda SEM görüntüleri
Figure 9. SEM images of dried powder of pomegranate peel inner membrane

SONUÇ

Bu çalışma, nar kabuğundan biyoaktif bileşenlerin ultrason destekli ekstraksiyon (UDE) yöntemiyle başarılı bir şekilde elde edilmesini ve karakterizasyonunu sağlamıştır. Elde edilen sonuçlar, dış kabuğun fenolik bileşikler açısından daha zengin olduğunu (en yüksek 58.73±0.79 mg GAE/g KM, %100 güç, 10 dakika) ve iç zarın ise polisakkarit içeriğinin daha yüksek olduğunu göstermiştir. UDE uygulamasında, nar kabuğu dış kabuğunda en yüksek antioksidan kapasite 3177.75±6.06 ppm TEAC/g KM değerine %100 güç ve 20 dakika kombinasyonunda ulaşılmıştır. İç zar örneklerinde ise en yüksek fenolik madde içeriği 7.31±0.03 mg GAE/g KM ile %100 güç ve 10 dakika sürede elde edilmiştir. FTIR ve SEM analizleri, ultrasonik işlemin nar kabuğunun yapısal bütünlüğünü korurken aktif bileşenlerin salınımını kolaylaştırdığını göstermiştir. Bu bulgular, nar kabuklarının sıfır atık yaklaşımıyla değerlendirilmesi ve gıda ile ilaç endüstrilerinde katma değerli ürünlerin geliştirilmesi açısından önemli bir potansiyele sahip olduğunu ortaya koymaktadır.

TEŞEKKÜR

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KAYNAKLAR

[1] Qu, W., Pan, Z., Ma, H. (2010). Extraction modeling and activities of antioxidants from pomegranate marc. *Journal of Food Engineering*, 99(1), 16-23.

[2] Caruso, A., Barbarossa, A., Tassone, A., Ceramella, J., Carocci, A., Catalano, A., Basile, G., Fazio, A., Iacopetta, D., Franchini, C., Sinicropi, M.S. (2020). Pomegranate: Nutraceutical with promising benefits on human health. *Applied Sciences*, 10(19), 6915.

[3] Akhtar, S., Ismail, T., Fraternale, D., Sestili, P. (2015). Pomegranate peel and peel extracts: Chemistry and food features. *Food Chemistry*, 174, 417-425.

[4] Sharayei, P., Azarpazhooh, E., Zomorodi, S., Ramaswamy, H.S. (2019). Ultrasound assisted extraction of bioactive compounds from pomegranate (*Punica granatum* L.) peel. *LWT-Food Science and Technology*, 101, 342-350.

[5] Demir, T., Akpınar, Ö., Kara, H., Güngör, H. (2019). Nar kabuğundan antimikrobiyal ve antioksidan aktiviteye sahip fenolik bileşiklerin ekstraksiyon koşullarinin optimizasyonu. *Gıda*, 44(2), 369-382.

[6] Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., Jahurul, M.H.A., Ghafoor, K., Norulaini, N.A.N., Omar, A.K.M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, 117(4), 426-436.

[7] Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., Cheng, S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry*, 96(2), 254-260.

[8] Ghafoor, K., Park, J., Choi, Y.H. (2010). Optimization of supercritical fluid extraction of bioactive compounds from grape (Vitis labrusca B.) peel by using response surface methodology. *Innovative Food Science & Emerging Technologies*, 11(3), 485-490.

[9] Mason, T.J., Chemat, F., Vinatoru, M. (2011). The extraction of natural products using ultrasound or microwaves. *Current Organic Chemistry*, 15(2), 237-247.

[10] Pan, Z., Qu, W., Ma, H., Atungulu, G.G., McHugh, T.H. (2012). Continuous and pulsed ultrasound-assisted extractions of antioxidants from pomegranate peel. *Ultrasonics Sonochemistry*, 19(2), 365-372.

[11] Kaderides, K., Goula, A.M., Adamopoulos, K.G. (2015). A process for turning pomegranate peels into a valuable food ingredient using ultrasound-assisted extraction and encapsulation. *Innovative Food Science & Emerging Technologies*, 31, 204-215.

[12] Vilkhu, K., Mawson, R., Simons, L., Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry-A review. *Innovative Food Science & Emerging Technologies*, 9(2), 161-169.

[13] Mphahlele, R.R., Fawole, O.A., Makunga, N.P., Opara, U. L. (2016). Effect of drying on the bioactive compounds, antioxidant, antibacterial and

antityrosinase activities of pomegranate peel. BMC complementary and alternative medicine, 16, 1-12.
[14] Official Methods of Analysis, Association of Official Analytical Chemist. *Gaithersburg*: 2005.
[15] Singleton, V.L. (1999). Lamuela-Raventos: Analysis of total phenoles and other oxidation substartes and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, 299, 152.
[16] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9-10), 1231-1237.
[17] Al-Rawahi, A.S., Rahman, M.S., Guizani, N., Essa, M.M. (2013). Chemical composition, water sorption isotherm, and phenolic contents in fresh and dried pomegranate peels. *Drying Technology*, 31(3), 257-263.
[18] Chen, X.D., Mujumdar, A.S. (Eds.) (2009). Drying technologies in food processing. *John Wiley & Sons*.
[19] Galaz, P., Valdenegro, M., Ramírez, C., Nuñez, H., Almonacid, S., Simpson, R. (2017). Effect of drum drying temperature on drying kinetic and polyphenol contents in pomegranate peel. *Journal of Food Engineering*, 208, 19-27.
[20] Derakhshan, Z., Ferrante, M., Tadi, M., Ansari, F., Heydari, A., Hosseini, M. S., Sadrabad, E. K. (2018). Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. *Food and Chemical Toxicology*, 114, 108-111.
[21] El-Hamamsy, S.M.A., El-Khamissi, H.A.Z. (2020). Phytochemicals, antioxidant activity and identification of phenolic compounds by HPLC of pomegranate (*Punica granatum* L.) peel extracts. *Journal of Agricultural Chemistry and Biotechnology*, 11(4), 79-84.
[22] Campos, L., Seixas, L., Henriques, M.H., Peres, A. M., Veloso, A.C. (2022). Pomegranate Peels and Seeds as a Source of Phenolic Compounds: Effect of Cultivar, By-Product, and Extraction Solvent. *International Journal of Food Science*, 2022(1), 9189575.
[23] Suleria, H.A., Barrow, C.J., Dunshea, F.R. (2020). Screening and characterization of phenolic compounds and their antioxidant capacity in different fruit peels. *Foods*, 9(9), 1206.
[24] Derakhshan, Z., Ferrante, M., Tadi, M., Ansari, F., Heydari, A., Hosseini, M.S., Conti, G.O., Sadrabad, E. K. (2018). Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. *Food and Chemical Toxicology*, 114, 108-111.
[25] Yan, L., Zhou, X., Shi, L., Shalimu, D., Ma, C., Liu, Y. (2017). Phenolic profiles and antioxidant activities of six Chinese pomegranate (*Punica granatum* L.) cultivars. *International journal of food properties*, 20(sup1), S94-S107.
[26] Elfalleh, W., Hannachi, H., Tlili, N., Yahia, Y., Nasri, N., Ferchichi, A. (2012). Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower. *Journal of Medicinal Plants Research*, 6(32), 4724-4730.
[27] Safdar, M., Naqvi, S.A., Anjum, F., Pasha, I., Shahid, M., Waliullah, Jaskani, M.J., Khan, I.A., Aadil, R.M. (2021). Microbial biofilm inhibition, antioxidants, and chemical fingerprints of Afghani pomegranate peel extract documented by gas chromatography–mass spectrometry and Fourier transformation infrared. *Journal of Food Processing and Preservation*, 45(7), e15657.
[28] Heredia-Guerrero, J.A., Benítez, J.J., Domínguez, E., Bayer, I.S., Cingolani, R., Athanassiou, A., Heredia, A. (2014). Infrared and Raman spectroscopic features of plant cuticles: a review. *Frontiers in Plant Science*, 5, 305.
[29] Hu, Y., Qian, W., Fan, S., Yang, Y., Liao, H., Zhuang, G., Gao, S. (2024). Ultrasonic-assisted extraction of phenolic compounds from Lonicera similis flowers at three harvest periods: Comparison of composition, characterization, and antioxidant activity. *Molecules*, 29(14), 3280.
[30] Altemimi, A., Watson, D. G., Choudhary, R., Dasari, M.R., Lightfoot, D.A. (2016). Ultrasound assisted extraction of phenolic compounds from peaches and pumpkins. *Plos One*, 11(2), e0148758.
[31] Soltanzadeh, M., Peighambardoust, S.H., Ghanbarzadeh, B., Mohammadi, M., Lorenzo, J.M. (2021). Chitosan nanoparticles as a promising nanomaterial for encapsulation of pomegranate (*Punica granatum* L.) peel extract as a natural source of antioxidants. *Nanomaterials,* 11(6), 1439.
[32] Jabrail, F.H., Hameed, A. R., Majdoub, H. (2023). Effects of Surface Morphology and Type of Cross-linking of Chitosan-Pectin Microspheres on their Degree of Swelling and Favipiravir Release Behavior.
[33] Safdar, M., Naqvi, S.A., Anjum, F., Pasha, I., Shahid, M., Waliullah, Jaskani, M.J., Khan, I.A., Aadil, R.M. (2021). Microbial biofilm inhibition, antioxidants, and chemical fingerprints of Afghani pomegranate peel extract documented by gas chromatography–mass spectrometry and Fourier transformation infrared. *Journal of Food Processing and Preservation*, 45(7), e15657.
[34] Dias, R.F. (2005). Spectroscopy: How do we know what organic molecules look like. *Organic Chemistry* (CHEM311) fall, 48-58.
[35] Ben-Ali, S., Akermi, A., Mabrouk, M., Ouederni, A. (2018). Optimization of extraction process and chemical characterization of pomegranate peel extract. *Chemical Papers*, 72(8), 2087-2100.
[36] Siddiqui, V.U., Almehjani, T.M., Sapuan, S.M., Jamal, T., Ilyas, R. A., Eldin, S.M., Khan, A., Jameel, Y. (2024). Isolation and characterization of cellulose from pomegranate (*Punica granatum*) peel. *Journal of Natural Fibers*, 21(1), 2299943.
[37] Emam-Djomeh, Z., Moghaddam, A., Yasini Ardakani, S.A. (2015). Antimicrobial activity of pomegranate (*Punica granatum* L.) peel extract, physical, mechanical, barrier and antimicrobial properties of pomegranate peel extract-incorporated sodium caseinate film and application in packaging for ground beef. *Packaging Technology and Science*, 28(10), 869-881.
[38] Ali, A., Chen, Y., Liu, H., Yu, L., Baloch, Z., Khalid, S., Zhu, J., Chen, L. (2019). Starch-based

antimicrobial films functionalized by pomegranate peel. *International journal of biological macromolecules*, 129, 1120-1126. Zhong, C., Zu, Y., Zhao, X., Li, Y., Ge, Y., Wu, W., Zhang, Y., Li, Y., Guo, D. (2016). Effect of superfine grinding on physicochemical and antioxidant properties of pomegranate peel. *International Journal of Food Science & Technology*, 51(1), 212-221.

[39] Elnjikkal Jerome, R., Dwivedi, M. (2022). Microwave vacuum drying of pomegranate peel: Evaluation of specific energy consumption and quality attributes by response surface methodology and artificial neural network. *Journal of Food Processing and Preservation*, 46(3), e16325.

[40] Ali, A., Chen, Y., Liu, H., Yu, L., Baloch, Z., Khalid, S., Zhu, J., Chen, L. (2019). Starch-based antimicrobial films functionalized by pomegranate peel. *International journal of biological macromolecules*, 129, 1120-1126.

[41] Elnjikkal Jerome, R., Dwivedi, M. (2022). Microwave vacuum drying of pomegranate peel: Evaluation of specific energy consumption and quality attributes by response surface methodology and artificial neural network. *Journal of Food Processing and Preservation*, 46(3), e16325.

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Review Paper / Derleme Makale

Strains and Sugar Contents of Food Products Fortified with Probiotics

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ABSTRACT

An increasing interest in probiotic-fortified foods today can be attributed to factors such as the pandemic, climate change, and modern lifestyles. In the process of incorporating probiotics into foods, several crucial factors must be considered, including the selection of the appropriate strain, ensuring survival in the gut, production techniques, and storage conditions. The sugar content of these products is equally important, as it can significantly affect the survival and effectiveness of probiotics. Some products in the market may contain excessive amounts of sugar to make them more appealing to consumers. This study aims to determine the specific strains and sugar content in commercially available probiotic-fortified foods. Additionally, it seeks to investigate the impact of sugar on probiotics and evaluate the sugar levels in these products based on recommended dietary guidelines. Literature review indicated that strain selection and sugar content in probiotic-fortified foods could play a crucial role in the viability of probiotics and their health effects. High sugar content could negatively impact gut microbiota balance and reduce probiotic survival, emphasizing the necessity for consumers and food manufacturers to carefully assess total and added sugar levels. Further investigation into the interactions between different probiotic strains and sugar types is highly recommended for improving product formulations and ensuring the long-term health benefits of probiotics.

Keywords: Probiotic-fortified food, Probiotic, Sugar, Viability, Health effects

Probiyotiklerle Desteklenmiş Gıdalardaki Suşlar ve Şeker İçerikleri

ÖZ

Günümüzde probiyotiklerle desteklenmiş/güçlendirilmiş gıdalara olan ilginin artışı, pandemi, iklim değişikliği ve modern yaşam tarzı gibi faktörlere bağlanabilir. Probiyotiklerin gıdalara dahil edilmesi sürecinde, doğru suşun seçilmesi, bağırsakta hayatta kalma yeteneğinin sağlanması, üretim teknikleri ve saklama koşulları gibi önemli faktörler dikkate alınmalıdır. Bu ürünlerde bulunan şeker miktarı da eşit derecede önemlidir, çünkü probiyotiklerin hayatta kalmasını ve etkinliğini önemli ölçüde etkileyebilir. Piyasadaki bazı ürünler, tüketicilere daha cazip hale gelmesi için aşırı miktarda şeker içerebilmektedir. Bu çalışma, ticari olarak erişilebilir probiyotik ilaveli gıdalardaki spesifik suş çeşitlerini ve şeker içeriğini incelemeyi amaçlamaktadır. Ayrıca, şekerin probiyotikler üzerindeki etkisini araştırarak bu ürünlerdeki şeker seviyelerini önerilen kılavuzlara göre değerlendirmeyi hedeflemektedir. Literatür derlemesi, probiyotiklerle desteklenmiş gıdalarda suş seçimi ve şeker içeriğinin, probiyotiklerin canlılığı ve sağlık üzerindeki etkileri açısından önemli bir rol oynadığını ortaya koymaktadır. Yüksek şeker içeriği, bağırsak mikrobiyota dengesini olumsuz yönde etkileyebilir ve probiyotiklerin hayatta kalmasını azaltabilir, bu da tüketiciler ve gıda üreticileri için toplam ve ilave şeker seviyelerinin dikkatle değerlendirilmesi gerekliliğini vurgulamaktadır. Farklı probiyotik suşları ve şeker türleri arasındaki etkileşimin daha ayrıntılı incelenmesi, ürün formülasyonlarının geliştirilmesi ve probiyotiklerin uzun vadeli sağlık faydalarının sağlanması açısından önerilmektedir.

Anahtar Kelimeler: Probiyotikli gıda, Probiyotik, Şeker, Canlılık, Sağlık etkileri

INTRODUCTION

The ecosystem of microorganisms living in a particular environment is called the “microbiota”. Similar to the human microbiota, the soil microbiota is the living ecosystem of the earth and consists of a wide variety of organisms. Environmental pollutants introduced in modern life have a negative impact on soil microbiota. A decline in the nutritional value of foods is a result of these negative alterations in the soil. Macro and micronutrient deficiencies are gradually increasing in modern people who are fed low-nutritional-value foods [1]. In addition, industrial toxins, a western-style diet, and a sedentary lifestyle cause dysbiosis in which the balance of the intestinal microbiota is disturbed [2]. In the last century, the need for foods that provide benefits beyond nutritional function has increased to modulate nutritional deficiencies and impair gut microbiota. In addition, consumer demand for high-quality meals and awareness of healthy living have led to the development of functional foods. Functional foods can be created by changing the content of food using different engineering and biotechnological methods or by adding bioactive components, such as phenolic compounds, vitamins, minerals, and fiber [3]. Probiotics, which are beneficial bacteria, have also been used for this purpose. Probiotics are known to strengthen the immune system and improve overall health by regulating human gut microbiota [4]. Food formulations containing probiotic bacteria have an important place among functional foods due to the numerous proven health benefits they provide in addition to their traditional nutritional functions [5].

Owing to functional food technology, foods that are not contain probiotic bacteria (such as tea, coffee, chocolate, and granola) are used as probiotic-fortified products. However, the selected probiotic must be well defined at the species and strain levels and be safe for consumption. Additionally, probiotic strains must be compatible with the food matrix, resistant to production processes, and maintain their viability under storage conditions. Active strains must survive until the end of the product’s shelf life to provide the expected benefits from probiotics. In addition, they must survive in the low pH, bile salt, and enzymatic environment of the gastrointestinal tract to reach and colonize the intestine [6]. Therefore, not all probiotics are suitable for use in food. When choosing which probiotics to use in food formulations, the manufacturer should consider many factors [7]. The most common microorganisms found in probiotic foods are *Lactobacillus* and *Bifidobacterium* spp. However, because of their tolerance to harsh environments, spore-forming bacteria, such as *Bacillus coagulans* and *Bacillus subtilis*, are becoming more common in the food industry [8].

Sugar is added to food products to ensure the consumer acceptability of food and to improve its sensory properties [9]. Sugar is added to make nutrient-dense foods, including probiotic foods, appropriate for general consumption (Figure 1).

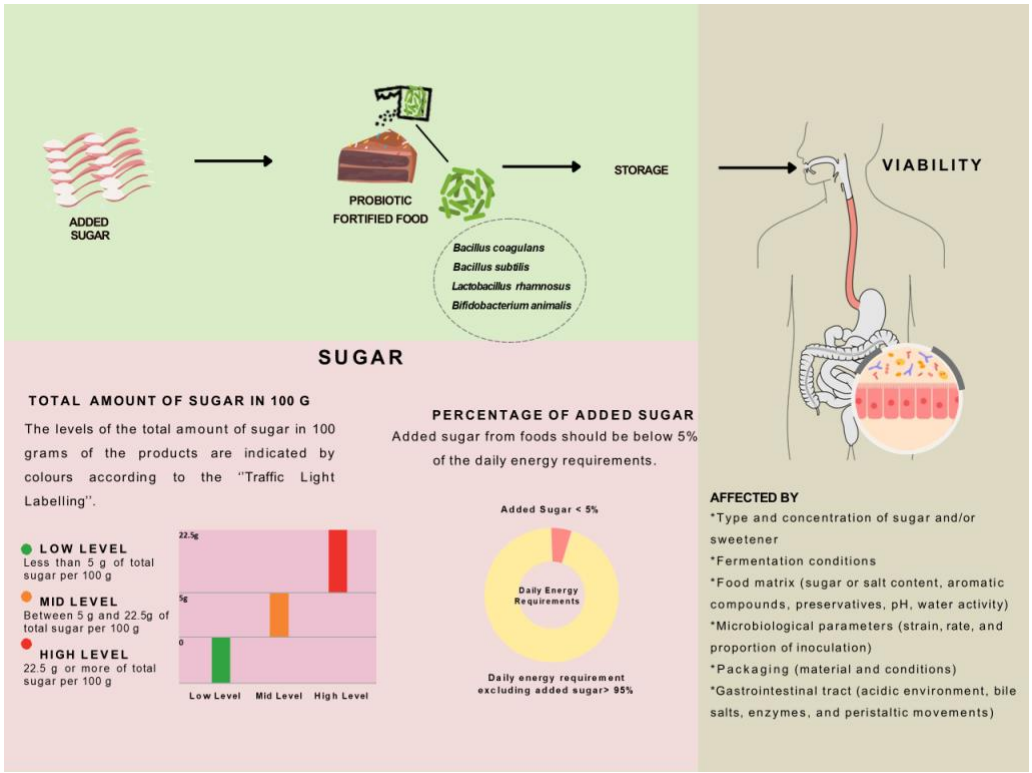

Figure 1. The effect of sugar amount on viability in probiotic-fortified foods

Health authorities agree that people can consume some sugar within their daily recommendations [10]. However, it should be noted that the nutritional composition of probiotic foods and the added active strains interact [3]. Considering the addition of different types and amounts of sugar sources to foods, the impact of the consumed

sugar quantity on probiotic activity is often overlooked. The sugar content in probiotic food products should be evaluated to ensure their effective use in a healthy diet. In our study, the compositions of probiotic-containing food samples from almost every sector available on the market were analyzed and assessed in terms of their sugar content (Table 1 and Table 3). Given that studies have shown a decline in the survival rates of probiotic bacteria in dairy products produced using different sugar sources or amounts [11], the potential risks of sugar's impact on the viability of probiotic bacteria in food are a significant public health concern.

PROBIOTIC-FORTIFIED FOODS

Viability of Strains

Viability is a basic requirement for the mechanism of action and functionality of probiotics. The viability of probiotic microorganisms can be divided into two parts: food and the human body [3]. Microorganisms must survive primarily under food production and storage conditions [7]. Processing parameters, such as the strain selected during food production, fermentation conditions, food matrix (sugar or salt content, aromatic compounds, preservatives, pH, water activity), heat treatment, incubation temperature, and microbiological parameters (strain of probiotics employed, rate, and proportion of inoculation) are among the factors affecting probiotic viability. The packaging material used, and packaging conditions should also be considered as they affect the conditions of microorganisms during storage [6].

The next step is to survive the passage through the gastrointestinal tract. The acidic environment, bile salts, enzymes, and peristaltic movements in the intestinal tract are the major challenges for microorganisms [12]. Different encapsulation methods are used to protect microorganisms from harsh conditions in the gastrointestinal tract. However, there are a few important factors: the capsules used should not affect the sensory properties of the food; they should provide cell release and be protective in the gastrointestinal tract. Thus, probiotics can reach the intestine and show the expected effects [13].

The type and concentration of sugar and/or sweetener added to the product during food processing can affect probiotic viability. In a study, the impact of varying sugar and inulin levels on the viability of probiotic bacteria was investigated. They utilized different formulations of probiotic ice cream containing freeze-dried mixed cultures of *Streptococcus salivarius* spp. *thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus* La-14, and *Bifidobacterium lactis* BL-01. This study focused on ice cream samples prepared with three different sugar concentrations (15%, 18%, and 21%) and two levels of inulin (1% and 2%). Over a 90-day storage period, the viability of probiotic bacteria in these formulations was evaluated. The results indicated that the viability of yoghurt and probiotic bacteria was influenced by inulin and sugar levels. Inulin, which serves as a food source for probiotic microorganisms, increases bacterial viability. Ice cream samples with 18% sugar concentration exhibited the highest number of viable bacteria. Conversely, the viability of the bacteria was lower at 15% and 21% sugar concentrations. Furthermore, this study emphasized that different bacterial strains react differently to the same sugar and inulin levels. *S. thermophilus* demonstrated the highest viability during storage, whereas *L. acidophilus* and *B. lactis* showed a rapid decrease in viability. In fact, at the end of the 90-day storage period, the viable numbers of *L. acidophilus* and *B. lactis* (5 log CFU/g) fell below acceptable levels (≥6 log CFU/g) [13].

Another study investigated the effects of low-calorie sweeteners as substitutes for sugar on the bacterial vitality in ice cream. Kalicka et al. [14], examined the viability of *Bifidobacterium animalis* subsp. *lactis* BB-12 strain before and after storage in nine different ice cream formulations containing various amounts of polyols (maltitol, erythritol, xylitol) and sugar. The control group was treated with an ice cream containing sucrose (15%). After 28 days of storage, all samples had a live cell count above 8 log CFU/g. The lowest vitality was observed in the sample containing 7.5% sucrose and 8.38% erythritol, whereas the highest vitality was consistently maintained in the control group. A sugar ratio of 15% yielded good vitality. This study supports the use of polyols in appropriate doses as a substitute for sugar.

To maximize the benefits of probiotic-fortified foods, pH, NaCl, sugar type/concentration, fat concentration, and storage conditions should be considered. When discussing the relationship between sugar and viability, it is important to consider that higher sugar concentrations can negatively impact both bacterial viability and sensory characteristics. In addition, the selection of specific probiotic bacterial strains plays a vital role in determining their response to these factors. Therefore, when determining the sugar levels in probiotic-fortified product formulations, it is necessary to consider the specific viability requirements of the chosen bacterial strains to ensure optimal survival. This is crucial for preserving the desired probiotic benefits in the final product and providing functional food to consumers. The number of bacteria (>7 log CFU/g) in probiotic-fortified foods must be maintained throughout their shelf life. Hence, the production process of probiotic foods should be designed to preserve the viability of probiotic microorganisms and maintain the specified bacterial count until the end of the shelf life [15].

Strain Content of Probiotic-fortified Foods

The dynamics and stabilization of the human gut microbiome during the first year of life are influenced by the mother's microbiota, diet, lifestyle, and genetics [16]. The diversity of the microbiota, which is affected by diet, lifestyle, ethnicity, geography, and environmental factors during the lifetime. Packaged probiotic- fortified products and their strain content in the current food market are listed in Table 1 as model products.

Table 1. Probiotic- fortified products and strain contents (Products in the table were found by searching the word "probiotic food" in the online database)

Probiotic Product	Brand Name	Active Strain Content
Grain And Grain Products		
Bread	The Grain Seed'licious	*B. coagulans*
Cracker	Kuna Pops	*B. coagulans*
Chickpea Granola- Wholesome Raspberry	Effi Foods	*B. coagulans*
Probiotic Oatmeal & Banana Baby Cereal	Gerber	*B. lactis*
Special K® Probiotics Berries & Peaches Cereal	Kellogs	*B. lactis*
Muesli	Sunsol	*B. coagulans*
Bar	Fropie	*B. coagulans*
Oat Bar	Nice & Natural	*B. coagulans*
Gluten Free Pancake Waffle Mix	Enjoy Life	*B. coagulans*
Baking mix Gluten Free, Nut Free, Vegan, Pizza Crust Mix	Enjoy Life	*B. coagulans*
Dairy Product		
Milk	Moringa Caldus	*Bifidobacterium longum* ssp. *longum* BB536
Lowfat Yoghurt	Nancy's	*Bifidobacterium lactis* BB-12, *L. acidophilus* LA-5, *L. rhamnosus* LB-3, *L. casei* (2 isolated strains), *L. rhamnosus* (2 isolated strains), *L. acidophilus* (2 isolated strains), *B. lactis* (2 isolated strains)
Yoghurt	Activia	*B. lactis*
Dairy Free Yoghurt	Califia Farms	*Bifidobacterium* BB-12, *S. thermophilus, L. bulgaricus*
Drinkable Yoghurt	Danactive	*Lactobacillus casei* CNCM I-1518
Kefir	Nancy's	*B. lactis BB-12, L. acidophilus LA-5, L. rhamnosus LGG, L. rhamnosus LB-3, L. casei (*2 isolated strains), *L. rhamnosus (*2 isolated strains), *L. acidophilus* (2 isolated strains), *B. lactis* (2 isolated strains)
Probiotic Drink	Yakult	*L. casei Shirota*
Probiotic Shot	Activia	*L. bulgaricus, L. lactis, S. thermophilus*
Sour Cream	Good Culture	*L. acidophilus, B. lactis*
Cottage Cheese	Sek	*L. acidophilus, B. lactis*
Products Containing Fruit and Vegetables		
Organic Vegetable and Fruit Mix	Garden of Life	*L. gasseri, L. plantarum, L. casei, L. acidophilus*
Non-Dairy Smoothie	Love Grace	*Bacillus coagulans*
Fruit Gel	Welch's	*B. subtilis*
Juice	Biola	*L. rhamnosus*
Juice	Tropicana	*B. lactis*
Juice	Valio Gafilus	*L. rhamnosus* GG
Fruit Drink	Danone Proviva	*L. plantarum*
Sorghum Cauliflower Puffs	Vegan Rob's	*Bacillus coagulans*
Fruit kombucha	Humm	*Bacillus coagulans, B. subtilis*
Soup	Presserys	*Bacillus coagulans*
Antioxidant Berry mix	Nature's garden	*L. rhamnosus* GG
Sugar Product		
Chocolate	Healthy Delights Naturals	*B. coagulans*
Lollipop	Dr. John's	*B. subtilis*
Chocolate Shake	Lyfe Fuel	*Lactobacillus acidophilus* DDS, *L. plantarum, L. rhamnosus, B. breve, B. longum*
Sugar	Sugar 2.0	*B. coagulans*
Herbal tea sweetened with molasses	Vita Biosa 10+	*B. animalis, B. lactis, L. acidophilus, L. casei, L. rhamnosus, Lactococcus lactis* ssp., *Lactis biovar. Diacetylactis, L. pseudo mesenteroides, S. thermophilus*
Drinks		
Green tea	Doğadan	*B. coagulans*
Black tea	Doğadan	*B. coagulans*
Coffee	Gut Power	*B. coagulans*
Water	Karma	*B. coagulans*
Hot chocolate	Cocosutra	*B. coagulans*
Dairy free drinkable yogurt	Califia farms	*Bifidobacterium lactis* BB-12, *S. thermophilus, L. bulgaricus*
Choco cinnamon banana vegan protein powder	Vega	*B. coagulans*
Pear digestion shot	Goodbelly	*L. plantarum* 299V
Mojita lime mint coconut sparkling drink	Kevita	*B. coagulans*
Metabolism tea	Super organics	*B. coagulans*
Turmeric ginger shot	Welo	*B. subtilis*

Table 1 presents a compilation of commercially available probiotic-fortified products categorized into grain-based items, dairy and non-dairy products, fruit and vegetable-containing foods, sugar products, and beverages. The active probiotic strains identified in these products include various species of *Bacillus*, *Lactobacillus*, and *Bifidobacterium*, which are known for their potential health benefits, particularly in gut microbiota modulation. The products in Table 1 were selected as examples from almost every category in the food and beverage industry. When the strain content of the products was examined, it was observed that *Bacillus coagulans,*

Bacillus subtilis, Bifidobacterium lactis, and *Lactobacillus rhamnosus* strains were frequently used in the food market.

Characteristic of Strains

Bacillus coagulans, Bacillus subtilis, Bifidobacterium lactis, and *Lactobacillus rhamnosus* are the probiotic microorganisms most frequently employed in probiotic-fortified products, as demonstrated in Table 1, and they accepted GRAS status. The origins of these probiotic microorganisms, their bacterial attributes, health impacts, preferred food sources, and the benefits of their utilization are comprehensively outlined in Table 2. The strains, such as *Bacillus coagulans*, *Bacillus subtilis*, *Bifidobacterium lactis*, and *Lactobacillus rhamnosus*, demonstrate unique attributes such as resistance to stomach acid and bile, spore formation, and lactic acid production, which contribute to their viability in various food matrices. These probiotics are associated with significant health benefits, including gastrointestinal health improvement, immunomodulation, and prevention of infections, making them valuable components in both dairy and non-dairy probiotic products. Although probiotic strains generally promote gut health, specific species also provide specific benefits [17]. However, when it comes to a probiotic that provides benefits to people in food and beverages, the characteristics of probiotic bacteria might be variable [7].

Sugar in Probiotic- Fortified Products

According to WHO, the sugar naturally present in fruits and vegetables is named ‘intrinsic sugars’ and "free sugar" includes all monosaccharides and disaccharides added to foods and sugars naturally found in honey, molasses, and fruit juices [52]. Intrinsic sugars take longer to enter the bloodstream and have less of an impact on blood sugar than added sugar, owing to beneficial components such as fiber and various phytochemicals found in fruits and vegetables. In addition, while sugar increases the energy content of the diet, it also decreases the quality of the diet as it decreases the nutrient density [9].

Sugar is an essential taste component of packaged products. In a consumer report from FONA, a specific subsidiary, it was found that 70% of consumers prioritize taste over high sugar content in a product. Despite this, 50% of consumers aim to cut down on their sugar intake. As a result, the market for new products with reduced sugar has surged by 54% since 2017 [53].

Additional sugar consumption is influenced by factors such as age, sex, ethnicity, and income. Men, young adults, low-income individuals, and adolescents consume more sugar than the average population. However, in recent years, the importance of specifying the sugar levels and sources in packaged foods has increased considerably. According to the results of the survey studies, consumers want to know how much sugar is in the product and what its source is, and taste is still the most important parameter [54]. This indicates that consumers’ choices are flavor-based rather than health based. Consumer preferences for probiotic food products are also similar. Probiotic- fortified products containing added sugars have been preferred over sugar-free products. This affects the ingredients of packaged probiotic products containing added sugar [9]. However, excessive sugar (more than 5% of total daily calories from added sugar, according to WHO) consumption leads to many non-communicable diseases such as obesity, diabetes, and heart disease [10]. The negative effects of sugar on health and its increasing prevalence have resulted in organizations such as the WHO and NHS (National Health Service) making consumption recommendations on sugar and added sugar.

In this case, the content and nutritional values of packaged foods (such as calories, total carbohydrates, total fat, protein, salt, and sugar) should be known to consumers. Consumers choose food according to the nutrition labels written on the package. Therefore, nutrition labels are of great importance in influencing consumer behavior. Anastasiou, Miller, and Dickinson [55] published a systematic review of the relationship between food label use and dietary intake in adults. The presence of health claims and use of nutrition labels have been reported to result in healthier decisions. A new FDA regulation requires added sugars to be listed on the ‘nutrition facts label’ so that you can make decisions based on your unique needs and preferences. There is information such as added sugar and fiber under the term carbohydrates on the label. The term “total carbohydrates” on the labels refers to the total amount of dietary fiber, sugars that are naturally found in the foods they contain and added sugars. When reviewing nutrition labels, the primary focus should be on how many carbohydrates come from added sugar [56].

According to the traffic light labelling, a product’s total sugar content of 100 g should be less than 5 g for a low level, between 5 and 22.5 g for a medium level, and 22.5 g or more for a high level. Products with low, medium, and high total sugar are colored green, amber, and red, respectively [57].

According to the WHO [58], "Sugar Intake for Adults and Children" guidelines, added sugar from foods should be below 5% of the daily energy requirements. In Table 3, the percentage of added sugar is presented according to the percentage of daily value (DV) in the label information. Daily values are the reference amounts of nutrients to be consumed or not exceeded each day. From this point of view, as seen in Table 3, the sugar contents of probiotic- fortified products available on the market were given according to WHO guideline and traffic light labelling.

Table 2. Characteristics of strains used in packaged probiotic- fortified products

Probiotics	Origin	Properties	Health Effects	Food Products	Advantages	Ref
Bacillus coagulans	Plant-based sources	Facultative anaerobe, Gram positive, Spore forming, Lactic acid production, Growth temperature: 35-50°C (live up to 65°C), Growth pH:5.5-6.5, (grow at pH 4.5), GRAS Level	Gastrointestinal disorders (such as IBS, constipation), Preventing the high serum lipid profile, increase nutrient absorption, promoting lactose digestibility (with enzyme production), improving intestinal function in the elderly	Grain-based products (such as pasta), heat-treated vegan probiotic food products, yogurt, Fermented beverage	Heat resistant spore forms, resistant to low pH and bile, no effects on sensorial properties	[8, 18-23]
B. subtilis	GI tract (ruminant and human), Soil	Facultative anaerob, Gram positive, Spore forming, biofilm forming, enzyme production (such as amylase, protease), Growth temperature:10-55°C, (Optimum growth temperature: 37°C), Growth pH: 2.5-9, GRAS Level	Childhood diarrhea, Improving IB symptoms, Antimicrobial activity in GI and other infections, Regulation of metabolic profile, Antioxidant activity	Natto (japanese food), A wide variety of foods and beverages in addition to dairy products	Spore forming, Durable biofilm layers, No refrigeration needed for vitality, Resistant to low pH,	[24-34]
Bifidobacterium lactis	Human and mammalian large intestine	Anaerob,Gram positive, Does not form spores, Lactic acid production, Proteolytic activity, Growth temperature:25-45°C, Growth pH: 4-7, GRAS Level	Antibiotic-associated diarrhea, Immunomodulation, Preventing respiratory infection, Improving gut functions, Improving atopic eczema in infant	Probiotic dairy products, Baby foods, Dietary supplements Fermented milk products	Tolerance to high oxygen level, Resistance to stomach acid and bile salt	[35-44]
Lactobacillus rhamnosus	A wide range of habitats (dairy products, fermented meat, fish, vegetables and cereals, sewage, human gastrointestinal tract, vagina)	Homofermentative facultative anaerobe, Gram positive, Does not form spores, Production of lactic acid and bacteriocin, Growth temperature: 6-41°C, Growth pH: 4.5-6.5, GRAS Level	Preventing urinary tract infection, Increasing insulin sensitivity, Diarrhea (AAD and gastroenteritis), preventing certain allergic symptoms	Yogurt, cheese, milk, kefir and non-dairy probiotic products such as juice	Resistance to stomach acid and bile, Adhesion to mucosal cells, Extending shelf life, Ripening of cheese	[45-51]

Table 3. Packaged probiotic-fortified products and sugar contents (Prepared using the product information of the manufacturers.)

Probiotic Product	Product	Serving Size /Calorie	Sugar Amount (T.S / A.S)	Added Sugar Amount (WHO 5% Criteria)	Total Sugar/100 g	Total Sugar Level (Traffic Light Labelling
Chickpea Granola- Wholesome Raspberry	Effi Foods	30g / 140 kcal	4g / 3g	7%	13.3g	Med Level
Probiotic Oatmeal & Banana Baby Cereal	Gerber	15g / 60 kcal	2g / <1g	<1%	13.3g	Med Level
Special K® Probiotics Berries & Peaches Cereal	Kellog's	42g / 160 kcal	13g / 12g	24%	30.9g	High Level
Muesli	Sunsol	50g /196 kcal	7.7g / U	U	15.4g	Med Level
Cashew & Chia Probiotic Bar	Fropie	35g /138 kcal	13.7g / 0g	0%	39.1g	High Level
Oat Bar	Nice & Natural	35g /153 kcal	6g / U	U	17.1g	Med Level
Gluten Free Pancake Waffle Mix	Enjoy Life	40g /140 kcal	5g / U	U	12.5g	Med Level
Lowfat Yogurt Blueberry	Nanc'y	150g /120 kcal	14g / 7g	14%	9.3g	Med Level
Yogurt (plain)	Activia	100g /100 kcal	11g / 0g	0%	11g	Med Level
Trail Mix, Probiotic Fruit & Yogurt	Archer Farms	35g /160 kcal	21g / 19g	38%	60g	High Level
Danactive Drinkable Yogurt (vanilla)	Danone	93mL /80 kcal	13g / 10g	20%	14g	Med Level
Kefir (plain)	Nancy's	240mL /180 kcal	8g / 0g	0%	3.3g	Low Level
Probiotic Drink	Yakult	80mL /50 kcal	10g / 9g	18%	12.5g	Med Level
Probiotic Daily Shot (plain)	Activia	80mL /27.2 kcal	3.7g / 0g	0%	4.6g	Low Level
Peach & Tea Probiotic Daily Shot	Activia	80mL / 45 kcal	6.9g / U	U	8.6g	Med Level

*(T.S: Total Sugar, A.S: Added Sugar, U: Unknown)

Table 3 lists probiotic-fortified products categorized by low, medium, and high sugar levels. It does not provide definitive information on how these sugar levels affect the benefits of probiotics, but high sugar consumption is known to reduce intestinal bacterial diversity, potentially leading to microbiota dysbiosis. Consequently, selecting probiotic products with low or medium sugar levels, as indicated in Table 3, may support a healthier diet. Furthermore, the traffic-light labeling for sugar levels is determined based on total sugar content, making it essential to consider the amount of added sugar in each product. For instance, Table 3 shows that a low-fat blueberry yogurt contains 9.3 grams of total sugar, placing it in the medium sugar category, yet 14% of this sugar comes from added sources, which should be taken into account when making dietary choices. Another example is a drinkable yoghurt, which contains a total of 14 g of sugar and is considered a product with medium sugar levels; however, 20% of this sugar is added sugar. Both probiotic yoghurts exceeded the WHO's sugar recommendations. This situation can be a risk factor for a decrease in probiotic bacteria and increase in pathogenic bacteria. Therefore, it is important to consider both the total sugar content and amount of added sugar in probiotic- fortified products. At this point, the effect of sugar on the viability of probiotics in the products and how this effect reflects the beneficial effects of probiotics has become significant.

The impact of added sugar on the viability of probiotics in functional foods varies depending on factors such as the type and concentration of sugar, the specific probiotic strains used, and the processing conditions of the food product. As a result, research studies have been conducted using different sugar levels, various strains, and diverse food matrices to investigate the relationship between sugar and the viability of probiotics. Table 4 presents the selected research studies concerning the correlation between sugar and viability in probiotic- fortified products.

As shown in Table 4, ice cream is frequently utilized in probiotic studies due to its ability to support probiotic viability. In such products, high sugar concentrations can induce osmotic stress, potentially leading to cell damage or death. Conversely, sugar may also exert a cryoprotective effect, mitigating freezing-induced damage [59].

Shahsavan et al. [61] tested this issue using ice cream formulations with different quantities of sugar (14%, 16%, and 18%) and fat (5%, 7.5%, and 10%). The study found that the lowest number of *L.casei* cells was observed in the sample with 14% sugar and 10% fat, while the sample with 16% sugar and 5% fat had the highest number of cells. As the amounts of sugar and fat increased, the viability of *L. casei* was negatively affected. Similarly, Akın et al. [13] also examined ice cream formulations with varying sugar concentrations (15%, 18%, and 21% w/w) and observed that a relatively high sugar content could have a negative impact on probiotic viability due to excessive osmotic pressure. The best viability was observed in the sample with 18% sugar. Both studies indicate that optimal *L. casei* viability is achieved at moderate sugar concentrations.

Table 4. Studies on strain viability in probiotic- fortified products

Formulation	Probiotic Bacteria	Sugar/Amount	Probiotic Strain (log cfu/g)	Viability (log cfu/g)	Storage Conditions	Ref.
Ice cream Fresh milk =45%; cream= 15%; skimmed milk powder= 7.5%; sucrose=15%; aroma =0.1%; water =10%	*Bifidobacterium animalis subsp. lactis* BB-12	Sucrose =15%	8.38	8.31	28 days at -22 °C	[60]
Ice cream Fresh milk =45%; cream= 15%; skimmed milk powder= 7.5%; erythritol=8.38%; sucrose=7.5%; aroma =0.1%; water =10%		Erythritol=8.38% Sucrose=7.5%	8.13	8.03		
Ice cream Skim milk, cream, skim milk powder, vanilin, stabilizer and emulsifier, sugar =14%	*Lactobacillus casei*	Sucrose =14% (fat=10%)	8.80	6.23	90 days at -24ºC	[61]
Ice cream Skim milk, cream, skim milk powder, vanilin, stabilizer and emulsifier,sugar =16%		Sucrose =16% (fat=5%)	8.95	7.38		
Ice cream Whole milk=45%; fat=15%; skim milk powder=7.4%; stabilizer=0.5% vanilin=0.1%; Sugar=18%; inulin=2%	*Streptococcus salivarius* spp. *thermophilus, Lactobacillus delbrueckii* ssp. *bulgaricus Lactobacillus acidophilus* LA-14, *Bifidobacterium lactis* BL-01	Sugar=18% (inulin=2%)	*S. thermophilus*: 8.59 *L. delbrueckii* ssp. *bulgaricus*:7.79 *L. acidophilus* LA-14: 8.08 *B. lactis* BL-01: 8.14	*S. thermophilus*: < 8 *L. delbrueckii* ssp. *bulgaricus*: <7 *L. acidophilus* LA-14: <7 *B. lactis* BL-01: < 7	90 days at -18ºC	[11]
Ice cream Whole milk=45%; fat=15%; skim milk powder=7.4%; stabilizer=0.5% vanilin=0.1%; Sugar=21%; inulin=0%		Sugar=21% (without inulin)	*S. thermophilus*: 7.99 *L. delbrueckii* ssp. *bulgaricus*:7.52 *L. acidophilus* LA-14: 7.64 *B. lactis* BL-01: 7.73	*S. thermophilus*:< 8 *L. delbrueckii* ssp. *bulgaricus*: 7 *L. acidophilus* LA-14: <5 *B. lactis* BL-01: < 5		
Chocolate Cocoa butter=30%; cocoa mass;whole milk=13.95%; skim milk powder=7.80%; sugar =38.75% soy lecithin =0.30%, polyglycerol polyricinolate (PGPR)=0.20%	*L.acidophilus* LA-14	Sugar=38.75%	8.92		90 days at 20 °C	[62]
Chocolate Cocoa butter=30%; cocoa mass;whole milk=13.95%; skim milk powder=7.80%;maltitol =38.75% soy lecithin =0.30%, polyglycerol polyricinolate (PGPR)=0.20%			8.16			
Cocoa juice Water, cacao pulp =34%, sucrose=9.7%	*Lactobacillus casei* NRRL B-442	Sucrose=9.7%	8.76		42 days at 4 °C	[63]
Cocoa juice Water, cacao pulp = 34%, sucralose=0.48%		Sucralose=0.48%				
Chokeberry juice Sugar= 28.8 g/L; chokeberry juice = 100 ml;L. paracaseiSP5 =1 g freeze-dried bacterial biomass	*L. paracasei* SP5	Sugar=28.8 g/L	7.4		28 days at 4 °C	[64]
Chokeberry juice Sugar= 40.3 g/L; chokeberry juice = 100 ml;L. paracaseiSP5 =1 g freeze-dried bacterial biomass		Sugar=40.3 g/L	9.5			
Ice cream Fat= 12% ; milk solids nonfat= 11%; stabilizer emulsifier=0.32%; sugar=12.5%; corn syrup solids=4.5%	*Lactobacillus acidophilus*	Sugar=12.5% Corn syrup=4.5%	8		17 weeks at -29ºC	[65]
Ice cream Fat= 12% ; milk solids nonfat= 11%; stabilizer emulsifier=0.32%; sugar=12.5%; corn syrup solids=4.5%	*Bifidobacterium bifidum*	Sugar=12.5% Corn syrup=4.5%				

In addition to sugar, the impact of low-calorie sweeteners on probiotic activity is also a subject of interest. Therefore, sweeteners are commonly used in studies that monitor probiotic viability in food products. A study conducted on white chocolate investigated the viability of *Lactobacillus acidophilus* and *Lactobacillus paracasei* in sugar-containing and sugar-free (containing maltitol) chocolates. The study found that *L. acidophilus*-maintained cell counts above 7 log CFU/g during a 90-day storage period*. L. paracasei* initially exhibited a faster decline but stabilized at around 6.61-6.89 log CFU/g. Throughout the study, although sucrose provided better results, the cell counts in white chocolate containing maltitol was still reported to be within the therapeutic range (>6 log CFU/g) [62]. Similarly, Kalicka et al. [60] investigated the effects of sweetener and sugar on viable cell count in ice cream containing *Bifidobacterium animalis* subsp. BB-12. In the study, the highest number of viable cells was found in the control sample containing 15% sucrose, while the lowest numbers were found in the sample containing a combination of erythritol and sucrose. At the end of the 28-day storage period, it was reported that the cell counts of all ice cream samples indicated promising results, with a consistent presence of 8 log CFU/g. Gündoğdu et al [66] reported 7.49 log CFU/g for the same contidions for *Bifidobacterium animalis* subsp. BB-12.

In summary, the varying sugar content of the products determines the viability of probiotic bacteria and the sensory properties of the product. However, higher sugar concentrations do not always result in a lower count of viable bacteria. It is the responsibility of food manufacturers to find the right concentrations for both the sensory and physical properties of the product, as well as its probiotic activity. In fact, the inclusion of higher sugar amounts in probiotic-fortified products intended for health benefits is not deemed appropriate when evaluated in the context of a healthy diet [67].

In contrast, a notable portion of individuals face challenges in embracing sugar-free variants of probiotic-fortified products. For such individuals, an advisable approach might involve selecting the products featuring sugar content below 5% during the initial phase of acclimatization. The integration of these specific probiotic items, known to enhance overall health, into their dietary regimen could be deemed an advantageous strategy.

CONCLUSION

In this review, we evaluated probiotic-fortified foods across different categories available in the market based on their strain composition and sugar content. The sugar levels found in these foods often surpass the recommended daily limits for added sugar and total sugar intake as advised by the World Health Organization (WHO) and the National Health Service (NHS). According to the guidelines, opting for low-sugar options can increase both consumption and the intake of probiotics. However, the precise threshold at which excessive sugar consumption leads to dysbiosis and inhibits probiotic activity remains uncertain.

The viability of a particular probiotic strain can vary depending on factors such as the type of sugar, sugar concentration, strain type, product formulation, and food processing conditions. Conducting research that focuses on specific strains would be invaluable in understanding how different amounts of sugar impact viability – either supporting or inhibiting it. Such strain-specific investigations will shed light on the intricate interplay between sugar content and probiotic viability, offering critical insights for optimizing these foods' benefits.

REFERENCES

[1] Davis, D.R. (2009). Declining fruit and vegetable nutrient composition: what is the evidence? *HortScience*, 44(1), 15-19.

[2] Tungland, B. (2018). Dysbiosis of the Microbiota: Therapeutic Strategies Utilizing Dietary Modification, Pro- and Prebiotics and Fecal Transplant Therapies in Promoting Normal Balance and Local GI Functions. In Human Microbiota in Health and Disease, Edited by B. Tungland, Academic Press, Cambridge, USA, 381-419p.

[3] Palanivelu, J., Thanigaivel, S., Vickram, S., Dey, N., Mihaylova, D., Desseva, I. (2022). Probiotics in functional foods: survival assessment and approaches for improved viability. *Applied Sciences*, 12(1), 455.

[4] Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., and Salminen S., Calder, P.C., Sanders, M.E. (2014). The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology and Hepatology*, 11, 506–514.

[5] Jones, P.J., Jew, S. (2007). Functional food development: concept to reality. *Trends in Food Science and Technology*, 18(7), 387-390.

[6] Terpou, A., Papadaki, A., Lappa, I.K., Kachrimanidou, V., Bosnea, L.A., Kopsahelis, N. (2019). Probiotics in food systems: significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. *Nutrients*, 11(7), 1591.

[7] Mortazavian, A.M., Mohammadi, R, Sohrabvandi, S. (2012). Delivery of probiotic microorganisms into gastrointestinal tract by food products. In New Advances in the Basic and Clinical Gastroenterology, Edited by T. Brzozowski, InTech, Rijeka, Croatia, 121-146p.

[8] Konuray, G., Erginkaya, Z. (2018). Potential use of *Bacillus coagulans* in the food industry. *Foods*, 7(6), 92.

[9] Acton, R.B., Vanderlee, L., Hobin, E.P., Hammond, D. (2017). Added sugar in the packaged foods and beverages available at a major Canadian retailer in 2015: a descriptive analysis. *Canadian Medical Association Open Access Journal*, 5(1), E1-E6.

[10] Johnson, R.J., Segal, M.S., Sautin, Y., Nakagawa, T., Feig, D.I., Kang, D.-H., Gersch, M.S., Benner,

S., Sánchez-Lozada, L.G. (2007). Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. *The American Journal of Clinical Nutrition,* 86(4), 899-906.

[11] Akın, M., Akın, M., Kırmacı, Z. (2007). Effects of inulin and sugar levels on the viability of yoghurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. *Food Chemistry*,104(1), 93-99.

[12] Blaiotta, G., La Gatta, B., Di Capua, M., Di Luccia, A., Coppola, R., Aponte, M. (2013). Effect of chestnut extract and chestnut fiber on viability of potential probiotic *Lactobacillus* strains under gastrointestinal tract conditions. *Food Microbiology*, 36(2), 161-169.

[13] Heidebach, T., Först, P., Kulozik, U. (2012). Microencapsulation of probiotic cells for food applications. *Critical Reviews in Food Science and Nutrition*, 52(4), 291-311.

[14] Kalicka, D., Znamirowska, A., Pawlos, M., Buniowska, M., Szajnar, K. (2019). Physical and sensory characteristics and probiotic survival in ice cream sweetened with various polyols. *International Journal of Dairy Technology*, 72(3), 456-465.

[15] Francavilla, R., Piccolo, M., Francavilla, A., Polimeno, L., Semeraro, F., Cristofori, F., Castellaneta, S., Barone, M., Indrio, F., Gobbetti, M., De Angelis, M. (2019). Clinical and microbiological effect of a multispecies probiotic supplementation in celiac patients with persistent IBS-type symptoms: a randomized, double-blind, placebo-controlled, multicenter trial. *Journal of Clinical Gastroenterology*, 53(3), e117.

[16] Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., Li, Y., Xia, Y., Xie, H., Zhong, H., Khan, M.T., Zhang, J., Li, J., Xiao, L., Al-Aama, J., Zhang, D., Lee, Y.S., Kotowska, D., Colding, C., Tremaroli, V., Yin, Y., Bergman, S., Xu, X., Madsen, L., Kristiansen, K., Dahlgren, J., Wang, J. (2015). Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe*, 17(5),690-703.

[17] Ciorba, M.A. (2012). A gastroenterologist's guide to probiotics. *Clinical Gastroenterology and Hepatology*, 10(9), 960-968.

[18] Altun, G.K., Erginkaya, Z. (2021). Identification and characterization of *Bacillus coagulans* strains for probiotic activity and safety. *LWT,* 151, 112233.

[19] Aminlari, L., Shekarforoush, S.S., Hosseinzadeh, S., Nazifi, S., Sajedianfard, J., Eskandari, M.H. (2019). Effect of probiotics *Bacillus coagulans* and *Lactobacillus plantarum* on lipid profile and feces bacteria of rats fed cholesterol-enriched diet. *Probiotics and Antimicrobial Proteins*, 11, 1163-1171.

[20] Batra, N., Singh, J., Banerjee, U.C., Patnaik, P.R., Sobti, R.C. (2002). Production and characterization of a thermostable β-galactosidase from *Bacillus coagulans* RCS3. *Biotechnology and Applied Biochemistry,* 36(1), 1-6.

[21] Kimmel, M., Keller, D., Farmer, S., Warrino, D. (2010). A controlled clinical trial to evaluate the effect of GanedenBC (30) on immunological markers. *Methods and Findings in Experimental and Clinical Pharmacology*, 32(2), 129-132.

[22] Kobus-Cisowska, J., Szymanowska, D., Maciejewska, P., Szczepaniak, O., Kmiecik, D., Gramza-Michałowska, A., Kulczyński, B., Cielecka-Piontek, J. (2019). Enriching novel dark chocolate with *Bacillus coagulans* as a way to provide beneficial nutrients. *Food and Function*, 10(2), 997-1006.

[23] Minamida, K., Nishimura, M., Miwa, K., Nishihira J. (2015). Effects of dietary fiber with *Bacillus coagulans lilac*-01 on bowel movement and fecal properties of healthy volunteers with a tendency for constipation. *Bioscience, Biotechnology and Biochemistry*, 79(2), 300-306.

[24] Ayala, F.R., Bauman, C., Cogliati, S., Leñini, C., Bartolini, M., Grau, R. (2017). Microbial flora, probiotics, *Bacillus subtilis* and the search for a long and healthy human longevity. *Microbial Cell*, 4(4), 133.

[25] Errington, J., Wu, L.J. (2017). Cell Cycle Machinery in *Bacillus subtilis*. *Subcellular Biochemistry*, 84, 67-101.

[26] Hong, H.A., Khaneja, R., Tam, N.M., Cazzato, A., Tan, S., Urdaci, M., Brisson, A., Gasbarrini, Barnes, A.I., Cutting, S.M. (2009). *Bacillus subtilis* isolated from the human gastrointestinal tract. *Research in Microbiology,* 160(2), 134-143.

[27] Jeżewska-Frąckowiak, J., Seroczyńska, K., Banaszczyk, J., Jedrzejczak, G., Żylicz-Stachula, A., Skowron, P.M. (2018). The promises and risks of probiotic *Bacillus* species. *Acta Biochimica Polonica*, 65 (4), 509-519.

[28] Lefevre, M., Racedo, S.M., Denayrolles, M., Ripert, G., Desfougeres, T., Lobach, A.R., Simon, R., Pélerin, F., Jüsten, P., Urdaci, M.C. (2017). Safety assessment of *Bacillus subtilis* CU1 for use as a probiotic in humans. *Regulatory Toxicology and Pharmacology*, 83, 54-65.

[29] Lei, K., Li, Y.L, Wang, Y., Wen, J., Wu, H.Z., Yu, D.Y., Li.W. (2015). Effect of dietary supplementation of *Bacillus subtilis* B10 on biochemical and molecular parameters in the serum and liver of high-fat diet-induced obese mice. *Journal of Zhejiang University. Science B*, 16(6),487-495.

[30] Lu, Z., Guo, W., Liu, C. (2018). Isolation, identification, and characterization of novel *Bacillus subtilis*. *Journal of Veterinary Medical Science*, 80(3), 427-433.

[31] Olmos, J., Paniagua-Michel. J. (2014). *Bacillus subtilis* a potential probiotic bacterium to formulate functional feeds for aquaculture. *Journal of Microbial and Biochemical Technology*, 6(7) ,361-365.

[32] Piggot, P. (2009). *Bacillus subtilis*. In Encyclopedia of Microbiology, Edited by M. Schaechter, Academic Press, Oxford, UK, 45-56p.

[33] Su, Y., Liu, C., Fang, H., Zhang, D. (2020). *Bacillus subtilis*: a universal cell factory for industry, agriculture, biomaterials, and medicine. *Microbial Cell Factories*, 19(1), 1-12.

[34] Tompkins, T., Xu, X., Ahmarani, J. (2010). A comprehensive review of post-market clinical

studies performed in adults with an Asian probiotic formulation. *Beneficial Microbes*, 1(1), 93-106.

[35] Candela, M., Turroni, S., Centanni, M., Fiori, J., Bergmann, S., Hammerschmidt, S., Brigidi, P. (2011). Relevance of *Bifidobacterium animalis* subsp. *lactis* plasminogen binding activity in the human gastrointestinal microenvironment. *Applied and Environmental Microbiology*, 77(19), 7072-7076.

[36] de Souza Oliveira, R.P., Perego, P., de Oliveira, M.N., Converti, A. (2012). Growth, organic acids profile and sugar metabolism of *Bifidobacterium lactis* in co-culture with *Streptococcus thermophilus*: The inulin effect. *Food Research International*, 48(1),21-27.

[37] Janer, C., Arigoni, F., Lee, B., Peláez, C., Requena, T. (2005). Enzymatic ability of *Bifidobacterium animalis* subsp. *lactis* to hydrolyze milk proteins: identification and characterization of endopeptidase O. *Applied and Environmental Microbiology*, 71(12), 8460-8465.

[38] Jungersen, M., Wind, A., Johansen, E., Christensen, J.E., Stuer-Lauridsen, B., Eskesen, D. (2014). The science behind the probiotic strain *Bifidobacterium animalis* subsp. *lactis* BB-12®. *Microorganisms*, 2(2), 92-110.

[39] Kim, N.Y., Ji., G.E. (2012). Effects of probiotics on the prevention of atopic dermatitis. *Korean Journal of Pediatrics*, 55(6), 193.

[40] López, P., Gueimonde, M., Margolles, A., Suárez, A. (2010). Distinct *Bifidobacterium* strains drive different immune responses *in vitro*. *International Journal of Food Microbiology*, 138(1-2), 157-165.

[41] Meile, L., Ludwig, W., Rueger, U., Gut, C., Kaufmann, P., Dasen, G., Wenger, S., Teuber, M. (1997). *Bifidobacterium lactis* sp. nov., a moderately oxygen tolerant species isolated from fermented milk. *Systematic and Applied Microbiology*, 20(1), 57-64.

[42] Shah, N.P., Lankaputhra, W.E.V. (2002). *Bifidobacterium* spp: Morphology and Physiology. In Encyclopaedia of Dairy Sciences, Edited by H. Roginski, Academic Press, USA, 141-146p.

[43] Solano-Aguilar, G., Dawson, H., Restrepo, M., Andrews, K., Vinyard, B., Urban Jr, J.F. (2008). Detection of *Bifidobacterium animalis* subsp. *lactis* (Bb12) in the intestine after feeding of sows and their piglets. *Applied and Environmental Microbiology,* 74(20), 6338-6347.

[44] Ku, S., Yang, S., Lee, H.H., Choe, D., Johnston, T.V., Ji, G.E., Park, M.S. (2020). Biosafety assessment of *Bifidobacterium animalis* subsp. *lactis* AD011 used for human consumption as a probiotic microorganism. *Food Control*, 117, 106985.

[45] Gharbi Yahyaoui, A., Bouzaiene, T., Aouidi, F., Aydi, A., Hamdi, M. (2017). Traditional cereal food as container of probiotic bacteria "*Lb. rhamnosus* GG": optimization by response surface methodology. *Journal of Food Quality*, 1-12.

[46] Gorbach, S., Doron, S., Magro. F. (2017). *Lactobacillus rhamnosus* GG. In The Microbiota in Gastrointestinal Pathophysiology, Edited by M.H. Floch, Y. Ringel, W.A. Walker, Academic Press, San Diego, USA. 79-88p.

[47] Johnston, B.C., Goldenberg, J.Z., Parkin, P.C. (2016). Probiotics and the prevention of antibiotic-associated diarrhea in infants and children. *Jama*, 316(14), 1484-1485.

[48] Kim, S.W., Park, K.Y., Kim, B., Kim, E., Hyun, C.K. (2013). *Lactobacillus rhamnosus* GG improves insulin sensitivity and reduces adiposity in high-fat diet-fed mice through enhancement of adiponectin production. *Biochemical and Biophysical Research Communications*, 431(2), 258-263.

[49] Lazzi, C., Turroni, S., Mancini, A., Sgarbi, E., Neviani, E., Brigidi, P., Gatti, M. (2014). Transcriptomic clues to understand the growth of *Lactobacillus rhamnosus* in cheese. *BMC Microbiology*, 14(1),1-14.

[50] Segers, M.E., Lebeer, S. (2014). Towards a better understanding of *Lactobacillus rhamnosus* GG-host interactions. *Microbial Cell Factories*, 13(1), 1-16.

[51] Zheng, J., Wittouck, S., Salvetti, E., Franz, C.M.A.P, Harris, H.M.B., Mattarelli, P., O'toole, P.W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G.E., Ganzle, M.G., Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus beijerinck* 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology,* 70(4), 2782-2858.

[52] Mann, J. (2014). The science behind the sweetness in our diets. World Health Organization. *Bulletin of the World Health Organization*, 92(11), 780.

[53] FONA International (2021). Sugar: the voice of the consumer. Accessed November 29, 2023. https://www.fona.com/articles/2021/06/sugar-the-voice-of-the-consumer.

[54] Sollid, K., Webster, A.D., Paipongna, M., Smith, K. (2022). Food perceptions, beliefs, and behaviors amid a global pandemic: results of the international food information council 2021 food & health survey. *Nutrition Today*, 57(1), 26-33.

[55] Anastasiou, K., Miller, M., Dickinson, K. (2019). The relationship between food label use and dietary intake in adults: A systematic review. *Appetite*, 138, 280-291.

[56] Food and Drug Administration (2023). Generally recognized as safe (GRAS) determination for the intended use of *Bifidobacterium animalis* ssp. *lactis* BB-12®. Accessed July 18, 2023. https://www.fda.gov/media/134330/download.

[57] Kunz, S., Haasova, S., Rieß, J., Florack, A. (2020). Beyond healthiness: the impact of traffic light labels on taste expectations and purchase intentions. *Foods*, 9(2), 134.

[58] World Health Organization (2015). In Guideline: sugars intake for adults and children, WHO Press, Geneva, Switzerland,14-26p.

[59] Mohammadi, R., Mortazavian, A.M., Khosrokhavar, R., da Cruz, A.G. (2011). Probiotic ice cream: viability of probiotic bacteria and sensory properties. *Annals of Microbiology*, 61, 411-424.

[60] Kalicka, D., Znamirowska, A. Pawlos, M., Buniowska, M., Szajnar, K. (2019). Physical and sensory characteristics and probiotic survival in ice cream sweetened with various polyols. *International Journal of Dairy Technology*, 72(3), 456-465.

[61] Shahsavan, A., Pourahmad, R., Rajaei, P. (2018). Effect of different amounts of sugar and fat on the viability of *Lactobacillus casei*, physical, chemical and sensory properties of probiotic ice cream. *International Journal of Biology and Biotechnology*, 15(1), 63-69.

[62] Konar, N., Palabiyik, I., Toker, O.S., Polat, D.G., Kelleci, E., Pirouzian, H.R., Akcicek, A., Sagdic, O. (2018). Conventional and sugar-free probiotic white chocolate: Effect of inulin DP on various quality properties and viability of probiotics. *Journal of Functional Foods*, 43, 206-213.

[63] dos Santos Filho, A.L., Freitas, H.V., Rodrigues, S., Abreu, V.K.G., de Oliveira Lemos, T., Gomes, W.F., Narain, N., Pereira, A.L.F. (2019). Production and stability of probiotic cocoa juice with sucralose as sugar substitute during refrigerated storage. *LWT*, 99, 371-378.

[64] Bontsidis, C., Mallouchos, A., Terpou, A., Nikolaou, A., Batra, G., Mantzourani, I., Plessas, S. (2021). Microbiological and chemical properties of chokeberry juice fermented by novel lactic acid bacteria with potential probiotic properties during fermentation at 4ºC for 4 weeks. *Foods*, 10(4),768.

[65] Hekmat, S., Mcmahon, D.J. (1992). Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in ice cream for use as a probiotic food. *Journal of Dairy Science,* 75(6), 1415-1422.

[66] Gündoğdu, E., Ertem, H., Çakmakçı, S. (2022). Effect of using green tea (*Camellia sinensis* L.) powder and probiotic bacteria on probiotic shelf life and quality properties of ice cream. *Akademik Gıda*, 20(2), 138-144.

[67] Ashwin, D., Ke, V., Taranath, M., Ramagoni, N.K., Nara, A., Sarpangala, M. (2015). Effect of probiotic containing ice-cream on salivary mutans *Streptococci* (SMS) levels in children of 6-12 years of age: a randomized controlled double-blind study with six-months follow up. *Journal of Clinical abd Diognostic Research: JCDR*, 9(2), ZC06-09.

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Review Paper / Derleme Makale

Effect of Advanced Cooking Methods on Formation of Toxic Compounds in Meat

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ABSTRACT

Meat is an essential component of human nutrition and is prepared using various cooking techniques to ensure its microbiological safety and enhance its sensory qualities. However, conventional cooking methods like grilling, deep-frying, and pan-searing often involve extended heat exposure, which can result in the production of harmful toxic compounds such as polycyclic aromatic hydrocarbons, heterocyclic amines, and acrylamide that may pose health risks. Moreover, the high temperatures and prolonged cooking times, typical of these conventional methods, can cause a reduction in the nutritional content of meat. In recent years, research has focused on innovative methods that can minimize the formation of toxic substances in meat while preserving its nutritional value and offering safer cooking alternatives. Among these advanced cooking technologies, microwave heating, ohmic heating, sous-vide cooking, and air-frying have attracted increasing interest. Compared to conventional methods, these techniques present important benefits, including improved product quality and efficiency, faster processing times, energy savings, and lower production costs. This review discusses advanced cooking technologies and their principles, highlighting their influence on the formation of toxic compounds in meat in comparison with conventional cooking practices.

Keywords: Meat, Cooking methods, Quality, Advanced techniques

İleri Pişirme Tekniklerinin Ette Toksik Bileşik Oluşumuna Etkisi

ÖZ

İnsan beslenmesinde önemli bir besin kaynağı olan et, mikrobiyolojik güvenirliliğinin sağlanması ve organoleptik özelliklerinin geliştirilmesi amacıyla çeşitli yöntemlerle pişirilerek tüketilmektedir. Ancak etin pişirilmesinde kullanılan ızgara, yağda kızartma, tavada pişirme gibi geleneksel yöntemlerde uzun süre yüksek ısı uygulanmasından dolayı, polisiklik aromatik hidrokarbonlar, heterosiklik aminler ve akrilamid gibi insan sağlığı açısından tehlikeli toksik bileşikler oluşabilmektedir. Bunun yanı sıra geleneksel pişirme yöntemlerinde kullanılan uzun süre ve yüksek sıcaklık etin besleyici değerinde kayıplara neden olmaktadır. Son zamanlarda etin besin değerinde minimum kayıpla birlikte toksik bileşiklerin oluşumunu azaltacak daha güvenli pişirme sağlayacak ileri teknikler ile ilgili çalışmalar popülerlik kazanmıştır. İleri pişirme yöntemleri arasında mikrodalga ısıtma, ohmik ısıtma, sous-vide pişirme ve air-fryer teknolojileri dikkat çekmektedir. Geleneksel yöntemlere kıyasla bu yöntemlerin ürün kalitesini ve verimliliğini arttırması, işlem süresinin kısa olması, enerji tasarrufu sağlaması, işlem maliyetini azaltması gibi avantajlarından dolayı önemli bir potansiyele sahip olduğu ifade edilmektedir. Bu derlemede ileri pişirme teknikleri, uygulama mekanizmaları, ette toksik bileşik oluşumu üzerine etkileri geleneksel yöntemlerle kıyaslanarak irdelenmiştir.

Anahtar Kelimeler: Et, Pişirme yöntemleri, Kalite, İleri teknikler

INTRODUCTION

During cooking, the tenderness of meat increases due to the shrinkage of muscle fibers and the reduction of adhesion between fibers, resulting in the collagen tissue dissolving to form gelatin, especially at temperatures between 40-60°C [1]. However, when the cooking temperature rises to a temperature between 60 and 90, longitudinal shrinkage of muscle fibers increases water loss, negatively affecting the texture [2]. Furthermore, high temperatures during cooking accelerate oxidation reactions. Lipid oxidation occurs as unsaturated fatty acids undergo oxidation during meat cooking, shortening the shelf life of meat, releasing unwanted odor and flavor compounds, causing nutrient loss, and leading to color and texture abnormalities, which adversely affect quality [3].

Throughout the cooking process, chemical contaminants such as polycyclic aromatic hydrocarbons, heterocyclic amines, and acrylamide, which pose significant risks to human health, can also form in the composition of the meat depending on many factors such as method, temperature and application time [4]. Polycyclic aromatic hydrocarbons (PAHs) are organic compounds released as a result of incomplete combustion of organic materials, which can persist in the environment for extended periods, causing environmental pollution and disrupting biological balance [5]. PAHs are a major concern due to their confirmed or potential adverse health impacts. Regulatory agencies have classified high molecular weight PAHs, such as benzo[a]pyrene (BaP), as priority pollutants because of their significant toxicological relevance [6]. Among foods, meat is considered one of the highest-risk sources of PAHs formation. PAHs levels in cooked meats vary depending on factors such as cooking method, type of fuel, cooking temperature and duration, distance from the heat source, and fat content of the food [7-9]. Studies have shown that smoking, frying, and grilling methods result in higher levels of PAHs formation, whereas boiling and baking methods result in lower levels of PAHs formation [10, 11]. Heterocyclic amines (HAs) are mutagenic/carcinogenic compounds formed when meat is cooked at high temperatures, especially during pan frying or charcoal grilling [12]. It has been reported that HAs can be 100 times more mutagenic than aflatoxin B1 and 2,000 times more mutagenic than BaP on an equal mass basis [13]. HAs level can be reduced by avoiding prolonged exposure to high temperatures and preventing the surface from coming into direct contact with flames while cooking meat [14]. Acrylamide, chemically known as 2-propenamide (C_3H_5NO), is a water-soluble compound commonly present in foods with high carbohydrate content like potato and grain-based products. Acrylamide is found in various quantities in many foods, except for boiled or raw food. Cooked carbohydrate-rich foods were found to contain higher levels of acrylamide (150-4000 µg/kg) compared to cooked protein-rich foods (5-50 µg/kg) [15]. Many foods, particularly those high in carbohydrates (starch), amino acids (asparagine), and oils (glycerol is converted to acrolein) produce acrylamide when they are heated to temperatures above 120°C during the thermal frying process [16]. The International Agency for Research on Cancer (IARC) [17] has identified acrylamide as a potential human carcinogen, and it is also formed during the frying of chicken [18].

In conventional (traditional) cooking methods, the use of prolonged time and high temperatures generally leads to losses in the nutritional value of the meat. Additionally, the cooking method and duration may result in the formation of toxic compounds that may pose a threat to human health. To eliminate these risks, recent studies have focused on the use of alternative advanced cooking methods such as microwave heating, ohmic heating, sous-vide, and air-frying technologies to produce safer, minimally processed foods with high nutritional value [19, 20]. In advanced cooking methods, thermal energy is produced directly within the food, ensuring homogeneous distribution of heat throughout the food. The aim of using these methods is to improve product quality, shorten processing times, reduce costs, and increase efficiency by decreasing water and energy consumption [21]. In this study, the effects of traditional and advanced cooking technologies on formation of toxic compounds in meat were reviewed.

EFFECT OF TRADITIONAL MEAT COOKING METHODS ON FORMATION OF TOXIC COMPOUNDS

Grilling

Grilling is a dry cooking method that utilizes direct radiant heat. In this method, heat sources such as oven grills, electric grills, or charcoal grills can be used. Since the heat spreads from one direction, it is necessary to turn the meat during cooking [22]. In the charcoal grilling method, one of the biggest risks that arise from cooking at very high temperatures with charcoal is the formation of PAHs [23]. It has been reported that in grilled meat products cooked at high temperatures, PAHs compounds are formed as a result of pyrolysis of fat dripping onto the charcoal and these compounds are transferred to the meat surface along with the smoke [23, 24]. The levels of PAHs in cooked meats vary depending on the preparation and cooking method, temperature, duration, distance of the meat from the heat source (direct or indirect), and fat content (Table 1) [8, 25].

To reduce or prevent the formation of PAHs in grilled meat, avoiding direct contact with flames, minimizing exposure to smoke, preventing the burning of fat, removing dripping fat, avoiding excessively high cooking temperatures, and selecting the appropriate distance from the heat source are crucial [9]. In a study examining the effects of different cooking methods (boiling, frying, oven grilling, electric grilling and charcoal grilling) on the formation of PAHs compounds in various types of meat (beef, lamb, chicken, turkey), the concentration of PAH4 compounds BaP, benzo(a)anthracene (BaA), benzo(b)fluoranthene (BbFlu), chrysene (Chr)) was found to vary depending on the structure of the meat and the cooking methods. The highest PAH4 concentrations ranged between 1.10

and 3.30 µg/kg was found in chicken meats cooked on the grill [11]. Another way to reduce PAHs compounds in meat is by applying different marinades before the cooking process, which has been shown to be quite effective in reducing the formation and concentration of PAHs in cooked meats [27, 28]. In a study examining the effect of different tea plant marinades on the formation of PAHs in grilled chicken drumsticks cooked using different methods (charcoal and electric), it was found that marinated drumsticks with white tea and cooking them on an electric grill significantly reduced the formation of HCAs (from 13.15 ng/g to 10.08 ng/g) and BaP (from 13.15 ng/g to 0.17 ng/g) compared to wood charcoal grilled drumsticks ($P<0.05$) [29].

Table 1. The major factors influencing the PAH formation in meat [26, 27]

Type of grill	Temperature and time
Electric < Gas ≤ Briquettes=Charcoal < Wood	Low temperature < High temperature
No dripping fat < Dripping fat	Short grilling time < Long grilling time
Long distance from heat < Short distance	
Vertical heat source < Horizontal heat source	
Type of food	**Preparation of the food**
Low fat < High fat	Frequent turning < No turning
Smooth/sealed surface < Rough surface	Marination with antioxidants < No marination
Low protein < High protein	Precooking of food < No precooking

Saute Cooking

Saute cooking is a method where small pieces of meat are cooked in a pan with a small amount of oil. In this method, the pan is heated to 160-240°C before cooking, thus preventing the meat from sticking to the pan and the water in the meat from leaking out during the sealing process [23]. It is stated that factors such as cooking method, temperature, duration, pH, and fat content play significant roles in the formation of mutagenic and carcinogenic heterocyclic amines in saute cooking method [30].

In a study, various cooking methods (deep fat frying, pan frying, charcoal grilling, and roasting) on the formation of heterocyclic amines in chicken and duck breast meats were investigated. The results showed that chicken breast cooked by charcoal grilling had the highest total HAs content (112 ng/g), followed by pan-fried (27.4 ng/g) and deep fried (21.3 ng/g), while the lowest formation of HAs was observed in roasted chicken breast (4 ng/g). Similarly, for duck breast pan frying resulted in the highest HAs content (53.3 ng/g), followed by charcoal grilling (32 ng/g), deep frying (13.9 ng/g), while the lowest HAs formation was observed in roasted duck breast (6.8 ng/g) [31].

Deep Fat Frying

Deep fat frying is a method of cooking meat by immersing it in oil at 150-190°C [32]. During frying, various physical and chemical changes occur in the color and structure of the oil [33]. The primary degradation products in frying oil are non-volatile polar compounds, triacylglycerol dimers, and polymers. The change in color of the oil is associated with oxidation, polymerization, and the formation of carbonyl compounds [34]. Deep fat frying is a method commonly used in large-scale food production operations. The oils used in this process tend to degrade more quickly than those used for home frying, primarily due to their prolonged use [32].

Since oil can break down and produce smoke at high temperatures, oil with low smoke points should not be used for frying [35]. Due to the application of high temperatures, carcinogenic compounds such as PAHs, HAs and acrylamide can form in meat [23]. In the frying process, the formation of carcinogenic compounds increases as the time increases [34]. Additionally, the toxic compounds produced by the repeatedly using cooking oil at high temperatures during frying pose a health risk [34]. HAs are formed during frying in oil and repeated use of oil leads to an increase in the amount of toxic components [32, 36].

In a study conducted by Ghasemian et al. [37], hamburgers prepared from beef were fried in sunflower oil at 180°C and 200°C for 4 and 6 min. Their results indicated that acrylamide levels reached 40 ppb in a beef burger fried in sunflower oil at 180°C for 4 min, while 85 ppb of acrylamide was detected in a beef burger fried at 200°C for 6 min. As a result of the study, it was reported that an increase in temperature and cooking time led to an increase in acrylamide content of hamburgers. In another study, various cooking methods (microwave, baking, deep fat frying, and pan frying) were applied to heat-treated croquettes filled with meat. As a result, acrylamide concentrations of 360 µg/kg, 285 µg/kg, and 298 µg/kg were detected in baked, pan fried, and deep fat fried croquettes, respectively, while the highest level of acrylamide formation (420 µg/kg) was reported in the microwave cooked croquettes [38].

Boiling

Boiling is a method of cooking food in a liquid at temperatures above 100°C. This can be achieved by rapidly boiling the liquid or by gently simmering it at temperatures below the boiling point. It is known that the formation of carcinogenic heterocyclic amines is lower in boiled meats compared to grilling, frying, and sauteing methods. In the boiling method, the aim is to prevent drip loss and physical changes in the meat by retaining its juice. However, the addition of more water than

necessary during cooking causes vitamin and mineral losses, as well as color and flavor deterioration [35].

Solyakov and Skog [39] reported that the content of HAs in poultry meat samples was lower (0.5 ng/g) when boiled at low temperatures (<100°C) compared to cooking at higher temperatures, and HAs formation increased with rising cooking temperature. In another study, the effect of different cooking methods (smoking, grilling, and boiling) on the formation of PAHs in processed meats was examined. As a result, it was reported that the highest level of PAHs (2.79 µg/kg) was found in smoked meats and the lowest level of PAHs (0.99 µg/kg) was determined in boiled meats [40].

Baking

Baking is a method that evenly cooks food by transferring heat directly or through convection. The baking method is widely used in the food industry [41]. The quality characteristics and microbial safety of meat are affected by the baking process [42]. High cooking temperatures enhance the color and flavor of meat, shorten the cooking time, but reduce its juiciness and cause it to gain a hard texture [43]. In a study, the formation of PAHs in beef and pork during grilling and baking were investigated. The results indicated that the concentration of PAHs depended on the cooking method and the type of heat source used, with the highest concentration of PAHs (10.2 µg/kg) observed in charcoal grilling [44].

EFFECT OF ADVANCED MEAT COOKING METHODS ON FORMATION OF TOXIC COMPOUNDS

Microwave Heating

Microwave heating is a dielectric heating method commonly used in industry for cooking or reheating food quickly in households. Microwave energy is a non-ionizing electromagnetic radiation with a frequency range of 300 MHz to 300 GHz [21]. In microwave heating method, the formation of a homogeneous electromagnetic field allows energy to penetrate directly into the food, enabling volumetric and rapid heating. In food processing, operations such as heating, drying, sterilization, and thawing can be performed more efficiently with microwave technology, allowing for greater energy efficiency and shorter processing times [45]. The principle of microwave cooking involves converting electromagnetic energy into thermal energy within the meat. During the cooking process, microwave energy is absorbed through the rotation of water molecules and the movement of ionic components in the meat. Therefore, the water content and the dissolved ion content play crucial roles [46]. Compared to traditional heating methods, microwave heating provides homogeneous heat treatment [47].

In a study, the effect of different cooking methods on the concentration of HAs in chicken and beef satays was investigated. Beef and chicken satays were subjected to grilling, microwave pre-treatment before grilling, and microwave followed by deep frying. The results indicated that chicken satays cooked by charcoal grilling had the highest total HAs content (126.59 ng/g), followed by microwave-charcoal grilled (81.69 ng/g), while the lowest formation of HAs was observed in microwave-deep fried chicken satays (3.44 ng/g). Similarly, for beef satays charcoal grilling resulted in the highest HAs content (140.68 ng/g), followed by microwave-charcoal grilling (81.31 ng/g), while the lowest HAs formation was observed in microwave-deep fried beef satays (2.51 ng/g) [48].

Ohmic Heating

Ohmic heating is an advanced electro-heating technique in which alternating electric current is passed through food to achieve heating. In ohmic heating, food is used as a resistor, allowing alternating current is passed through it, thereby converting the electrical energy into heat energy [49]. Generally, ohmic heating is applied to foods at frequencies between 50-60 Hz [21]. Ohmic heating offers many advantages such as preserving product quality, reducing cooking loss, requiring low-cost, high-energy efficiency, and being environmentally friendly due to its ability to provide rapid and uniform heating [20]. The heat generation that occurs in the ohmic heating method varies depending on the thermal conductivity of the food and the voltage gradient used during heating. In ohmic heating, as the applied voltage gradient increases, the time required for the sample to reach the desired temperature decreases. Foods with high electrical conductivity heat up more quickly [50].

It has been stated that ohmic heating is effective on color stability as well as increasing protein coagulation and aggregation in meat [51]. In a study reported by Zell et al. [20], the effects of ohmic heating and steam cooking on beef quality were investigated. The results indicated that samples cooked with ohmic heating had a lighter color and less cooking loss compared to those cooked with steam, but they exhibited a firmer texture. Another study explored the impact of low-temperature long-time (72°C, 15 min), high-temperature short-time (95°C, 8 min) ohmic heating, and steam heating (80°C, 105 min) on meat quality. The findings revealed that meats subjected to ohmic heating had higher L^* and lower cooking loss values compared to those subjected to steam heating [52].

Sous Vide

Sous vide is a cooking technique of French origin where vacuum-sealed meat, either raw or pre-cooked, is cooked in a controlled environment of low temperature (60-95°C) with circulating water or both heat and steam [53, 54]. After cooking, the product can be served directly or grilled or pan fried [55]. Compared to traditional methods, the sous-vide method uses lower temperatures and longer cooking times [56]. Studies have reported that this combination enhances the activity of proteolytic enzymes in meat, leading to the release of free amino acids and increased tenderness [57, 58]. It has been reported that in sous vide cooking, there is less loss of nutritional components like vitamins

and minerals in meat compared to traditional cooking methods such as frying, microwaving, oven cooking, and grilling [59, 60]. Cooking meat at high temperatures can lead to the formation of toxic and harmful substances such as HAs, as well as a loss of nutritional value [61]. In sous-vide cooking technology, the formation of harmful substances is reduced, and the nutritional value of meat is preserved, as cooking takes place in a vacuum packaging environment and at low temperature [62].

There are two main points that distinguish sous-vide cooking from other methods. First, the food being cooked is vacuum-sealed, which minimizes contact with air and thus preventing oxidative spoilage during cooking or subsequent storage. Second, the food is cooked under controlled conditions at a constant temperature, ensuring that the temperature remains the same throughout the cooking process, both at the center and on the outer surface [62, 63]. Traditional cooking methods often result in temperature differences between the center and the surface of the food. In the sous-vide method, controlled temperature and time parameters are used, allowing products to be easily prepared to the desired level of doneness and ensuring consistent food quality every time [64]. Additionally, because the meat is cooked in a vacuum-sealed package, the heterogeneous temperature and color distribution observed in traditional cooking methods are eliminated, and no additional processes such as stirring or flipping are needed to ensure uniform cooking [65]. The biggest problem in cooking meat at low temperatures for a long time using the sous-vide method is its pink appearance due to the lack of Maillard Reaction on the surface of the cooked meat [66]. To prevent this, the surface of the meat cooked with sous-vide is fried to induce the Maillard reaction [56].

In a study, the formation of HAs was examined in beef samples cooked using sous-vide, boiling, and frying methods at different temperatures and durations. The results showed that total HAs content of the samples ranged from 0.032 ng/g to 0.940 ng/g. The highest level of HAs was detected in samples cooked using the frying method, while the lowest level was found in samples cooked using sous-vide at 75-85°C for 120 min. Additionally, it was reported that maintaining a constant temperature and increasing the cooking time in sous-vide cooking resulted in higher levels of HAs in the samples [67].

Air-Frying

Air-frying is a new technology that cooks food by circulating hot air around it, ensuring even contact between the food and the oil droplets [68]. The mechanism of the air-frying is based on the transfer of heat from the circulating hot air to the food. During the cooking process, dehydration from the surface occurs, resulting in a crispy texture and unique sensory and textural properties in the final product. Air-frying is considered a relatively healthier frying method compared to traditional methods due to lower oil absorption [69]. Compared to deep fat frying, foods cooked in an air fryer contain 70-80% less fat [70].

Recent studies have reported that excessive fat intake increases the risk of hypertension and obesity [71]. Therefore, new methods have been investigated to reduce the fat content of fried products without compromising product quality, and it has been reported that air-fryer cooking method is effective in maintaining food quality and safety [70].

In a study conducted by Lee et al. [72], the formation of acrylamide and PAHs in chicken breast, thigh, and wing samples thawed using different methods (microwave, refrigerator and immersion in water) and cooked using air frying and deep fat frying methods was investigated. Their results revealed that deep-fat-fried chicken meat had higher levels of acrylamide (n.d.-6.19 µg/kg) and total PAH (2.64-3.17 µg/kg) compared to air-fried chicken meat (n.d.-3.49 µg/kg and 1.96-2.71 µg/kg). It was also observed that the thawing method did not significantly affect the formation of acrylamide or PAHs ($P>0.05$) in chicken meat. They concluded that the air-frying method could reduce the formation of acrylamide and PAHs in chicken compared to the deep fat frying method.

TRADITIONAL VERSUS ADVANCED COOKING METHODS

Traditional cooking methods can lead to the formation of harmful compounds (HAs, PAHs etc.) as previously mentioned. In contrast, advanced cooking methods may help reduce the production of these undesirable substances [73]. Therefore, various advanced technologies have been developed and applied to meat to mitigate the harmful effects of high temperatures while enhancing meat quality, as shown in Table 2.

CONCLUSION

Meat, which is an essential source for a balanced nutrition and requires thermal processing for consumption, plays a significant role in the intake of carcinogenic compounds into the body. Exposure of meat to high temperatures for extended periods can lead to the formation of carcinogenic compounds such as PAHs, HAs and acrylamide, which adversely affect the eating quality of meat. Cooking conditions are one of the most important parameters affecting the formation of these compounds. For this reason, it is very important to choose the appropriate cooking method, apply the controlled cooking process at low temperatures, avoid cooking directly over the flame, prevent the burning of meat fat and pay attention to the distance from the heat source. It is predicted that advanced cooking methods may be preferred as alternative safe cooking methods offering advantages over traditional methods including energy savings due to shorter processing times, protection of product quality, reduction or prevention of toxic compound formation, decreased cooking losses, lower oil absorption, and more homogeneous temperature, and color distribution.

Table 2. Comparison of the advantages and disadvantages of different meat cooking methods

Cooking Methods	Advantages and Disadvantages	References
Grilling	Processing at temperatures above 200°C poses a risk of HAs formation, while prolonged cooking further enhances the likelihood of PAHs formation, including higher BaP levels	[23]
Saute Cooking	PAHs and HAs formation from prolonged cooking at high temperature	[23]
Deep Fat Frying	The acrylamide levels increase when frying oil is reused multiple times	[33]
Baking	Dry air flow causes the evaporation of surface water, leading to surface drying and formation of harmful compounds such as acrylamides, PAHs and HAs, which accumulate on the meat surface	[43]
Microwave Heating	Fast processing time reduces PAHs formation	[45]
Ohmic Heating	Reduction of PAHs and HAs formation due to direct and homogeneous cooking	[20]
Sous Vide	Reduction in PAHs and HAs formation due to prolonged cooking at temperatures between 60-95 °C	[56]
Air-frying	Reduction in PAHs and HAs formation due to lack of direct flame contact	[70]

REFERENCES

[1] Roldan, M., Loebner, J., Degen, J., Henle, T., Antequera, T., Ruiz-Carrascal, J. (2015). Advanced glycation end products, physico-chemical and sensory characteristics of cooked lamb loins affected by cooking method and addition of flavour precursors. *Food Chemistry*, 168, 487-495.

[2] Serdaroglu, M., Derin, E. (2020). Sous-vide cooking technique: Its effects on meat quality (in Turkish). *Turkish Journal of Agriculture-Food Science and Technology*, 8, 1320-1330.

[3] Sabuncular, G., Akbulut, G., Yaman, M. (2021). Factors affecting fat oxidation and lipid peroxidation (in Turkish). *European Journal of Science and Technology*, 27, 362-369.

[4] Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., Törnqvist, M. (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *Journal of Agricultural and Food Chemistry,* 50, 4998-5006.

[5] Kılıç, Ö., Dinçer, A. E., Erbaş, M. (2017). The presence of polycyclic aromatic hydrocarbon compounds in foods and their effects on health (in Turkish). *Gıda*, 42, 127-135.

[6] Basançelebi, O., (2024). Polycyclic Aromatic Hydrocarbons (PAHs) contamination levels in some commercial olive oils sold on the markets in Giresun, Türkiye. *Akademik Gıda*, 22(2), 86-95.

[7] Jiang, D., Wang, G., Li, L., Wang, X., Li, W., Li, X., Lijun, S., Li, F. (2018). Occurrence, dietary exposure, and health risk estimation of polycyclic aromatic hydrocarbons in grilled and fried meats in Shandong of China. *Food Science & Nutrition*, 6, 2431-2439.

[8] Ghorbani, M., Saleh, N.H., Barjasteh-Askari, F., Nasseri, S., Davoudi, M. (2020). The effect of gas versus charcoal open flames on the induction of polycyclic aromatic hydrocarbons in cooked meat: A systematic review and meta-analysis. *Journal of Environmental Health Science and Engineering*, 18, 345-354.

[9] Karslıoğlu, B. (2022). Mechanisms of formation and mitigation approaches for polycyclic aromatic hydrocarbons in meat and meat products (in Turkish). *The Journal Food*, 47, 1032-1045.

[10] Yıldız-Turp, G., Sengun, I.Y., Kendirci, P., Icier, F. (2013). Effect of ohmic treatment on quality characteristic of meat: A review. *Meat Science*, 93, 441-448.

[11] Aydın, Ö.Ş., Şahan, Y. (2018). The effect of different cooking methods on the formation of polycyclic aromatic hydrocarbons in certain types of meat (in Turkish). *Akademik Gıda*,16(4), 387-394.

[12] Çiçek, Ü., Bulgan, A. (2013). Heterocyclic amines in meat and meat products (in Turkish). *Journal of Agricultural Faculty of Gaziosmanpasa University*, 30(1), 25-32.

[13] Acar Çolakoğlu, B., Özdestan Ocak, Ö. (2022). Effect of cooking method on heterocyclic aromatic amines contents of sucuk. *Akademik Gıda*, 20(2), 114-121.

[14] Sugimura, T. (2002). Food and cancer. *Toxicology*, 181-182, 17-21.

[15] Hee, P.-T.E., Liang, Z., Zhang, P., Fang, Z. (2024). Formation mechanisms, detection methods and mitigation strategies of acrylamide, polycyclic aromatic hydrocarbons and heterocyclic amines in food products. *Food Control*, 158, 110236.

[16] Seilani, F., Shariatifar, N., Nazmara, S., Khaniki, G.J., Sadighara, P., Arabameri, M. (2021). The analysis and probabilistic health risk assessment of acrylamide level in commercial nuggets samples marketed in Iran: Effect of two different cooking methods. *Journal of Environmental Health Science and Engineering*, 19, 465–473.

[17] International Agency for Research on Cancer (IARC). (1994). Some industrial chemicals. In IARC Monographs on the Evaluation of Carcinogenic Risks to Humans (Vol. 60, pp. 435–453). IARC, Lyon, France.

[18] Lee, J.-S., Han, J.-W., Jung, M., Lee, K.-W., Chung, M.-S. (2020). Effects of thawing and frying methods on the formation of acrylamide and polycyclic aromatic hydrocarbons in chicken meat. *Foods*, 9, 573.

[19] İçier, F. (2005). Alternative heating method in food processing-ohmic heating (in Turkish). *Gıda*, 30(2), 139-143.

[20] Zell, M., Lyng, J.G., Cronin, D.A., Morgan, D.J. (2009). Ohmic cooking of whole beef muscle –

Optimisation of meat preparation. *Meat Science*, 81, 693-698.
[21] Candan, T., Bağdatlı, A. (2018). Alternative heating methods in meat technology (in Turkish). *El-Cezerî Fen ve Mühendislik Dergisi*, 5(2), 656-670.
[22] Aşçıoğlu, Ç., Şevik, R. (2019). The effects of cooking methods on the properties of beef longissimus dorsi. *Gıda*, 44(1), 173-183.
[23] Babür, T.E., Gürbüz, Ü. (2015). The effects of traditional cooking methods on meat quality (in Turkish).*Journal of Tourism and Gastronomy Studies*, 3(4), 58-64.
[24] Oz, E. (2020). Effects of smoke flavoring using different wood chips and barbecuing on the formation of polycyclic aromatic hydrocarbons and heterocyclic aromatic amines in salmon fillets. *PLoS One*, 15(1), e0227508.
[25] Kim, H-J., Cho, J., Jang, A. (2020). Effect of charcoal type on the formation of polycyclic aromatic hydrocarbons in grilled meats, *Food Chemistry*, 343(1), 128453.
[26] Mariussen, E., Alexander, J., Bukhvalova, B.A., Dahl, L., Olsen, A.-K.H., Kvalem, H.E., Schlabach, M., Amlund, H., Hannisdal, R., Ruus, A., Samdal, I.A., Knutsen, H.K. (2024). Risk assessment of grilled and barbecued food. *Food Safety and Risk Assessment*, 2(2), 0024E.
[27] Duedahl-Olesen, L., Ionas, A.C. (2022). Formation and mitigation of PAHs in barbecued meat–A review. *Critical Reviews in Food Science and Nutrition*, 62(13), 3553-3568.
[28] Büyükkurt, K.Ö., Dinçer, A.E., Çam, İ.B., Candal, C., Erbaş, M. (2020). The influence of cooking methods and some marinades on polycyclic aromatic hydrocarbon formation in beef meat. *Polycyclic Aromatic Compounds*, 40(2), 195-205.
[29] Yao, M., Khan, I.A., Cheng, Y., Ang, Y., Zhou, X., Huang, M. (2020). Effects of cooking methods and tea marinades on the formation of heterocyclic amines and benzo[a]pyrene in grilled drumsticks. *Journal of Food Protection*, 83(2), 365-376.
[30] Yıldız, O., Şahin, H., Kara, M., Aliyazıcıoğlu, R., Tarhan, Ö., Kolaylı, S. (2010). The importance of Maillard reactions and reaction products in foods (in Turkish). *Akademik Gıda,* 8(6), 44-51.
[31] Liao, G.Z., Wang, G.Y., Xu, X.L., Zhou, G.H. (2010). Effect of cooking methods on the formation of heterocyclic aromatic amines in chicken and duck breast. *Meat Science*, 85, 149-154.
[32] Demircigil, G.Ç. (2011). Formation of toxic compounds during the frying process, filtration methods, and legal regulations (in Turkish). *Gıda*, 36(2), 105-112.
[33] Teruel, M.R., Gordon, M., Linares, M.B., Garrido, M.D., Ahromrit, A., Niranjan, K. (2015). Comparative study of the characteristics of French fries produced by deep fat frying and air frying. *Journal of Food Science,* 80(2), E349-E358.
[34] Bhattacharya, A.B., Sajilata, M.G., Tiwari, S.R., Singhal, R.S. (2008). Regeneration of thermally polymerized frying oils with adsorbents. *Food Chemistry,* 110, 562-70.
[35] Gümüş, A.B., Yardımcı H. (2019). Mutagenic-carcinogenic compounds formed as a result of cooking (in Turkish). *Anadolu Hemşirelik ve Sağlık Bilimleri Dergisi*, 22(2), 136-141.
[36] Terzi, G., Çelik, T.H. (2006). The presence of polycyclic aromatic hydrocarbons in certain foods and their effects on human health (in Turkish). *Gıda*, 295-301.
[37] Ghasemian, S., Rezaei, K., Abedini, R., Poorazarang, H. (2011). Acrylamide formation during the frying of beef burger: effect of temperature and time. *Chemical Engineering Research Bulletin*. 15, 39-44.
[38] Michalak, J., Gujska, E., Czarnowska-Kujawska, M., Nowak, F. (2017). Effect of different home-cooking methods on acrylamide formation in pre-prepared croquettes. *Journal of Food Composition and Analysis,* 56, 134-139.
[39] Solyakov, A., Skog, K. (2002) Screening for heterocyclic amines in chicken cooked in various ways. *Food and Chemical Toxicology*, 40, 1205-1211.
[40] Olatunji, O.S., Opeolu, B.O., Fatoki, O.S., Ximba, B.J. (2013). Concentration profile of selected polycyclic aromatic hydrocarbon (PAH) fractions in some processed meat and meat products. *Journal of Food Measurement and Characterization,* 7, 122–128.
[41] Mora, B., Curti, E., Vittadini, E., Barbanti, D. (2011). Effect of different air/steam convection cooking methods on turkey breast meat: Physical characterization, water status and sensory properties. *Meat Science*, 88, 489-497.
[42] Goñi, S.M., Salvadori, V.O. (2010). Prediction of cooking times and weight losses during meat roasting. *Journal of Food Engineering*, 100, 1-11.
[43] Rinaldi, M., Killackey, E., Smith, J., Shepherd, G., Singh, S.P., Craig, T. (2010). First episode psychosis and employment: A review. *International Review of Psychiatry*, 22(2), 148-162.
[44] Chung, S.Y., Yettella, R.R., Kim, J.S., Kwon, K., Kim, M.C., Min, D.B. (2011). Effects of grilling and roasting on the levels of polycyclic aromatic hydrocarbons in beef and pork. *Food Chemistry*, 129, 1420-1426.
[45] Ekezie, F-G.C., Sun, D-W., Han, Z., Cheng, J-H. (2017). Microwave-assisted food processing technologies for enhancing product quality and process efficiency: A review of recent developments. *Trends in Food Science & Technology,* 67, 58–69.
[46] Vadivambal, R., Jayas, D.S. (2010). Non-uniform temperature distribution during microwave heating of food materials: A review. *Food Bioprocess Technology*, 3, 161-171.
[47] Bejerholm, C., Tørngren, M.A., Aaslyng, M.D. (2004). Cooking of meat. *Encyclopedia of Meat Sciences*, Elsevier.
[48] Jinap, S., Mohd-Mokhtar, M.S., Farhadian, A., Hasnol, N.D.S., Jaafar, S.N., Hajeb, P. (2013). Effects of varying degrees of doneness on the formation of Heterocyclic Aromatic Amines in chicken and beef satay. *Meat Science*, 94, 202-207.
[49] Pathare, P.B., Roskilly, A.P. (2016). Quality and energy evaluation in meat cooking. *Food Engineering Reviews,* 8, 435-447.

[50] Kaya, O., İçier, F. (2019). Comparison of the applicability of induction and ohmic heating processes to foods (in Turkish). *Akademik Gıda*, 17, 111-20.
[51] Dai, Y., Miao, J., Yuan, S-Z., Liu, Y., Li, X-M., Dai, R-T. (2013). Colour and sarcoplasmic protein evaluation of pork following water bath and ohmic cooking. *Meat Science*, 93, 898-905.
[52] Zell, M., Lyng, J.G., Cronin, D.A., Morgan, D.J. (2010). Ohmic cooking of whole turkey meat – Effect of rapid ohmic heating on selected product parameters. *Food Chemistry,* 120, 724-729.
[53] Aguilera, J.M. (2018). Relating food engineering to cooking and gastronomy. *Comprehensive Reviews in Food Science and Food Safety,* 17, 1021-1039.
[54] Ayub, H., Ahmad, A. (2019). Physiochemical changes in sous-vide and conventionally cooked meat. *International Journal of Gastronomy and Food Science*, 17, 100145.
[55] Erdem, N., Karakaya, M. (2021). Application of sous vide technology in foods (in Turkish). *Turkish Journal of Agriculture-Food Science and Technology*, 9(9), 1618–1630.
[56] Ruiz-Carrascal, J., Roldan, M., Refolio, F., Perez-Palacios, T., Antequera, T. (2019). Sous-vide cooking of meat: a maillarized approach. *International Journal of Gastronomy and Food Science*, 16, 100138.
[57] Clausen, M.P., Christensen, M., Djurhuus, T.H., Duelund, L., Mouritsen, O.G. (2018). The quest for umami: Can sous vide contribute? *International Journal of Gastronomy and Food Science*, 13, 129-133.
[58] Dominguez-Hernandez, E., Salaseviciene, A., Ertbjerg, P. (2018). Low-temperature long-time cooking of meat: Eating quality and underlying mechanisms. *Meat Science*, 143, 104-113.
[59] da Silva, F.L.F., de Lima, J.P.S., Melo, L.S., da Silva, Y.S.M., Gouveia, S.T., Lopes, G.S., Lopes, G.S. Matos W.O. (2017). Comparison between boiling and vacuum cooking (sous-vide) in the bioaccessibility of minerals in bovine liver samples. *Food Research International*, 100, 566-571.
[60] Falowo, A.B., Muchenje, V., Hugo, A. (2017). Effect of sous-vide technique on fatty acid and mineral compositions of beef and liver from Bonsmara and non-descript cattle. *Annals of Animal Science*, 17 (2), 565–580.
[61] Cui, Z., Zhang, N., Lou, W., Manoli, T. (2022). Application of sous vide cooking to aquatic food products: A review. *Food Science and Technology*, 42, e108021.
[62] Cui, Z., Yan, H., Manoli, T., Mo, H., Bi, J., Zhang, H. (2021). Advantages and challenges of sous vide cooking. *Food Science and Technology Research*, 27(1), 25-34.
[63] Baltalı, B., Akoğlan, K.M. (2021). Evaluation of the cooking process within the scope of the "Sous-Vide" technique (in Turkish). *Aydın Gastronomy*, 5(1), 13-33.
[64] Baldwin, D.E. (2012). Sous vide cooking: A review. *International Journal of Gastronomy and Food Science*, 1, 15-30.
[65] Vaudagna, S.R., Sánchez, G., Neira, M.S., Insani, E.M., Picallo, A.B., Gallinger, M.M., Lasta, J.A. (2002). Sous vide cooked beef muscles: effects of low temperature-long time (LT-LT) treatments on their quality characteristics and storage stability. *International Journal of Food Science and Technology*, 37, 425-441.
[66] Kathuria, D., Dhiman, A.K., Attri, S. (2022). Sous vide, a culinary technique for improving quality of food products: A review. *Trends in Food Science & Technology*, 119, 57-68.
[67] Oz, F., Zikirov, E. (2015). The effects of sous-vide cooking method on the formation of heterocyclic aromatic amines in beef chops. *LWT-Food Science and Technology*, 64, 120-125.
[68] Liu, L., Huang, P., Xie, W., Wang, J., Li, Y., Wang, H., Xu, H., Bai, F., Zhou, X., Gao, R., Zhao, Y. (2022). Effect of air fryer frying temperature on the quality attributes of sturgeon steak and comparison of its performance with traditional deep fat frying. *Food Science & Nutrition*, 10, 342-353.
[69] Tian, J., Chen, S., Shi, J., Chen, J., Liu, D., Cai, Y., Ogawa, Y., Ye, X. (2017). Microstructure and digestibility of potato strips produced by conventional frying and air-frying: An in vitro study. *Food Structure*, 14, 30–35.
[70] Song, G., Li, L., Wang, H., Zhang, M., Yu, X., Wang, J., Xue, J., Shen Q. (2020). Real-time assessing the lipid oxidation of prawn (*Litopenaeus vannamei*) during air-frying by iKnife coupling rapid evaporative ionization mass spectrometry. *Food Control*, 111, 107066.
[71] Saguy, I.S., Dana, D. (2003). Integrated approach to deep fat frying: engineering, nutrition, health and consumer aspects. *Journal of Food Engineering*, 56, 143-152.
[72] Lee, J.S., Han, J.W., Jung, M., Lee, K.W., Chung, M.S. (2020). Effects of thawing and frying methods on the formation of acrylamide and polycyclic aromatic hydrocarbons in chicken meat. *Foods*, 9(5), 573.
[73] Sulemana, R., Wang, Z., Aadil, R.M., Hui, T., Hopkins, D.L., Zhang, D. (2020). Effect of cooking on the nutritive quality, sensory properties and safety of lamb meat: Current challenges and future prospects. *Meat Science*, 167, 108172.

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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.

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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.

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

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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.

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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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- 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.

Yayın Ücreti

Akademik Gıda dergisinde makalelerin yayınlanması için talep edilen ücret yazım kuralları bölümünde verilmiştir.

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.

Destekleyen/Finans Sağlayan Kuruluşlar

Araştırmanın tüm finans kaynaklarına ilişkin detaylar, Teşekkür bölümünde belirtilmelidir. Yazarlar, resmi finansman kurum/larının tam isimlerini ve proje/hibe numaralarını belirtmelidir.

Yazarlarda Değişiklik

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.

Makalenin Geri Çekilmesi

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.

Etik Hususlar

Çıkar çatışması:

- Yazar/lar başvuru sırasında herhangi bir çıkar çatışması varsa beyan etmelidir. Yazar/ların başvuru sırasında bilimsel değerlendirme için en az üç potansiyel hakem önermeleri istenir. Önerilen hakemler çalışma arkadaşları, ortak çalıştıkları kişiler veya çalıştıkları kurumların üyeleri olamazlar.
- Hakemler makaleyi değerlendirmelerini önleyen herhangi bir çıkar çatışması olması durumunda Editörleri bilgilendirmesi ve bu konuda COPE kurallarına uyması tavsiye edilmektedir.
- Editörler Kurulu üyeleri veya kurul üyelerinin ortak çalıştıkları kişiler tarafından dergiye gönderilen makaleler için, değerlendirme sırasındaki önyargıları en aza indirgemek amacıyla, değerlendirme süreci ilgili kurul üyelerini dışarıda tutacak şekilde değiştirilerek uygulanır.
- Düzeltmeler (revizyonlar) sırasında, editörler Dergi Editörleri İçin Davranış Kuralları ile En İyi Uygulama Kılavuzu ve Dergi Yayıncıları İçin Davranış Kurallarını (Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers) takip ederler.

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.
- Hayvanlar üzerinde yapılacak her türlü araştırma kurumsal, ulusal veya uluslararası kurallara uygun olmalı ve etik kurul tarafından onaylanmalıdır.
- 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.

Yayın suistimali

- 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.
- Makaledeki benzerlik oranı tek bir kaynaktan %10'dan fazla olmamak üzere en fazla %25 ile sınırlandırılmıştır. Bu koşula uymayan makaleler reddedilir. Bu şartların ihlal edilmesi durumunda, COPE (COPE recommendations) tavsiyeleri izlenecek ve ilgili tüm taraflara bildirilecektir.

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