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PISTACIA TEREBINTHUS L. OIL EXTRACTION BY LIQUID and SUPERCRITICAL CARBON DIOXIDE MODIFIED WITH A CO-SOLVENT AND EVALUATION OF PHENOLIC COMPOUNDS, FATTY ACIDS PROFILE AND TOCOPHEROLS

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ABSTRACT
The *Pistacia terebinthus L* was extracted using liquid and supercritical carbon dioxide together with a co-solvent (ethanol). The effect of different temperatures (30 and 50°C), pressure (250, 300, and 350 bar), extraction time (60 and 120 min), and different percentages of co-solvent (0, 5, and 10%) was investigated. The amount of phenolic compounds, tocopherols, and fatty acid composition was determined. HPLC, UHPLC and GC were used for analysis of phenolic compounds, tocopherols, and fatty acid composition respectively. Quercetin was the main phenolic compound. The oil was rich in unsaturated fatty acids which were between 69.68 – 75.47%. Oleic acid was the predominant unsaturated fatty acid, and the main saturated fatty acid was palmitic acid. Total tocopherol content of the oil was between 13.07-245.3 ppm and the main tocopherol was β-tocopherol. The study showed that the amount of phenolic compounds, fatty acid composition, and tocopherol content were changed according to the parameters.
Keywords: *Pistacia terebinthus* L, supercritical carbon dioxide, phenolic compounds, tocopherols, fatty acid composition

PISTACIA TEREBINTHUS L. YAĞININ KOSOLVENT ILE MODİFİYE EDİLMİŞ SIVI ve SUPERKRİTİK KARBONDİOKSİT İLE EKSTRAKSİYONU ve FENOLİK BİLEŞİKLER, YAĞ ASİDİ PROFİLİ VE TOKOFEROLLERIN ANALİZİ

ÖZ
Çalışmada *Pistacia terebinthus L*, sıvı ve süperkritik karbondioksit ile yardımcı çözücü (etanol) kullanılarak ekstrakte edilmiştir. Bu amaçla farklı sıcaklık (30 ve 50°C), basınç (250, 300 ve 350 bar), ekstraksiyon süresi (60 ve 120 dakika) ve yardımcı çözücü yüzdelerinin (0, 5 ve 10%) etkisi

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araştırılmıştır. Ekstraksiyon sonrasında elde edilen yağların fenolik bileşik ve tokoferol içerikleri ve yağ asidi kompozisyonu belirlenmiştir. Fenolik bileşiklerin analizi HPLC, tokoferollerin analizi UHPLC ve yağ asidi kompozisyonunun analizi için GC kullanılmıştır. Fenolik bileşikler arasında en yüksek oran Kuersetine ait bulunmuştur. Elde edilen yağın doymamış yağ asitleri bakımından zengin olduğu bulunmuştur (69.68 – 75.47%.). Doymamış yağ asitlerince zengin olan yağda oleik asit baskın, doymuş yağ asitleri içinde ise palmitik asidin ana bileşen olduğu bulunmuştur. Yağın toplam tokoferol içeriğinin 13.07-245.3 ppm arasında ve ana tokoferolün β-tokoferol olarak bulunmuştur. Çalışma, fenolik bileşiklerin miktarının, yağ asidi kompozisyonunun ve tokoferol içeriğinin parametrelere göre değiştiğini göstermiştir.
Anahtar kelimeler: *Pistacia terebinthus* L, süperkritik karbondioksit, fenolik bileşikler, tokoferoller, yağ asidi kompozisyonu

INTRODUCTION

Terebinth (*Pistacia Tehrebinthus* L.) is a member of the *Anacardiaceae* family and is native to Asia and the Mediterranean widely grown in the southeast parts of Türkiye (Gecgel and Arici 2009; Kizil and Turk 2010). It is known as Menengiç or Melengic in Türkiye. The plant's fruits are small and globular and have a dark greenish color when ripe (Gecgel and Arici 2009). Menengiç paste is consumed as a hot beverage like coffee and the powder of the fruit is utilized in seasoning mixtures, especially in the Southeast Anatolian region of Türkiye (Kavak et al. 2010). Various parts and extracts of this fruit have long been used for different purposes including the treatment of gastric issues, cough, rheumatism (externally) and gastralgia (internally) (Kavak et al. 2010). Further, species of Pistacia can be used for treating eczema, renal stones, and throat infections, also they have anti-inflammatory, antibacterial, antiviral, and antipyretic effects (Giner-Larza et al. 2002). Studies have indicated that terebinth fruits are rich in oil (35-47%) and composed of mainly unsaturated acids which are oleic and linoleic acid (Gecgel and Arici 2009; Kizil and Turk 2010). Furthermore, the fruits are rich in carotenoids, phenolic compounds, tocopherols, tannins, and dietary fiber (10%) (Matthaus and Özcan 2006). Researchers have investigated the bioactive characteristics, fatty acid composition, mineral content, volatile compounds, physicochemical properties, and anti-microbial properties of terebinth fruits or leaves in various studies (Kordali et al. 2003; Matthaus and Özcan 2006; Kavak et al. 2010; Durmaz and Gökmen, 2011; Orhan et al. 2012). However, in these studies, the extractions were performed by classical extraction methods using different solvents such as ethanol, chloroform, and ethyl acetate. The main drawbacks of solvent extraction methods include high consumption of solvent, long extraction time, and matrix interactions between the extract and the solvent which makes it difficult to remove the solvent from the extract. Additionally, these methods may require high temperatures leading to the degradation of heat-sensitive compounds such as (phenolic compounds. Supercritical fluid extraction (SFE) is a promising method for the extraction of oils from different matrices as it eliminates the degradation of heat-sensitive compounds and minimizes solvent residues in extracts (Senyay-Oncel et al. 2011). Carbon dioxide is widely utilized in supercritical fluid extractions due to its non-toxic, cheap, and has non-flammable properties. When extracting phenolic compounds, a co-solvent like ethanol or methanol should be used in supercritical carbon dioxide extraction since carbon dioxide is non-polar and phenolic compounds cannot be extracted without a co-solvent.

In this study the extraction of phenolic compounds and tocopherols along with the oil from *Pistacia terebinthus* L by using $scCO_2$ with ethanol as a co-solvent was performed. Also, the fatty acid composition of the oils obtained was investigated. The effect of different parameters, such as temperature (30 and 50°C), pressure (250, 300, and 350 Bar), co-solvent percentage (0, 5, and 10%), and extraction time (60 and 120 min.) was investigated.

MATERIALS AND METHODS

Materials

Terebinth fruits (*Pistacia terebinthius*) were obtained from a local bazaar in Gaziantep during the

growing season. The fruits were cleaned to remove foreign materials such as dirt, stones, and chaff. Prior to each extraction, 30 g of fruits were crushed to homogenize the samples and increase the surface area for supercritical extraction. The standards used for HPLC analysis were obtained from Sigma-Aldrich (Steinheim, Germany). Ethanol, used as a co-solvent, and methanol, utilized for the extraction of phenolic compounds from the terebinth fruit oil was obtained from Riedel de-Haen (Germany). HPLC grade acetic acid (100%) was from Sigma-Aldrich (Germany) and Acetonitrile was from Merck (Darmstadt, Germany).

Supercritical fluid extraction of terebinth fruits

The extraction of oil and the phenolic compounds from the terebinth fruits was performed by using an analytical supercritical fluid extractor (SFE-100-2-FMC10, Thar Instruments, PA, USA). The instrument was equipped with an Automated back pressure Regulator, 100ml extraction vessel, 500 ml collection vessel, six-zone temperature controller, high-pressure P-50 Series pump, cooling systems filled with glycol, and a series III pump for co-solvent (which cannot operate over 400 bar). The co-solvent pump was purged before each extraction to ensure that co-solvent entered the system (Thar Instruments, Series III pump, Manuel)

The terebinth fruits obtained from the local market were cleared of foreign substances. 30 g of terebinth fruits were weighed for extraction, and they were smashed in a porcelain mortar for 10 min, to increase the surface area before extraction. Then they were sieved and the ones that were between 425 and 230 μm were used for extraction. The smashed fruits were then placed in the extraction vessel. The flow rate of CO_2 was set to 5 g/min and was the same for all the extraction parameters. Since CO_2 is a non-polar solvent, it is not possible to extract the phenolic compounds without a polar co-solvent. In this study, CO_2 was modified by using ethanol as a co-solvent. Three different co-solvent (ethanol) percentages were tested which were 0, 5, and 10% (weight %). Other parameters studied in the research to observe their effects on phenolic compounds, fatty acid profile, and tocopherol contents, were temperature (30 and 50ºC), pressure (250, 300, and 350 Bars), and extraction time (60 and 120 min.). The parameters were chosen based on the studies of Eyiler-Yılmaz et al. (2011). The oil extracted was collected in a 250ml volumetric flask along with the co-solvent and the ethanol in the collected samples was removed with a rotary evaporator (Büchi, B465, Switzerland) at 45°C, 110rpm. After removal of the ethanol, the samples were stored at 4°C until further analysis. The extractions with SC-CO_2 were performed in duplicate.

Phenolic compound analysis of terebinth extracts.

The samples obtained after the extraction process were analyzed by RP-HPLC according to the method of Pirisi et al. (2010). According to this method, 1 ml of oil sample was transferred to a centrifuge tube and 1 ml of methanol was added. The mixture was vortexed for 2 min before being centrifuged at 3000 rpm for 10 min (Nüve, NF 1215, Istanbul, Turkey). The supernatant obtained was then transferred to a test tube for further analysis.

The samples were analyzed using HPLC (Agilent 1100 RP-HPLC) equipped with a nucleosil C18 HPLC column (250*4.6 mm, Supelco Inc., Bellefonte, PA, USA) and a DAD detector set at 280 nm for catechin and gallic acid, 370nm for myricetin and quercetin. The flow rate of the mobile phase was maintained at 1ml/min and the injection volume was 20 μl. A gradient flow method was used with 2 mobile phases which are 2% acetic acid (in water Mobile Phase A) and 100 % acetonitrile as phase B, the total analysis time was 30 min. The gradient program was as follows: the concentration of acetonitrile was increased to 50% in 20 min, isocratic for 5 min, then the concentration of acetonitrile was decreased to 0% in 1 min and was isocratic for the last 4 min. The retention times for gallic acid, catechin, myricetin and quercetin were 4.4, 9.7, 15.7 and 18.2 minutes respectively. Each experiment was performed in 2 parallels.

Tocopherol analysis

The analysis of α-, β-, γ- and δ-tocopherol was performed with ultra-high-performance liquid chromatography (UHPLC, Dionex Ultimate 3000), by using LiChrosorb SI 60-5 column (4.6 mm internal diameter * 250 mm length and 5μm particle size). 2 g of oil samples were weighed into 25 ml volumetric flask and dissolved with some hexane then completed to 25 ml with hexane. The mixture was vortexed for 15 min then the prepared mixture was passed through 0.45 μm syringe-type HPLC filters (PVDF, Millipore Millex-HV) and transferred to an HPLC vial. The tocopherols were differentiated from each other with isopropanol: hexane (0.5:99.5%, v/v) mobile phase under isocratic conditions at a flow rate of 1ml/min, at 292 nm and the temperature of the column was 30°C. The analysis time was 30 min and the injection volume was 100μl. the tocopherol isomers were identified according to the retention times of the prepared standards and the amount was determined as ppm level by using the area under the peaks obtained from UHPLC (AOAC 2017). The retention times for α, β, γ and δ -tocopherol were 8.01, 14.12, 15.87, and 28.77 minutes respectively. The experiments were performed in 2 parallels.

Determination of fatty acid composition

The fatty acid composition of the samples was determined by using TRACE Ultra GC (Thermo Fisher Scientific, Waltham, MA, USA) gas chromatography equipped with flame ionization detector and capillary column (0.25 mm i.d. × 100 m, 0.25μm film thickness) (European Commission Regulation 2017). The injection detector temperatures were set to 240 and 250°C respectively. The initial temperature was 100°C and the temperature ramp rate was 4°C/min. Helium was used as carrier gas with a flow rate of 1mL/min. The split ratio was set as 40:1. For the preparation of samples, 0.1 g of extracted oil sample was weighed, and 2ml heptane and 0.2 ml 2 M of methanol (11.2 g KOH dissolved in 100 ml HPLC grade methanol) were added. The mixture was vortexed for 15 min. then was centrifuged at 5000 rpm for 5 min. the supernatant obtained after centrifugation was used for the GC analysis. The experiments were performed in 2 parallels.

Statistical analysis

The results of the research were evaluated with factorial design variance analysis using SAS 9.4 statistical program. The means that were significantly different were compared by applying the Tukey multiple comparison tests.

RESULTS AND DISCUSSION

Phenolic compounds determined in the Terebinth oil samples.

To comprehensively assess the combined impact of pressure, co-solvent percentage, temperature, and extraction time on phenolic compounds, Table 1 presents the average values obtained across all parameters corresponding to the same pressure, co-solvent percentage, temperature, and extraction time for each specific compound.

The results indicate that changes in pressure and temperature did not significantly affect the amount of quercetin (Table 1, $P > 0.05$). However, an increase in co-solvent percentage and extraction time significantly increased the amount of quercetin ($P < 0.05$). These findings highlight the significance of co-solvent percentage and extraction time as the primary factors influencing quercetin levels in terebinth oil. These results align with the findings of Martino and Guyer (2004), who emphasized that co-solvent was the most influential parameter for quercetin extraction.

The results indicate that changes in temperature, pressure, and extraction time did not significantly affect the amount of catechin (Table 1). However, increasing the co-solvent percentage from 0% to 5% resulted in a significant increase in catechin levels ($P < 0.05$). Murga et al. (2000) discovered in their study that when the co-solvent level was below 5%, only gallic acid could be extracted from grape seeds. Although our study involved a different matrix, the results in Table 1 suggest that catechin extraction without co-solvent is possible at 50°C and 350 bars of pressure which is the supercritical condition. At high pressure and temperature, it becomes feasible to disrupt

analyte-matrix interactions and extract catechin from terebinth, which is consistent with the findings of Berna et al. (2001). (Table 1). According to the studies by Spencer Chatwell et al. (2021) and Bassing and Siegfried Braeuer (2021) when CO_2 is mixed with ethanol the critical conditions changes because the critical condition for ethanol is higher. Therefore, in our study the mixture obtained at 10% ethanol might not reach critical conditions which could be the reason for the decrease in the level of phenolic compounds.

Table 1. Effect of Pressure, Co-Solvent Percentage, Temperature and Extraction Time on Quercetin and Catechin

Main Factors	Quercetin (QUE) (mg/kg)	Catechin (CAT) (mg/kg)
Pressure (Bar)[1]		
250	72.66±20.65[a]	30.45±5.47[a]
300	79.87±27.64[a]	21.06±6.11[a]
350	72.75±23.07[a]	30.67±6.31[a]
p	0.8564	0.2668
Co-Solvent Percentage (%)[2]		
0	15.90±1.16[a]	11.12±5.59[a]
5	36.23±3.13[a]	41.42±2.18[b]
10	172.90±19.78[b]	29.61±6.15[ab]
p	0.0001	0.0005
Temperature (°C)[3]		
30	78.79±19,34[a]	25.88±4.92[a]
50	71.42±19.19[a]	29.14±4,97[a]
p	0.5460	0.5716
Extraction Time (min)[4]		
60	55.25±10.67[a]	31.57±4,69[a]
120	94.96±24.17[b]	23.46±5,03[a]
p	0.0026	0.1654

a-b: Means within the same factor and the same column with different letters are different ($p < 0.05$). ND: Not Detected.

[1] Each number represents the average value of each parameter for all samples with the same pressure.

[2] Each number represents the average value of each parameter for all samples with the same Co-solvent percentage.

[3] Each number represents the average value of each parameter for all samples with the same temperature.

[4] Each number represents the average value of each parameter for all samples with the same extraction time.

The results of gallic acid were not included in the factorial design statistical analysis because the obtained results were not suitable for the design. It was not possible to extract gallic acid in most of the extraction parameters.

The solvation power of CO_2 is a function of temperature and pressure, and it is well-known that an increase in pressure increases the solvation of supercritical CO_2. On the other hand, increasing the temperature decreases the solvation of CO_2 (Brunner 2005). Additionally, it should be noted that the impact of temperature is more complex, such as it was reported in the literature that at low pressures (10 – 15 MPA), temperature negatively affects the SFE of phenolic compounds. However, above these pressure thresholds, it was stated that temperature has s positive effect on the extraction of polyphenols. In our study, the pressure values exceeded these thresholds. Nevertheless, increasing the temperature while holding all other parameters constant did not lead to a significant increase in the concentrations of quercetin, catechin, and gallic acid. Similar findings have been documented in the literature, with this behavior being attributed to the thermal degradation of phenolic compounds (Ferrentino et al. 2018). Furthermore, Chafer et al. (2007) found that increasing the temperature decreased the amount of gallic acid in their study.

Myricetin was also analyzed by HPLC from the extracted oil however it was not possible to detect the compound. This may be attributed to the possibility that myricetin was not extractable under the parameters employed in this study.

As mentioned above temperature and pressure are the primary factors influencing the solubility of CO_2 as they define the density of supercritical fluids (Lee et al. 2006). In supercritical and near-critical solvents, the solubility of low-volatility substances typically decreases with temperature at low pressures due to the rapid decrease in fluid density as temperature increases near critical pressures. However, at higher pressures (over 200 Bar), the effect of temperature on density is diminished, and vapor pressure becomes the predominant factor affecting the density of the

supercritical fluid (Brunner, 2005). It can be observed from the results that the higher levels of catechin were extracted at 50°C where CO_2 is in a supercritical state. There was a slight, albeit non-significant decrease in quercetin levels with increasing the temperature. Since different compounds could be extracted at their maximum levels at different parameters, extractions should be conducted according to the selected compound. It can be emitted from the obtained results that the percentage of the co-solvent used was the most effective parameter for the extraction of phenolic compounds from terebinth fruits.

Fatty acid profile of the Terebinth oil

Thirteen different fatty acids were observed in the extracted terebinth oils. However, only the results of the five predominant fatty acids: palmitic, stearic, palmitoleic, oleic, and linoleic acids were presented in Table 2. Table 2 provides an overview of the combined effects of pressure, co-solvent percentage, temperature, and extraction time on the fatty acid profile. The values represent the average value of all the parameters within the same pressure, co-solvent percentage, temperature, and extraction time for the specific compound. The results indicate that changes in pressure did not significantly affect the levels of saturated and unsaturated fatty acids ($P > 0.05$). However, oleic and stearic acid levels were significantly increased with higher co-solvent percentages (Table 2). On the other hand, the levels of palmitic, palmitoleic, and linoleic acids were decreased when the co-solvent percentage was increased. Both temperature and extraction time were found to be significant factors in reducing the palmitic acid level ($P<0.05$). A decrease in the level of palmitic acid is favorable because it is a saturated fatty acid and as mentioned above saturated fatty acids increase LDL and HDL cholesterol.

Table 2. Effect of Pressure, Co-Solvent Percentage, Temperature, and Extraction Time on Fatty acid composition of Terebinth oil.

Main Factors	Palmitic acid (%)	Stearic acid (%)	Palmitoleic acid (%)	Oleic acid (%)	Linoleic acid (%)
Pressure (Bar)[1]					
250	22.18±0.20^{a}	1.68±0.03^{a}	3.61±0.34^{a}	44.25±0.27^{a}	22.23±0.12^{a}
300	22.29±0.17^{a}	1.70±0.02^{a}	3.87±0.07^{a}	44.69±0.15^{a}	22.22±0.10^{a}
350	22.37±0.20^{a}	1.72±0.02^{a}	3.20±0.43^{a}	47.53±0,32^{a}	21.94±0.13^{a}
p	0.5699	0.1581	0.1946	0.4274	0.1235
Co-Solvent Percentage (%)[2]					
0	22.75±0.06^{a}	1.63±0.02^{a}	4.07±0.03^{a}	47.15±0.24^{a}	22.27±0.12^{a}
5	22.42±0.18^{a}	1.69±0.02^{b}	3.90±0.06^{a}	47.30±0.31^{ab}	22.28±0.08^{a}
10	21.67±0.14^{b}	1.78±0.01^{c}	2.70±0.47^{b}	48.03±0.11^{b}	21.85±0.12^{b}
p	0.0001	0.0001	0.0013	0.0343	0.0137
Temperature (°C)[3]					
30	22,31±0.16^{a}	1,71±0.02^{a}	3,47±0.30^{a}	47,47±0.21^{a}	22,14±0.11^{a}
50	22,26±0.15^{b}	1,69±0.02^{a}	3,66±0.22^{a}	47,52±0.21^{a}	22,13±0.09^{a}
p	0.7347	0.1887	0.5223	0.8555	0.9546
Extraction Time (min)[4]					
60	22,42±0.15^{a}	1,69±.0.02^{a}	3,24±0.35^{a}	47,35±0.27^{a}	22,13±0.27^{a}
120	22,14±0.15^{b}	1,71±0.02^{a}	3,88±0.06^{b}	47,64±0.13^{a}	22,13±0.13^{a}
p	0.0735	0.2111	0.0367	0.3095	0.9825

Means within the same factor and the same column with different letters are different ($p < 0.05$).

[1] Each number represents the average value of each parameter for all samples with the same pressure.

[2] Each number represents the average value of each parameter for all samples with the same Co-solvent concentration.

[3] Each number represents the average value of each parameter for all samples with the same temperature.

[4] Each number represents the average value of each parameter for all samples with the same extraction time.

These results were lower than the findings of Gecgel and Arici (2009) and Durak and Uçak (2015) which could be attributed to differences in climate, soil type, and environmental factors where the fruits were obtained. Sodeifian et al. (2016) investigated the extraction of fruit oil from *Pistacia khinjuk* stocks using supercritical carbon dioxide and reported that the main component of UFA was oleic acid consistent with our study. The oleic acid content in *Pistacia khinjuk* stocks fruit was approximately 57% which was higher than the findings of our study. It was stated by Satil et al. (2003) that the amount of oil and the fatty acid composition of the terebinth samples were influences by the climatic conditions and type of soil of the area they were grown.

Pistacia terebinthus (Terebinth) and Pistachio nuts belong to the same family. It was shown by Satil et al. (2003) that the amount of oleic acid in the pistachio nuts was nearly 60% which is higher than that in *Pistacia terebinthus*. Olive oil has long been known and used oil, especially in the Mediterranean because of its positive effects on health such as the prevention of coronary heart disease and certain cancer types (Visioli et al. 2018). It is believed that this health benefit of olive oil comes from the unsaturated fatty acid which is mainly oleic acid. According to the results of Belbaki et al. (2017), the olive oil extracted using supercritical CO_2 included fatty acids from C_{16} to $C_{20:1}$, and oleic acid was the main unsaturated fatty acid with a ratio of 59.3% which is higher than our results. The amount of linoleic acid in the extracts was between 21.85 – 22.28%. These results were found to be higher than its relative pistachio nuts (Satil et al. 2003) and olive oil (Belbaki et al. 2017).

Palmitic acid was the main saturated fatty acid found in the fatty acid profile analysis (21.67 – 22.37%), while stearic acid content was lower, between 1.63 – 1.78%. Similar findings were reported by Gecgel and Arici (2009). Saturated fatty acids have been linked to increased serum low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol levels. Lauric and myristic acids exhibit the strongest effect on LDL and HDL cholesterol levels among saturated fatty acids, whereas palmitic acid, although increasing cholesterol levels, does so to a lesser extent than lauric and myristic acid. On the other hand, stearic acid does not affect LDL and HDL cholesterol (Mensink 2013). Based on this knowledge, it is important to reduce the amount of saturated fatty acids in the diet. It was found by Chowdbury et al. (2007) that the amount of palmitic acid in palm oil was 41.78% which is higher than that in terebinth oil. Palm oil is one of the most used oils in the food industry. Given the lower saturated fatty acid content in terebinth oil compared to palm oil, it could serve as a viable alternative in food applications. Nevertheless, it should be noted that the amount of saturated fatty acids in the study was higher than that of pistachio nuts and olive oil (Satil et al. 2003; Belbaki et al. 2017). Olive oil is one of the most suggested oils for consumption due to its high unsaturated fatty acid content. The results of the study showed that the unsaturated fatty acid levels of terebinth oil are lower when compared to olive oil. While olive oil usage in the food industry is somewhat limited due to its higher cost, terebinth oil presents an attractive alternative due to its high oleic acid and low saturated fatty acid content.

The ratio of saturated fatty acids to unsaturated fatty acids (SFA/UFA) is a criterion for the evaluation of the nutritional and functional properties of the oil (Sodeifian et al. 2016). The average value of SFA/UFA ratios of the samples was between 0.31 – 0.38 which is close to the results of Sodeifian et al. (2016). However, these values are relatively low when compared to other vegetable oils such as soybean, peanut, and olive oil (Fasina et al. 2008). Besides the effect of saturated fatty acids on cholesterol levels, it is believed that a high intake of saturated fatty acids may cause cardiovascular diseases and it has been found that a high intake of saturated fatty acids can lower insulin sensitivity which is an important factor in metabolic disorder and diabetes (Nagao and Yanagita 2010). Due to this reason, the extraction parameters should be chosen carefully to decrease the amount of saturated fatty acids. Also as mentioned previously the fatty acid composition of terebinth oil can be affected by the climatic conditions, soil type, and

environmental factors where the plant is grown (Satil et al. 2003).

Tocopherol content of the terebinth oil

The average values of each parameter for all the samples were given in Table 3. The total tocopherol content of the oil samples was between 13.07-245.3 mg/kg which was lower than the findings of Matthaus and Özcan (2006) and Durmaz and Vural (2011). Unlike the findings of previous studies (Matthaus and Özcan 2006; Durmaz and Vural 2011), the main tocopherol found in the study was β- tocopherol.

Table 3. Effect of Pressure, Co-Solvent Percentage, Temperature and Extraction Time on Tocopherol Content of Terebinth oil

Main Factors	α – tocopherol (mg/kg)	β- tocopherol (mg/kg)	γ- tocopherol (mg/kg)	δ- tocopherol (mg/kg)
Pressure (Bar)[1]				
250	18.23±12.79[a]	90.06±19.33[a]	8.79±0.76[a]	5.67±1.29[a]
300	7.24±6.15[a]	103.99±18.64[a]	8.47±0.78[a]	2.06±0.80[b]
350	8.62±4.02[a]	70.87±19.40[a]	6.36±0.60[b]	3.70±0.72[ab]
p	0.5514	0.3462	0.0411	0.0282
Co-Solvent Percentage(%)[2]				
0	21.90±13.41[a]	33.99±8.70[a]	6.59±0.72[a]	1.86±0.76[b]
5	2.61±1.13[a]	127.41±16.79[b]	8.53±0.62[a]	4.61±0.91[a]
10	9.58±5.08[a]	103.53±19.02[b]	8.51±0.86[a]	4.96±1.27[a]
p	0.2158	0.0007	0.0987	0.0406
Temperature (°C)[3]				
30	19.43±9.38[a]	91,34±16.57[a]	8,14±0.51[a]	4,47±0.99[a]
50	3,28±1.04[a]	85,28±14.82[a]	7,61±0.73[a]	3,14±0.65[a]
p	0.0789	0.7433	0.5135	0.2099
Extraction Time (min)[4]				
60	21,30±9.22[a]	92,27±18.67[a]	7,69±0.70[a]	3,41±0.85[a]
120	1,42±0.34[b]	84,34±12.03[a]	8,06±0.56[a]	4,21±0.86[a]
p	0.0329	0.6682	0.6523	04488

a-b: Means within the same factor and the same column with different letters are different ($p < 0.05$).

[1] Each number represents the average value of each parameter for all samples with the same pressure.

[2] Each number represents the average value of each parameter for all samples with the same Co-solvent concentration.

[3] Each number represents the average value of each parameter for all samples with the same temperature.

[4] Each number represents the average value of each parameter for all samples with the same extraction time.

The amount of α-tocopherol decreased with increasing temperature while keeping other parameters constant. α-tocopherol is susceptible to oxidation at high temperatures, potentially leading to its degradation. Similarly, the extraction time had a negative effect on α- tocopherol levels. As depicted in Table 3 increasing extraction time significantly decreased the amount of α-tocopherol (P <0.05).

According to the results an increase in the co-solvent percentage significantly increased the amount of β- tocopherol (P <0.05, Table 3). Conversely, temperature, pressure, and extraction time showed no significant effect. Moreover, time, co-solvent concentration, and temperature had no significant effect on the γ- tocopherol content. However, increasing the pressure to 350 bars led to a significant decrease in the amount of γ- tocopherol (P <0.05). The only parameter that significantly reduced the δ- tocopherol content was pressure (P <0.05). while, temperature, time, and co-solvent percentage showed no significant effect on δ- tocopherol content. The results demonstrated that changing the parameters had diverse effects on each tocopherol type. Generally

increasing the temperature from 30°C to 50°C led to a decrease in tocopherol levels, which may indicate the degradation of these substances at higher temperatures. Therefore, if it is desired to obtain higher amounts of tocopherols in the extract, temperatures lower than 50°C could be selected for SFE.

The tocopherol contents of terebinth oil in our study were lower when compared to the results of Mathaus and Ozcan (2006) which could be due to the differences in the environmental and climatic conditions and the differences in the soil where the terebinth plants were grown. The terebinth oil's tocopherol content was lower compared with other oils like palm oil (Tan et al. 2009) and olive oil (Uluata et al. 2021).

CONCLUSION

In this study, the extraction of oil from *Pistacia terebintus* L with liquid CO_2 and $ScCO_2$, followed by the analysis of phenolic compounds, tocopherols, and fatty acid composition was conducted. It was observed that Supercritical Fluid Extraction (SFE) offers several advantages, such as requiring less or no solvent, operating at low temperatures, and being oxygen-free, which prevents the degradation of easily degradable substances during extraction. Terebinth oil has a lower amount of unsaturated fatty acids and tocopherols when compared to olive oil. However, olive oil is an expensive type of oil and due to this, it is difficult to use in the food industry. In comparison, terebinth oil emerges as a promising alternative to oils like palm, soy, or rapeseed oil. Additionally, due to its high content of unsaturated fatty acids and tocopherols, terebinth oil can be used in the meat industry in low-fat meat products. It is important to note that changing the extraction parameters changes the amount of the phenolic compounds, tocopherols, and fatty acid composition. Therefore, researchers or producers should choose the extraction parameters carefully based on the substance they aim to extract at the highest concentration. This ensures optimal extraction efficiency and product quality.

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AUTHOR CONTRIBUTIONS

Prof. Halil Vural was involved in the conceptualization and planning of the study, Atakan Sür was involved in planning the experiments, execution of experiments, generating the data. Esen Eyiler Kaya contributed to statistical analysis and interpretation of the results followed by manuscript writing and editing for publication.

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SHANKLİSH PEYNİRİNDEN İZOLE EDİLEN ENDOJEN MAYALARIN MOLEKÜLER TANIMLANMASI VE ENZİMATİK KARAKTERİZASYONU

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ÖZ

Bu çalışmada, Ortadoğu'da uzun yıllardan beri tüketilen ve son yıllarda Türkiye'de de üretilmeye başlanan Shanklish peynirlerinden mayaların izolasyonu, Start Codon Targeted (SCoT) markör yöntemi kullanılarak identifikasyonu ve enzimatik aktivitelerinin belirlenmesi amaçlanmıştır. Olgunlaştırılmış peynirlerden 24 adet maya izole edilmiş, SCoT markör yöntemiyle DNA parmak izleri elde edilerek gruplandırılmış ve her gruptan temsili izolatlar sekanslanarak identifikasyon sonuçları elde edilmiştir. Bu sonuçlara göre, 19 adet *Kluyveromyces lactis*, 2 adet *Pichia kudriavzevii*, 1 adet *Pichia fermentans*, 1 adet *Pichia membranifaciens* ve 1 adet *Clavispora lusitaniae* suşu tanımlanmış ve API-ZYM enzim test kiti yardımıyla enzimatik karakterizasyonları belirlenmiştir. Bu suşlar arasından *K. lactis* ANO17 suşu yüksek esteraz lipaz, lösin arilamidaz, valin arilamidaz, sistin arilamidaz, asit fostataz, Naftol-as-bi-fosfohidroliz, α-glukosidaz ve β-glukosidaz aktivitesi gösterirken orta seviyede esteraz, β-galaktosidaz ve düşük seviyede alkalin fostataz aktivitesi göstermiş ve bu suş enzimatik aktivite yönünden en umut verici suş olarak tespit edilmiştir. Çalışma sonuçlarına göre, *K. lactis* ANO17 suşunun olası starter/destek kültür kombinasyonlarında laktik asit bakterileriyle birlikte kullanımının teknolojik yönden üstün peynir elde edilmesinde faydalı olacağı düşünülmektedir.

Anahtar kelimeler: Shanklish peyniri, SCoT markör, destek starter, maya, enzimatik aktivite

MOLECULAR IDENTIFICATION AND ENZYMATIC CHARACTERIZATION OF ENDOGENOUS YEAST ISOLATED FROM SHANKLISH CHEESE

ABSTRACT

In this study, the isolation of yeasts originated from Shanklish cheeses produced and consumed in Turkey, their identification using the SCoT marker method, and the determination of their enzymatic activities were aimed. Twenty-four yeasts were isolated from these ripened Shanklish cheeses and they are grouped by obtaining DNA fingerprints using the SCoT marker method and then representative isolates from each group were sequenced for identification. Based on the identification results, 19 *Kluyveromyces lactis*, 2 *Pichia kudriavzevii*, 1 *Pichia fermentans*, 1 *Pichia membranifaciens* and 1 *Clavispora lusitaniae* strains were identified and their enzymatic characterizations were determined using the API-ZYM enzyme test kit. Among these strains, *K. lactis* ANO17 showed high esterase lipase, leucine arylamidase, valine arylamidase, cysteine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-glucosidase, and β-glucosidase activities, while showing moderate esterase, β-galactosidase, and low-level alkaline phosphatase activities and so this strain was identified as the

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most promising strain in terms of its enzymatic activity. According to these study results, it is considered that the use of *K. lactis* ANO17 strain in potential starter/adjunct culture combinations with lactic acid bacteria can be utilized to obtain technologically superior cheese.
Keywords: Shanklish cheese, SCoT marker, adjunt starter, yeast, enzymatic characterization

GİRİŞ

Fermantasyon ile üretilen peynirler ve süt ürünleri özellikle Akdeniz ülkelerinde yaşayan insanlar için beslenmenin önemli bir parçasıdır. Shanklish peyniri ise bunlardan birisi olup Lübnan, Suriye, Irak başta olmak üzere tüm Ortadoğu'da tüketilmektedir (Nehme vd., 2019). Bu bölgenin dışında 1860 Lübnan iç savaşı nedeniyle Arjantin'in Corrientes şehrine göç eden Lübnanlılar nedeniyle Arjantin'de de tüketilen bir peynir haline gelmiştir (Patino vd., 1999). Benzer şekilde 2010 yılında Suriye'de başlayan iç savaş nedeniyle ülkemize göç eden bölge halkı nedeniyle de ülkemizde tanınırlığı ve tüketimi artmıştır.

Shanklish peyniri, Türkiye'de üretilen ve coğrafi işareti alınmış Antakya Sürk peyniriyle benzerlikler taşısa da olgunlaşma periyodu ve üretim metotlarındaki farklılıklar ile Antakya Sürk peynirinden ayrılmaktadır. Shanklish üretimi için ilk olarak koyun sütünden yoğurt yapılır ve elde edilen yoğurt 2-3 gün buzdolabında dinlendirilir. Mevsimsel farklılıklara ve bölge tercihlerine göre koyun sütü yanında keçi ve inek sütleri de tercih edilebilmektedir. Dinlendirilen yoğurt proteinlerin koagülasyonu için ısıtılır ve çökelti toplanarak tülbent yardımıyla süzülür. Daha sonra tuz ilave edilerek top haline gelecek şekilde şekillendirilir. Hazırlanan taze peynirler kimyon, kekik ve kırmızıbiber tozu ile kaplanarak çeşnilendirilir. Son olarak çeşnilendirilen toplar toprak kavanozlara doldurularak birkaç hafta olgunlaşmaya bırakılır. Elde edilen sert peynir zeytinyağında 1-2 yıla kadar muhafaza edilmektedir (Nehme vd., 2019; Addas, 2013, Toufeili vd., 1995).

Shanklish peynirinin mikrobiyolojik ve fizikokimyasal özellikleri üzerine çeşitli araştırmalar devam etmektedir. Suriye'de geleneksel yöntemlerle üretilmiş Shanklish peynirlerinin laktik asit bakterileri (LAB) florasının polimeraz zincir reaksiyonu (Polymerase Chain Reaction-PCR) yöntemi kullanılarak belirlendiği bir çalışmada, araştırmacılar 82 adet LAB izole etmişlerdir. Bunlardan 35 tanesi *Lactobacillus paracasei*, 20 tanesi *Lb. plantarum,* 18 tanesi *Lb. lactis*, 7 tanesi *Lb. brevis* ve 2 tanesi *Lactobacillus* spp. olarak tanımlanmıştır (Abou Younes vd., 2018). Shanklish peynirlerinin fizikokimyasal özelliklerinin belirlendiği başka bir çalışmada, araştırmacılar koyun sütü kullanarak ürettikleri Shanklish peynirlerinde %55.97 nem, %32.15 protein, %6.06 yağ ve %2.99 kül miktarı tespit etmişlerdir. Ayrıca bu peynirlerin 70 günlük olgunlaşma periyodu sonunda pH değerlerinin 5.14 olduğunu bulmuşlardır (Toufeili vd., 1995). El Mayda (2007) tarafından yürütülen bir başka çalışmada ise keçi sütü kullanılarak üretilen Shanklish peynirlerinin nem içeriği %30.2, protein içeriği %46.6, yağ içeriği %5.4, kül miktarı %7 ve pH değeri 4.5 olarak tespit edilmiştir.

Bunlarla birlikte, Shanklish peyniri üretiminde henüz standart bir üretim yöntemi yoktur ve kullanılan starter kültür olmadığından son ürün üreticiden üreticiye değişim göstermektedir. Bu peynirin çeşitli baharatlarla kaplanması veya olgunlaşma süresinden ötürü karmaşık bir floraya sahip olduğu düşünülmektedir. Her ne kadar daha önce yapılan çalışmalarda LAB florası ortaya çıkarılmış olsa da Shanklish peynirinin sahip olduğu maya florası hakkında henüz bir çalışma gerçekleştirilmemiştir. Öte yandan mayaların peynirlerin olgunlaşma aşamasında faaliyete geçip, LAB tarafından üretilen laktik asidi parçalayarak ortamın pH'sını yükselttikleri ve böylece olgunlaşmada rol olan florayı destekledikleri bildirilmektedir (Suzzi vd., 2001). Dahası, peynirlerden izole edilen maya suşlarının lipolitik, proteolitik ve/veya enzimatik aktivitelere sahip olabileceği, böylece gerçekleşen lipoliz ve proteoliz sayesinde peynirin yapı ve aromasına katkı sunabileceği belirtilmektedir (Martin vd., 2001; Kesenkaş ve Akbulut, 2006; McSweeney, 2004). Üretilen peynirlerin kalite karakteristiklerini geliştirmeleri yanında son yıllarda probiyotik özellikler taşıyan mayalar da araştırmacıların dikkatini çekmiş ve bu konu üzerine yapılan çalışmalar giderek artmıştır

(Psomas vd, 2001). Mayaların aroma gelişime katkıları, olgunlaşmanın hızlandırılması ve probiyotik potansiyel barındırmalarından ötürü peynir üretiminde kullanılan starter LAB ile birlikte destek kültür olarak kullanılmaları birçok araştırmacı tarafından tavsiye edilmektedir (Tempel ve Jakobsen, 1998; Klein vd., 2002; Ferreira ve Viljoen, 2003).

Mayaların moleküler karakterizasyonunda kullanılan her DNA markör yönteminin bazı avantaj ve dezavantajları bulunmaktadır. Konu hakkında çalışan pek çok laboratuvarda bir markör yönteminin seçimi çalışılan materyale, teknik uzmanlığa, mevcut ekipmanlara ve araştırma bütçesine göre değişmektedir. Start Codon Targeted (SCoT) poliformizm markör yöntemi Collard ve Mackill (2009) tarafından bitki genomunun başlangıç kodonuna dayanarak geliştirilmiş bir yöntemdir. İleri ve geri primer olarak tek primer kullanılır ve bu açıdan bakıldığında RAPD veya ISSR markör yöntemlerine benzemektedir. SCoT markörleri ATG başlangıç kodonunu çevreleyen gen bölgelerini hedef alacak şekilde tasarlandığından diğer markör yöntemlerine göre daha çoğaltılabilir bir yöntemdir (Tikendra vd., 2021; Amom vd., 2020; Gogoi vd., 2020).

Dünya genelinde çok farklı peynir çeşidinin (Tulum, Otlu, Fossa, Serpa peynirleri) maya floraları tanımlanmış bu kültürlerin teknolojik/probiyotik özellikleri araştırılmıştır (Karasu-Yalcin vd., 2012; Güneş vd., 2021; Biagiotti vd., 2018; Dos Santos vd., 2017). Ancak yapılan literatür araştırmasında Shanklish peynirinin maya florası hakkında bir çalışmaya rastlanmamıştır. Bu çalışma, farklı şehirlerden toplanan Shanklish peynirlerinin maya florasının SCoT primerleri kullanılarak moleküler yöntemlerle tanımlanmasını ve enzimatik karakterizasyonunu belirlemeyi amaçlamaktadır.

MATERYAL VE YÖNTEM

Materyal

Çalışmada kullanılan beş adet Shanklish peyniri örneği farklı şehirlerde (Hatay, Gaziantep, İstanbul, Mersin, Kilis) evlerinde geleneksel yöntemlerle üretim yapan ailelerden toplanarak laboratuvara getirilmiştir. Toplanan peynirlerin üretiminde koyun sütü kullanıldığı ve oda sıcaklığında toprak kaplarda 1 ay süresince olgunlaşmaya bırakıldığı, olgunlaşma periyodunun sonunda peynirlerin cam kavanozlara alınarak kavanozların zeytinyağı ile doldurulduğu ve peynirlerin 3 ay boyunca zeytinyağı içerisinde muhafaza edildiği bilinmektedir. Olgunlaştırılmış peynir örneklerinden maya izolasyonu gerçekleştirilmiştir.

Yöntem

Mayaların izolasyonu

Shanklish peynirlerinin maya popülasyonunu belirlemek için Yeast Extract Glucose Chloramphenicol (YGC, Merck, Darmstadt, Almanya) agar kullanılmıştır. Örneklerden 10 gram alınarak 90 ml %0.90'lık steril fizyolojik tuzlu suya aktarılmıştır. Homojenizasyon işlemi bir stomacher (MAYO, hg-400, Avustralya) yardımı ile gerçekleştirilmiş ve daha sonra 10^{-5}'e kadar dilüsyonlar hazırlanmıştır. Hazırlanan dilüsyonlar YGC agara yayma kültür yönyemi ile ekilerek 28°C'de 48-72 saat inkübasyona bırakılmıştır. Moleküler karakterizasyon için saf kültür izole edilmesi amacıyla morfolojik olarak farklı görünen koloniler seçilmiş ve sürme yöntemiyle kolonilerin saflaştırılması sağlanmıştır. Son olarak izolatlar %20 gliserol içeren cryo tüplerde -80°C'de ve gliserol içermeyen %1.5 agar (Sigma Aldrich, ABD) eklenmiş besiyerlerinde yatık olarak stoklanmıştır.

Maya izolatlarının moleküler karakterizasyonu

DNA izolasyonu

Maya izolatlarının DNA ekstraksiyonu için Harju vd. (2004) tarafından önerilen metot minör değişiklikler yapılarak kullanılmıştır. Bu amaçla maya izolatları YPD (Merck, Darmstadt, Almanya) broth besiyerinde 28°C'de 24 saat inkübasyona bırakılarak aktifleştirilmiştir. Daha sonra YGC agar besiyerine ekimleri yapılarak 48-72 saat inkübasyon sonucunda kolonilerden 3 öze dolusu alınarak 2 ml'lik mikrosantrifüj tüplerine aktarılmıştır. Toplanmış kolonilerin üzerine 300 µL steril distile su eklenerek bir kuru blok ısıtıcıya (Dri-block DB-2A, Techne, Cambridge, BK) yerleştirilmiştir. İzolatlar kuru blok ısıtıcıda 85°C'de 15 dakika kaynamaya maruz bırakıldıktan sonra üzerlerine 650 µL ekstraksiyon miksi (200 mM Tris-HCl pH: 8.5, 25 mM NaCl, 25 mM

EDTA, %0.5 SDS) eklenmiş ve 65°C'de 1 saat inkübasyona bırakılmıştır. İnkübasyondan sonra tüplere eşit hacimde kloroform:izoamil alkol (24:1 v/v, AppliChem, Darmstadt, Almanya) eklenmiş ve 13000 rpm'de 15 dakika santrifüj işlemi uygulanmıştır. Santrifüj sonunda ayrılan üst fazdan yaklaşık 500 µL alınarak 500 µL izopropanol ile karıştırılmış ve yeni bir mikrosantrifüj tüpüne aktarılarak karışım 13000 rpm'de 15 dakika santrifüj edilmiştir. Elde edilen pellet 100 µL %70'lik etanol ile yıkanmış ve 50 µL steril iki kez damıtılmış (ddH_2O) su içerisinde süspanse edilmiştir. DNA konsantrasyonun belirlenmesinde bir nanospektrofotometre (DS-11 FX, DeNovix Inc., Wilmington, DE, ABD) kullanılmış ve son olarak izole edilen DNA'lar -20°C'de stoklanmıştır.

SCoT primerleri ile DNA amplifikasyonu

SCoT markörleri kullanılarak DNA amplifikasyon analizleri için Collard ve Mackill (2009) tarafından tasarlanan 36 primer arasından ekşi hamur örneklerinden izole edilen mayalarda ayrım gücü yüksek bulunan SCoT 12 primeri (ACGACATGGCGACCAACG) kullanılmıştır (Aydın vd., 2022). Daha sonra seçilen primer ile birlikte PCR reaksiyonları T100 termal cycler (Bio-Rad, Hercules, CA, ABD) cihazı kullanılarak gerçekleştirilmiştir. Bu amaç için öncelikle 10x DreamTaq DNA polimeraz tamponu (Thermo Fischer Scientific, ABD), 0.24 mM dNTPs, 1 mL $MgCl_2$,0.8 µM primer, 0.5 birim DreamTaq DNA polimeraz (Thermo Fisher Scientific, Waltham, MA, ABD) ve 20 ng DNA içeren PCR miksi hazırlanmıştır. Daha sonra 95°C'de 3 dakika denatürasyon işlemini takiben 35 döngü 95°C'de 60 saniye denatürasyon, 72°C'de 1 dakika bağlanma ve son uzama safhası 72°C'de 5 dakika olacak şekilde PCR koşulları belirlenmiştir. Elde edilen PCR ürünleri 1xTAE solüsyonunda %1.5'lik hazırlanmış agaroz jelde elektroforetik (90 dakika, 120 volt) ayrıma tabi tutulmuş ve süre sonunda etidyum bromür ile boyanarak PCR ürünlerinin varlığı jel görüntüleme sisteminde (G: BOX F3, Syngene, İngiltere) kontrol edilmiştir.

Internal Transcribed Spacer (ITS) sekanslama

SCoT primerleri kullanılarak elde edilen DNA parmak izlerine göre maya izolatları 5 grupta (19 adet *Kluyveromyces lactis* (Ana grup, temsilen 3 izolat), 2 adet *Pichia kudriavzevii* (temsilen 2 izolat), 1 adet *Pichia fermentans*, 1 adet *Pichia membranifaciens* ve 1 adet *Clavispora lusitaniae)* toplanmıştır. PCR ürünlerinin doğrulanması için ana gruptan 3 izolat ve diğer grupları temsilen 2 ve 1'er izolat seçilerek sekanslama hizmetine gönderilmiştir. Bu amaçla, ITS1 (5'-CCG TAG GTG AAC CTG CGG-3') ve ITS 4 (5'- TCC TCC GCT TAT TGA TAT GC-3') primer çiftleri genomik DNA'nın dahili transkript ayırıcı (ITS) bölgesini amplifiye etmek için kullanılmıştır (White vd., 1990). Hazırlanan PCR miksi, 10x Dream Taq tamponu, 2.5 mM dNTPs, 25 mM $MgCl_2$, 100 µM ITS1 primeri, 100 µM ITS4 primeri, 5 µL DreamTaq DNA polimeraz, 50 ng DNA ve steril distile su içermektedir. PCR koşulları ise 95°C'de 2 dakika denatürasyon ardından 95°C'de 30 saniyelik 30 döngü, 52°C'de 30 saniye, 72°C'de 1 dakika ve 72°C'de 5 dakikalık son uzatma şeklinde gerçekleştirilmiştir. Elde edilen PCR ürünleri ticari bir şirkete (Atlas Biyoteknoloji, Ankara, Türkiye) gönderilerek ITS1 primeriyle tek yönlü sekanslamaya tabi tutulmuştur. Elde edilen sekans verileri MEGA X programı kullanılarak analiz edilmiş (Kumar vd., 2018) ve son olarak Gen Bank (https://www.ncbi.nlm.nih.gov/) adresinde yer alan BLAST (http://blast.ncbi.nlm.nih.gov/) programı kullanılarak sonuçlar karşılaştırılmıştır. Tüm diziler, PP258023'den PP258030'e kadar erişim numaraları ile GenBank veri tabanına işlenmiştir.

Enzimatik karakterizasyon

Mayaların enzimatik aktivitesini belirlemek için API-ZYM (BioMérieux, Fransa) test kiti kullanılmıştır. Bu amaçla aktive edilmiş maya izolatları YGC besiyerine sürülerek geliştirilmiş ve tekli kolonilerden alınarak distile su yardımıyla 5-6 McFarland bulanıklık seviyesine kadar süspanse edilmiştir. Hazırlanan süspansiyondan 65 µL alınarak kuyucuklara inoküle edildi ve her kuyucuğa ZYM A ve ZYM B reaktifleri eklenerek 37°C'de 5 saat inkübasyona bırakılmıştır. Fast Blue'ya bağlı sarı renk oluşumunu engellemek için şeritler 1000 W'lık lamba altında 10 saniye tutuldu ve 5 dakika renk oluşumu için beklendi. Son olarak oluşan renkler API-ZYM renk reaksiyon kartıyla karşılaştırılarak 0'dan 5'e kadar

derecelendirildi. Aktivite olmayanlar (renk oluşmayan) 0 olarak kaydedilirken en yoğun renk 5 olarak kaydedilmiştir.

BULGULAR ve TARTIŞMA

Maya izolatlarının identifikasyon sonuçları

Olgunlaştırılmış Shanklish peynirlerinden izole edilmiş 24 adet endojen maya izolatı ITS1 bölgelerinin dizilenmesiyle tanımlanmıştır. Sekans sonuçlarına göre peynirlerden, 19 adet *Kluyveromyces lactis* (%79.16), 2 adet *Pichia kudriavzevii* (%8.33), 1 adet *Pichia fermentans* (%4.17), 1 adet *Pichia membranifaciens* (%4.17) ve 1 adet *Clavispora lusitaniae* (%4.17) mayası olmak üzere toplam 24 maya izole edilerek tanımlanmıştır. Elde edilen sekans dizileri NCBI web sitesinde bulunan BLAST veritabanında analiz edilmiş ve %99-100 arasında benzerlik göstermiştir. Çalışmadan izole edilen ve tanımlanan mayaların bilgileri ve erişim numaraları Çizelge 1 ve Çizelge 2'de verilmiştir.

Çizelge 1. Shanklish peynirlerinden izole edilen mayalara ait bilgiler
Table 1. Information on yeasts isolated from Shanklish cheeses

1	Hatay	ANO1	*K. lactis*		
		ANO2	*K. lactis*	PP258025	%99.38
		ANO3	*P. kudriavzevii*	PP258023	%99.80
		ANO4	*K. lactis*		
		ANO5	*K. lactis*		
		ANO6	*P. fermentans*	PP258028	%99.84
2	Gaziantep	ANO7	*K. lactis*		
		ANO8	*K. lactis*		
		ANO9	*P. membranifaciens*	PP258029	%99.34
		ANO10	*K. lactis*		
3	İstanbul	ANO11	*K. lactis*		
		ANO12	*K. lactis*	PP258026	%99.38
		ANO13	*K. lactis*		
		ANO14	*K. lactis*		
4	Mersin	ANO15	*K. lactis*		
		ANO16	*K. lactis*	PP258027	%99.38
		ANO17	*K. lactis*		
		ANO18	*C. lusitaniae*	PP258030	%98.37
		ANO19	*K. lactis*		
5	Kilis	ANO20	*K. lactis*		
		ANO21	*P. kudriavzevii*	PP258024	%99.80
		ANO22	*K. lactis*		
		ANO23	*K. lactis*		
		ANO24	*K. lactis*		

Çalışmadan elde edilen verilere göre *K. lactis* olgunlaştırılmış Shanklish peynirlerinde baskın maya türü olarak tespit edilmiştir. *Kluyveromyces* cinsi mayalar, özellikle *Kluyveromyces lactis*, endüstriyel biyoteknoloji için en önemli maya türlerinden biridir ve endüstriyel olarak başta β-galaktosidaz enzimi olmak üzere çeşitli metabolitlerin ve proteinlerin üretiminde kullanılmaktadır (Spohner vd., 2016). Bununla birlikte bu maya, sıklıkla süt ve süt ürünlerinden özellikle peynirlerden (Canastra peyniri, French peyniri, Fiore Sardo peyniri, Tulum peyniri) izole edilmektedir (Oliveira vd., 2019; Andrade vd., 2017; Ceugniez vd., 2017; Fadda vd., 2017; Karasu-Yalcin vd., 2012). Bu mayanın en çarpıcı özelliklerinden birisi ise probiyotik özellikler barındırma potansiyelidir. Çeşitli araştırmalar *K. lactis*'in mide-bağırsak yolunda canlı kalabildiğini,

bağırsak epitel dokusuna yapışabileceğini, kısa zincirli yağ asitleri üretimi bakımından üstün olduğunu, gıda patojenlerine karşı inhibisyon etkisi olduğunu ve kanserli hücrelerde pro-apoptotik aktivite göstermesi gibi fonksiyonel özellikler barındırdığını ortaya koymuştur (Oliveira vd., 2019). Peynir üretiminde uygulanan olgunlaştırma aşamasının da, maya florasının çeşitliliğini doğrudan etkileyen bir faktör olduğu ve ticari olarak satılan ve yüzeyi olgunlaştırılmış birçok peynirden de *K. lactis* mayasının izole edildiği rapor edilmektedir (Karasu Yalçın vd., 2011). *K. lactis*'in peynirlerde proteolitik aktivite gösterdiği ve aminoasit, amin grubu bileşikler, uzun zincirli ketonlar ve monogliserid sentezleyerek peynirlerin tatlarında aromatik acı tat gelişmesinde öncü olduğu belirtilmektedir (Ozmen Togay vd., 2020; Geronikou vd., 2020). Ayrıca, *K. lactis*'in laktozu asimile etme yeteneği ve aminopeptidaz aktivitesi gibi enzimatik potansiyeli olduğu da bilinmektedir (Lenoir, 1984).

Çizelge 2. Shanklish peynirlerinden izole edilerek tanımlanan maya suşları ve sayıları
Table 2. Yeast strains and total of numbers isolated from Shanklish cheeses

Maya türleri / *Yeast species*	İzolat sayıları / *Isolate number*
Kluyveromyces lactis	19
Pichia kudriavzevii	2
Pichia fermentans	1
Pichia membranifaciens	1
Clavispora lusitaniae	1
Toplam	24

K. lactis K. marxianus, Yarrowia lipolytica ve *Debaryomyces hansenii* peynirlerden izole edilen başlıca maya izolatları olmasına rağmen bunların yanında daha az sayıda olmakla birlikte *Pichia* spp., *Geotrichum candidum* ve *Saccharomyces cerevisiae* gibi mayalar da peynirlerden izole edilerek çeşitli teknolojik/probiyotik özellikleri sıklıkla araştırılmaktadır (Atanassova vd., 2016; Ceugniez vd., 2017; Aponte vd., 2010; Zheng vd., 2018). Çalışmadan elde edilen sonuçlara göre Shanklish peynirlerinden 2 adet *P. kudriavzevii*, 1 adet *P. fermentans* ve 1 adet *P. membranifaciens* olmak üzere toplam 4 adet *Pichia* spp. cinsi maya izole edilmiştir. *P. kudriavzevii* olası probiyotik özellikleri ve zorlu stres koşullarına karşı üstün performans göstermesinden ötürü son yıllarda artan bir ilgi görmektedir. Bu tür çok çeşitli fermente ürünlerden izole edilmekle birlikte, özellikle peynirlerde aromanın geliştirilmesinde önemli bir rol oynamaktadır. Ayrıca bu türün hücre dışı proteaz ve lipaz aktivitelerine sahip olduğu bilinmektedir (Chu vd., 2023) *P. kudriavzeii*'nin Kazak peynirlerinde destek kültür olarak kullanıldığı bir çalışmada, bu mayanın brendi, otsu ve soğan aromaları gibi hoş bir tat oluşturduğu tespit edilmiştir (Zheng vd., 2018). Öte yandan *P. fermentans* ise Lor peyniri, Otlu peynir ve Feta peyniri gibi çeşitli peynirlerden izole edilmiştir (Güneş vd., 2021; Tokak vd., 2019; Zheng vd., 2021). Merchán vd. (2020) tarafından yapılan bir araştırmada, Extremadura bölgesindeki yumuşak tip peynirlerden izole edilen *P. fermentans*'ın yapay mide ortamında hayatta kalabildiği, yüksek antioksidan aktiviteye sahip olduğu, antimikrobiyal potansiyelinin yüksek ve otoagregasyon/hidrofobisite değerlerinde diğer suşlara göre en üstün tür olduğu ortaya konulmuştur. Shanklish peynirlerinden izole edilen bir diğer maya ise *P. membranifaciens* olup bu maya Cabrales peyniri (Álvarez-Martín vd., 2017) ve Civil peyniri (Yildiz vd., 2021) gibi peynirlerinde florasında bulunmaktadır ve destek kültür veya starter kültür olarak kullanılabilecek önemli bir maya olduğu bildirilmektedir (Karasu-Yalcin vd., 2019).

C. lusitaniae geniş yayılım gösteren bir maya olup Mozarella peyniri (Facchin vd., 2013) ve Beyaz peynir (Gelen ve Ceylan, 2017) gibi süt ürünlerinde de izole edilmiştir. Bu maya hakkında çok sınırlı çalışma olmasına rağmen, probiyotik özellikler taşıdığı (Gürkan, 2018), lipid üretim yeteneğinin bulunduğu (Berikten vd., 2021) ve çeşitli enzim aktiviteleri gösterdiği (Müjdeci, 2012) yapılan araştırmalarla ortaya konmuştur.

Enzim aktiviteleri

İzolatların API-ZYM test kiti ile elde edilmiş enzim aktiviteleri Çizelge 3'te verilmiştir. *K. lactis* suşlarının tamamına yakını yüksek lösin arilamidaz, valin arilamidaz, sistin arilamidaz, asit fostataz, Naftol-as-bi-fosfohidroliz, β-

galaktosidaz, α-glukosidaz ve β-glukosidaz enzim aktivitesi göstermiştir. Suşların enzim aktiviteleri yüzdeleri Şekil 1'de verilmiştir.

P. kudriavzevii ANO21 suşu iyi derecede esteraz lipaz ve Naftol-as-bi-fosfohidroliz aktivitesi gösterirken, *P. kudriavzevii* ANO3 ve *P. fermentans* ANO6 suşları ise lösin arilamidaz, asit fosfataz ve Naftol-as-bi-fosfohidroliz aktivitelerini iyi derecede göstermiştir. *P. membranifaciens* ANO9 suşu ise diğer *Pichia* spp. cinsi suşlarla karşılaştırıldığında en yüksek asit fosfataz ve naftol-as-bi-fosfohidroliz aktivitesi gösteren suş olarak göze çarpmaktadır. *C. lusitaniae* ANO18 suşu ise yüksek lösin arilamidaz ve asit fosfataz aktivitesi göstermesine rağmen enzimatik aktivite yönünden diğer maya suşlarıyla rekabet edebilecek sonuçlar vermemiştir.

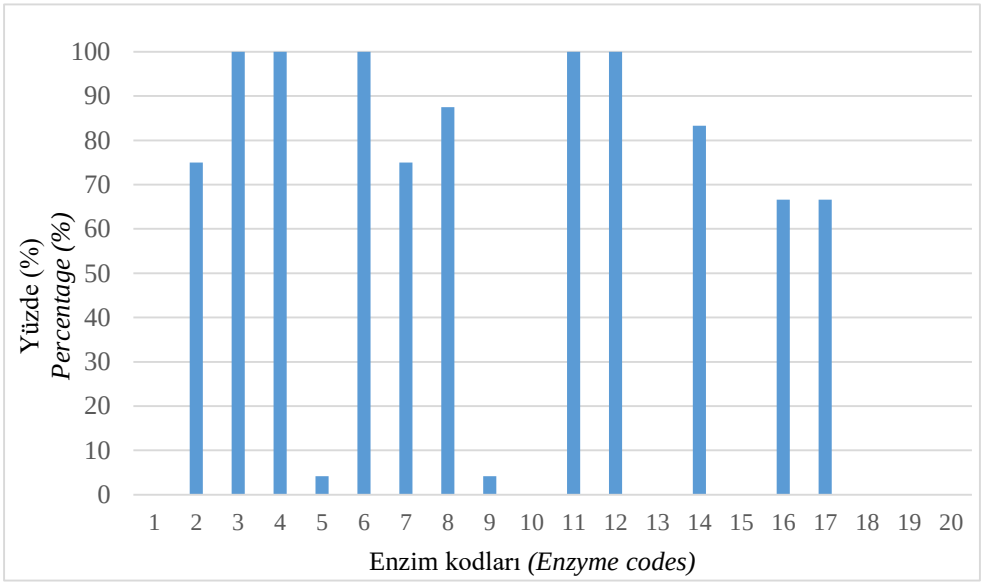
Çizelge 3. API-ZYM test kiti ile elde edilmiş mayaların enzimatik karakterizasyonu
Table 3. Enzymatic characterization of yeasts obtained with API-ZYM test kit

İzolat No*/ *Isolate number*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
ANO1	0	2	3	4	0	5	4	4	0	0	5	3	0	3	0	5	5	0	0	0
ANO2	0	1	3	3	0	4	4	3	0	0	5	3	0	2	0	5	4	0	0	0
ANO3	0	0	3	3	0	4	0	0	0	0	4	4	0	0	0	0	0	0	0	0
ANO4	0	2	3	4	0	5	5	4	0	0	4	4	0	3	0	5	5	0	0	0
ANO5	0	2	3	4	0	5	4	4	0	0	5	3	0	3	0	5	5	0	0	0
ANO6	0	2	3	3	0	5	2	3	0	0	4	4	0	0	0	0	0	0	0	0
ANO7	0	0	1	1	0	4	0	1	0	0	3	3	0	4	0	0	0	0	0	0
ANO8	0	0	2	3	0	4	0	1	0	0	3	3	0	4	0	0	0	0	0	0
ANO9	0	3	4	3	1	5	4	4	1	0	5	5	0	1	0	0	0	0	0	0
ANO10	0	2	3	4	0	5	4	4	0	0	5	3	0	3	0	5	5	0	0	0
ANO11	0	2	3	3	0	4	5	3	0	0	5	3	0	3	0	5	4	0	0	0
ANO12	0	2	3	4	0	5	5	4	0	0	4	4	0	3	0	5	5	0	0	0
ANO13	0	2	3	4	0	5	4	4	0	0	5	3	0	3	0	5	5	0	0	0
ANO14	0	0	2	2	0	4	0	2	0	0	3	3	0	4	0	0	0	0	0	0
ANO15	0	2	3	4	0	5	4	4	0	0	5	4	0	3	0	5	5	0	0	0
ANO16	0	2	3	3	0	4	4	3	0	0	5	3	0	2	0	5	5	0	0	0
ANO17	0	2	3	4	0	5	5	4	0	0	5	4	0	3	0	5	5	0	0	0
ANO18	0	0	2	1	0	5	1	0	0	0	5	1	0	0	0	3	3	0	0	0
ANO19	0	2	3	4	0	5	4	4	0	0	5	3	0	3	0	5	5	0	0	0
ANO20	0	1	2	3	0	4	0	4	0	0	4	3	0	4	0	0	0	0	0	0
ANO21	0	0	3	4	0	3	0	0	0	0	3	4	0	0	0	0	0	0	0	0
ANO22	0	2	3	3	0	5	5	4	0	0	5	3	0	3	0	5	5	0	0	0
ANO23	0	1	3	3	0	4	5	3	0	0	5	3	0	4	0	5	5	0	0	0
ANO24	0	2	3	4	0	5	3	4	0	0	4	4	0	3	0	5	5	0	0	0

1: Kontrol, 2: Alkalin fosfataz, 3: Esteraz, 4: Esteraz lipaz, 5: Lipaz, 6: Lösin arilamidaz, 7: Valin arilamidaz, 8: Sistin arilamidaz, 9: Tripsin, 10: Alfa simotripsin, 11: Asit fosfataz, 12: Naftol-as-bi-fosfohidroliz, 13: α-galaktosidaz, 14: β-galaktosidaz, 15: β-glukuronidaz, 16: α-glukosidaz, 17: β-glukosidaz, 18: N-asetil-β-glukozaminidaz, 19: α-mannosidaz, 20: α-fukosidaz

*ANO3 *P. kudriavzevii*, ANO6 *P. fermentans*, ANO9 *P. membranifaciens*, ANO18 *C. lusitaniae*, ANO21 *P. kudriavzevii,* diğer izolat kodları ise *K. lactis* suşlarına aittir. (ANO3 *P. kudriavzevii*, ANO6 *P. fermentans*, ANO9 *P. membranifaciens*, ANO18 *C. lusitaniae*, ANO21 *P. kudriavzevii*, and other isolate codes indicate *K. lactis* strains.)

Şekil 1. Maya izolatlarının enzim aktivitesi yüzdeleri
Figure 1. Enzyme activity percentages of yeast isolates



1: Kontrol, 2: Alkalin fosfataz, 3: Esteraz, 4: Esteraz lipaz, 5: Lipaz, 6: Lösin arilamidaz, 7: Valin arilamidaz, 8: Sistin arilamidaz, 9: Tripsin, 10: Alfa simotripsin, 11: Asit fosfataz, 12: Naftol-as-bi-fosfohidroliz, 13: α-galaktosidaz, 14: β-galaktosidaz, 15: β-glukuronidaz, 16: α-glukosidaz, 17: β-glukosidaz, 18: N-asetil-β-glukozaminidaz, 19: α-mannosidaz, 20: α-fukosidaz

Arilamidazlar peptid, ammid veya arilamidlerden N-terminal aminoasitlerin hidrolizini katalize etmektedirler (Dodor ve Tabatabai, 2007). Böylece, arilamidazların aminoasitlerin serbest bırakılmasında ve peynirde arzu edilen aromanın geliştirilmesinde önemli etkileri olduğu bulunmuştur. Ayrıca bu enzimlerin peynirin olgunlaşması aşamasında gelişen acılığın giderilmesinde rol aldıkları bildirilmiştir (Herreros vd., 2003).

Fosfatazların çeşitli fosfat esterlerinin C-O-P bağlarının hidrolizini katalize ettikleri bilinmektedir. Optimum pH değerlerine bağlı olarak asit veya alkalin olarak isimlendirilmektedirler. Peynirde her iki fosfataz enzimi bulunmasına rağmen peynirlerin düşük pH'ya sahip olmalarından ötürü asit fostatazlar daha aktif rol oynamaktadırlar (Magboul ve McSweeney, 1999). Peynirlerin olgunlaşma aşamasında proteolize dirençli olan fosfat açısından zengin peptidlerin üretildiği bildirilmektedir. Peynirdeki asit fosfataz ve proteolitik enzimlerin aktivasyonuyla küçük peptidler ve serbest aminoasitler geniş çapta üretilmektedir. Ayrıca, asit fostataz enziminin proteoliz aktivitesi olduğu ve bu nedenle peynirde aroma oluşumuna katkı sunduğu da bildirilmiştir (Akuzawa ve Fox, 2004).

Esteraz ve lipazlar, lipitlerin ester bağlarının hidrolizini katalizleyerek peynirlerdeki serbest yağ asitlerinin miktarını artırmaktadırlar. Düşük konsantrasyonlardaki serbest yağ asitlerinin proteoliz ürünleri ve diğer reaksiyonlarla doğru şekilde dengelendiklerinde aromaya ve lezzete katkı sunabilecekleri belirtilmektedir (Herreros vd., 2003). Yüksek esteraz veya esteraz lipaz aktivitesine sahip suşlarının peynirlerin olgunlaşma aşamasında lipolize katkıda bulunabilecekleri düşünülmektedir (Karasu-Yalcin vd., 2012).

β-galaktosidaz enzimi laktozu hidrolize etmekten sorumlu bir enzimdir. Peynir üretiminde bu enzim laktozu parçalayarak glikoz ve galaktoza dönüştürerek fermantasyon sürecinin hızlanmasına katkıda bulunur. Ayrıca peynirlerin

olgunlaşma aşamasında hem tekstürel özelliklerinin hem de tat profilinin geliştirilmesinde rol almaktadır (Saqib vd., 2017). α ve β-glukosidazların ana rolü ise süt ürünlerindeki glikozun ve glikoz içeren moleküllerin parçalamalarıdır. Böylece hem fermantasyon süreçlerine katkı verirler hem de olgunlaşma aşamasında peynirlerin tat profillerine katkıda bulunabilirler (de Morais vd., 2023).

Suşların enzimatik aktivite sonuçlarına göre, *K. lactis* ANO17 suşu en üstün enzimatik aktivite gösteren suş olarak tespit edilmiştir. Bu suş, yüksek esteraz lipaz, lösin arilamidaz, valin arilamidaz, sistin arilamidaz, asit fostataz, Naftol-as-bi-fosfohidroliz α-glukosidaz ve β-glukosidaz aktivitesi gösterirken orta seviyede esteraz, β-galaktosidaz ve düşük seviyede alkalin fostataz aktivitesi göstermiştir. Olgunlaştırılmış Shanklish peynirinden izole edilen bu suş enzimatik aktivite yönünden oldukça üstün performans gösterdiğinden, daha ileri çalışmalarda veya starter kültür kombinasyonlarında destek kültür olarak kullanılabilme potansiyeli göstermektedir.

SONUÇ

Türkiye'de son yıllarda üretilmeye ve tüketilmeye başlanan Shanklish peynirinin maya florası SCoT markör yöntemi kullanılarak moleküler düzeyde tanımlanmış ve tanımlanan suşların enzim aktiviteleri belirlenmiştir. Çalışma sonuçlarına göre Shanklish peynirlerinden 19 adet *K. lactis*, 2 adet *P. kudriavzevii*, 1 adet *P. fermentans*, 1 adet *P. membranifaciens* ve 1 adet *C. lusitaniae* olmak üzere toplam 24 adet maya suşu izole edilmiştir. Bu suşlardan *K. lactis* ANO17 suşu esteraz lipaz, lösin arilamidaz, valin arilamidaz, sistin arilamidaz, asit fostataz, Naftol-as-bi-fosfohidroliz α-glukosidaz ve β-glukosidaz aktivitesi göstermiştir. Böylece, bu suşun peynirin olgunlaşması aşamasında önemli yapı, aroma ve tat geliştirme potansiyeli bulunduğu ve destek kültür kombinasyonlarında kullanılabileceği düşünülmektedir. Bununla birlikte, bu suşun teknolojik ve probiyotik özelliklerinin araştırılarak ayrıca gıda güvenliği açısından değerlendirilmesine ve genel olarak Shanklish peyniri üzerine daha geniş ve kapsamlı çalışmaların gerçekleştirilmesine ihtiyaç duyulduğu düşünülmektedir. Ayrıca bu çalışmanın, maya izolasyonu ve tanımlamasının peynir üretiminde kullanılan hammaddelerden ve üretimin farklı aşamalarından alınan örneklerde gerçekleştirilerek suşların izolasyon kaynaklarının da belirlenebileceği çalışmalara katkı sağlayacağı öngörülmektedir.

ÇIKAR ÇATIŞMASI

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THE THERMAL STABILITY OF PHYTOCHEMICALS AND PHYSICOCHEMICAL PROPERTIES OF KIRAZ AND FINDIK CHERRY LAUREL FRUITS (*LAUROCERASUS OFFICINALIS* L.) AND THEIR MOLASSES

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ABSTRACT
This study aimed to investigate the thermal stability of certain phytochemicals in molasses at temperatures of 50°C, 60°C, and 70°C throughout 6 to 168 hours. Additionally, the chemical makeup of Kiraz (KCLM) and Fındık (FCLM) cherry laurel (*Laurocerasus officinalis* L.) fruits, as well as their molasses, was examined. The two molasses compositions were different due to the type of fruit used. The soluble dry matter (SDM) and dry matter (TDM) of the molasses ranged from 68.0-68.2% and 72.3-73.1%, respectively. The FCLM had higher values for titratable acidity (TA) (1.201%), hydroxymethylfurfural (HMF) (22.72 mg/kg), Vitamin C (66.83 mg/100 g), phenolics (TP) (5359 mg GAE/100 g), anthocyanin (ACN) (45.27 mg/kg), DPPH-RSA (80%), antioxidant capacity (AC) (33.74 µg TE/g), Hunter L* (31.34), a* (0.96), b* (-0.59), and browning level (BL) (15.20) compared to KCLM. The ANOVA results showed that cultivars, temperature, and storing time significantly affected phytochemicals and physicochemical properties ($P < 0.05$).
Keywords: Phytochemicals, molasses, *Laurocerasus officinalis* L., stability

KİRAZ VE FINDIK KARAYEMİŞ (*LAUROCERASUS OFFICINALIS* L.) MEYVE VE PEKMEZLERİNİN FİZİKOKİMYASAL ÖZELLİKLERİ VE FİTOKİMYASALLARININ ISI KARARLILIĞI

ÖZ
Bu çalışma, 6 ila 168 saat boyunca 50 °C, 60 °C ve 70 °C sıcaklıklarda pekmez içindeki bazı fitokimyasalların termal stabilitesini araştırmayı amaçlamıştır. Ek olarak, Kiraz (KCLM) ve Fındık (FCLM) karayemiş (*Laurocerasus officinalis* L.) meyvelerinin ve pekmezlerinin kimyasal yapısı incelendi. Kullanılan meyve türüne bağlı olarak iki pekmez bileşimi farklıydı. Pekmezlerin çözünebilir kuru maddesi (ÇKM) ve kuru maddesi (TKM) sırasıyla %68.0-68.2 ve %72.3-73.1 arasında değişmektedir. FCLM, KCLM'e göre titre edilebilir asitlik (TA) (%1.201), hidroksimetilfurfural (HMF) (22.72 mg/kg), C Vitamini (66.83 mg/100 g), fenolikler (TF) (5359 mg GAE/100 g), antosiyanin (ACN) (45.27 mg/kg), DPPH-RSA (%80), antioksidan kapasite (AK) (33.74 µg TE/g), Hunter L* (31.34),

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a* (0.96), b* (-0.59) ve kahverengileşme derecesi (KD) (15.20) bakımından daha yüksek değerlere sahipti. ANOVA sonuçları çeşit, sıcaklık ve depolama süresinin fitokimyasallar ile fizikokimyasal özellikleri önemli ölçüde etkilediğini gösterdi ($P < 0.05$).
Anahtar kelimeler: Fitokimyasallar, pekmez, *Laurocerasus officinalis* L., stabilite

INTRODUCTION

Cherry laurel (*Laurocerasus officinals* L.) is a popular fruit in the form of a bush or tree scattered along the Black Sea coast of Turkey and turns dark purple or black when ripe (Halilova and Ercişli, 2010). 'Oxygemmis', 'Globigemmis' and 'Angustifolia' were identified as three forms of fruits in the morphological and cytological studies on cherry laurel. The fruits of Oxygemmis are the biggest and have a bright black color when ripe, and the fruit taste is bitter and sour. It has been reported that the fruits of Globigemmis have a thinner mesocarp, are rigid and black when ripe, have a better taste and is less tart than Oxygemmis, and the fruits of this form are more preferred for fresh consumption. Angustifolia, which has a more extensive distribution than these forms, is used as an ornamental plant in Europe (Ayaz et al., 1997a; Akbulut et al., 2007).

Fruit composition varies among cultivars, likely due to ecological conditions, cultivar properties, climate, soil etc. (Sulusoglu et al., 2015). According to a study conducted by Şahan et al. (2012), the average content of cherry laurel fruits is as follows: 79.63% moisture, 0.75% ash, 0.95% protein, 11.61% sugar, and 0.16% oil. The study also found that the organic acids per 100g of fruits were: oxalic acid (1.02mg), malic acid (47.92mg), L-ascorbic acid (2.11mg), acetic acid (9.21mg), citric acid (1.28mg), succinic acid (3.84mg), and fumaric acid (5.53mg). Furthermore, the total phenolic (TP) and DPPH-radical scavenging activity (RSA) in methanol extracts of fresh fruits were 22.9mg GAE/100g and 26.70μmol trolox/g, respectively.

Akbulut et al. (2007) determined *TA* between 0.38-1.21% and *SDM* between 8.6-21.3% in 28 cherry laurel genotypes growing in the Black Sea Region. Also, Ayaz et al. (1997b) found dominant fructose, glucose, sorbitol and sucrose as sugar composition in cherry laurel cultivars. Furthermore, Alasalvar et al. (2005) reported sugars such as xylose and arabinose outside fructose, glucose and sorbitol in fruit cultivars and their molasses. Karahalil and Şahin (2011) demonstrated that phenolics such as gallic, protocatechuic, p-OH benzoic, chlorogenic, vanillic, p-coumaric, ferulic, syringic acids and catechin and rutin were in extracts, also found high the *AC* of the fruit extracts. Ergüney et al. (2015) reported that the main anthocyanins determined in cherry laurel are cyanidin-3-arabinoside and peonidin-3-arabinoside. Furthermore, Kolaylı et al. (2003) revealed that their fruits contain significant amounts of potassium (2215 mg/kg), magnesium (179 mg/kg), calcium (153 mg/kg), sodium (55 mg/kg), manganese (24.2 mg/kg) and traces of iron (8.3± 0.8 mg/kg), zinc (1.9±0.2 mg/kg) and copper (0.8±0.1 mg/kg). Additionally, Alasalvar et al. (2006) reported that between *α*-tocopherol 0.29-0.42 mg, *γ*-tocopherol 0.55-0.69 mg and *β*-sitosterol 192.5-222 mg per 100 g of oil obtained from the seeds.

Also, cherry laurel fruit is used as a medicinal plant to treat health issues like stomach ulcers, digestive problems, bronchitis, eczema, and hemorrhoids. The fruit also acts as a diuretic. Furthermore, they have anti-inflammatory, antinociceptive, antioxidant, neuroprotective, antidiabetic and anticarcinogenic effects (Karahalil and Şahin, 2011; Demir et al., 2017). Cherry laurel fruits are usually eaten fresh, but they can also be dried, pickled, and used to make jam, marmalade, and fruit juice, (Şahan et al., 2012). Although their fruits have been processed into molasses using traditional methods recently, their commercial production has not become widespread yet. It's important to diversify the usage of cherry laurel fruits, which is rich in phytochemicals, by turning it into different foods like molasses. This helps to increase the economic potential of this valuable fruit. Additionally, using it in different food formulations can increase the functionality of foods (Vahapoğlu et al., 2018).
Molasses that are traditional foods are sweet, delicious natural products produced by

concentrating sugar-rich fruit juices by boiling them without adding any food additives or sugar, thus extending their shelf life. Molasses are produced mainly from sugar-containing fruits such as grapes, mulberries, figs, apples, plums, carob, dates, apricots, cranberry, blueberries, pomegranate, black mulberry and watermelons, as well as from yields such as andız, juniper, sugar beet or cane, and sugar millet (Şimşek and Artık, 2002; Tosun and Üstün, 2003; Turhan et al., 2007; Kalaycıoğlu, 2023). Molasses is an important food that can immediately meet the required energy needs due to quickly mixing into the blood. The 100 g of molasses contains 55-80% sugar and 0.6-0.9% nitrogenous substance, 2.2-14 µg Vitamin B1, 150 µg Vitamin B2, 1.4 mg niacin (Vitamin B3) and provides approximately 280 kcal. In addition, it has been reported that molasses is a good source of mineral substances such as K, Ca, Mg, P, Na, Fe, Zn, Cu and Mn (Şimşek and Artık, 2002; Toker and Hayoğlu, 2004; Ekin and Çelikezen, 2015).

In the literature review, although there are a limited number of studies on cherry laurel fruit and especially its molasses, there are no studies for determining the possible changes in molasses compositions at different storage temperatures. The study aimed to demonstrate the effect of model storage temperatures and times on the changes in the phytochemical compounds and physical properties of molasses produced by vacuum using two cherry laurel fruits and to obtain mathematical equations explaining the thermal stability of phytochemicals.

MATERIAL AND METHODS

Material

A total of 15 kg of each Kiraz (KCL) and Fındık *(*FCL) cherry laurel cultivar (*Laurocerasus officinalis* L.) were collected on 24 July and 5 August 2017, respectively by the sampling rules from predetermined trees in Giresun province and its surroundings. The collected cherry laurels were then processed into two different types of molasses.

Methods

Production of cherry laurel molasses (FCLM, KCLM)

To remove or reduce the dust, soil, microorganisms, and agricultural pesticide residues on the harvested and sorted KCL and FCL fruits, washing was done with tap water. Following cleaning, drying, and crushing into small pieces by hand, tap water was added to the mix at a ratio of 1:1. Wort was subjected to short-term pre-heat treatment (3 min at 80-85 °C) to inactivate enzymes and facilitate extraction and cooled to room temperature (25 ±2°C). Then, worths waited for 2 hours at 25 ±2°C for extraction, and the coarse sediment and seeds were removed using a filter cloth for clear juice (repeated twice). The obtained extracts were kept in a refrigerator (4 °C) for 12 hours and filtered through coarse filter paper to remove the fine sediment. The obtained clear filtrates were concentrated in a laboratory rotary evaporator (Heidolph Laborota 4000, Germany) at 50±2 °C, under vacuum (500-600 mmHg), at a rotational speed of 60-120 rpm (60 rpm at the beginning, 120 rpm towards the end). Evaporation terminated when the soluble dry matter (*SDM*) was 68% by refractometer (Hanna HI 96800, Romania) (Fig. 1).

Packaging and storage of KCLM and FCLM samples

KCLM and FCLM samples produced from FCL and KCL cultivars were placed in 50 mL glass jars, and their lids were closed. The molasses samples in the jar were stored at three different temperatures (50-60 and 70 °C) according to the experimental plan, between 6 and 168 hours, for seven storage times. The samples, whose storage period was completed, were immediately cooled with ice water and kept in a deep freezer at -24 °C until analysis.

Physicochemical analyzes

Soluble dry matter *(SDM)* and Total dry matter *(TDM)*

The SDM of fresh fruit and their molasses were determined by a digital refractometer (Hanna HI 96800, Romania). *TDM* was determined by calculating the weight loss caused by keeping the fresh fruit and molasses in a certain amount in glass petri dishes in an oven (Ecocell, Germany) at 70 °C until they reach a constant weight.

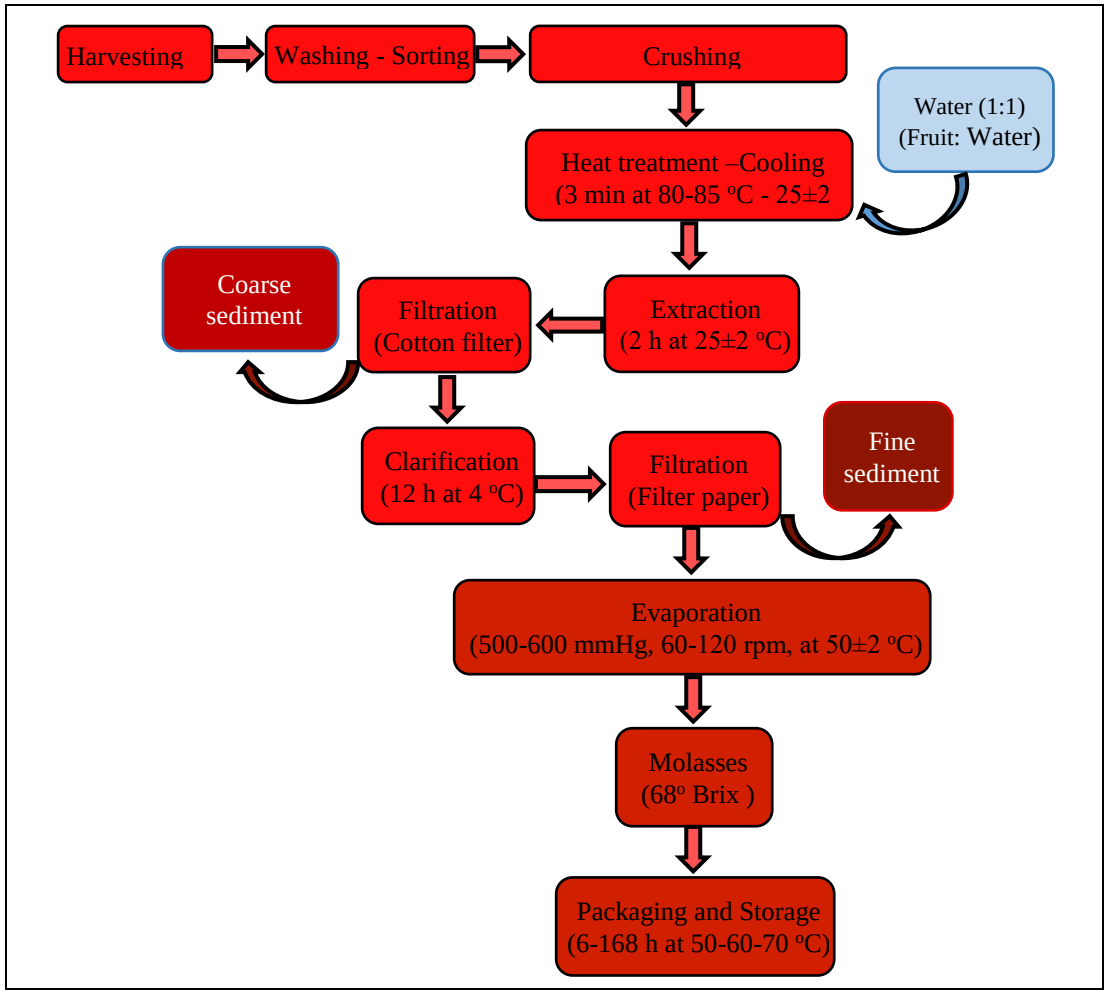

Figure 1 Production steps of KCLM and FCLM

pH and Titratable acidity (TA)

The pH of fruits and molasses was determined potentiometrically using a pH meter (Mettler Toledo-S210, Switzerland) calibrated with buffer solutions (pH 4.0 and pH 7.0). *TA* was determined by the titration of fresh fruit and molasses with 0.1 N NaOH solution using a pH meter up to pH 8.1 and expressed as g/100 g malic acid from the spent amount of NaOH.

5-Hydroxymethylfurfural (HMF*)*

For HMF, 1 g of molasses samples were diluted with distilled water in appropriate proportions, made up to 50 mL with 2 mL of Carrez I and Carrez II solutions, mixed by vortex and filtered with Whatman 42 filter paper. After taking 1 mL of the filtrate and adding 2.5 mL of p-toluidine and 0.5 mL of barbutyric acid solutions, homogenized samples' the absorbances (*Abs*) were read within 1-2 min against the witness sample prepared with distilled water instead of samples at 550 nm using *UV-VIS* spectrophotometer (Shimadzu UV mini-1240, Japan) (Cemeroğlu, 2010).

Vitamin C *(*Vit C)

The amount of Vit C in fresh fruit and their molasses was determined with the spectrophotometric method reported by Cemeroğlu (2010). After taking 1 g sample and making it up to 25 mL with 6% metaphosphoric acid solution, it was kept in the dark for two hours, centrifuged at 727 x g (2500 rpm) for 5 min (Sigma 2-6, Germany) and filtered. The 5 mL of acetate buffer (pH 4.0), 2 mL of 2.6 dichlorofernolindophenol solution and 10 mL of

xylene were added to 5 mL of the filtrate. Then, *Abs* readings were made against the blank prepared with metaphosphoric acid after 30 min at 500 nm using a *UV-VIS* spectrophotometer (Shimadzu UV mini-1240, Japan).

Total Phenolics (TP)

In determining TP, the colorimetric Folin-Ciocalteu method reported by Cemeroğlu (2010) was used with some modifications. Approximately 1 g of KCL and FCL were weighed and extracted using a shaker in 80% methanol (MeOH) containing 5 mL of 1% HCl for 2 hours and 10 min at 4000 rpm. Then, extracts were centrifuged and the current extraction process was repeated twice. 20 µL of the prepared and combined MeOH extracts of fresh fruit and molasses were transferred to disposable spectrophotometer cuvettes, on added 75 µL of Folin-Ciocalteu reagent and 750 µL of Na_2CO_3 (7.5%) solution and 765 µL of distilled water. The Abs of mixtures kept in the dark for 90 min at room temperature was measured at 725 nm using a UV-VIS spectrophotometer (Shimadzu UV mini-1240, Japan). The TP was calculated over mg GAE/kg fresh fruit and molasses using the calibration curve obtained from the solutions prepared from gallic acid.

Total Monomeric Anthocyanin (ACN)

The total monomeric ACN was determined according to the pH differential method given by Lee et al. (2005). Accordingly, MeOH (80% MeOH + 20% H_2O containing 1% HCl) extracts of fresh fruit and molasses diluted with 0.025 M KCl (pH 1.0) and 0.4 M $CH_3COONa*3H_2O$ (pH 4.5) buffer solutions at appropriate rates. The *Abs* of diluted samples hidden in the dark for 15 min were detected at 520 nm and 700 nm with a *UV-VIS* spectrophotometer (Shimadzu UV mini-1240, Japan). Results were expressed in mg/kg based on the cyanidin-3-glucoside equivalent.

DPPH Free Radical Scavenging Activity (DPPH-RSA) and Antioxidant Capacity (AC)

For DPPH-RSA analysis, 2.9 mL of DPPH radical solution (1 mM) was added to the 0.1 mL MeOH extracts of the samples obtained for phenolics. After keeping in a water bath at 30 °C for 30 min, Abs was measured using a UV-VIS spectrophotometer (Shimadzu UV mini-1240, Japan) at a wavelength of 517 nm. DPPH-RSA was calculated as % inhibition according to the formula below (Equation 1). It was also expressed as Trolox equivalent (mg TE /100 g fresh fruit and molasses) using the daily prepared Trolox standard calibration curve (Cemeroğlu, 2010).

$$DPPH - RSA(\%) = (1 - (Abs_s/Abs_c) \times 100 \quad (1)$$

where Abs_S = the Abs of the sample, Abs_C = the Abs of the control sample

Viscosity

The viscosity of the molasses samples in the homogenized glass jar was measured at 20 °C at a shear rate of 100 rpm using a Brookfield viscometer and probe no s-63.

Browning Level (BL)

Approximately 1.5 g of fresh fruit and their molasses were weighed into centrifuge tubes and made up to 10 mL with distilled water. After adding 20 mL of ethyl alcohol and homogenizing the samples by vortex, centrifuged (Sigma 2-6, Germany) for 5 min at 727 x g (2500 rpm). 5 mL samples were taken from the supernatant part and added 5 mL of distilled water and 1 mL of $K_2S_2O_5$. The *Abs* of centrifuged mixtures again at 1860 x g (4000 rpm) for 5 min and waited for 20 min were detected at 420 nm in a *UV-VIS* spectrophotometer (Shimadzu UV mini-1240, Japan). *BL*s were calculated by multiplying the read *Abs* value with the dilution factor (Cemeroğlu, 2010).

Hunter *L*, a** and *b** color values

The Hunter color values of fruit and molasses samples were measured by a color meter that was calibrated with the standard calibration plate of L*= 97.79, a*= -0.44 and b*= +2.04 (Konica Minolta CR-410, Japan).

Statistical analysis

The research was set up and conducted in a Factorial Experimental Desing (2 cherry laurel molasses (FCLM, KCLM) x 3 temperature (T) x 7 storage time (ST) x 2 replications, a total of 84 samples). The averages of the sources of variation found to be significant in the Analysis of Variance

(ANOVA) were compared using the Tukey Multiple Comparison Test (TMCT) (Düzgüneş et al., 1987). Also, the data of phytochemicals such as TP, ACN, and Vit C were subjected to multi-regression analysis to get mathematical models reflecting the changes with T and ST (Equation 2). The MINITAB 18 statistic program was used for data analysis.

$$\Upsilon = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^{\,2} + \Sigma\beta_{ij} X_i\, X_j \qquad (2)$$

where β_0, β_i, β_{ii}, and β_{ij} = regression coefficients for the intercept, linear, quadratic, and interaction terms, respectively, X_i (T) = the independent variable as temperature (°C), X_j (ST) = the independent variable as storage time (h)

RESULTS AND DISCUSSION

Some physicochemical properties of cherry laurel cultivars and their molasses

The physicochemical analysis results of cherry laurel cultivars and their molasses are shown in Table 1.

Table 1. Some physicochemical compounds of cherry laurel cultivars and their molasses (n=2)[α]

Properties	Cherry laurel cultivars and their molases			
	KCL	KCLM	FCL	FCLM
TDM (%)	19.78±0.74	72.60±0.43	23.70±0.64	72.79±0.31
SDM (%)	18.3±0.14	68.2±0.00	22.2±0.14	68.00±0.00
pH	4.57±0.014	4.53±0.014	4.42±0.00	4.36±0.007
TA (% as malic acid)	0.369±0.005	0.547±0.010	0.380±0.003	1.201±0.018
HMF (mg/kg)	-	9.94±1.24	-	21.07±2.33
Vit C (mg/100g)	64.01±6.67	59.98±0.52	81.52±1.07	63.17±5.17
TP (mgGAE/100g)	802.59±90.77	4287.60±20.24	1233.84±33.57	5083.20±390.25
ACN (mg/kg)	955.77±27.25	42.86±3.40	1201.22±112.20	36.03±3.08
DPPH-RSA (%)	71.39±0.00	66.50±2.15	75.11±1.95	77.94±3.66
AC (µg TE/mg sample)	29.91±0.00	27.85±0.90	31.47±0.82	32.65±1.54
Viscosity (at 100 rpm)	-	805.5±53.03		836.5±21.92
BL (A_{420} /mL)	4.291±0.862	14.529±0.131	4.960±0.200	15.181±0.029
Hunter Color values				
L*	26.42±0.09	31.25±0.13	21.84±0.01	30.73±0.16
a*	6.52±0.04	0.94±0.04	4.10±0.10	0.74±0.03
b*	4.00±0.08	-0.14±0.02	0.83±0.01	-0.47±0.18

α: as Mean±SD (Standard deviation)

Table 1 shows that significant differences existed between compositions of both fruits in terms of Vit C, TP and ACN. Also, the composition elements like TA, HMF, Hunter L* a* and b* values markedly changed during molasses production. While pH, Vit C, ACN, Hunter, a* and b* values of fruits generally decreased with molasses production, TA, TP, BI, and Hunter L* values increased. On the other hand, except for pH, Hunter L*, a* and b* values of FCL and FCLM, the SDM, TDM, TA, Vit C, TP, ACN, DPPH-RSA, AC, viscosity and BL values were higher than KCL and KCLM. Furthermore, the highest HMF was in KCLM compared to FCLM.

The pH values of KCL and FCL cultivars were 4.57 and 4.42, respectively, and in their molasses were slightly lower averages of 4.53 and 4.36, respectively. Concerning pH, the TA of both fruits varied between 0.369 and 0.380% as malic acid. The average TA of FCLM (1.201%) was higher than KCLM (0.547%). According to the Turkish Food Codex, both molasses were within the pH 3.5-5.0 limits specified for sour molasses (TFC 2017).

Due to the nature of the food industry, especially the chemical composition of processed raw materials, processing conditions and techniques increase 5-hydroxymethyl-2-furfural formation in foods. In the formation of HMF, not only simple sugars but also polysaccharides, proteins (amino acids), low pH and high temperatures applied

during the process are effective (Kowalski et al., 2013). The amount of HMF of the molasses vacuum evaporated showed an average value almost two-fold higher in FCLM, which has a lower pH and high TA than KCLM. HMF findings were consistent with between 0.15-166 mg/kg values that were reported for different molasses (Şimşek and Artık, 2002; Tosun and Üstün, 2003; Toker and Hayaloğlu, 2004; Turhan et al., 2007).

Compared to KCL, FCL contained more Vit C at 81.52 mg/100g. However, Vit C decreased to 59-63 mg in both molasses during the vacuum evaporating process. After all, FCLM had a higher Vit C degradation rate at 23%. Consistent with our results, Kuşçu and Bulantekin (2016) reported a gradual decrease of ascorbic acid in apple pekmez by open-pan evaporating (56%) and vacuum evaporating (23%).

In our samples, the TP of FCL was about 53% higher than KCL, and the TP of cultivars increased 5.34 fold in KCLM and 4.12 fold in FCLM due to concentration during molasses production. As can be seen, cherry laurel fruits are a good source of antioxidants such as Vit C, TP and ACN. In a previous study, TP in KCL, FCL and molasses samples was determined by Alasalvar et al. (2005) as 454, 651, and 1444 mg GAE/100 g, respectively. Celep et al. (2012) reported the average TP in dry extracts of cherry laurel fruits as 23.64 ± 0.84 mg GAE/g. Ayaz et al. (1997a), in the phenolic profile of cherry laurel fruits and the wild form, determined vanillic acid as the predominant phenolic acid besides cinnamic (p-coumaric and caffeic acids), benzoic (p-hydroxybenzoic, protocatechuic, and vanillic acids) acids. According to the literature, our data shows approximately 2-fold higher from fruit and 3-4-fold higher from molasses, except for Celep et al. (2012), where the dry extract was used. Furthermore, Tunç et al. (2021) reported that the total phenolic contents of the grape pekmez samples produced by ohmic heating-assisted vacuum evaporation varied between 1.36 and 1.67 mg GAE/g sample. As a result, it turned out that cherry laurel fruits are a good source of TP. Cherry laurel fruits and molasses' TP differences may be due to ecological conditions and harvest time. During the extraction, crushing and heat treatment stages of the fruits, bioactive compounds with antioxidant effects such as amygdalin, tocopherols and sterols pass through the crushed seeds and may have increased the AC in both molasses. As a matter of fact, in the previous study, Elmastas et al. (2013) determined that the ACs of amygdalin, prunasin and β-sitosterol isolated from cherry laurel seeds were higher than BHA and lower than BHT and α-tocopherol.

Alasalvar et al. (2005) reported a higher ACN in KCL, FCL variety and their molasses than our results to be 123, 174 and 9.3 mg/100 g, respectively. Although washing, sorting and pre-heating to remove contaminants for fruits and evaporating at low temperatures (vacuum) to prevent the color loss for molasses were made, discolorations might have been due to the effects of moderate light intensity and heat rather than endogenous and microbial enzymes (glycosides, peroxidases and polyphenol oxidases). Additionally, anthocyanin monomers might have polymerized into brownish oligomers, known as polymeric color. Also, researchers reported that the color losses catalyzed by high temperature, time, oxygen and metal ions were even more accelerated by ascorbic acid, glucose and fructose and their degradation products (Stintzing and Carle, 2003). In this study, increasing concentration may have affected the decrease of monomeric anthocyanin. Similarly, Kırca et al. (2006) reported that the degradation of monomeric anthocyanins in black carrots increased with increasing solid content during heating and decreased during storage. Furthermore, it is important to note that both molasses used in the study have a pH level above 4. This means that four different types of anthocyanins, namely flavylium cation, anhydrous quinoidal base, colorless carbinol base, and pale yellow chalcone, will likely coexist in KCLM and FCLM. These various forms of anthocyanins significantly impact the process of thermal degradation (Jiang et al., 2019). Additionally, it has been found that the stability of anthocyanins is affected by whether they are acylated or

unacylated. Studies have shown that colorants from red sweet potato and purple carrot, which are rich in acylated anthocyanins, exhibit higher stability than colorants from purple corn and red grape, which are rich in non-acylated anthocyanins, under different pH, temperature, and light conditions (Cevallos-Casals and Cisneros-Zevallos, 2004).

DPPH-RSA varies between 71.39% and 76.49% among fruits. FCL exhibited a higher mean DPPH-RSA (75.11±1.95%), due to its higher TP and Vit C content DPPH-RSA increased in FCLM, reaching 80.53%, but decreased to 66.50% in KCLM. AC of FCL varied between 30.89 and 32.05 μg/mg over TE, higher than KCL (29.91 μg/TE mg sample). Furthermore, AC (27.85±0.90) decreased in KCLM but increased in FCL (32.65±1.54). Additionally, Liyana-Pathirana et al. (2006) found AC to be higher in cherry laurel molasses on a fresh weight basis and hydrogen peroxide and DPPH-RSA in fruit on a dry weight basis. This situation explained by the authors that it was due to the moisture content of both samples and the possible destruction of antioxidant compounds during molasses production. They calculated the inhibition values of their fruits as 23.4, 20.7 and 14.0% at concentrations of 400, 200 and 100 mg/kg, respectively. On the other hand, many phenolics in nature are shown AC due to their reducing, singlet oxygen-scavenging and metal-chelating properties (Robards et al., 1999). Additionally, AC has reflected several phytochemicals substantial in the fruit and their synergistic effects. Also, studies have shown a direct relationship between the TP and AC of many fruits and vegetables (Jacobo-Velázquez and Cisneros-Zevallos, 2009; Matthes and Schmitz-Eiberger, 2009). Indeed, regarding the issue, Kolaylı et al. (2003) found that the cherry laurel fruits' AC of TP were higher than the reference ascorbic acid.

The BL of the fruit extracts was partially higher in FCL than in KCL as Abs/mL at 420 nm. BL value increased in KCLM and FCLM and reached the values of 14.621 and 15.201, respectively. The most significant change in color values was that the Hunter a* value, which is an index of the red color in fruits, decreased, and the positive Hunter b* value increased in a negative trend (-b*) with molasses production. That is, while the red color tone decreased in molasses, the violet-purple color tone became dominant.

Thermal changes in some physicochemical properties and phytochemical compounds of molasses during storage at different temperatures and times

Total dry matter (TDM) and Soluble dry matter (SDM)

ANOVA results showed that only the amount of TDM and SDM was affected by C at the P <0.01 level. T and ST with CxT, CxST, TxST, and CxTxST interactions did not affect TDM and SDM. When comparing TDM averages of molasses using the TMCT, the FCLM had the highest average value of 72.85%, while the KCLM had the lowest average of 72.42%. According to the TMCT results of SDM for C, SDM had significantly different values in KCLM (67.83%) and FCLM (67.68%).

pH and Titratable acidity (TA)

ANOVA showed that variations in C, T and ST sources, besides CxT, TxST, CxST and CxTxST, had a statistically significant impact on the pH value and TA of all molasses at P <0.01. Comparing the pH and TA averages based on TMCT, KCLM molasses experienced a reduction in pH (from 4.53 to 4.41) and an increase in TA (from 0.515 to 0.714%) as the T and ST increased. Additionally, similar changes in FCLM occurred in the form of a decrease from 4.36 to 4.18 for the pH and an increase from 1.12 to 1.48% for TA. Similarly, Buckow et al. (2010), when pasteurized blueberry juice was stored at 4, 25, and 40 °C for approximately 6, 2, and 0.5 weeks, respectively, the pH decreased from 3.0 to 2.85, and the latter remained steady. The authors suggested that this reduction may be due to the rise in phenolic acids and *ACN* degradation products. In addition, increasing *T* and *ST* may cause the release of galacturonic acid from the breakdown of pectin, leading to an increase in *TA* and a decrease in pH value (Anthon and Barrett, 2012).

Vit C and HMF

ANOVA analysis indicated that Vit C was affected significantly by C and ST factors. Additionally, all sources of variation and interaction effects on the HMF level were statistically significant at P <0.01. When comparing the averages of Vit C with TMCT for C and ST, Vit C in KCLM had a higher value of 55.01 mg/100 g than FCLM's 51.09 mg/100 g. Also, the Vit C averages of both molasses samples decreased with ST compared to the control samples, with a range of 37.1-43.7 mg/100 g (30-41%) (Fig. 2). Similarly, Kuşçu and Bulantekin (2016) determined that after 4 months of storage, the loss in ascorbic acid was 69.54% in vacuum evaporation for apple pekmez. Beşe and Polatoğlu (2017) reported that oxygen and light in the environment are the most significant factors in the loss of Vit C at the rate of 51.1% after sun drying the cranberry fruit.

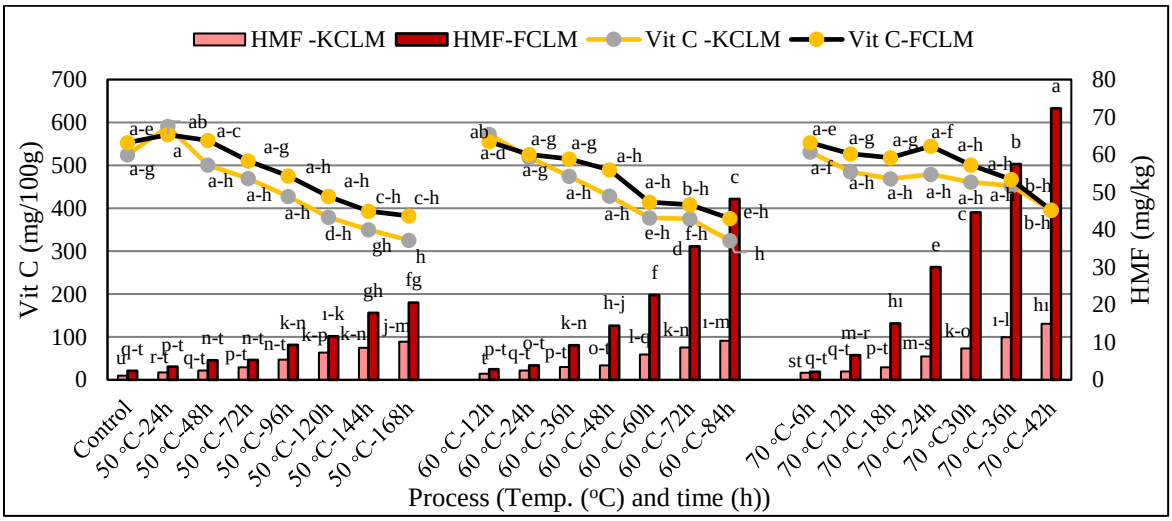

Figure 2 Thermal change of Vit C and HMF

Means shown with the same capital letter (a-h for Vit C, a-u for HMF) in the bar chart are not statistically different from each other ($P < 0.05$, Tukey's test).

HMF is a cyclic aldehyde produced by ascorbic acid and sugar deterioration. It can also form through the Maillard reaction, which occurs during food processing or long-term food storage (Torribio and Lozano, 1984; Cemeroğlu, 2013; Shapla et al., 2018). While the HMF content of FCLM was approximately 2-fold (21.07 mg/kg) at the start of storage compared to KCLM, the difference reached up to 5-fold with increasing T and ST (Fig. 3). Babsky et al. (1986) explained the increase of HMF during 111 days of storage of concentrated apple juice at 37 °C, the first period including an induction time of about two weeks, a rapid increase in HMF within 50 days, the second period, and the decrease in the rate of HMF formation in 3 periods as the third period. A study conducted by Burdurlu and Karadeniz (2003) discovered that two apple juice concentrates kept at 5 °C and 20 °C did not experience a significant increase in HMF levels. However, when stored at 37 °C, the HMF levels at the end of storage had reached 963 and 190 mg/kg in Golden Delicious and Amasya apple juice concentrates, respectively. Similarly, a study by Simsek et al. (2006) determined HMF levels in two grape juice concentrates after thermal storage. Results showed that HMF amounts were affected by grape type, concentration, temperature, and duration of storage. Most researchers reported that the increase of HMF is related to heat processing, temperature and storage, and the presence of simple sugars (like glucose and fructose), acids (with low pH), aw (water activity), protein, and minerals (such as Ca, K, Mg, Na+2, Fe+2, and Fe+3 (Torribio and Lozano, 1984; Burdurlu and Karadeniz, 2003; Şimşek et al., 2007; Cemeroğlu, 2013; Karataş and Şengül, 2018; Shapla et al., 2018). These results are

similar to the research findings and the highlighted results on the subject.

Total Phenolics (TP) and DPPH-RSA

According to the ANOVA results, the effects of T, ST and CxT on TP and C, T, ST, CxT and CxST on DPPH-RSA were found significant ($P < 0.05$). After comparing the statistically significant CxT interaction averages with the TMCT, it is found that the TPs of KCLM and FCLM decreased due to an increase in T and ST in all thermal processes. The TP averages reached during the last storage period at 50 and 60 °C were statistically similar in both molasses, but the TP of both molasses differed at the end of the storage period at 70 °C. After all, it has been found that FCLM TP's stored for 6-42 h at 70 °C are better preserved than other samples. Furthermore, it showed that the ST had a higher effect on the TP of both FCLM and KCLM than the T (Fig. 3).

Insert Fig. 3 here

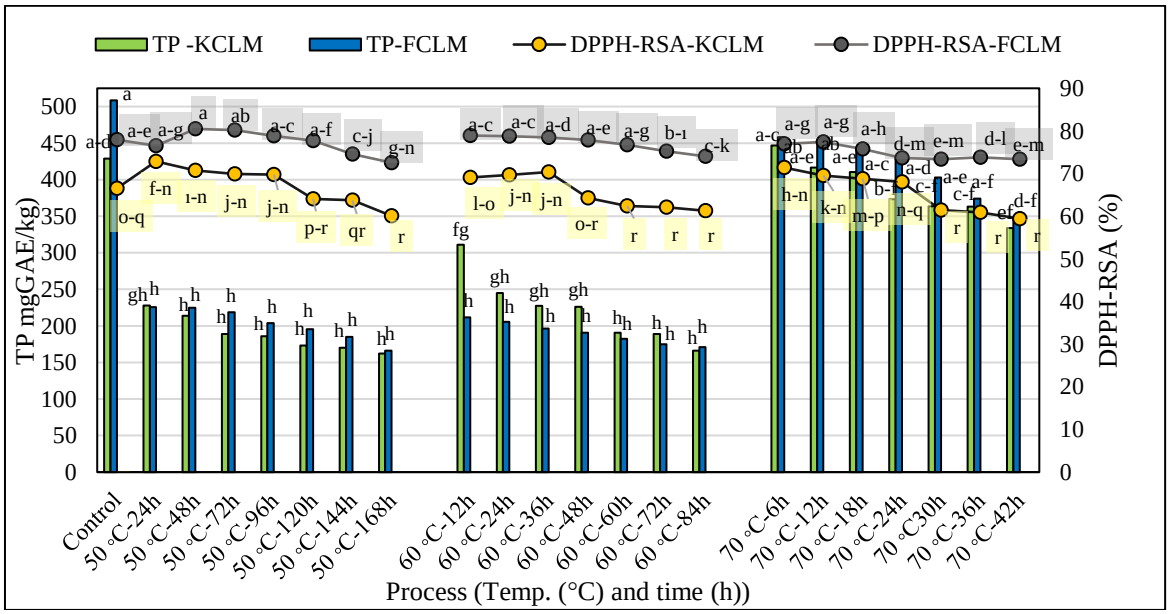

Figure 3 Thermal change of TP and DPPH-RSA with process

Means shown with the same capital letter (a-h for TP, a-r for DPPH-RSA) in the bar chart are not statistically different from each other ($P < 0.05$, Tukey's test).

Kuşçu and Bulantekin (2016) reported that the highest TP in apple molasses produced by two different methods (boiler and vacuum) was in molasses produced by vacuum. However, the amount of TP as epicatechin (114-138 to 92-112 mg/kg) and catechin (94-115 to 83-97 mg/kg) decreased during storage compared to the initial value. Moldovan et al. (2016) discovered that extracts' TP decreased slightly (between 3.1% and 22.6%) after being stored at 2 °C in the dark for 10-60 days. However, the TP of stored extracts at room temperature (22 °C) decreased significantly after 60 days (25.4%). Furthermore, TP deterioration was 8 and 16 times higher at 55 °C and 75 °C, respectively, compared to 2 °C. The researchers explained that high temperatures cause the polyphenolic content of the extracts to decrease due to an increase in the oxidation rate of bioactive components. Similar decreases in TP (16.31-9.31 μg GAE/mg sample) and AC (21.29-17.38%) were observed by Karataş and Şengül (2018) in mulberry molasses stored at 20±2 °C for 6 months. The findings of this study coincide with the literature findings reported above.

When comparing the DPPH-RSA mean values of CxT interaction to TMCT, the highest and lowest values were observed at 50 and 70 °C, respectively. Additionally, the mean value of FCLM is greater than that of KCLM. Comparing

CxST averages with TMCT, DPPH-RSA of both molasses reduced as the storage process progressed. However, the DPPH-RSA value of FCLM was higher than KCLM throughout the same storage period (Fig. 3).

Total monomeric anthociyanin (ACN) and Antioxidant capacity (AC)

As a result of ANOVA, the effect of CxTxST interaction on the amount of ACN and AC was significant at the P <0.01 level. The ACN amount of the control samples was higher (42.86 mg/kg) in KCLM than in FCLM. When the averages of CxTxST ACNs data are compared according to TMCT, they decreased with increasing time of 50, 60 and 70 °C. While these decreases were between 71.60-80.91% at the end of 50 °C-168 h in both molasses and 85% in KCLM at the end of 60 °C-84 h, it completely disintegrated in FCLM at the end of 60 °C-60 h. At the end of the highest temperature of storage, at 70 °C-42 h, the amount of ACN decreased in KCLM to 3.73 mg/kg with a loss of 91%, while ACN completely degraded in FCLM at the end of 70 °C- 24 h (Fig. 4).

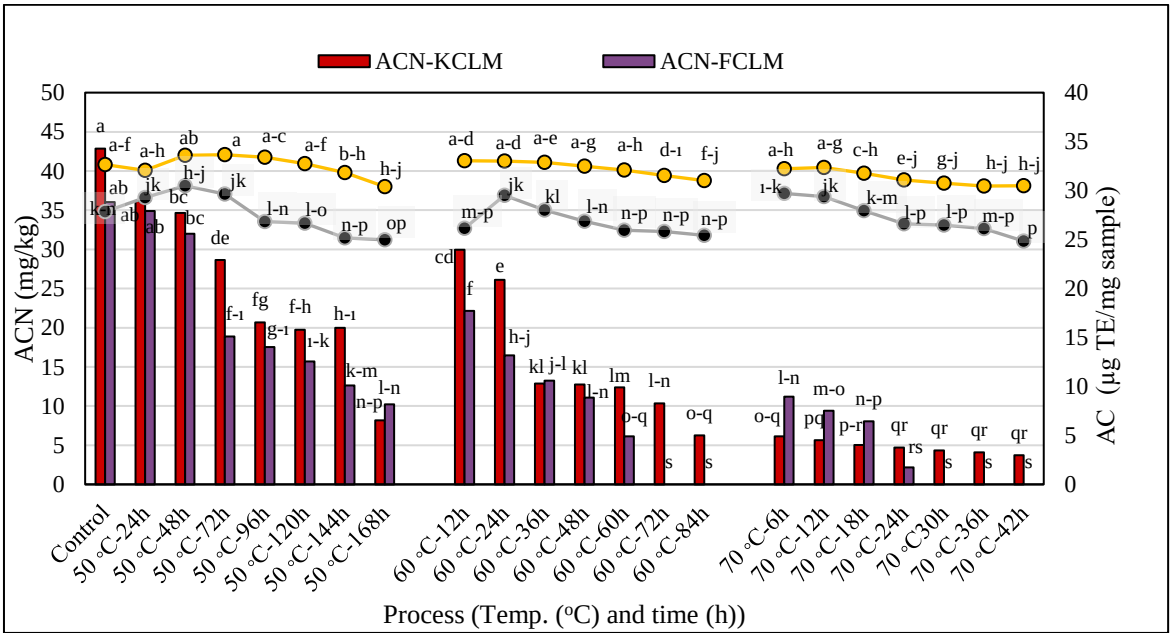

Figure 4 Thermal change of ACN and AC with process

Means shown with the same capital letter (a-s for ACN, a-p for AC) in the bar chart are not statistically different from each other ($P < 0.05$, Tukey's test).

The AC of FCLM and KCLM samples were increased slightly at baseline relative to their controls but decreased with increasing time at 50, 60 and 70 °C. Additionally, FCLM's AC decrease was higher than KCLM's. The fact that the compounds such as Vit C, TFM and ACN, which have AC in both molasses, decreased with increasing T and ST probably may have contributed to the slight decrease in AC with extension ST. However, the slight increases seen in the first process applications may be related to the HMF increase, which is known to have AC, as well as phloroglucinaldehyde and protocatechic acid, which have higher antioxidant activity revealed by ACN degradation (Sadilova et al., 2007). According to Karataş and Şengül (2018), mulberry molasses stored at 20±2 °C for 6 months showed a decrease in AC (from 21.29% to 17.38%) and TP (from 16.31 µg GAE/mg sample to 9.31 µg GAE/mg sample).

Hunter L, a*, b* values and Browning Levels (BL)*

As a result of the ANOVA, the effect of CxT interaction on Hunter L* and BL, CxTxST interaction on Hunter a* value and CxT, CxST and TxST interactions on Hunter b* value were

found significantly at the P <0.05 level. When the Hunter L* value averages belonging to C were compared with TMCT, it was determined that KCLM had a lighter color tone than FCLM. Hunter L* value of both molasses cultivars was affected the most during storage at 50 and 70 °C, Insert Fig. 5 here

and this effect was partially high in KCLM. Presumably, the differences in the composition elements of the cultivars (Vit C, ACN concentration, HMF, amount of mineral matter, etc.) caused the differences in the Hunter L* value of molasses (Fig. 5).

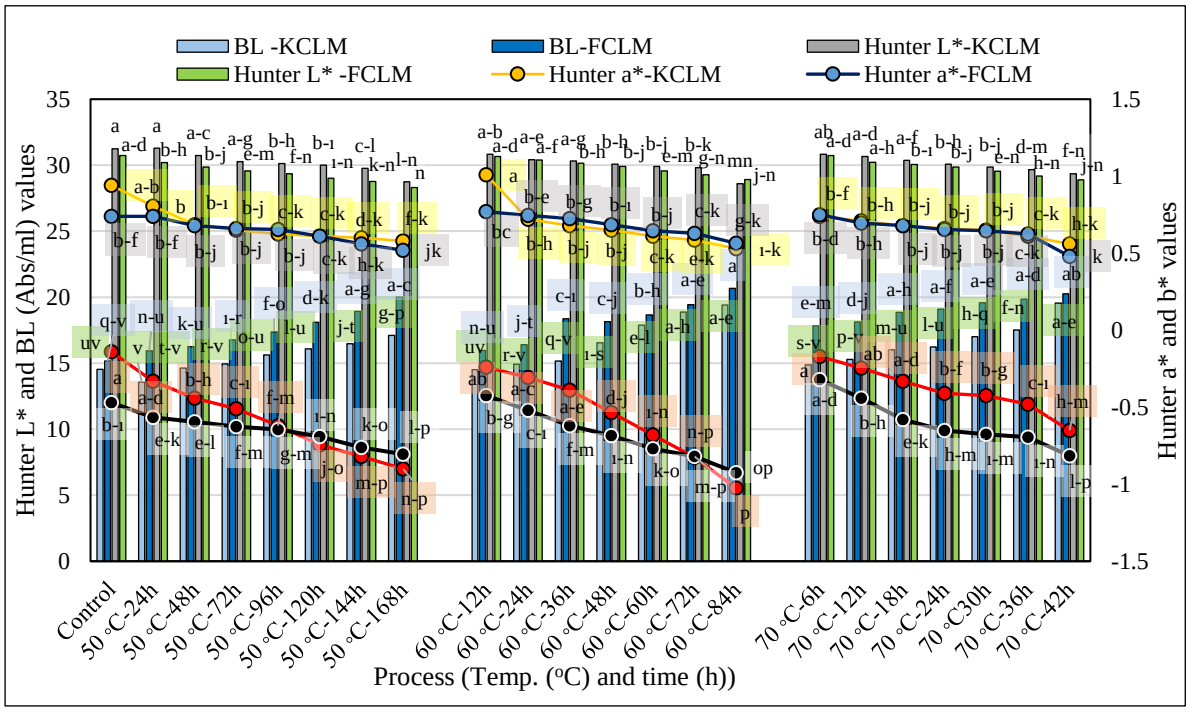

Figure 5 Thermal change of Hunter L*, a*, b* values and BI

Means shown with the same capital letter (a-n for Hunter L*, a-k for Hunter a*, a-p for Hunter b*, a-v for BI) in the bar chart are not statistically different from each other ($P < 0.05$, Tukey's test).

TMCT results showed a reduction in cyanidin derivatives that provide the red color (Hunter a* value) due to increased T and ST. The read Hunter a* value after 168 hours of storage at 50 °C varied between 0.520 and 0.580, and the effect of 72-84 hours at 60 °C and 42 hours at 70 °C on Hunter a* values were similar for both molasses (Fig. 5).

Additionally, according to the statistically significant CxT interaction, the difference between increasing T and Hunter b* value was highest in KCLM (-0.3871). Also, CxST co-interaction decreased the Hunter b* value of molasses cultivars, and the blue color tone turned into purple-violet (increase in delphinidin derivative) tones and reached the highest average values in the last storage period. Hunter b* values of both molasses were statistically indifferent (P <0.05) at the end of the ST. According to the TMCT compared results of SxDS Hunter b* means for both molasses, the highest increase was at 60 °C in the negative direction, followed by increases at 50 °C and 70 °C, respectively. However, there was no difference between these increases at the statistical significance level (P <0.05).

According to the TMCT results of the CxT interaction found to be important in the BL data, BL increased significantly with increasing T based on C. The highest BL values were determined at

70 °C (19.08) for FCLM, at 60 °C (16.76) and 70 °C (16.64) for KCLM, but there was no statistical difference between both T.

Viscosity

According to the ANOVA analysis, factors T and ST had a significant effect, along with the interactions CxT, CxST, and TxST, on the viscosity of KCLM and FCLM at a statistical level of P <0.05. However, the C and CxTxST did not have any effect on viscosity. According to the TMCT results of viscosity averages of CxT, while the measured viscosity of KCLM at a 100 rpm shear was 718 at 50 °C, it reduced with the increased T compared to the control to 653 and 522 at 60 and 70 °C, respectively. FCLM also showed similar decreases, but the reductions at 50 and 70 °C were no different from each other statistically (P <0.05) (Fig. 6).

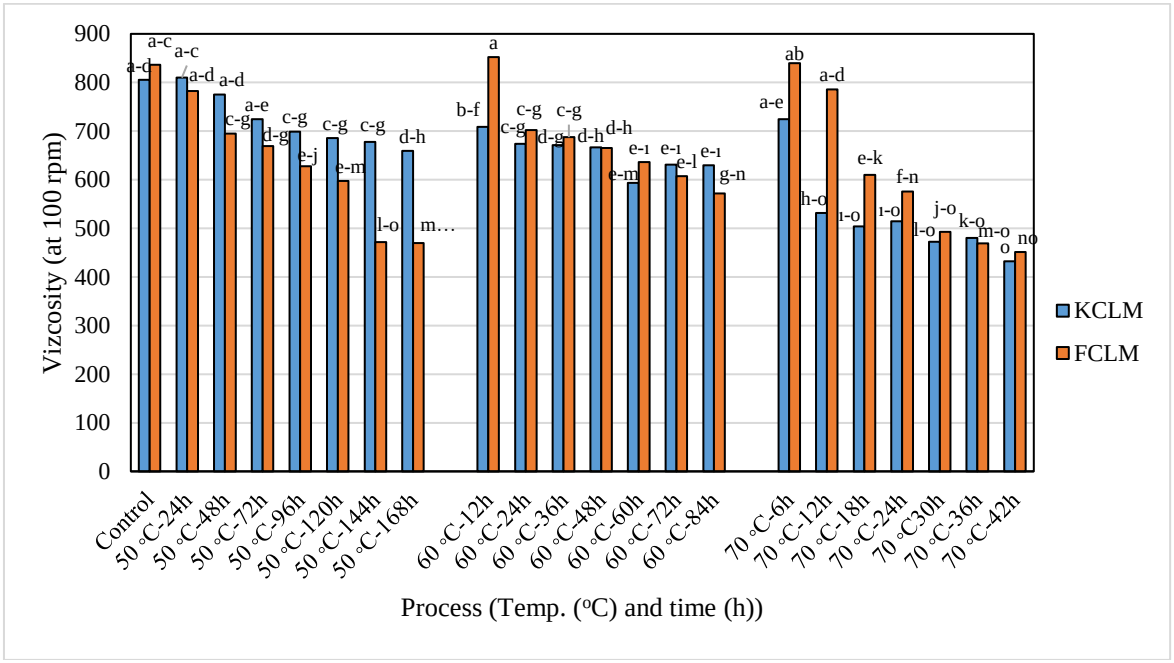

Figure 6 Thermal change of viscosity

Means shown with the same capital letter (a-o) in the bar chart are not statistically different from each other ($P < 0.05$, Tukey's test).

The viscosity averages of two different molasses were significantly affected by the interaction of CxST, as revealed by TMCT. The increase in ST led to a decrease in viscosity, and this decrease varied depending on the type of molasses. The KCLM and FCLM's viscosities decreased by 29% and 40%, respectively, compared to the control sample. Differences in viscosity between the molasses are likely due to the characteristics of each cultivar. However, while pectin remaining in molasses initially provided a specific consistency to the samples with the presence of sugar, increased T may have caused a decrease in viscosity by disrupting the structure of pectin (decomposition into galacturonic acid) and sucrose content. Studies indicate that low pH values and high temperatures can cause the breakdown of glycosidic bonds and the deterioration of pectin structure (Sundar Raj et al., 2012). The interaction between SxDS had an impact effect on the change in molasses viscosity. When comparing the averages based on TMCT, the highest viscosity value was observed after 42 h of storage at 70 °C compared to the control sample, followed by 168 h at 50 °C and 84 h at 60 °C, respectively. However, there was no statistical difference found between the last two applications.

Mathematical equations reflecting the thermal change of some phytochemicals

One of the critical factors to consider in food processing is nutrient loss. Therefore, kinetic studies are needed to minimize undesirable variation and optimize the quality of certain foods. Kinetic models are frequently used to ensure safe food production is objective, fast and economical. Kinetic models are also used to predict and examine the impact of the application and process on critical quality parameters (Patras et al., 2009). We took the average values of Y (Vit C, TP, and ACN) from two separate experiments and used them to create a quadratic polynomial model. As a result of the multi-regression analysis, it determined that the relationship between Vit C, TP and ACN phytochemicals and the T and ST could be disclosed by a three-dimensional polynomial or paraboloid regression equation that had a high R^2 value. The R^2 value (74-94.7 %) of calculated regression equality for the average values of molasses phytochemicals using regression analysis was less than the equations formed according to the KCLM (76-96.4 %) and FCLM (84-97.2 %). This model predicts the amount of phytochemicals in mg/100 g, mg GAE/100 g, and mg/kg, respectively.

$$\text{Vit C} = 148.6 - 2.31\,T + 0.121\,ST + 0.016\,T^2 - 0.00049\,ST^2 - 0.0806\,TST$$

(R^2 = 74.55%)

$$TP = 21798 - 749\,T + 49.2\,ST + 7.192\,T^2 - 0.0020\,ST^2 - 1.056\,TST$$

(R^2 = 94.73%)

$$ACN = 192 - 3.86\,T - 0.310\,ST + 0.0185\,T^2 + 0.00076\,ST^2 - 0.00047\,TST$$

(R^2 = 90.40%)

However, TP and ACN equations with high regression coefficients (R^2), which vary within the same limits as the regression equations created according to data belonging to cultivars, can be used to determine optimum conditions in kinetic calculations belonging to thermal stability. In the models of phytochemicals such as Vit C, TP and ACN of molasses, besides the linear (primary) effect of T and ST, the quadratic (secondary or T^2, ST^2) and interaction (TxST) effects were also found to be significant ($P < 0.001$).

As observed from equalities, the thermal change of phytochemical compounds such as Vit C, TP and ACN in different applications (including the T and ST) didn't show compliance with the first-degree reaction kinetics. Consistently, Kanner et al. (1982) reported that the fragmentation course did not comply with the first-order reaction kinetic can be used in temperatures of 25 ^{o}C and below because ascorbic acid decomposed at 36 ^{o}C, but in the decomposition of ascorbic acid during the storage of orange juice concentrate. Similarly, Moldovan and David (2014) explained the degradation of ascorbic acid in the same conditions with the Arrhenius equation. In a previous study, the reaction kinetics reflecting the thermal decomposition of cranberry fruit polyphenols during storage has shown compliance with the Arrhenius equation (Moldovan et al., 2016). Furthermore, Buckow et al. (2010) demonstrated the relationship between ACN degradation and pressure, T and application time (ST) factors in blueberry juice using a nonlinear regression equation.

CONCLUSIONS

Cherry laurel (*Laurocerasus officinalis* L.) is a fruit whose consumption is limited in quantities due to its astringent taste, processed into pickles, jams, dried fruit, juices, molasses and brine products with traditional methods in the Blacksea region of Turkey. Considering the nutrients it contains, such as anthocyanins, phenolic substances and *Vit C*, and its relations with health, this fruit should be delivered to most consumers as converted into molasses with appropriate technology in modern factories and evaluated in different foods as an additive. As a result, a significant reduction of nutrient losses is possible by determining thermal changes in some physicochemical properties and phytochemical compounds during molasses production and storage under different *T* and *ST* conditions. The results of this study demonstrated that phytochemicals such as Vit C, TP and ACN were quality parameters in KCLM and FCLM, and the equations reflected the change with T and ST of Vit C, TP, and ACN may be used to calculate the phytochemicals thermal stability.

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AUTHORS' CONTRIBUTION
Vesile Başar: Investigation, formal analysis, original draft. Atilla Şimşek: Project administration, supervision, conceptualization, methodology, formal analysis, data curation, validation, writing - review and editing. Emre Turan: Supervision, formal analysis, resources, validation, writing, original draft.

DECLARATION OF CONFLICTING INTERESTS
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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DATA AVAILABILITY STATEMENT
The data supporting this study's findings are available from the corresponding author upon reasonable request.

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ULTRASOUND-ASSISTED ENZYMATIC EXTRACTION OF ANTIOXIDATIVE PROTEIN EXTRACTS FROM *SARGASSUM VULGARE*: OPTIMIZATION OF EXTRACTION PARAMETERS USING RSM

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ABSTRACT

In this study, extraction conditions of proteins from *Sargassum vulgare* were optimized. The Box-Behnken design (BBD)-based Response Surface Methodology (RSM) was used to investigate and optimize the protein content (PC), total phenolic content (TPC), and antioxidant activity (AOA), which were affected by extraction parameters (ultrasonic probe time: 0.09-2.91 min and enzyme/substrate ratio (E/S): 0.18-1.02). The optimal extraction was achieved while applying an ultrasonic probe for 2.5 min and using an E/S of 0.90. Under this optimum conditions PC and TPC were found to be as 248.30 mg protein/g dry weight (dw) and 38.03 mg gallic acid equiavalent (GAE)/g dw, respectively. Moreover, AOA was determined to be 53.77 mg Trolox equivalent (TE)/g dw by CUPRAC and 19.88 mg TE/g dw by ABTS methods. These findings provide a good basis for future research into the potential of macroalgae protein extracts, which have a high protein content and antioxidant potential for food industry.

Keywords: *Sargassum vulgare*, macroalgae, extraction, protein extract, antioxidant activity

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ULTRASON DESTEKLİ ENZİMATİK YÖNTEMLE *SARGASSUM VULGARE*'DEN ANTİOKSİDAN PROTEİN EKSTRAKSIYONU: RSM İLE EKSTRAKSİYON PARAMETRELERİNİN OPTİMİZASYONU

ÖZ

Bu çalışmada, *Sargassum vulgare*'den proteinlerin ekstraksiyon koşulları optimize edilmiştir. Ekstraksiyon parametrelerinin (ultrases prop süresi: 0.09-2.91 dk ve enzim/substurat oranı (E/S): 0.18-1.02), protein miktarı (PM), toplam fenolik madde miktarı (TFMM) ve antioksidan aktivite (AOA) üzerine etkisini araştırmak ve optimizasyon çalışmalarını gerçekleştirmek için Box-Behnken Tasarım-Yanıt Yüzey Metodolojisi kullanılmıştır. Optimum protein ekstraksiyon koşulları, 2.5 dk ultrases prop uygulama süresi ve 0.90 E/S oranıdır. Optimum ekstraksiyon koşullarında, PM ve TFMM sırasıyla 248.30 mg protein/g kuru madde (km) ve 38.03 mg gallik asit eşdeğeri (GAE)/g km olarak bulunmuştur. Ayrıca AOA, CUPRAC yöntemi ile 53.77 mg Trolox eşdeğeri (TE)/g km ve ABTS yöntemi ile 19.88 mg TE/g km olarak belirlenmiştir. Bu bulgular, yüksek protein miktarı ve antioksidan aktivitesiye sahip makroalg protein ekstraktlarının gıda endüstrisi için potansiyelini araştıracak yeni çalışmalara bir temel oluşturabilir.

Anahtar kelimeler: *Sargassum vulgare*, makroalg, ekstraksiyon, protein ekstraktı, antioksidan aktivite

INTRODUCTION

Macroalgae are considered a viable source of protein with an essential amino acid composition, and their use for protein synthesis has several advantages over the traditional use of protein-rich plants in terms of productivity and nutritional content (Taboada, 2010; Sirbu, 2019; Bleakley and Hayes, 2017). Alternative sources and techniques for protein production are needed to meet consumer demand and the projected global protein demand (Bleakley and Hayes, 2017). Numerous health benefits of brown algae are attributed to their protein hydrolysates and bioactive peptides for the control, treatment, and risk reduction of degenerative and chronic diseases (Alvarez-Vinas, 2021).

The brown seaweed *Sargassum vulgare* is a member of the *Phaeophyceae* family, which includes many species found in both tropical and temperate waters worldwide. Shallow waters and coral reefs are the most important habitats for these algae (Karkhane et al., 2020; Mahmoud et al., 2019). It is known that the total protein content of *S. vulgare* varies greatly depending on the growth environment and ranges from a relatively low to a high content (10-15% dry weight) (Field et al., 2017). On the other hand, *S. vulgare* contains vital components such as polyphenols, carotenoids, vitamins, unsaturated fatty acids, and free amino acids (Karkhane et al., 2020). The stimulating effect of the macroalgae extract could be related to all these different substances contained in the extract (Mahmoud et al., 2019; Khan et al., 2009). *S. vulgare* is widely distributed along the Mediterranean coasts, but this species originating from Türkiye has not been studied yet.

The extraction of algal proteins has received less research attention than that of proteins from other plants. The traditional methods for extracting algal proteins are aqueous, acidic, and alkaline (Bleakley and Hayes, 2017). One of the novel extraction techniques is ultrasound-assisted extraction (UAE), which produces a final product with higher purity while reducing the need for downstream processing due to its fast-processing time, non-thermal properties, and minimal solvent consumption (Bleakley and Hayes, 2017). The sonicated liquid and its components are chemically excited by the violent implosion of the bubbles formed by UAE, resulting in the formation of microscopic zones of high pressure and temperature. This facilitates degradation of the target compound and disruption of the particles (Mason et al., 1996).

In this study, the UAE of proteins from *S. vulgare* collected from the Mediterranean coast of Türkiye was described. The extraction conditions were optimized using response surface methodology (RSM). The objectives of the study are (i) to establish a protocol for the extraction of *S. vulgare* protein extract (SVPE) with high protein

content; (ii) to optimize the conditions for the enzymatic UAE of proteins from *S. vulgare* in terms of protein content (PC), total phenolic content (TPC), and antioxidant activity (AOA); and (iii) to compare protocols for the extraction process with different extraction time, sonication time, and amounts of added enzyme (hemicellulose). Within our knowledge, this is the first study in the literature detailing the extraction procedures of protein extracts from *S. vulgare* from the Türkiye seas using RSM.

MATERIAL AND METHOD

Collection and preparation of algae

Sargassum vulgare was collected on the Aegean coast of Türkiye (coordinates: 40°1'35.90 "N and 26°19'49.49 "E). The collected algae samples were prepared for analysis according to the procedures of Bozdemir et al. (2022). The dried and pulverized algae, which had a 8% moisture content and a particle size of less than 500 μm, were carefully packed to protect them from light and air and stored at -20 °C for further analysis.

Chemicals

The phenol reagent Folin-Ciocalteu was purchased from Merck (Merck, Darmstadt, Germany). The hemicellulase (HSP 50000) supplied from Bakezyme. All the other chemicals and solvents were obtained from Sigma-Aldrich (Sigma-Aldrich Chemie, St. Louis, Missouri, USA). All of the solvents and chemicals used in this study were of the analytical grade.

Ultrasound-assisted enzymatic protein extraction

To extract proteins from *S. vulgare*, a combination of ultrasound pretreatment and carbohydrase addition was used as described by Bozdemir et al. (2022) based on the design of the experiment (Table 1). In brief, 0.5 g algae sample was mixed with 50 mLcitrate buffer (0.1 N, pH 4.5). The suspension sonicated with an ultrasonic probe (53 kHz and 65% amplitude) (Sonopuls HD 2200, Bandelin Electronic GmbH & Co. KG, Berlin, Germany) at ~25 ºC for the given time periods under RSM settings. Hemicellulase was then added to the mixture and kept in shaking water at 1 g-force and 35 °C for 24 h. Finally, the samples were kept in the shaking water bath at 85°C for 10 min for enzyme inactivation. At the end of the procedures, the samples were centrifuged at 4100 rpm, 4 °C, 15 min and the supernatant (*S. vulgare* protein extract) was collected, then stored in a dark place at -20 °C until further analysis.

Table 1: Actual and coded levels of independent variables for central composite design.

Independent variables for extraction of SVPE*	Codded levels				
	-α	-1	0	1	+α
X_1; Ultrasound probe time (min)	0.09	0.50	1.50	2.50	2.91
X_2; Enzyme/substrat ratio	0.18	0.30	0.60	0.90	1.02

*: SVPE: *Sargassum vulgare* protein extracts.

Determination of protein content

The protein content of *S. vulgare* extract was determined using a modified Lowry method (trichloroacetic acid (TCA)-Lowry). In this method, the proteins are separated from the samples using TCA in order to eliminate possible interfering substances (Moein et al., 2015). Using the method of Lowry et al. (1951), the protein content of the extracts was determined by using UV spectrophotometer (Scilogex, USA). A standard curve had a linear equation (R^2= 0.99) was generated using bovine serum albumin (BSA) at concentrations ranging from 0.05 to 0.1 mg/mL. The protein content was expressed as milligrams per gram of sample dry weight (dw) using bovine serum albumin as a standard.

Total phenolic content (TPC)

The TPC of the SVPE was determined according to the Folin-Ciocalteu's method (Toor and Savage, 2006) as described by Bozdemir et al (2022). The samples' absorbance was measured at 765 nm by using the UV spectrophotometer. The TPC was computed using a linear equation (R^2= 0.99) derived from a calibrated curve at five different point including 0.01 to 0.1 mg/mL, with gallic acid functioning as the standard. The results

are expressed in mg of gallic acid equivalents (GAE) per gram of dw.

Antioxidant activity (AOA)

The cupric reducing antioxidant capacity (CUPRAC) method

The CUPRAC assay was carried out in accordance with the method of Apak et al. (2005), as described in our previous study (Bozdemir et al., 2022). Using the UV spectrophotometer, the absorbance of the samples was determined at 450 nm. Trolox prepared at 5 different points ranging from 0.2 to 0.01 was utilized as a standard on the calibration curve (R^2= 0.99). The Trolox equivalent (TE) in milligrams per gram of dry weight was used to express the results.

2,2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS) method

The ABTS assay was performed according to Miller and Rice-Evans (1997), as described in our previous study (Bozdemir et al., 2022). The absorbance was measured at 734 nm and results were given in mg TE/g dw. Trolox prepared at 5 different points ranging from 0.2 to 0.01 was utilized as a standard on the calibration curve (R^2= 0.97).

Experimental design and statistical analysis

The extraction conditions are optimized by the application of RSM. The effects of two independent variables (ultrasonic probe time and enzyme/substrate ratio) for optimization at 5 levels (-α, -1, 0, 1. +α) were investigated using Central Composite Design (CCD). In the present study, ultrasonic probe time (0.5-2.5 min) and enzyme/substrate ratio (0.30-0.90), coded as X_1 and X_2, were chosen as independent variables. The experimental design consists of 13 (run) conditions comprising five center points, four factorial points, and four axis points.

As shown in Table 1, the parameters for extraction were standardized as coded variables. Response functions (Y) were PC (mg protein/g dw sample), TPC (mg GAE/g dw sample) and AOA (mg TE/g dw sample). Surface response with a second-order polynomial was used to determine the relationship between the independent factors and the response (Tekin et al., 2015). The mathematical model used for the bivariate central composite design is given in Equation 1.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{12} X_1 X_2 \quad (1)$$

The regression coefficients for the second-order polynomial model are as follows: β_0 represents the constant term, β_i represents a linear effect, β_{ii} represents a quadratic effect and βi_j represents an interaction effect. The fit of the model was assessed using statistical significance analysis of variance and regression coefficients. Surface responses and contour plots of the polynomial regression equations to visualize the relationship between the responses and the independent variables and optimum conditions for the target responses were obtained using the trial version of Design Expert 7.1 software (Stat-Ease, Inc., USA). The results were statistically tested at the statistical significance level p=0.05. The fit of the model was determined based on the model analysis, the coefficient of determination (R^2), and the model error. Mathematical models were created to describe the interaction effects of a single parameter and/or multiple parameters on each response studied.

Validation of model for the optimum conditions

Protein extraction was carried out using the optimum extraction conditions given by RSM, and the predicted and actual values were compared.

RESULT AND DISCUSSION

Fitting model

13 combinations of two independent variables (ultrasonic probe time and enzyme/substrate ratio) were used to determine the protein, phenolic and antioxidant content of SVPEs and the results were given in Table 2. Table 3 shows the analysis of variance and model coefficients (R^2) for each dependent variable. The *P*-value was used to calculate the significance of each coefficient. The most significant factor is the enzyme/substrate ratio (P<0.05). Previous studies showed a comparable result (Liadakis et

al., 1995; Morais et al., 2015; Yucetepe et al., 2022). In addition, the interaction effect of E/S ratio and ultrasonic probe time was significant ($P< 0.05$).

Table 2. Box–Behnken experimental design with natural and coded extraction conditions and experimentally obtained values of all investigated responses.

	Independent variables					Responses			
	Run	A: Ultrasound application time (min)		B: Enzyme/ substrate		PC (mg protein/g extract, dw)	TPC (mg GAE/g extract, dw)	CUPRAC (mg TE/g extract, dw)	ABTS (mg TE/g extract, dw)
	1	1.50	0	1.02	+α	248.53	41.57	55.01	16.98
	2	1.50	0	0.18	-α	34.92	20.79	37.60	19.90
	3	1.50	0	0.60	0	147.00	31.97	53.66	20.62
	4	2.91	+α	0.60	0	143.10	32.86	52.63	19.70
PC:	5	1.50	0	0.60	0	146.70	32.42	47.35	17.55
	6	1.50	0	0.60	0	152.50	33.06	49.90	18.94
	7	0.50	-1	0.90	1	194.11	37.62	53.20	12.60
	8	1.50	0	0.60	0	161.97	34.17	43.70	19.70
	9	2.50	1	0.30	-1	85.95	25.45	36.61	18.66
	10	2.50	1	0.90	1	258.55	39.37	53.30	17.85
	11	1.50	0	0.60	0	176.30	31.98	44.35	14.72
	12	0.50	-1	0.30	-1	100.00	23.46	32.50	20.70
	13	0.09	-α	0.60	0	157.76	31.40	40.80	20.90

Protein content, TPC: Total phenolic content, CUPRAC: Cupric reducing antioxidant capacity; ABTS: 2,2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt.

Table 3: Analysis of variance (ANOVA) of the fitted second-order polynomial model

Source	Sum of squares	DF	Mean square	F-value	p-value
Protein content					
Model	42216.30	5	8443.26	33.17	<0.0001*
Linear	110.02	1	110.02	0.43	0.5319
$ß_1$	40440.67	1	40440.67	158.88	<0.0001*
$ß_2$					
Quadratic	0.19	1	0.19	7.384E-004	0.9791
$ß_{11}$	122.03	1	122.03	0.48	0.5110
$ß_{22}$					
Interaction $ß_{12}$	1540.00	1	1540.00	6.05	0.0435*
Residual	1781.70	7	254.53	-	-
Lack of fit	1157.67	3	385.89	2.47	0.2011
Pure error	624.03	4	156.01	-	-
Cor total	43998.00	12	-	-	-
R^2=0.96; C.V.(%)=10.33					

Table 3: Analysis of variance (ANOVA) of the fitted second-order polynomial model

Source	Sum of squares	DF	Mean square	F-value	p-value
Total phenolic content					
Model	421.90	5	84.38	145.74	<0.0001*
Linear					
$ß_1$	4.20	1	4.20	7.25	0.0310*
$ß_2$	412.70	1	412.70	712.82	<0.0001*
Quadratic					
$ß_{11}$	0.80	1	0.80	1.38	0.2784
$ß_{22}$	4.60	1	4.60	7.95	0.0258*
Residual	4.05	7	0.58	-	-
Lack of fit	0.63	3	0.21	0.24	0.8622
Pure error	3.43	4	0.86	-	-
Cor total	425.95	12	-	-	-
R^2=0.9905; C.V.(%)= 2.38					

Table 3 (cont.): Analysis of variance (ANOVA) of the fitted second-order polynomial model.

Source	Sum of squares	DF	Mean square	F-value	p-value
Antioxidant activity (CUPRAC)					
Model	556.11	5	111.22	6.36	0.0154*
Linear					
$ß_1$	54.83	1	54.83	3.13	0.1199
$ß_2$	476.72	1	476.72	27.25	0.0012*
Quadratic					
$ß_{11}$	10.10	1	10.10	0.58	0.4721
$ß_{22}$	13.07	1	13.07	0.75	0.4160
Interaction					
$ß_{12}$	4.04	1	4.04	0.23	0.6454
Residual	122.44	7	17.49	-	-
Lack of fit	54.74	3	18.25	1.08	0.4532
Pure error	67.70	4	16.93	-	-
Cor total	678.55	12			
R^2=0.82; C.V.(%)=9.05					
Antioxidant activity (ABTS)					
Model	36.17	5	7.23	1.47	0.3096
Linear					
$ß_1$	0.29	1	0.29	0.059	0.8144
$ß_2$	17.59	1	17.59	3.58	0.1005
Quadratic					
$ß_{11}$	2.22	1	2.22	0.45	0.5228
$ß_{22}$	2.22	1	2.22	0.45	0.5235
Interaction					
$ß_{12}$	13.18	1	13.18	2.68	0.1457
Residual	34.43	7	4.92	-	-
Lack of fit	13.33	3	4.44	0.84	0.5378
Pure error	21.10	4	5.28	-	-
Cor total	70.60	12			
R^2=0.51; C.V.(%)=12.12					

*significant at $P \leq 0.05$, β_1: Ultrasound probe application time (sec), β_2: Enzyme/substrate ratio.

The R^2 values were 0.96, 0.99, 0.82, and 0.51 for PC, TPC, CUPRAC, and ABTS, respectively (Table 3). Apart from ABTS (<0.80), TPC, CUPRAC, and ABTS had high R^2 values for the models. The model's fit is indicated by the high R^2 values (Moorthy et al., 2015). A low coefficient of variation (CV) in the model indicates that the evaluated systems are highly reproducible. Similarly, PC (CV=10.33%), TPC (CV=2.38%), CUPRAC (CV=9.05%) and ABTS (CV=12.12%) showed low variation in their mean scores (Table 3). The lack of fit has no significance for PC and all AOA methods measured (P>0.05, Table 3). The results indicate that the PC, TPC, and AOA models (by CUPRAC method) can be utilized to optimize the parameters for protein extraction from *S. vulgare* (P<0.05). Statistically significant linear effects of E/S on PC, TPC, and CUPRAC were observed (P<0.05, Table 3).

Protein content

RSM was used to determine the best method for extracting proteins from *S. vulgare* and to investigate optimum extraction conditions. Two different factors that were investigated in relation to protein content are the ultrasonic probe time and the enzyme/substrate ratio. The effect of these factors on protein content is shown in Figure 1. According to the results, the protein content of *S. vulgare* was determined between 34.92-258.55 mg protein/g dw (3.50% and 25.86%) depending on the extraction parameters. Similar to the current study, the PC of some *Sargassum* spp. in the study by Bonilla Loaiza (2022) and Perumal et al. (2019) ranged from 4.13% to 15.42%. On the other hand, the PC of some *Gracilaria* species ranged from 5.6% to 30.2% (Chan and Matanjun, 2017; Rodrigues et al, 2015; Gressler et al, 2010). De Melo et al. (2021) found that the highest protein content was between 22.93 and 21.27% in dw for *C. corneus*, followed by *U. fasciata* between 17.97 and 11.42% in dw and *S. vulgare* between 14.02 and 10.32% in dw. Vasquez et al. (2018) reported that the protein content obtained by enzyme-assisted extraction was 7.39% g protein in dried sample for *M. pyrifera* and 6.35% g protein in dried sample for *C. chamissoi*. Our results were in agreement with those of these authors, although we obtained protein contents in a wider range depending on the extraction parameters.

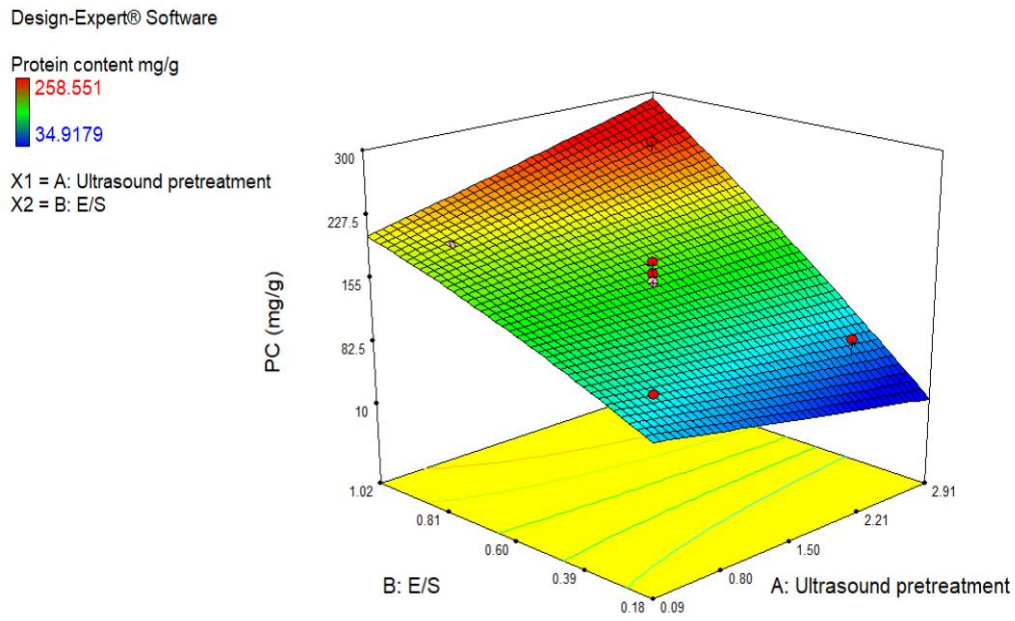

Figure 1. 3D contour plot response surface for the effect of cross-interaction between ratio of enzyme/substrate ratio and ultrasonic probe pretreatment on protein content.

The response equation (Table 4) demonstrates that two independent factors, ultrasonic probe time (β_1) and enzyme/substrat ratio (β_2) had a positive effect on protein content. The quadratic effect of enzyme/substrat ratio had a negative effect on protein content, whereas the interaction between ultrasonic probe time and enzyme/substrat ratio, as well as the quadratic effect of ultrasonic probe time, had a positive effect on protein content. Increasing the enzyme/substrate ratio and the application time of the ultrasonic probe led to an increase in protein content during extraction (Fig. 1). As shown in Table 2, the maximum PC content was 258.55 mg/g under the defined extraction conditions (ultrasonic probe application time of 2.5 seconds, E/S of 0.90). Table 3 shows that the linear effect of E/S on PC showed statistically significance (P<0.05). This could be due to the fact that the enzyme utilized breaks down the cell wall of the algae and releases more protein into the solvent. Similarly, Joubert and Fleurence (2008) investigated the effects of specific enzymes, including xylanase and cellulase, and the concentration of the enzymes on the PC of *P. palmata* and found that PC increased proportionally to the amount of enzyme. Similar results were reported by Harnedy and FitzGerald (2013), who used polysaccharidase to disrupt the cell wall increased the efficiency of protein extraction from macroalgae. In addition, Suwal et al. (2019) showed that protein content increased by 17% when the enzyme cellulase was utilized in the extraction process. They also reported that the extraction yield for *P. palmata* increased from 9 to 37% when a cell wall-dissolving enzyme was used (Suwal et al., 2019).

Table 4: Estimated coefficients of the fitted second-order polynomial model for all response variables.

Regression coefficient	PC (mg protein/g extract, dw)	TPC (mg GAE/g extract, dw)	CUPRAC (mg TE/g extract, dw)	ABTS (mg TE/g extract, dw)
β_0	156.89	32.72	47.79	18.30
Linear				
β_1	3.71	0.72	2.62	0.19
β_2	71.10	7.18	7.72	-1.48
Quadratic				
β_{11}	0.16	-0.34	-1.21	0.57
β_{22}	-4.19	-0.81	-1.37	-0.56
Interaction				
β_{12}	19.62	-0.062	-1.01	1.82

β_1: Ultrasound pretreatment, β_2: Enzyme/substrate

Protein content was generally sensitive to especially proteolytic enzyme treatment. In an ideal reaction, enzyme and substrate react continuously until the central equilibrium was reached. According to Ramakrishnan et al. (2013), there was a clear correlation between increasing enzyme concentration and protein yield, with the highest enzyme concentration resulting in 76.30% of protein extracted. The amount of enzyme used and the duration of extraction had a positive relationship that improved protein extraction (Bozdemir et al., 2022). When the E/S value was above 1, the protein content was basically increased (Fig. 1). Once it approached 2.5, the protein content increased by a considerable percentage more than without enzyme treatment (Bozdemir et al., 2022). Thus, the ratio of E/S 1.60 in our study seems to be the ideal value for extraction. This result is in line with previous findings by Joubert and Fleurence (2008) and Suwal et al. (2019). A similar result was obtained by Bozdemir et al. (2022), who showed that the optimal E/S ratio for protein extraction from *G. dura* was higher than 1.50. These results indicate

that protein content was significantly affected by the two variables. The appropriate range of the variables was found using the previously discussed one-factor test, which provided significant support for the RSM.

TPC and AOA

According to our results, the TPC of the protein extracts ranged from 20.79 to 41.57 mg GAE/g dw, depending on the extraction conditions listed in Table 1. Similarly, Nursid et al. (2020) determined the TPC value of 23.37 mg GAE/g for *G. salicornia*, 24.97 mg GAE/g for *Laurencia sp.*, and 24.38 mg GAE/g for *G. latifolium*. On the other hand, Khaled et al. (2012) determined the TPC of *S. vulgare* as 12.71±0.03 mg GAE/g and as 10.55 mg GAE/g for *P. pavonica*. In addition, Arguelles et al. (2019) reported that the TPC of *S. vulgare* was 10.55 mg GAE/g. Prasedya et al. (2021) investigated the TPC of ethanolic extracts of some *Sargassum* spp. and reported TPC of 66.13 mg GAE/g for *S. cristaefolium*, 39.83 mg GAE/g for *S. aquifolium*, 38.93 mg GAE/g for *S. polycystum* and 52.90 mg GAE/g for *S. crassifolium*.

The lowest TPC was determined for an ultrasonic probe time of 1.50 and an E/S ratio of 0.18 (Table 2). The TPC increased under experimental conditions with an ultrasonic probe time of about 1.50 and an E/S of about 1.02 (Figure 2). The breakdown of phenolic compounds in response to extended exposure to ambient conditions may be responsible for the statistically significant (P<0.05) decreased in TPC with time (Thoo et al., 2010).

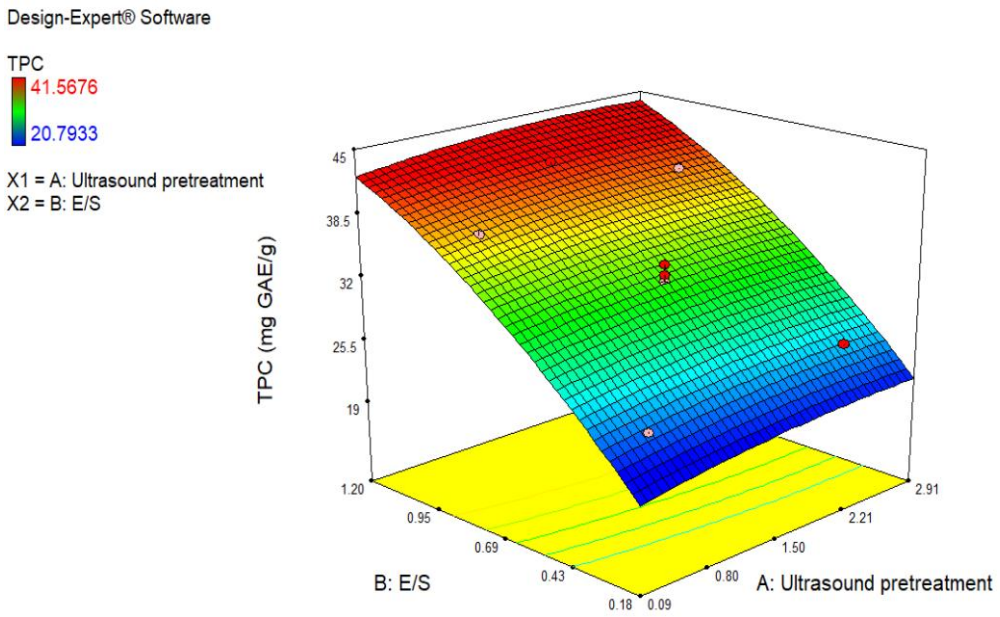

Figure 2. 3D contour plot response surface for the effect of cross-interaction between ratio of enzyme/substrate ratio and ultrasonic probe pretreatment on total phenolic content.

The AOA of the protein extracts ranged from 32.50 to 55.01 mg TE/g dw (by CUPRAC method) and from 12.60 to 20.90 mg TE/g dw (by ABTS method), as shown in Table 2. Yuan et al. (2018) reported the highest AOA with the ABTS assay for *L. nigrecens* at 0.95±0.01 mg TEAC/g dry sample. Kumar et al. (2020) reported the AOA of some algae species using the FRAP method as 8.21 mg TE/g for *S. wightii*, 6.90 mg TE/g for *U. rigida*, and 1.06 mg TE/g for *G. edulis*. According to Nursid et al. (2020), the season, the location, the time of harvest and the type of algae can have an influence on the

fluctuations in polyphenol concentration and antioxidant activity.

According to the results, the linear effect of the E/S ratio on TPC and AOA (by CUPRAC method) of the extracts was significant, and the quadratic effect of the E/S ratio on TPC was significant (P<0.05, see Table 3). The AOA of the extracts increased with increasing enzyme amounts, similar to TPC, as the phenols have high antioxidant activity. These results were compatible with the studies of Barclay and Vinqvist (2003) and Ferruzzi and Green (2006). In the TPC assay, the effect of E/S was significant (P<0.05), while ultrasonic probe time had no significant effect (P<0.05, Table 3). The response equation shows that two independent factors, ultrasonic application time (β_1) and enzyme/substrat ratio (β_2) had a substantial effect onTPC, but all other interaction and quadratic terms are negative (Table 4). Since phenolics are covalently bound to proteins, there was a strong effect of the E/S ratio on TPC, similar to PC (Acosta-Estrada et al., 2014). There was statistical significance in the TPC overall model (P<0.05, Table 3). As with CUPRAC assay, the effect of E/S was significant (P<0.05), while ultrasonic probe time had no significant effect (P<0.05, Table 3, Figure 3). In addition, the model for CUPRAC was statistically significant (P<0.05, Table 3). ABTS was not significantly different from the linear effects of the individual variables examined (P>0.05, Table 3, Figure 4). According to response equation of CUPRAC, only ultrasonic application time (β_1) and enzyme/substrat ratio (β_2) showed a positive effect. On the other hand, linear and quadratic effect of ultrasonic application time (β_1) had a positive effect on the ABTS (Table 4).

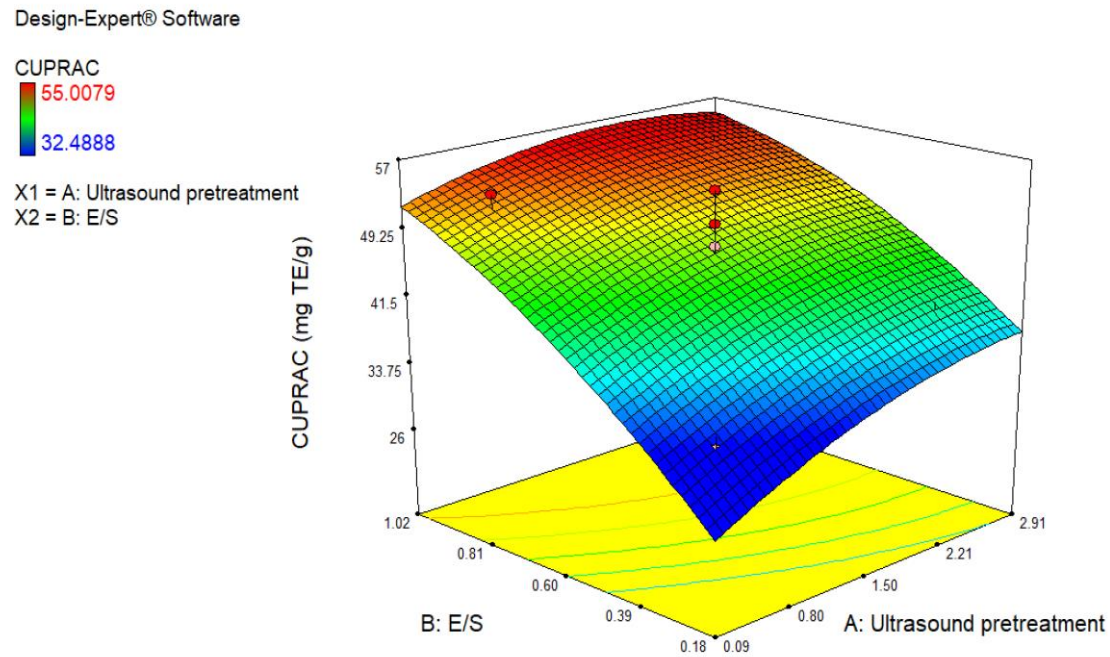

Figure 3. 3D contour plot response surface for the effect of cross-interaction between ratio of enzyme/substrate ratio and ultrasonic probe pretreatment on antioxidant activity by CUPRAC.

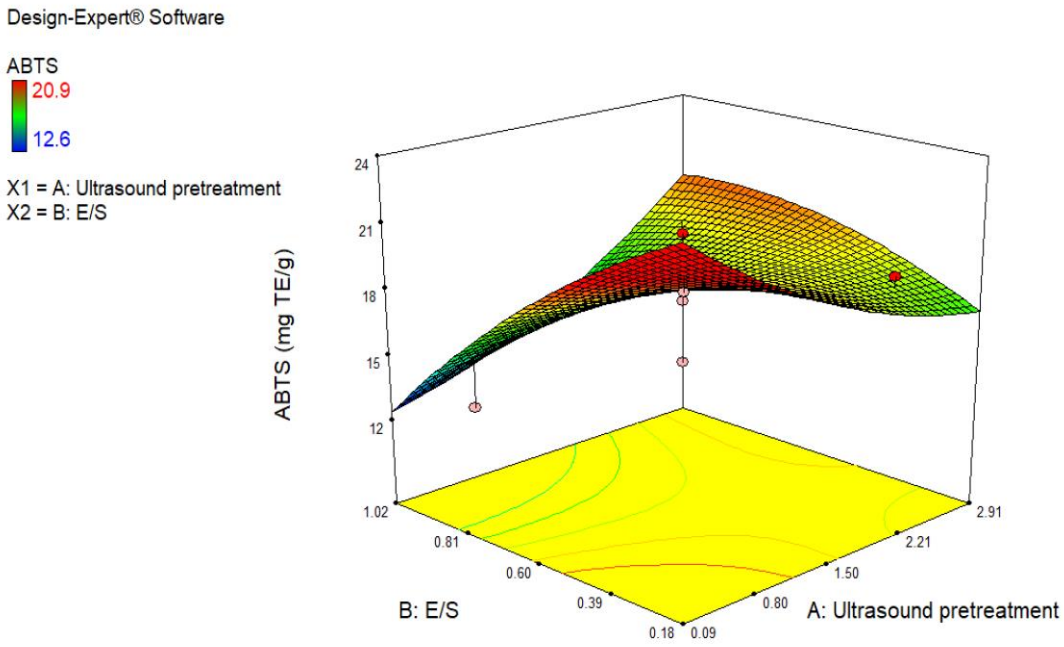

Figure 4. 3D contour plot response surface for the effect of cross-interaction between ratio of enzyme/substrate ratio and ultrasonic probe pretreatment on antioxidant activity by ABTS.

Optimization and verification

Macroalgae are high-protein alternative protein sources but their complex cell wall limits protein extraction. Therefore, the aim of optimization process is to obtain macroalgal proteins with higher yield. In order to determine the ideal level of the independent variables and obtain the highest values for PC, TPC, and AOA, optimization processes were performed. Under the optimum conditions (ultrasonic probe time of 2.5 min and E/S of 0.90), the predicted PC value was 247.29 mg/g dw, while the predicted TPC and AOA values (by CUPRAC and ABTS methods) were 39.41 mg GAE/g dw, 54.55 mg TE/g dw, and 18.83 mg TE/g dw, respectively, corresponding to a "desirability" of 0.89. The AOA by CUPRAC (53.77 mg TE/g dw), PC (248.30 mg protein/g dw), TPC (38.03 mg GAE/g dw) and ABTS (19.88 mg TE/g dw) showed no statistically significant difference from the mean and predicted values of the experiment at the 5% significance level. The limitations of optimization include the need to test different enzymes due to the structure's complexity.

CONCLUSION

According to the results of the study, RSM was successfully applied to determine the optimum extraction conditions for the brown macroalgae *S. vulgare*. The optimum conditions for extraction were as follows: an ultrasonic probe time of 2.5 minutes and an E/S ratio of 0.90. It was found that the factor that had the greatest effect on PC and AOA was the E/S ratio, and the effect of ultrasonic probe time was also significant. The R^2 values above 0.80 obtained for PC, TPC, and CUPRAC indicated that the extraction model applied was appropriate. Compared to other algal sources in the literature, the protein extract obtained under optimal conditions showed higher antioxidant activity as well as phenolic content. Consequently, further studies may provide the utilization of the protein-rich *S. vulgare* as a potential protein source in the protein development of new products and as a viable food ingredient.

CONFLICT OF INTEREST

Authors declare that they have no known conflict of interest.

CONTRIBUTIONS

H. Dinç is responsible for carrying out all experiments. E. Şensu is responsible for some experiments, interpretation of the results, writing original draft preparation. Ü. Altuntaş is responsible for data analysis, interpretation of the results, writing original draft preparation. E. Ş. Okudan is responsible for collection of macroalgae samples. B. Özçelik is responsible for providing research infrastructure and interpretation of the results. A. Yücetepe is responsible for planning of the study, some experiments, data analysis, interpretation of the results, reviewing, and coordination.

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TAHIL VE PSEUDO-TAHILLARIN B VİTAMİNLERİ: BİYOERİŞİLEBİLİRLİK VE BİYOYARARLILIKLARI

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ÖZ
B vitaminleri, enerji, bağışıklık, sinir sistemi, hücre bölünmesi ve homosistein metabolizmalarında rol oynayan, suda çözünür, organik besinlerdir. Tam tahıllar, B_1, B_2, B_3, B_5, B_6, B_9 vitaminleri bakımından zengindirler. B vitaminleri, tahılların ruşeym, tohum kabuğu ve alöron tabakalarında bulunur. B vitaminlerinin önerilen günlük alım miktarı 0.1-20 mg/gün arasındadır. Tahıl işleme yöntemlerinin vitamin içeriğini etkilediği bildirilmiştir. B vitaminleri yetersiz beslenmenin önlenmesinde başvurulan besin ögeleridir. Biyoerişilebilirlik, sindirim sisteminde, gıdadan enzim hidrolizi ile salınan bir besin maddesinin ince bağırsakta emilim için hazır bulunmasıdır. Biyoyararlılık, sindirilen gıdalardaki besin ögelerinin ince bağırsak epitel hücrelerinden absorbe edildikten sonra kan dolaşımına geçmesidir. Kinoa, amarant ve karabuğday glutensiz pseudo-tahıllardır. Pseudo-tahılların B vitamini miktarı bakımından iyi bir kaynak olduğu belirtilmiştir. Tahıl ürünlerinin B vitaminleri bakımından zenginleştirilmesi için fortifikasyon işlemi uygulanmaktadır. Biyofortifikasyon, tahılların B vitamini içeriklerinin artırılması için son yıllarda uygulanan yeni bir yöntemdir. Bu derlemenin amacı, tahıl ve pseudo-tahıllarda bulunan B vitaminlerinin biyoerişilebilirliği ve biyoyararlılığını son bilimsel çalışmalara göre incelemektir.
Anahtar kelimeler: Tahıl, pseudo-tahıl, B vitaminleri, biyoerişilebilirlik, biyoyararlılık

B VITAMINS OF CEREAL AND PSEUDOCEREALS: THEIR BIOACCESSIBILITY AND BIOAVAILABILITIES

ABSTRACT
B vitamins, which are water soluble, organic nutrients, play roles in energy, immunity, neural system, cell division and homocystein metabolisms. Wholegrain cereals are rich of B_1, B_2, B_3, B_5, B_6, B_9 vitamins. B vitamins are available in the germ, seed coat and aleurone layers of cereals. Recommended dietary allowance of B vitamins are in the range of 0.1-20 mg/day. It is reported that cereal processing methods influence the vitamin contents of cereals. B vitamins are referred nutrient compounds in the prevention of malnutrition. Bioaccessibility is defined as releasing of nutrient from the food matrix with enzyme hydrolysis in the gastrointestinal system and available in small intestine to be absorbed. Bioavailability is defined as transportation of nutrients liberated from foods through the

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blood stream after absorption from the epithelial cells of small intestine. Quinoa, amaranth and buckwheat are gluten-free pseudocereals. It was indicated that pseudocereals are good sources in terms of B vitamin contents. Cereal products are fortified for enrichment of B vitamins. Biofortification is a new method practiced recently in order to increase B vitamin contents of cereals. The aim of this review is to investigate bioaccessibility and bioavailability of B vitamins available in cereal and pseudocereals according to recent scientific works.
Keywords*:* Cereal, pseudocereal, B vitamins, bioaccessibility, bioavailability

GİRİŞ

Vitaminler, büyüme ve gelişme, metabolizma, üreme ve genel sağlık memnuniyeti için gerekli mikro besin ögeleridir. Vitaminler çözünürlüklerine göre ikiye ayrılırlar: yağda çözünen vitaminler (A, D, E, K vitaminleri) ve suda çözünen vitaminler (B ve C vitaminleri). B vitamini grubunda, tiamin (B_1), riboflavin (B_2), niasin (B_3), pantotenik asit (B_5), pridoksin/pridoksal/pridoksamin (B_6), biyotin (B_7), folat/folik asit (B_9) ve kobalamin/ siyanokobalamin (B_{12}) olmak üzere 8 farklı vitamin bulunduğu bildirilmiştir. Beslenme ile vitaminlerin alımı çok önemlidir, çünkü D ve B_1 vitaminleri dışında diğerlerini insanların sentezleyemediği, bitkilerin ise B_{12} vitamini dışında diğer B vitaminlerini sentezleyebildiği bildirilmiştir. Ayrıca, aynı B vitamini grubunda yapısal olarak birbirine benzeyen ve aynı vitamin aktivitesini gösterenlere ise vitamer denilmiştir, örneğin tiamin difosfat, tiamin vitamininin vitamer bileşiğidir. Bazı B vitaminleri, enzimin biyolojik olarak aktif formu olan apoenzimine bağlanarak holoenzim oluşturmak suretiyle koenzim görevi gördüğü açıklanmıştır. Kimyasal olarak birbirine benzemeyen B vitaminlerinin katabolik ve anabolik reaksiyonlarda koenzim olarak işlevlerinin olduğu bildirilmiştir. Örneğin, B_1, B_2, B_3 ve B_5 vitaminlerinin özellikle aktif formlarının hücresel enerji üretiminde temel koenzimler olduğu, sitrik asit döngüsünde ve elektron taşıma zincirinde doğrudan rollerinin bulunduğu belirtilmiştir. B_6 vitamininin beyin gelişimi ve fonksiyonlarında, B_9 vitamininin kırmızı kan hücreleri ve yeni hücrelerin yapımında ve B_3 vitamininin ise sindirim sisteminde, deri ve sinir sistemi sağlığında etkili olduğu açıklanmıştır (Kerns vd., 2015; Lindschinger vd., 2019; Fitzpatrick ve Chapman, 2020; Yaman vd., 2021; Huda vd., 2021).

Tahıllar, tüm dünyada yaygın ekim alanına sahip, ucuz ve beslenmemize önemli katkıları bulunan gıdalardır. İnsan metabolizmasında gerekli olan enerjinin yarısı tahıllar tarafından karşılandığı bildirilmiştir. Dünyada ve ülkemizde en çok tüketilen tahıllar buğday, mısır, pirinç, arpa, çavdar ve yulaftır. Bu tahılların, insan sağlığı için önemli vitamin ve mineralleri içerdikleri belirtilmiştir. Tam tahılların, A, B_1, B_2, B_3, B_5, B_6, B_9, E ve K vitaminleri bakımından zengin fakat B_{12}, C ve D vitaminlerini içermedikleri gösterilmiştir. B_1, B_2, B_3, B_5, B_6 ve B_9 vitamini eksikliklerinin sırasıyla beriberi, kalp yetmezliği, pellegra, fiziksel koordinasyonsuzluk, sinirsel rahatsızlık ve megaloblastik anemi hastalıklarına sebep olduğu ifade edilmiştir. B_1, B_2, B_3, B_6, B_9 ve B_{12} vitamin eksikliği ile depresyon, anksiyete ve stres arasında bağlantı tespit edilmiştir. B vitaminlerinin önerilen günlük alım miktarı (*Recommended Dietary Allowance, RDA veya Recommended Daily Intake, RDI*) 0.1-20.0 mg/gün arasında değiştiği bildirilmiştir (Vega-Gálvez vd., 2010; Onyambu vd., 2021; Garg vd., 2021).

Tahılların B vitamini içeriğinin çeşit, ekim yeri, hasat zamanı, depolama yöntemi ve işleme yöntemine (öğütme, parlatma, patlatma) bağlı olarak değiştiği bildirilmiştir. B vitaminleri tahılların başlıca ruşeym, tohum kabuğu ve alöron tabakalarında bulunduğu belirtilmiştir (Onyambu vd., 2021). Tahıllar, tam tane veya öğütülerek tüketilirler. Valsli değirmende öğütme ile rafine un üretiminde vitamin içeriğinin önemli ölçüde azaldığı (%50-90) bildirilmiştir (Garg vd., 2021).

B vitaminlerinin ayrıca, yetersiz beslenmede (gizli açlıkta) başvurulan önemli besin ögeleri olduğu ifade edilmiştir. "Gizli açlık", kalori alımı bakımından yeterli fakat zihinsel ve fiziksel gelişme için gerekli vitamin ve/veya minerallerin eksikliğinden kaynaklanan yetersiz beslenmedir (Acar vd., 2023). Yetersiz beslenmenin, dünyada

iki milyardan fazla insanı etkilediği, özellikle gelişmekte olan ülkelerdeki kadın ve çocukları daha fazla etkileyerek alarm verdiği bildirilmiştir (Onyambu vd., 2021).

Tahıl unlarının genellikle B_1, B_2, B_3 ve B_9 vitaminleri ile bilerek zenginleştirilmesi veya güçlendirilmesine fortifikasyon denilmektedir. Amerika Birleşik Devletleri'nin Gıda ve İlaç İdaresi (USA Food and Drug Administration, FDA), buğday ununun besin kalitesinin artırılması için B_1, B_2, B_3 ve B_9 vitaminleri ile zenginleştirilmesini sırasıyla 6.4 mg/kg, 3.9 mg/kg, 52.9 mg/kg ve 1.5 mg/kg olarak tavsiye etmiştir. Tahıl ürünlerinde maya kullanımının, B_9 vitamini miktarını artırdığı da bildirilmiştir (Garg vd., 2021). Biyofortifikasyon ise, vitamin ve mineral bakımından zengin tahıl ürünlerinin yetiştirilmesi için tarlada uygulanan, sürdürülebilir, yeni bir yaklaşımdır. Biyofortifikasyonun, tahılların mikro besin ögesi eksikliğini gidererek gizli açlığın önlenmesinde önemli bir yöntem olduğu bildirilmiştir. Biyofortifikasyonun, zirai uygulamalar, konvansiyonel bitki ıslahı, genetik mühendisliği ve modern biyoteknolojik uygulamalar ile yapılabileceği ifade edilmiştir. Biyofortifiye edilmiş tahıl bir kez geliştirildiğinde uzun yıllar ekilebileceği ve böylece gıdaların mikro besin ögesi miktarı bakımından sürekliliğinin sağlanabileceği belirtilmiştir (Garg vd., 2018; Onyambu vd., 2021; Acar vd., 2023). Bu derlemenin amacı, tahıl ve pseudo-tahıllarda bulunan B vitaminlerinin biyoerişilebilirliği ve biyoyararlılığını son bilimsel çalışmalara göre incelemektir.

B VİTAMİNLERİ

Tiamin (B_1 Vitamini)

B_1 vitamininin, biyolojik dokularda, tiamin monofosfat (TMP), tiamin difosfat (TDP) veya tiamin pirofosfat (TPP), tiamin trifosfat (TTP) vitamerleri halinde ve serbest formda bulunduğu bildirilmiştir. Canlı dokularda, tiaminin %90'dan fazlasının fosforlanmış formda yani TPP olarak bulunduğu, bağırsak lümeninde alkalin fosfataz gibi farklı fosfatazlar tarafından serbest tiamine hidrolize edildiği ifade edilmiştir (Ball, 2006; Hrubša vd., 2022). Tiamin emiliminin, ince bağırsağın özellikle jejunum bölgesinde gerçekleştiği belirtilmiştir. B_1 vitamininin emiliminin, ortamdaki konsantrasyonuna bağlı olarak aktif veya pasif formda olduğu, 5 mg/gün'den az olduğunda, Na^+ iyonuna bağlı olarak, aktif taşıma yoluyla kolayca emilebildiği açıklanmıştır. Başlıca aktif form olan TDP'nin vücutta ince bağırsak mukozasında, karaciğerde ve böbrekte fosforilasyona uğradığı belirtilmiştir (Demir vd., 2023). Tiaminazlar ve polifenolik bileşiklerin, anti-tiamin faktörleri olduğu ve sindirim sisteminde biyoyararlılığı azalttığı söylenmiştir. Tiamin eksikliğinde, karbonhidrat metabolizmasının bozulduğu, kas ve sinir sisteminin etkilendiği, Wernick-Korsakoff sendromunun ve beriberi hastalığının görüldüğü belirtilmiştir (Yaman vd., 2021). Amerika Birleşik Devletleri (ABD)'nde tiaminin önerilen günlük alım miktarı, kadınlarda 1.1 mg/gün ve erkeklerde 1.2 mg/gün olarak belirlenmiştir (Kennedy, 2016; Yaman, 2019).

Riboflavin (B_2 Vitamini)

Gıdalarda serbest, organizmada koenzim flavin mononükleotit (FMN) ve flavin adenindinükleotit (FAD) olmak üzere 2 farklı formda bulunduğu bildirilmiştir. Sindirim sisteminde, FMN ve FAD'ın fosfatazlar tarafından hidrolize edilerek, serbest riboflavinin ince bağırsağın mukozal (epitel) hücrelerinden absorbe edildiği gösterilmiştir. Safra tuzlarının, riboflavinin absorpsiyonunu iki şekilde artırdığı söylenmiştir: Birincisi, safra tuzlarının ince bağırsakta riboflavinin çözünürlüğünü artırarak fırça kenarlı hücrelerden riboflavinin geçişini sağladığı; ikincisinin ise safra tuzlarının gastrik boşalmayı geciktirerek ince bağırsakta FMN ve FAD'ın riboflavine defosforilasyonu için gereken zamanı sağlayarak artırdığı bildirilmiştir. Önerilen günlük B_2 vitamini alım miktarının kadınlarda 1.1 mg/gün ve erkeklerde 1.3 mg/gün olduğu, depolanmadığı için fazla alındığında toksisite etkisinin gözlemlenmediği, idrarla atıldığı ifade edilmiştir (Suwannasom vd., 2020; Yaman vd., 2021).

Niasin (B_3 Vitamini)

Gıdalardaki nikotinamid ve nikotinik asitin toplamı niasin olarak ifade edilir ve nikotinamid bir koenzim olarak nikotinamid adenindinükleotid (NAD) ve nikotinamid

adenindinükleotid fosfat (NADP) vitamerleri halinde canlı hücrelerde bulunduğu bildirilmiştir (Yaman vd., 2021). NAD ve NADP'nin, hücrelerdeki redoks reaksiyonlarında önemli koenzimler olarak görev yaptıkları belirtilmiştir. NAD'nin, elektronlarını elektron taşıma zincirinde oksijene aktardığı, NADP'nin ise amino asitlerin, yağ asitlerinin ve pentoz şekerlerin biyosentezinde indirgeyici molekül olarak görev yaptığı açıklanmıştır (Kurek vd., 2017). Vitaminin nikotinik asit formunun tahıllarda polisakkaritlere ve glikopeptitlere bağlı olduğu ve emilmeden önce serbest hale getirilmesi gerektiği bildirilmiş ve moleküldeki bağların bir kısmının mide özsuyu tarafından hidrolize edilerek, aktif forma dönüştürüldüğü ifade edilmiştir. Nikotinik asit ve nikotinamidin biyoerişilebilirliği ve biyoyararlılığının aynı olmadığı; nikotinamidin, nikotinik aside göre daha fazla biyoerişilebilirliğe sahip olduğu belirtilmiştir (Ball, 2006). Önerilen günlük B_3 vitamini alım miktarının kadınlarda 14.0 mg/gün ve erkeklerde 16.0 mg/gün olduğu bildirilmiştir (Kennedy, 2016; Mikkelsen ve Apostolopoulos, 2019; Yaman vd., 2021).

Pantotenik Asit (B_5 Vitamini)

Koenzim A (CoA) ve açil taşıyıcı proteinin (ACP) yapısında bağlı formda bulunduğu, ayrıca biyolojik dokularda serbest formda da bulunabileceği gösterilmiştir. Kolesterol sentezi, kortizol hormonu salgılanması ve asetil-CoA'nın sentezinde ve bundan ise beyinde önemli fonksiyonları olan asetil kolin bileşiğinin sentezinde etkin rol oynadığı bildirilmiştir. Gıdalarda bulunduğu gibi bağırsak mikrobiyatasında da sentezlendiği belirtilmiştir. Pantotenik asidin biyoyararlılığının, emilmeden önce, bağırsak lümeninde yapısından CoA'nın ayrılmasını sağlayan enzimlerin aktivitesine bağlı olduğu ve ayrıca bağırsaktaki alkali ortamın pantotenik asidin biyoerişilebilirliğinin azalmasına sebep olabileceği belirtilmiştir. Son yıllarda B_5 vitamininden immün sistem uyarıcısı olarak faydalanılması amacıyla yapılan çalışmaların yaygınlaştığı kaydedilmiştir. Önerilen günlük B_5 vitamini alım miktarı 5.0 mg/gün olarak açıklanmıştır (Ball, 2006; Xu vd., 2020; Yaman vd., 2021; Bourgin vd., 2022; Roy vd., 2023).

Piridoksin/Piridoksal/Pridoksamin (B_6 Vitamini)

B_6 vitamininin; piridoksin (PN), piridoksal (PL), piridoksamin (PM) ve bunların ilgili 5'-fosfatları (sırasıyla PNP, PLP ve PMP) ve pridoksin glikozit (PNG) olmak üzere yedi adet vitamerden meydana geldiği bildirilmiştir. Hayvan dokularındaki ana formlarının PLP ve PMP, bitkilerde ise genellikle PN ve PNP formlarında olup bazen PNG formunda da bulunduğu belirtilmiştir (Ball, 2006; Etcheverry vd., 2012). PNG formunun bir kısmının bağırsak lümeninde hidrolize edildiği ve bozulmadan emilebildiği, kalan büyük bir kısmının ise hidroliz olmadan idrarla atıldığı gösterilmiştir. PNG formunun biyoyararlılığının, PLP formuna göre daha düşük olduğu açıklanmıştır. B_6 vitamininin biyoyararlılığını azaltan faktörler arasında; gıda işleme yöntemleri, amino asitlerin varlığında meydana gelen reaksiyon ürünleri, besinsel lif tipi ve içeriği ve B_6 glikozitinin varlığının olduğu bildirilmiştir. Doğal olarak meyve, sebze ve tahıl tanelerinde bulunan bir ana form olan PNG'nin, B_6 vitamini kaynağı olarak kullanıldığı ve biyoyararlılığının enzim hidroliz derecesine bağlı olduğu gösterilmiştir. Pridoksin hidroklorit (PN.HCl) ise bebek mamalarında takviye (0.35 mg/100 kcal) amaçlı kullanılan ticari formdur. Önerilen günlük B_6 vitamini alım miktarı 1.3 mg/gün olarak açıklanmıştır (Yaman ve Mızrak, 2019; Mikkelsen ve Apostolopoulos, 2019; Stach vd., 2021; Roy vd., 2023).

Biyotin (B_7 Vitamini)

Biyotin, önceleri vitamin H veya koenzim-R olarak biliniyordu. Bitkiler, çoğu bakteri ve bazı mantarlar biyotini sentezleyebilirken, memelilerin sentezleyemedikleri görülmüştür, bunun için çeşitli besinler (süt, yumurta, kanatlı etleri, karaciğer, bitkisel gıdalar) ile alınmak zorunda oldukları belirtilmiştir (Carling ve Turner, 2019). Beslenmeyle alınan biyotinin ince bağırsakta emildiği, mikrobiyota tarafından üretilen biyotinin ise kalın bağırsakta emildiği gösterilmiştir. Biyotinin, bazı karboksilazların işlevlerinde koenzim görevi gördüğü, ayrıca gen ekspresyonunu da düzenlediği bildirilmiştir. Biyotinin, yetişkinler için önerilen günlük alım miktarının (*RDA/RDI*) Avrupa Birliği (AB)

ülkelerinde 50 µg/gün, ABD'nde yeterli alım (*Adequate Intake, AI*) miktarının 30 µg/gün olduğu rapor edilmiştir (Carling ve Turner, 2019; Ramamoorthy vd. 2021; Roy vd., 2023).

Folat/Folik Asit (B_9 Vitamini)

Gıdalardaki folatın, pteroyilglutamik asit formunda bulunduğu ve gıdalardaki başlıca pteroyilglutamatların tetrahidrofolat (THF), 5-metil-THF ve 10-formil-THF olduğu bildirilmiştir. Gıdalarda doğal olarak bulunan folatın protein ve polisakkaritlere bağlı olarak bulunduğu belirtilmiştir. Folik asitin ise, vitaminin sentetik formu olduğu ve takviye edicilerde ve fortifiye gıdalarda monoglutamat formunda kullanıldığı ifade edilmiştir. Folat biyoyararlılığının; poliglutamat folatın ince bağırsaktaki dekonjugasyonuna, vitaminin sindirim öncesi ve sindirim sırasındaki stabilitesine, stabiliteye etki eden bileşiklere ve gıda matrisine bağlı olduğu söylenmiştir (Etcheverry vd., 2012). Emiliminin, ince bağırsak mukozasındaki fırça kenarlı hücrelerde bulunan glutamat karboksipeptidaz II enziminin, poliglutamat formunu monoglutamat formuna dekonjugasyonuna veya hidroliz yeteneğine bağlı olduğu bildirilmiştir (Etcheverry vd., 2012). Organik asitlerin (malik, sitrik ve fitik asit) bu enzimin çalışmasını inhibe ettiğini, dolayısıyla poliglutamil folatların biyoyararlanımını azalttığını bildirmişlerdir. Askorbik asitin ise vitaminin biyoerişilebilirliğini artırdığı söylenmiştir. Besinsel liflerin ise folik asitin biyoyararlanımını etkilemediği ifade edilmiştir (Etcheverry vd., 2012). Biyolojik membranlara folat taşınımının; folat reseptörü-α (FRα), protona bağlı folat taşıyıcısı ve indirgenmiş folat taşıyıcısı ile olmak üzere üç yolla başarıldığı belirtilmiştir (Alam vd., 2020). Yetişkin erkek ve kadınlarda önerilen günlük folat alım miktarı 400 µg/gün olarak belirtilmiştir (Kennedy, 2016; Lyon vd., 2020). Avrupa Gıda Güvenliği Otoritesi (European Food Safety Authority, EFSA)'ne göre önerilen günlük folat alım miktarı yetişkinlerde 330 µg/gün, hamilelerde 600 µg/gün olarak tanımlanmıştır (Buffière vd., 2021).

Kobalamin (B_{12} Vitamini)

B_{12} vitamini, yapısındaki kobalttan dolayı kobalamin olarak adlandırılmıştır. B_{12} vitamininin dokulardaki aktif formlarının 5-deoksiadenozil kobalamin ve metilkobalamin olduğu belirtilmiştir (Ede ve Ayaz, 2016). Diğer B vitaminleri ile kıyaslandığında, önerilen günlük alım miktarı en düşük (2.4 µg/gün) hayvansal kaynaklı elzem bir vitamin olup, ayrıca takviye edilmiş gıdalarda da bulunduğu açıklanmıştır. B_{12} vitamini; tek karbon metabolizmasında (metil vericisi) örneğin homosisteinden tekrar metiyoninin meydana getirilmesinde, metiyonin sentaz enziminin koenzimi olarak, DNA sentezinde (DNA nükleotidlerinin metilasyonu), enerji üretim metabolizmasında, eritrosit yapımında ve nörolojik işlevlerde, büyüme ve gelişmenin düzenlenmesinde önemli görevlerinin olduğu belirtilmiştir (Ede ve Ayaz, 2016; Afonso vd., 2023). Besinlerle alınan B_{12} vitamini mideye ulaştığında mide asidi ve pepsin ile serbest kaldıktan sonra, tükürük bezlerinden ve mide mukozasından salgılanan R proteini ile birleşen kobalamin, paryetal hücrelerden intrinsik faktörün salınımını uyardığı bildirilmiştir. Daha sonra, ince bağırsağın duodenum bölgesinde pankreatik enzimler ile R proteininden ayrılan kobalamin, intrinsik faktör ile birleştiği ve sonrasında ince bağırsağın ileum bölgesinde intrinsik faktörden ayrılarak transkobalamin-II'ye transfer edilerek, özgül reseptörlerin yardımıyla aktif taşınım ile emildiği ve kan dolaşımına katıldığı bildirilmiştir (Etcheverry vd., 2012; Ede ve Ayaz, 2016; Sezgin, 2019; Afonso vd., 2023).

TAHILLARIN B VİTAMİNLERİ

Buğdayın, B_1, B_2, B_3, B_6 ve B_9 vitaminlerini önemli düzeyde içerdiği, özellikle buğdayın "alöron tabakasının" B_1 ve B_3 vitaminlerine sahip olması sebebiyle besleyici özellikte olduğu bildirilmiştir. Fakat valsli değirmende, kepek ve ruşeymin ayrılması sonucunda rafine buğday ununda B grubu vitaminlerin miktarının azaldığı açıklanmıştır (Zaupa vd., 2014; Tekin vd., 2018). Tekin vd. (2018), 36 einkorn ve 49 emmer kavuzlu buğday hatlarının B vitamini değerleri üzerine yaptıkları bir çalışmada; emmer buğdayı hatlarının ortalama B_1, B_2, B_5, ve B_6 vitamini değerlerini sırasıyla ortalama 4.22 mg/kg, 0.36 mg/kg, 3.60 mg/kg ve 2.06 mg/kg olarak bulurken, einkorn buğday hatlarında sırasıyla ortalama 0.80 mg/kg,

0.29 mg/kg, 0.32 mg/kg ve 0.29 mg/kg olarak bulmuşlardır.

Karakas vd. (2021) buğday çimi ve einkorn (Iza), emmer (Gernik), durum (Kunduru-1149) ve ekmeklik buğday (Kıraç-66) örneklerinin B vitaminleri (B_1, B_2, B_5, and B_9) miktarlarını LC-ESI-MS/MS yöntemiyle incelemişlerdir. Buna göre, kavuzlu ata buğdayları ile kavuzsuz modern buğdaylar arasında B vitamini miktarları bakımından önemli farklılığın olduğu tespit edilmiştir. Çalışmaya göre en yüksek B_1 vitamini einkorn buğdayında, en yüksek B_2 vitamini einkorn ve emmer buğdaylarında, en yüksek B_5 vitamini Kıraç-66 buğdayında ve einkorn buğday çiminde tespit edilmiştir. Test edilen buğday çimleri arasında en yüksek B vitamini içeriği einkorn buğday çiminde gözlenmiştir. Araştırmalarında B_2 vitamini tüm buğday çimlerinde, buğday tanesinden daha fazla bulunmuştur.

Liang vd. (2020), 8 ay depoladıkları buğdaydaki folat (THF) miktarında %26 oranında kayıp belirlemişlerdir. Buğdayın, %70 randımanda öğütülmesi ile %71 oranında folat kaybı gözlemlemişlerdir. Ürettikleri eriştelerde, folatı, %78 oranında muhafaza edebilmişlerdir. Ayrıca, maya fermantasyonu ile ürettikleri ekmeklerdeki folat miktarında 1.5-4 kat iyileşme sağlamışlardır.

Arpanın B_1, B_2 ve B_3 vitaminlerini içerdiği ve arpadaki B vitamini miktarının olgun ve olgunlaşmamış tanelerde farklılık gösterdiği belirtilmiştir. Kavuzsuz arpa tanesi olgunlaştıkça B vitamini miktarının azaldığı açıklanmıştır. Olgun kavuzsuz arpa tanesinde B_1 vitamini miktarı (0.34 mg/100 g), olgunlaşmamış taneden daha fazla (0.26 mg/100 g) bulunmuştur. Fakat B_2 ve B_3 vitamini miktarları bakımından farklılık gözlendiği, olgunlaşmamış kavuzsuz arpada sırasıyla 0.25 ve 7.11 mg/100 g bulunurken, olgun tanede sırasıyla 0.23 ve 3.95 mg/100 g olarak gözlemlenmiştir. Aynı araştırmacılar, benzer bir durumu tritikale tahılı için de doğrulamışlardır. Olgun ve olgunlaşmamış tritikalede B_1 vitamini miktarı sırasıyla 0.52 ve 0.46 mg/100 g olarak bulunmuştur. Olgun tritikalede B_2 ve B_3 vitamini miktarı ise sırasıyla 0.21 ve 3.75 mg/100 g bulunurken, olgunlaşmamış tritikalede sırasıyla 0.40 ve 7.05 mg/100 g olarak tespit edilmiştir (Petrovska-Avramenko vd., 2017).

Rybicka ve Gliszczyńska-Świglo (2017), glutensiz tahıl ve pseudo-tahıl unlarının B vitaminleri üzerine yaptıkları bir araştırmada, yulaf unundaki B_1, B_2, B_3 ve B_6 vitaminlerinin miktarlarını sırasıyla 0.25 mg/100 g, 0.04 mg/100 g, 0.72 mg/100 g ve 0.10 mg/100 g olarak belirlemişlerdir. Aynı çalışmada, darı ununun B_1, B_2, B_3 ve B_6 vitamini miktarlarını sırasıyla 0.40 mg/100 g, 0.20 mg/100 g, 6.02 mg/100 g ve 0.09 mg/100 g olarak bulmuşlardır. Bulunan sonuçların gluten içeren tahıllara (buğday, arpa, çavdar) göre benzer veya daha düşük olduğu gösterilmiştir.

Çavdar, tam çavdar unu olarak kullanıldığı için B vitamini içeriği yüksek bir tahıldır. Tam çavdar unu, siyah çavdar unu ve kepeksiz çavdar unu kullanılarak yapılan ekşi mayalı çavdar ekmeklerinde tiamin (B_1) miktarı %20-45, nikotinik asit miktarı %25-50, pridoksal miktarı %45-65 oranında azalmıştır. Kepeksiz çavdar ununda ise riboflavin miktarı %50, piridoksin miktarı %15 oranında azalmıştır. Nikotinamid miktarının, ekşi hamur fermantasyonundaki mikrobiyal aktivite sonucu 10 kat arttığı rapor edilmiştir (Mihhalevski vd., 2013).

Nemeth ve Tömösközi (2021) çavdarda tiamin, riboflavin, niasin, pantotenik asit, pridoksin ve folat miktarlarını sırasıyla 4.0-4.6 mg/kg, 1.8-1.9 mg/kg, 12.0-15.0 mg/kg, 10.0 mg/kg, 3.0-3.4 mg/kg ve 0.48-0.52 mg/kg arasında bildirmiştir. Buğdayda aynı vitaminleri sırasıyla 5.0-12.0 mg/kg, 1.0-3.1 mg/kg, 41.0-64.0 mg/kg, 7.7-9.1 mg/kg, 3.0-4.7 mg/kg ve 0.35-0.56 mg/kg arasında belirtmişlerdir. Arpada söz konusu vitaminleri sırasıyla 2.0-2.6 mg/kg, 0.9-1.0 mg/kg, 45.0-50.0 mg/kg, 3.5 mg/kg, 3.0-3.2 mg/kg ve 0.19-0.25 mg/kg arasında açıklamışlardır. Tritikalede aynı vitaminleri sırasıyla 3.8-9.8 mg/kg, 1.3 mg/kg, 29.0 mg/kg, 6.5-8.8 mg/kg, 4.0 mg/kg ve 0.7 mg/kg olarak bildirmişlerdir. Yulafda ise sırasıyla 5.0-8.0 mg/kg, 1.0-1.4 mg/kg, 9.6-16.0 mg/kg, 13.4 mg/kg, 2.0-2.4 mg/kg ve 0.45-0.60 mg/kg olarak açıklamışlardır.

Roy vd. (2023) 309 adet *indica* yerli pirinç çeşitlerindeki B vitamini miktarlarını modern pirinç çeşitleri ile karşılaştırmıştır. Buna göre, yerli pirinç çeşitlerinin dikkate değer ölçüde B vitamini içerdiğini gözlemlemişlerdir. Yerli *indica* pirinç çeşitlerindeki B_1 vitamini miktarını 0.01-10.55 mg/100 g, B_2 vitaminini 0.01-2.63 mg/100 g, B_3 vitaminini 0.20-4.52 mg/100 g, B_5 vitaminini 0.01-18.55 mg/100 g, B_6 vitaminini 0.01-0.86 mg/100 g ve B_7 vitaminini 0.01-5.90 mg/100 g arasında belirlemişlerdir. Araştırmalarında, modern pirinç çeşitlerindeki B vitamini miktarlarının önemli düzeyde düşük olduğunu gözlemlemişlerdir.

PSEUDO-TAHILLARIN B VİTAMİNLERİ

Pseudo-tahıllar, tahıl benzeri glutensiz bitki tohumlarıdır. Kinoa, amarant ve karabuğday pseudo-tahıl grubunu oluşturduğu bildirilmiştir. Pseudo-tahılların, protein kalitesi ve miktarı, esansiyel yağ asitleri, vitamin ve mineral madde miktarının yüksek olduğu açıklanmıştır (Bock vd., 2021).

Karabuğdayın, B_1, B_2, B_3, B_6 ve B_9 vitaminlerini diğer tahıllara göre daha fazla içerdiği belirtilmiştir. Karabuğdaydaki tiaminin, tiamin bağlayıcı proteine sıkı bir şekilde bağlandığı bildirilmiştir. Karabuğdayın çimlendirilmesi ile C, B_1 ve B_6 vitamini içeriğinin artırılabileceği ifade edilmiştir (Rodriguez vd., 2020; Huda vd., 2021). Joshi vd. (2019) karabuğdaydaki tiamin, riboflavin, niasin, pantotenik asit ve kolin miktarlarını sırasıyla 3.3 mg/100 g, 10.6 mg/100 g, 18.0 mg/100 g, 11.0 mg/100 g ve 440.0 mg/100 g olarak bildirmişlerdir.

Amerika Birleşik Devletleri'nin Ulusal Bilimler Akademisi (United States National Academy of Sciences), amarantın yüksek besinsel kalitesini ve potansiyel tarımsal özelliklerini açığa çıkarmasından sonra amarant, gıda teknolojistlerinin ilgisini çekmeye başlamıştır. Amarantın tohum ve yapraklarının B grubu vitaminler açısından oldukça zengin olduğu belirtilmiştir. Amarantın, tiamin (0.07-0.10 mg/100 g un), riboflavin (0.19-0.23 mg/100 g un), niasin (1.17-1.45 mg/100 g un) ve askorbik asit (4.5 mg/100 g un) gibi suda çözünen vitaminler bakımından iyi bir kaynak olduğu gösterilmiştir (Rodriguez vd., 2020). Murakami vd. (2014), amarant tohumlarına 260°C'de patlatma işlemi uygulayarak B vitamini içeriğindeki değişimi araştırmışlardır. Buna göre, işlem uygulanmamış amarant tohumlarında B_2, B_3, B_5 B_6, B_7 ve B_9 vitamini miktarları sırasıyla 147.0 µg/100 g, 3.230 µg/100 g, 1.150 µg/100 g, 454.0 µg/100 g, 24.8 µg/100 g, 152.0 µg/100 g olarak tespit edilmiştir. İşlem uygulanmış amarant tohumlarında ise aynı vitamin miktarları sırasıyla 140.0 µg/100 g, 3.080 µg/100 g, 991.0 µg/100 g, 408.0 µg/100 g, 25.2 µg/100 g, 137.0 µg/100 g olarak bulunmuştur. Çalışmada, uygulanan işlemin B vitamini içeriğini önemli düzeyde etkilemediği rapor edilmiştir.

Kinoanın, birçok bitkinin yetişemeyeceği marjinal ekolojik koşullarda yetişebilen, abiyotik stress koşullarına (tuzlu toprak, kurak ve soğuk iklimler, yüksek sıcaklık farkı örneğin -8°C'den +35°C'ye sıcaklığın yükselmesi) dayanıklı, glutensiz, düşük glisemik indekse ve yüksek besinsel kaliteye sahip bir pseudo-tahıl olduğu bildirilmiştir. Kinoada B_1, B_2, B_3, B_5 ve B_6 vitamin miktarlarının 0.21-1.70 mg/kg arasında değiştiği, B_7 ve B_9 vitamini miktarlarının ise sırasıyla 0.62 µg/kg ve 1.73 µg/kg olduğu açıklanmıştır (Rodriguez vd., 2020).

B VİTAMİNLERİNİN BİYOERİŞİLEBİLİRLİĞİ VE BİYOYARARLANIMLARI

Biyoerişilebilirlik, sindirim sisteminde gıda matrisinden asidik koşullarda ve enzim hidrolizi ile salınan bir besin maddesinin ince bağırsakta emilim için hazır bulunmasıdır (Garg vd., 2021; López-Gámez vd., 2021). Sindirim sisteminde bulunan suda çözünen vitaminlerin biyoerişilebilirliğinin, pH'ya, sıcaklığa, polipeptit, polisakkarit, metal iyonları ve enzim inhibitörlerinin varlığına, besinsel lif miktarına ve öğütme yöntemine bağlı olduğu bildirilmiştir (Yaman vd., 2021). Biyoerişilebilirliği ayrıca, gıdanın tipi ve matrisi, vitaminin çeşidi, hasat sonrası depolama, işleme ve ambalajlama yöntemleri gibi faktörlerin de etkilediği ifade edilmiştir (Onyambu vd., 2021; Garg vd., 2021; López-Gámez vd., 2021).

Bitkilerde vitaminlerin, besin dokusunda kompleks halde bulunduğu ve absorpsiyonları için dokunun parçalanarak biyoerişilebilirliğinin artırılması gerektiği bildirilmiştir. Besinsel liflerin ise sindirim sisteminde çok fazla suyu absorbe etmelerinden dolayı, sindirimi ve absorpsiyonu yavaşlattığı, B vitaminlerinin biyoerişilebilirliğini azalttığı söylenmiştir. Tahıl içeren bebek gıdalarındaki B_1, B_2, B_3, B_6, B_9 vitaminlerinin ortalama biyoerişilebilirliği sırasıyla %81, %79, %45, %60, %52 olarak verilmiştir. Biyoerişilebilirliğin, tahıl ürünlerindeki vitaminlerin sağlık etkilerinin tahmin edilmesinde önemli olduğu belirtilmiştir (Akça vd., 2019; Onyambu vd., 2021; Garg vd, 2021).

Biyoyararlılık, sindirilen gıdalardaki besin ögelerinin ince bağırsak epitel hücrelerinden absorbe edildikten sonra kan dolaşımına geçmesi olarak tanımlanmıştır. Gıda matrisi, gıda işleme yöntemi ve sindirilme oranı başta olmak üzere birçok faktör biyoyararlılığı etkilediği bildirilmiştir. Makro ve mikro besin ögelerinin vücuttaki absorpsiyon verimliliği, gıdanın kolay bir şekilde sindirilebilme kabiliyetine bağlı olduğu kaydedilmiştir. Sindirilme oranı yüksek bir gıda sağlıklı bir gıdadır ve organizmanın sağlık ve enerji durumunu iyileştirdiği söylenmiştir (Rodriguez vd., 2020).

Suda çözünen vitaminler, ince bağırsakta, yağda eriyen vitaminler gibi misel oluşturmak yerine, aktif taşınım ile direkt emilirler ve ayrıca ışık, sıcaklık, ortam pH'sına oldukça duyarlı olduklarından, besinlerin vitamin içeriklerinde önemli ölçüde kayıplar olabileceği belirtilmiştir. Aynı zamanda, suda çözünen vitaminlerin, özellikle B_1, B_2 ve B_6 vitaminlerinin, gıdalarda polisakkaritlere ve polipeptitlere kovalent olmayan bağlar ile bağlı olabilecekleri gösterilmiştir. Düşük pH değerlerinde (mide), protein parçalanması daha fazla olacağı için daha çok B_1 ve B_2 vitaminlerinin salınacağı ifade edilmiştir (López-Gámez vd., 2021).

Yaman (2019), farklı ekmek çeşitlerinde (beyaz, tam buğday, kepekli ekmek ve yulaflı ekmek) B_1, B_2 ve B_6 vitaminlerinin in vitro ortamda biyoerişilebilirliğini incelediği çalışmasında, B_1 vitamini için en düşük biyoerişilebilirliği yulaf ekmeğinde (%45), en yüksek biyoerişilebilirliği ise tam buğday ekmeğinde (%73) gözlemlemiştir. Yulaf ve arpada bulunan β-glukanın, vitamin ve minerallerin biyoerişilebilirliğini azalttığı kaydedilmiştir. Yaman (2019), B_2 vitamininin biyoerişilebilirliğini en yüksek tam buğday ekmeğinde (%66), en düşük ise yulaflı ekmekte (%56) bulmuştur. Araştırmasında, piridoksin (PN), piridoksal (PL), piridoksamin (PM) için biyoerişilebilirliğin %17-82 arasında değiştiğini saptamıştır. En yüksek biyoerişilebilirlik, kepekli ekmekteki PN formunda gözlenmiş olup, en düşük biyoerişilebilirlik ise yulaflı ekmekteki PL formunda bulunmuştur. Çalışmada, PL ve PM formlarının düşük biyoerişilebilirliği, bu formların sindirim sırasında proteinlerden ayrılıp tam olarak serbest hale geçememeleri şeklinde açıklanmıştır (Yaman, 2019).

Akça vd. (2019), 13 farklı tahıl içerikli ticari bebek mamalarındaki tiamin, riboflavin, nikotinik asit ve nikotinamidin ortalama biyoerişilebilirliğini in vitro koşullarda ve gastrik pH 1.5'da sırasıyla %81, %79, %39 ve %51 olarak, gastrik pH 4'te ise aynı vitaminlerin ortalama biyoerişilebilirliğini sırasıyla %65, %67, %33 ve %41 olarak tespit etmişlerdir. Araştırmalarında, B_1, B_2 ve B_3 vitaminlerinin biyoerişilebilirliklerinin stabilite, sıcaklık, gastrik pH değeri, besinsel lif içeriği, polisakkarit ve polipeptitlere bağlanma durumundan önemli derecede etkilenebileceği aktarılmıştır. Sonuç olarak suda çözünür vitaminlerin biyoerişilebilirliğinin in vivo koşullarda daha düşük olabileceği bildirilmiştir.

Fărcaş vd. (2022) farklı bira üretimlerinden gelen biracılık artığı küspeleri ile oluşturdukları 4 farklı biracılık artığı küspesinin in vitro sindirimi sonucunda, B vitaminlerindeki (B_1, B_3, B_6, B_{12}) değişimi incelemişlerdir. Buna göre, in vitro sindirimden sonra her bir biracılık artığı küspesindeki B vitaminlerinin kontrole göre azaldığı tespit edilmiştir. B_1, B_3, B_6, B_{12} vitaminleri için ulaşılan en yüksek biyoerişilebilirlik değerleri sırasıyla %72.45, %68.44, %52.94 ve %83.57 olarak bulunmuştur.

Bitkisel kaynaklı gıdalarda bulunan B_1, B_2 ve B_3 vitaminlerinin biyoerişilebilirlik ve biyoyararlılığının, düşük protein sindirilirliği ve yüksek besinsel lif içeriği sebebiyle genellikle azaldığı aktarılmıştır (Akça vd., 2019; Demir vd., 2023). Kurek vd. (2017), farklı boyutlardaki besinsel lif partikülleri ilave ederek yapmış oldukları buğday ekmeklerinde, tiamin, riboflavin ve niasin vitaminlerinin biyoerişilebilirliğini sırasıyla %69.1-91.2, %40.9-50.2 ve %60.2-70.2 arasında tespit etmişlerdir. Optimum biyoerişilebilirlik için besinsel lif miktarı ve partikül boyutunu sırasıyla %6.17 ve 124.12 μm olarak belirtmişlerdir. Besinsel lifin partikül boyutunun küçültülmesiyle, daha fazla hidroksil grubunun ortaya çıkması ve bunların B vitaminleri ile etkileşime girerek biyoerişilebilirliği azalttığı rapor edilmiştir.

Çatak (2019), 10'ar farklı hayvansal ve bitkisel gıdada yaptığı bir çalışmada, nikotinik asit ve nikotinamid miktarları toplamında (mg/100 g), hayvansal gıdalardaki ortalama oranları sırasıyla %30 ve %70, bitkisel gıdalardaki ortalama oranları sırasıyla %87 ve %13 olarak tespit etmiştir. Araştırmasında, nikotinik asit miktarını yulafta 1.025 mg/100 g, çavdarda 4.168 mg/100 g, arpada 4.523 mg/100 g, pirinçte 1.767 mg/100 g, ekmeklik buğdayda 5.483 mg/100 g ve durum buğdayında 6.668 mg/100 g olarak bulmuş ve aynı tahıllarda nikotinamid formuna rastlamamıştır.

Zaupa vd. (2014), in vitro koşullarda makarnalık durum buğdayının kepek ve alöron tabakasının iç ve dış kısımlarını ve ayrıca bu kısımlardan hazırladıkları mikronize iç ve dış alöron kısımlarındaki tiamin ve niasin vitaminlerinin biyoerişilebilirliği üzerine yaptıkları çalışmada, bu fraksiyonlardaki tiamin vitamininin miktarını ve biyoerişilebilirliğini sırasıyla 4.44-27.84 mg/100 g ve %62.64-99.42 arasında, niasin vitamininin ise sırasıyla 14.15-21.86 mg/100 g ve %10.61-55.94 arasında bulmuşlardır. Sonuç olarak partikül boyutunun küçülmesi ile biyoerişilebilirliğin de genellikle arttığını ve buğdayın alöron tabakasının iç kısmındaki tiamin ve niasinin biyoerişilebilirliğinin en yüksek olduğunu göstermişlerdir.

B_6 vitamininin proteinlere kovalent olmayan bağ ile bağlı olduğu ve B_6 vitamininin proteinlerden ayrılmasının gastrik ve ince bağırsak pH'sına bağlı olduğu aktarılmıştır. Bununla birlikte, PLP formunun biyoerişilebilirlik düzeyinin gastrik ve ince bağırsak asitliğine göre değişebildiği bildirilmiştir (Ball, 2006). Bebeklerdeki yüksek gastrik pH'nın, PLP formunun biyoerişilebilirliğini büyük bir olasılıkla etkilediği ifade edilmiştir (Yaman ve Mızrak, 2019). On üç farklı tahıl içerikli bebek mamasında B_6 vitamininin in vitro biyoerişilebilirliği üzerine yapılan bir çalışmada, PL ve PM'nin biyoerişilebilirliğinin gastrik pH 1.5 ve 4.0'te PN'den daha düşük ve birbirine benzer olduğu gözlemlenmiştir. Tüm formların biyoerişilebilirliğinin gastrik pH 4'te azaldığı fakat her iki gastrik pH'da düşük olduğu tespit edilmiştir (Yaman ve Mızrak, 2019).

Yaşlılarda, B_6 ve B_{12} vitamin eksikliği sık karşılaşılan bir durumdur ve sırasıyla kardiovasküler hastalıklar ve megaloblastik anemi ile bağlantılı olduğu açıklanmıştır. Lee vd. (2022), B_{12} vitamininin, bağırsak mikrobiyotası tarafından in vitro sindiriminin yaşlılarda (%45.08), yetişkinlere (%35.96) göre daha yüksek olduğunu belirlemişlerdir. B_6 ve B_{12} vitaminlerinin biyoaktivitesinin yaş, sindirim yeri ve bağırsak mikrobiyotasından etkilendiğini, özellikle bağırsak mikrobiyotasının etkili olduğunu rapor etmişlerdir.

Gıda matrisinin, plazmadaki folat biyoyararlılığını değiştirdiği açıklanmıştır. Bir araştırmada en yüksek biyoyararlılık pudingte belirlenirken, pandispanya kekinde daha az oranda tespit edilmiştir. Krem karamel ve bisküvide folat biyoyararlılığı en düşük düzeyde bulunmuştur (Buffière vd., 2021). Neves vd. (2019), folik asit ile fortifiye edilmiş buğday unundan üretilmiş Fransız tipi ekmeklerin, folik asitin biyoerişilebilirliği ve biyoyararlanımının artırılmasında iyi bir araç olduğunu açıklamışlardır. Araştırmalarında, buğday ununun homojenize edildikten sonra serbest folik asitin %85'nin buğday ununda kaldığını, Fransız tipi ekmekte ise %75'inin tutulduğunu bildirmişlerdir. Fransız tipi ekmeklerin in vitro sindiriminden

sonra, folik asitin tamamının ince bağırsaklarda absorbe edilmek üzere hazır bulunduğunu keşfetmişlerdir. Bationo vd. (2020), tahıl içerikli fermente gıdalar (7 farklı ürün) üzerine yaptığı çalışmada, fermantasyonun folat içeriğini artırdığını tespit etmişlerdir. Çalışmada, folat biyoerişilebilirliği %23-81 arasında bulunmuş ve bu durumun folat stabilitesinden veya besin matrisinden kaynaklanabileceği belirtilmiştir.

SONUÇ

Tahıllar ve pseudo-tahıllar, B vitaminleri bakımından zengin gıda kaynaklarıdır. B vitaminlerinin, metabolizmanın düzenlenmesi ve işleyişi bakımından kritik öneme sahip, suda çözünür, organik bileşikler olduğu açıklanmıştır. Günlük beslenmede, yeterli alım miktarlarının tanımlanması ile birlikte, enerji metabolizması ve bağışıklık sistemi için önemli koenzimler olduğu da gösterilmiştir. B vitaminlerinin biyoerişilebilirliği ve biyoyararlılığının belirlenmesinde, genellikle, "in vitro sindirim sistemi modelleri" çalışılmıştır. Bu yöntemin ucuz, hızlı ve deneysel parametrelerin kontrolünün sağlanması bakımından in vivo yöntemlere göre avantajlarının bulunduğu bildirilmiştir. B vitaminlerinin biyoerişilebilirliği, sindirilirliğe ve gıda matrisinden serbest kalmasına bağlıyken; biyoyararlılıklarının sindirilirliğe, gıda matrisinden serbest kalmalarına, ince bağırsak hücrelerinden absorpsiyon ve vücut hücrelerine taşınmalarına bağlı olduğu kaydedilmiştir. Tahıl ve pseudo-tahıl ürünlerinde B vitamini fortifikasyonunun bazı ülkelerde zorunlu olduğu ve biyofortifikasyon yönteminin B vitaminleri bakımından zengin tahılların yetiştirilmesinde yeni bir uygulama olduğu gösterilmiştir. Bu derlemede, tahıl ve pseudo-tahılların B vitaminlerinin biyoerişilebilirliği ve biyoyararlılığındaki son çalışmalar incelenmiş olup, in vivo sonuçların in vitro sonuçlar ile doğruluğunun karşılaştırılması için daha fazla doğrulama testlerinin yapılması gerektiği görülmüştür.

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PRODUCTION OF ALKALINE PROTEASE BY A NOVEL ANAEROBIC BACTERIUM ISOLATED FROM A MUNICIPAL ANAEROBIC TREATMENT SYSTEM

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ABSTRACT

In this study, alkaline protease enzyme production by a bacterial strain isolated from sludge samples collected from an anaerobic treatment system was investigated. According to the 16S rDNA sequence analysis, the isolate was identified as *Thermoanaerobacter thermohydrosulfuricus* (98.52%). Enzyme activity analyses revealed an optimum pH value of 10, an incubation time of 64 h, and a temperature of 35°C. Arabinose and casein hydrolysates were found to be the best carbon and nitrogen sources, respectively. Maximum protease activity was recorded (864.68 U/mL) when arabinose was used instead of glucose. Moreover, the addition of 1 g/L $MgSO_4.7H_2O$ and 0.25 g/L Tween-80 to the medium increased the enzyme activity. Therefore, it can be concluded that *T. thermohydrosulfuricus* is a significant producer of alkaline protease enzymes in the culture medium. To the best of our knowledge, this is the first study to investigate the optimization of alkaline protease production by *T. thermohydrosulfuricus*.

Keywords: Protease, novel strain, *Thermoanaerobacter thermohydrosulfuricus*, anaerobic

BELEDİYE ANAEROBİK ARITMA SİSTEMİNDEN İZOLE EDİLEN YENİ BİR ANAEROBİK BAKTERİ İLE ALKALİ PROTEAZ ÜRETİMİ

ÖZ

Bu çalışmada, anaerobik arıtma sisteminden toplanan çamur örneklerinden izole edilen bir bakteri suşunun alkali proteaz enzim üretimi araştırılmıştır. 16S rDNA dizi analizine göre, izolatın *Thermoanaerobacter thermohidrosulfuricus* (%98.52) olduğu tespit edilmiştir. Enzim aktivitesi analizleri, optimum pH değerinin 10, inkübasyon süresinin 64 saat ve sıcaklığın 35°C olduğunu ortaya çıkarmıştır. Arabinoz ve kazein hidrolizatlarının sırasıyla en iyi karbon ve nitrojen kaynakları olduğu bulunmuştur. Maksimum proteaz aktivitesi (864.68 U/mL) glikoz yerine arabinoz kullanıldığında kaydedilmiştir. Ayrıca, besiyerine 1 g/L $MgSO_4.7H_2O$ ve 0.25 g/L Tween-80 ilavesi enzim aktivitesini artırmıştır. Bu nedenle, *T. thermohidrosulfuricus*'un kültür ortamında önemli bir alkali proteaz enzimi

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üreticisi olduğu sonucuna varılabilmektedir. Bildiğimiz kadarıyla bu çalışma, *T. thermohidrosulfuricus*'un alkali proteaz üretiminin optimizasyonunu araştıran ilk çalışmadır.
Anahtar kelimeler: Proteaz, yeni tür, *Thermoanaerobacter thermohydrosulfuricus*, anaerobik

INTRODUCTION

Enzymes are biological macromolecules that have a wide range of analytical, scientific, and industrial applications. Microbial enzymes are superior to inorganic catalysts because of their economic production, convenient handling, easy recovery from reaction media, and repeated reusability in industrial processes (Bashir et al., 2018). Proteases are a group of enzymes that catalyze the hydrolysis of proteins into peptides and amino acids (Sharma et al., 2017). Proteases are used in the food, leather, pharmaceutical, cosmetic, silk degumming, silver recovery, chemical, and wastewater treatment industries (Naveed et al., 2021). Microbial proteases are of significant interest, as they represent approximately 60% of the total enzyme market. These enzymes play a role in the synthesis of small peptides, the production of food ingredients by the hydrolysis of food proteins, and the improvement of the digestibility of proteins or amino acids (Dorra et al., 2018).

Most characteristics, including pH, temperature, active-site specificity, substrate specificity, catalytic activity, and stability profiles, tend to vary depending on the protease source. Proteases can be classified based on their optimal pH range, which includes acidic (pH 2-6), neutral (pH 7) and alkaline (pH 8-13) (Arya et al., 2021). Alkaline proteases have attracted considerable attention, accounting for approximately 89% of the protease market (Akhter et al., 2024). Alkaline proteases are important components in the detergent industry. They enhance cleaning efficacy by eliminating proteins such as milk, blood, and food stains (Mahakhan et al., 2023). Several alkaline proteases are produced by yeast, bacteria, actinomycetes, fungi, and plants. On the other hand, commercially produced microbial proteases of extracellular origin are resistant to physical and chemical environmental changes (Al-Dhabi et al., 2020).

Recently, there has been an increased demand for alkaline and thermostable proteases as industrial biocatalysts for biotechnological applications. In addition to their tolerance to pH and temperature, alkaline proteases exhibit high catalytic activity, substrate specificity, environmentally friendly byproduct formation, and cost-effective large-scale production (Datta et al., 2017). *Bacillu*s species are preferred for producing thermostable alkaline proteases because of their stability at high temperatures and pH values. These bacterial enzymes have higher activity in the pH range of 8-12 and temperature range of 50-70°C, which are ideal for producing enzymes on an industrial scale (Thakur et al., 2018). A wide variety of soil microorganisms (Aftab et al., 2006; Palsaniya et al., 2012; Sinha et al., 2013; Chauhan et al., 2020; Jadhav et al., 2020; Farooq et al., 2021; Hashmi et al., 2022) and strains isolated from mangrove ecosystems can produce proteases. Microbes isolated from mangrove ecosystems have a rapid growth rate in a limited space and are easy to genetically manipulate to produce new modified protease enzymes (Kharadi et al., 2020). Moreover, strains isolated from soda lakes and deserts can grow at an extremely alkaline pH and produce naturally stable alkaline enzymes. In addition, investigating the effects of certain parameters, such as cost-effective carbon and nitrogen sources, pH, temperature, agitation, and incubation time, is necessary for designing an effective process (Rathod and Pathak, 2016).

Bacillus, *Aspergillus*, and *Streptomyces* have been counted as predominant and prolific sources of alkaline proteases; however, new strain discovery studies are still important. Working with thermophiles offers some advantages, such as a reduced risk of contamination, owing to their ability to be used at high temperatures during the process. Although there is a study using *Thermoanaerobacter thermohydrosulfuricus* to produce heat-active lipase (Royter et al., 2009), there are no reports on the production of alkaline protease from *T. thermohydrosulfuricus*. In the present study, the optimum conditions (pH, incubation time, temperature, carbon and nitrogen sources, and addition of various compounds) were determined

for the alkaline protease produced by *T. thermohydrosulfuricus* G12 isolated from the sludge samples.

MATERIAL and METHODS

Source of sample collection

In this study, sludge samples collected from an anaerobic treatment system at the Ankara Metropolitan Municipality Tatlar Wastewater Plant were used. The sludge samples were stored in sterile bottles and transported to the laboratory at Ankara University, Turkey. The samples were stored at 4°C until subsequent analysis.

Isolation of bacteria and culture conditions

Skim milk (SM) medium had the following composition (g/L): nutrient broth 8, SM 100, and resazurin 1. The medium was prepared by adding resazurin, a colorimetric pH indicator based on redox potential, to SM and boiling until the resazurin-induced blue color turned pink. The medium distributed in the Hungate anaerobic culture tubes was cooled under an N_2 atmosphere to remove all dissolved oxygen, sealed with a butyl rubber stopper, and sterilized for 15 min. at 121°C. The SM medium was inoculated with 1% sludge sample and incubated at 65°C for 24 h. The cultures were then transferred to the SMNB medium.

The microorganisms were incubated for 24 h at 65°C in SMNB medium and prepared for bacterial isolation. The cultures were then transferred to Petri dishes containing skim milk nutrient agar (SMNA) in an anaerobic environment (Glove Box-Labconco) using the spreading method. After 48-72 h of incubation at 65°C, colonies forming the largest diameter transparent zone were identified and transferred to the SMNB medium (Ibrahim et al., 2007).

Genomic DNA was isolated using DNA isolation kits (Macherey Nagel), and DNA amplification by polymerase chain reaction (PCR) and sequence analysis were performed by a commercial company (REFGEN, Ankara, Turkey). The sequences of the microorganisms that were most similar to the isolate were aligned using CLUSTAL W 1.8 (multiple sequence alignment method) to perform molecular phylogenetic analysis of the isolate. Thus, the genetic diversity and degree of differentiation between species were determined. Evolutionary relationships were detected using the neighbor-joining method (Saitou and Nei, 1987). The evolutionary distance was calculated using the Maximum Composite Likelihood method and the results were obtained in the form of the number of base changes in each region (Tamura et al., 2004). Ten nucleotide sequences were used in the analysis, and gaps and positions containing missing data were eliminated. Phylogenetic analysis was performed using the MEGA5 (Molecular Evolutionary Genetics Analysis software version 5.0) package program (Tamura et al., 2011).

Medium for alkaline protease enzyme production

Horikoshi-I medium had the following composition (g/L): glucose 10, yeast extract 5, peptone 5, KH_2PO_4 1, $MgSO_4.7H_2O$ 0.2, and Na_2CO_3 5 (Horikoshi, 1996) and was used for protease enzyme production. The pH values of both media were adjusted to 9 using 5 M Na_2CO_3.

Alkaline protease activity assay

The modified Cupp-Enyard (2008) method was performed for the protease activity assay, and casein was used as the substrate. Five milliliters of 0.65% (w/v) casein solution were added to each test tube. The solutions were placed in a water bath at 37°C for 5 min. Cell-free supernatants were obtained by centrifugation at 15.000 rpm at 4°C for 5 min. One milliliter of the supernatant was added to the casein solutions, and the solutions were incubated in a water bath at 37°C for 10 min (1 mL of distilled water was used for the blank sample). The reaction was stopped by adding 5 mL of 110 mM trichloroacetic acid, and the solutions were incubated at 37°C for 30 min in a water bath. After incubation, each test solution was filtered using a 0.45 μm filter. Subsequently, 5 mL of 500 mM Na_2CO_3 and 1 mL of Folin's Phenol Reagent (1:4 Folin-distilled water) were added to 2 mL of the test filtrate and incubated at 37°C for 30 min. The absorbance of the samples was measured at 660 nm and extrapolated against a tyrosine standard curve.

One unit of protease activity was defined as the amount of enzyme liberating 1 μg of tyrosine per minute under assay conditions.

Units/ml enzyme = (μmole tyrosine equivalents released) x (A)/ ((B) x (C) x (D))
A= Total volume (mL) of assay
B= Time of assay (minutes) as per the Unit definition
C= Volume of Enzyme (mL) of enzyme used
D= Volume (mL) used in colorimetric determination

To prepare the standards, a solution containing 1.1 mM tyrosine was prepared as a stock solution and then transferred to tubes in certain volumes.

The enzyme activity of the control sample was calculated using samples collected from cultures incubated at 65°C for 24 h in Horikoshi-I medium. In this study, all inoculations were performed at a rate of 1%.

Optimization of cultural conditions for alkaline protease production

Enzyme activity was measured in samples collected at 16 h and every 8 h thereafter to determine the optimum incubation time. To determine the optimum pH for enzyme production, the pH value of the medium was adjusted to values between 6.5 and 10.5 using 5 M Na_2CO_3. The optimum temperature was determined in the samples that were obtained by incubating the cultures at 30-65°C. To select the carbon source for maximum enzyme activity, instead of 10 g/L glucose in Horikoshi-I medium, the medium was prepared by adding different carbon sources at the same concentration. To find the best nitrogen source for maximum enzyme activity, instead of a total of 10 g/L peptone, and yeast extract in Horikoshi-I medium, casein hydrolysate, soy flour (defatted), peptone, and casein mixture (5 g/L + 5 g/L), gelatin, yeast extract and peptone were used. $(NH_4)_2SO_4$ was used as an inorganic nitrogen source and added at a rate of 5 g/L. $CaCl_2$ (0.1 g/L and 1 g/L), KH_2PO_4 (0.5 g/L, 2 g/L), $MgSO_4.7H_2O$ (0.1 g/L, 1 g/L), and Tween-80 (0.25 g/L) were added to the Horikoshi-I medium to determine the effects of different minerals and surfactant on enzyme production.

Statistical analysis

Significant differences between factor levels were determined according to the least significant differences (LSD) at the $P \leq 0.05$ level of probability, and standard deviations (±SD) were shown as column bars in the figures.

RESULTS AND DISCUSSION

Molecular identification of the isolate

The cultures in NBSM were spread on the NASM medium under anaerobic conditions. Thus, a bacterial isolate was obtained and was coded as G-12. This isolate was determined to be a Gram-negative rod-shaped facultative anaerobic bacterium.

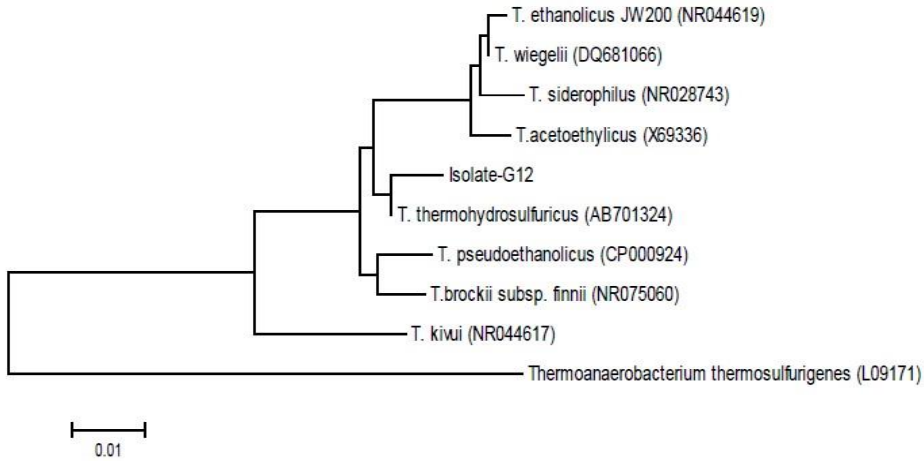

Figure 1. A phylogenetic tree created using MEGA5 with the G-12 isolate and its closest species.

DNA sequence analysis revealed 879 bases in the 16S rDNA region of the G-12 isolate. The DNA base sequences were compared with all DNA sequences from the GenBank BLAST database (www.ncbi.nlm.nih.gov/blast). The results obtained from the database showed that G-12 isolate belonged to *Thermoanaerobacter thermohydrosulfuricus* at a rate of 98.52%. Figure 1 shows the phylogenetic tree created using MEGA5.

Factors affecting enzyme production

Time course of protease production

The enzyme activity was calculated to be 641.58 U/mL in the samples collected at 64 h. However, the differences in enzyme activity between 64 h and 18, 24, and 32 h were statistically significant ($P \leq .05$). There was no statistical difference between the enzyme activity measured in samples taken at 64 h and the samples taken at 72 h. There was also no statistically significant difference in enzyme activity between samples taken at 40, 48, and 56 h and those taken at 64 h ($P \geq .05$). Therefore, 40 h to reach maximum enzyme activity may be sufficient, as prolonging the production time would only result in increased costs, particularly on an industrial scale (Figure 2). Prakasham et al. (2006) investigated the properties of alkaline proteases produced by *Bacillus* sp. and calculated the maximum enzyme activity after 60 h. Dhandapani and Vijayaragavan (1994) examined the thermophilic *Bacillus stearothermophilus* AP-4 to produce thermostable alkaline protease, and after 36 h of incubation, they achieved the maximum protease activity (250 U/mL).

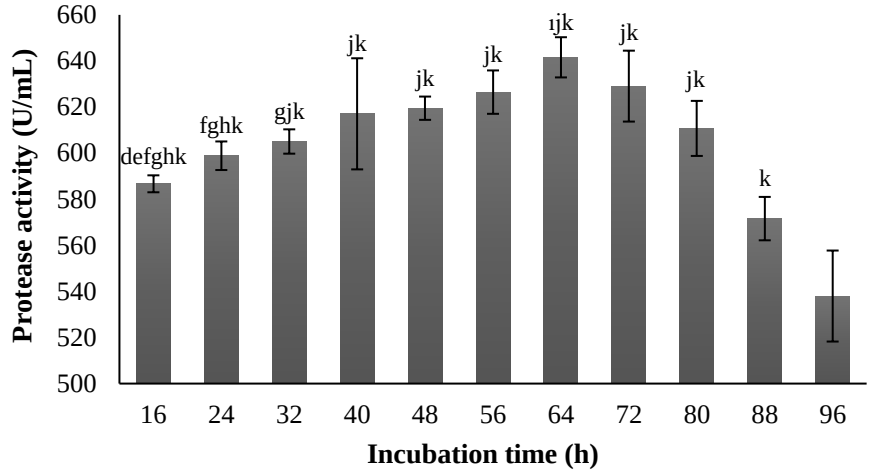

Figure 2. The time course of alkaline protease production (incubations were performed at an initial pH of 9 and at 65°C)

Vertical bars indicate standard deviations of the means and values are the means of two replicates
(a) 16, (b) 24, (c) 32, (d) 40, (e) 48, (f) 56, (g) 64, (h) 72, (ı) 80, (j) 88 and (k) 96 h
Different letters indicate significant differences according to the least significant difference (LSD) test at $P \leq .05$.

Effects of initial pH and incubation temperature on enzyme production

As shown in Figure 3, alkaline protease activity increased until it reached pH 10 and then decreased at pH 10.5. The highest enzyme activity (863.69 U/mL) was observed at pH 10. The differences between enzyme activities at pH 10 and all tested pH values were statistically significant ($P \leq .05$). Studies have shown that the optimum pH value for alkaline proteases ranges from 9 to 11 (Banerjee et al., 1999; Denizci et al., 2004; Gençkal and Tari, 2006; Patel et al., 2006; Sellami-Kamoun et al., 2008; Wilson and Remigio, 2012; Asha and Palaniswamy, 2018). In contrast, other studies have shown that the optimum pH value range is 8-9 (Mohamedin, 1999; Hutadilok-Towatana et al., 1999; Chi et al., 2007; Silva et al., 2007; Elbanna et al., 2015).

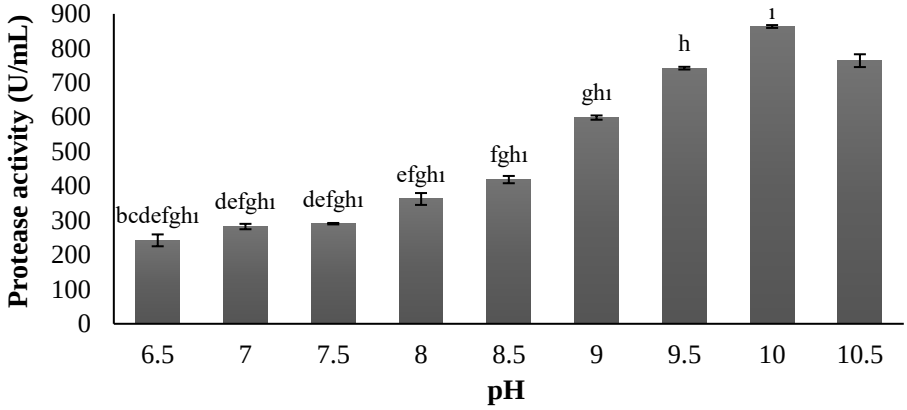

Figure 3. Effect of initial pH on alkaline protease production

Vertical bars indicate standard deviations of the means and values are the means of two replications
(a) 6.5, (b) 7.0, (c) 7.5, (d) 8.0 (e) 8.5, (f) 9.0, (g) 9.5, (h) 10.0, and (ı) 10.5
Different letters show significant differences according to least significant difference (LSD) test at $P \leq .05$.

The highest enzyme activity was determined to be 713.51 U/mL for samples incubated at 35°C (Figure 4). However, there was no statistically significant difference between the enzyme activities calculated at 35 and 40°C ($P \geq .05$). Although some researchers have reported similar results (Asha and Palaniswamy, 2018; Charles et al., 2008), others have found an optimum temperature of 37°C (Gençkal and Tari, 2006; Patel et al., 2006; Olajuyigbe and Ehiosun, 2013). However, many studies have reported optimum temperatures between 50 and 80°C (Denizci et al., 2004; Mohamedin, 1999; Hutadilok-Towatana et al., 1999; Silva et al., 2007; Aqel et al., 2012).

In a previous study, *Bacillus* sp. isolated from saline-alkali soils was selected for its ability to produce alkaline proteases in a milk agar medium. Maximum enzyme activity was obtained in the presence of glucose (1% w/v) and NH_4Cl (1% w/v) at pH 10.5, at a temperature of 40°C, and after 20 h (Mehrotra et al., 1999). In another study, the highest enzyme activity was observed in *Bacillus* sp. B18 under extreme conditions, with optimum pH and temperature values of 12-13°C and 85°C, respectively (Fujiwara et al., 1993).

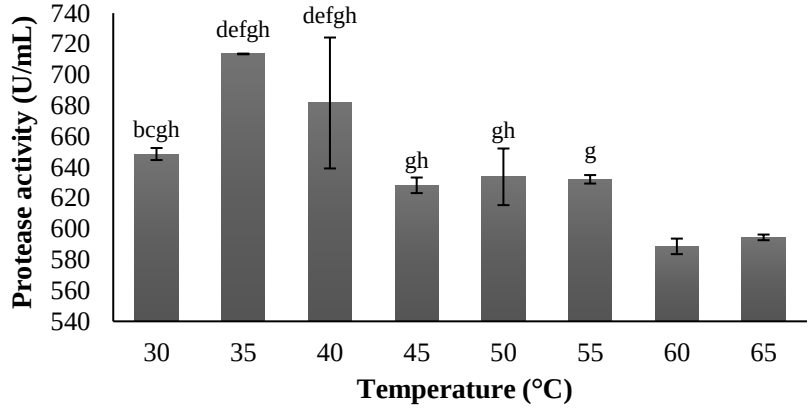

Figure 4. Effect of incubation temperature on alkaline protease production

Vertical bars indicate standard deviations of the means, and values are the means of two replications
(a) 30, (b) 35, (c) 40, (d) 45, (e) 50, (f) 55, (g) 60, and (h) 65°C
Different letters show significant differences according to least significant difference (LSD) test at $P \leq .05$.

Effect of carbon and nitrogen sources on enzyme production
Enzyme production could not be achieved because of the inability of microorganisms to grow well when inulin, sorbitol, starch, sucrose, and raffinose were added to the medium. Various carbon sources were used to determine the maximum enzyme production, as shown in Table 1. The highest enzyme activity (864.68 U/mL) was observed in the presence of arabinose. Following arabinose, the addition of xylose, sorbose, galactose, and fructose also increased enzyme activity. The difference between the enzyme activities obtained when arabinose and xylose were used in the medium was not statistically significant ($P \geq .05$). Maltose resulted in the lowest enzyme production. Kanekar et al. (2002) obtained the second highest enzyme activity using *Arthrobacter ramosus* (89.73 U/mL) in the presence of xylose. The activity of protease produced by the thermophilic *Bacillus* sp. SMIA-2 was 0.530 U/mg protein when glucose was used (9 h of incubation at 50°C and an initial pH of 7) (Nascimento et al., 2004). Prakasham et al. (2006) investigated certain properties of the alkaline protease that was produced by *Bacillus* species and reported that the enzyme activity increased with the addition of xylose and maltose to the medium at a rate of 1%. Akcan and Uyar (2011) reported that the maximum alkaline protease activity produced by *Bacillus subtilis* RSKK96 was 4688.2 U/mg, which was obtained by the addition of 1% arabinose.

Examination of the effects of different nitrogen sources on enzyme production revealed that the highest enzyme activity was 656.03 U/mL when casein hydrolysate was present in the medium. The difference between enzyme activities obtained when casein hydrolysate and yeast extract were used in the medium was not statistically significant ($P \geq .05$). Compared with the control, the addition of soy flour caused a large decrease in enzyme activity, followed by gelatin and $(NH_4)_2SO_4$ (Table 1). Johnvesly and Naik (2001) investigated the effects of various inorganic and organic nitrogen sources on alkaline protease production by thermophilic and alkalophilic *Bacillus* sp. JB-99. The researchers found that the highest enzyme activity (12780 U/mL) was obtained in the presence of $NaNO_3$, followed by samples containing yeast extract (10850 U/mL). Asha and Palaniswamy (2018) reported that casein was the best nitrogen source for producing alkaline proteases from *Bacillus cereus* FT 1. Lazim et al. (2009) found that $(NH_4)_2SO_4$ and yeast extract increased protease activity compared with the control. In contrast, malt extract, peptone, NH_4NO_3, $NaNO_3$, and casein decreased enzyme production. Some studies have reported that yeast extract is the best nitrogen source for enzyme production (Nadeem et al., 2008; Salihi et al., 2017; Rekik et al., 2019). Joo et al. (2002) isolated *Bacillus horikoshii* to investigate extracellular alkaline protease production and obtained the highest enzyme activity (115.3 U/mL) using soybean meal (1.5%, w/v) and casein (1%, w/v) at pH 9 and 34°C after 18 h.

Effects of minerals and Tween-80 on enzyme production
The medium components most likely to affect alkaline protease production were K_2HPO_4, $MgSO_4.7H_2O$, and $CaCl_2$ (Jadhav et al., 2020). The addition of 1 g/L $MgSO_4.7H_2O$ to the medium yielded the highest increase in enzyme activity compared to the control sample, and the activity was calculated to be 726.50 U/mL ($P \leq .05$). Enzyme activity increased when the concentration of KH_2PO_4 was increased from 0.5 to 2 g/L. Compared with the control sample, protease activity decreased with the addition of 0.1 g/L and 1 g/L $CaCl_2$, respectively. Moreover, using 2 g/L KH_2PO_4 and 0.1 g/L $MgSO_4.7H_2O$ in the medium did not create a statistically significant effect compared to the control sample ($P \geq .05$).

To ensure optimal performance during the washing process, alkaline proteases must demonstrate compatibility and stability with various components commonly found in detergents, including surfactants, oxidizing agents, and other additives (Hammami et al., 2017). Therefore, the efficacy of the enzyme was evaluated using Tween-80, which is a potential constituent of the formulation. The addition of Tween-80 to the production medium caused a statistically significant increase in enzyme activity compared with that of the control sample ($P \leq .05$) (Table 2).

Table 1. Effects of carbon and nitrogen sources on alkaline protease production

Carbon sources (g/L)	Protease activity (U/mL)	Relative activity (%)	Nitrogen sources (g/L)	Protease activity (U/mL)	Relative activity (%)
Glucose (Control)^{cdfghij}	598.88±6.20	100	Peptone+yeast extract (Control)^{bcdefg}	598.88±6.20	100
Mannose^{cdfij}	553.57±8.27	92.43	$(NH_4)_2SO_4$^{cdefgh}	379.83±11.02	63.42
Galactose^{deghij}	722.77±11.48	120.69	Casein hydrolysate^{defh}	656.03±11.25	109.54
Xylose^{efgh}	830.91±4.59	138.74	Soy flour^{efgh}	106.06±1.84	17.71
Lactose^{fhij}	583.77±7.81	97.48	Peptone+casein hydrolysate^{fh}	625.83±9.42	104.50
Fructose^{ij}	723.42±16.07	120.79	Gelatine^{gh}	227.36±4.82	37.96
Rhamnose^{je}	542.69±5.74	90.62	Yeast extract^{h}	641.74±6.2	107.16
Maltose^{ij}	518.66±48.91	86.60	Peptone	591.73±1.61	98.81
Arabinose^{j}	864.68±32.15	144.38			
Sorbose	792.59±14.24	132.35			

Carbon sources: (a) Control, (b) Mannose, (c) Galactose, (d) Xylose, (e) Lactose, (f) Fructose, (g) Rhamnose, (h) Maltose, (ı) Arabinose and (j) Sorbose.
Nitrogen sources: (a) Control, (b) $(NH_4)_2SO_4$, (c) Casein hydrolysate, (d) Soybean flour (e) Peptone+casein hydrolysate, (f) Gelatine, (g) Yeast extract, and (h) Peptone.
Different letters show significant differences according to least significant difference (LSD) test at $P \leq .05$.

Table 2. Effects of minerals and surfactant on alkaline protease production

Minerals and surfactant (g/L)	Protease activity (U/mL)	Relative activity (%)
Horikoshi-I (Control) ^{bcdgh}	598.88±6.20	100
$CaCl_2$ (0.1 g/L)^{cefgh}	576.47±1.15	96.26
$CaCl_2$ (1 g/L)^{defgh}	517.52±1.84	86.41
KH_2PO_4 (0.5 g/L)^{efgh}	564.29±5.97	94.22
KH_2PO_4 (2 g/L)^{g}	613.16±12.63	102.38
$MgSO_4.7H_2O$ (0.1 g/L)^{gh}	596.76±16.07	99.65
$MgSO_4.7H_2O$ (1g/L)^{h}	726.50±3.90	121.31
Tween 80 (0.25)	628.27±0.46	104.91

(a) Control, (b) $CaCl_2$ (0.1 g/L), (c) $CaCl_2$ (1 g/L), (d) KH_2PO_4 (0.5 g/L), (e) KH_2PO_4 (2 g/L), (f) $MgSO_4.7H_2O$ (0.1 g/L), (g) $MgSO_4.7H_2O$ (1g/L), (h) Tween 80 (0.25)
Different letters show significant differences according to least significant difference (LSD) test at $P \leq .05$.

Datta et al. (2017) obtained the highest protease production (0.128 U/mL) by *Aeromonas caviae* P-1-1 at pH 8 and 37°C after 42 h of incubation in a medium containing Tween-40. Zanphorlin et al. (2011) examined a new alkaline serine protease from the thermophilic fungus *Myceliophthora* sp. The researchers reported that the addition of Tween-80 caused an 80% decrease in enzyme activity. In another study investigating the effect of surfactants on the activity of proteases produced by *Bacillus cereus*; Tween-20, Tween-40, Tween-60, Tween-80, and Triton X-100 caused an increase in enzyme activity compared with the control sample (Esakkiraj et al., 2009).

CONCLUSION

Our results revealed that the concentration of alkaline protease produced by *T. thermohydrosulfuricus* isolated from sludge samples was greater than that of enzymes produced by many *Bacillus* and thermoalkaliphilic bacteria species. However, control and optimization of other process parameters are required to increase enzyme activity during large-scale production.

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CONFLICT of INTEREST
There is no content conflict of interest.

AUTHOR CONTRIBUTIONS
Bilge Sayın Börekçi: Formal analysis, Writing-original draft, Sedat Dönmez: Supervisor, Writing-review & editing, Conceptualization. Ayşe Avcı: Formal analysis, Writing-review & editing.

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ET ÜRÜNLERİNDE YAĞ İKAME MADDELERİNİN KULLANIMI

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ÖZ
Et ürünleri, üretimde kullanılan bileşenlere ve üretim tekniklerine bağlı olarak yüksek oranda hayvansal yağ içerebilmektedir. Hayvansal yağlar et ürünlerinin fizikokimyasal, tekstürel ve duyusal özelliklerinin gelişmesinde önemli roller üstlenebilmekte, ayrıca ürünlerin besleyici değerine katkıda bulunmaktadır. Bununla birlikte yüksek oranda doymuş yağ asitleri ve kolesterol içeren hayvansal yağların yüksek oranda tüketiminin bazı sağlık sorunlarına yol açabildiği bildirilmektedir. Bu kapsamda beslenme ve sağlık arasındaki ilişkiye yönelik artan tüketici bilinci yağı azaltılmış veya ikame edilmiş ürünlere olan ilgiyi artırmakta, bu nedenle et ürünlerinde hayvansal yağın azaltılması veya belirli oranlarda ikame edilmesi üzerine yapılan çalışmaların sayısı da gün geçtikçe artmaktadır. Et ürünlerinde hayvansal yağın ikame edilmesinde diyet lifleri, tahıllar, hayvansal proteinler, yenilebilir mantarlar ve organojeller gibi ikame maddeleri kullanılabilmektedir. Bu kapsamda kullanılan ikame maddelerinin hayvansal yağın üründe sağladığı olumlu etkileri karşılayabilmesi önemlidir. Mevcut bu çalışmada et ürünlerinde hayvansal yağ ikame maddelerinin kullanımı üzerinde durulmuş ve bu alandaki son araştırmalar hakkında ayrıntılı ve güncel bilgiler sunulmuştur.
Anahtar kelimeler: Et ürünleri, hayvansal yağ, yağ ikamesi, diyet lifi, oleojel

THE USE OF FAT REPLACERS IN MEAT PRODUCTS

ABSTRACT
Meat products may contain high amounts of animal fat, depending on the components used in production and production techniques. Animal fats can play an important role in the development of physicochemical, textural, and sensory properties of meat products, and contribute to the nutritional value of the products. However, it is reported that high consumption of animal fats containing high levels of saturated fatty acids and cholesterol may cause some health problems. In this context, rising consumer awareness of the relationship between nutrition and health increases the interest in fat-reduced or substituted products, therefore, the number of studies on reducing or substituting animal fat in meat products at certain rates is increasing day by day. Substitutes such as dietary fibers, grains, animal proteins, edible mushrooms, and organogels can be used to replace animal fat in meat products. It is important that the substitutes used in this context will be able to meet the positive effects of animal fat on the product. This current study focuses on the use of animal fat substitutes in meat products and provides detailed and up-to-date information on the latest research in this field.
Keywords: Meat products, animal fat, fat replacement, dietary fiber, oleogel

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GİRİŞ

Et ürünleri, kullanılan formülasyon ve işleme tekniğine bağlı olarak yüksek oranlarda hayvansal yağ içerebilmektedir. Bileşimde yer alan hayvansal yağ, et ürünlerine lezzet katmasının yanı sıra teknolojik ve arzu edilen tekstürel ve duyusal özellikleri geliştirmesi nedeniyle son ürün kalitesi üzerinde etkili olmaktadır (Ferro vd., 2021). Bununla birlikte yüksek oranda doymuş yağ asitleri ve kolesterol içeren hayvansal yağın fazla tüketiminin obezite, kalp-damar hastalıkları ve kanser gibi bazı sağlık sorunlarına neden olduğu bildirilmektedir. Dünya Sağlık Örgütü tarafından toplam enerji alımının en fazla %30'unun yağlardan alınması gerektiği, bu kapsamda doymuş yağların %10'u, trans yağların ise %1'i geçmemesi gerektiği ifade edilmiş ve tüketicilere hayvansal yağ alımını azaltmaları tavsiye edilmiştir (WHO, 2020; Manzoor vd., 2022). Beslenme ve sağlık arasındaki ilişkiye yönelik artan tüketici bilinci, yağı azaltılmış veya ikame edilmiş ürünlere olan ilgiyi artırmakta, bu kapsamda et ürünlerinde hayvansal yağın azaltılması veya belirli oranlarda ikame edilmesi üzerine yapılan çalışmaların sayısı da gün geçtikçe artmaktadır.

Birçok et ürününün hayvansal yağ içeriği, kullanılan hammadde, formülasyon ve işleme koşulları gibi faktörlere bağlı olarak değişkenlik gösterebilmektedir. Yağsız etle yapılan ürünlerde hayvansal yağ içeriği genellikle %10'dan daha az olmasına rağmen çoğu et ürününde daha yüksek seviyelerde hayvansal yağ bulunmaktadır (Dominguez vd., 2022). Örneğin, burgerler, kurutulmuş veya emülsifiye edilmiş et ürünleri %20-35 arasında hayvansal yağ içerebilmektedir. Ayrıca, bu tip ürünlerde kullanılan işleme tekniğine bağlı olarak, özellikle kurutulmuş ürünlerde meydana gelen nem kaybı nedeniyle son ürünün yağ oranı daha yüksek seviyelere de ulaşabilmektedir (Franco vd., 2020; Vargas-Ramella vd., 2020a; Ozaki vd., 2021; Öztürk-Kerimoğlu vd., 2021).

Et ürünlerinde hayvansal yağ içeriğinin azaltılması veya ikame edilmesi, hayvansal yağın üründe sağladığı olumlu etkiler mümkün olduğunca korunarak ve üretim maliyeti üzerinde önemli bir etkiye neden olmadan gerçekleştirilmelidir. Bu kapsamda et ürünlerinde hayvansal yağın kısmen veya tamamen diyet lifleri, tahıllar, hayvansal proteinler, hidrokolloidler, mantarlar veya bunların kombinasyonları ile değiştirildiği birçok çalışma bulunmaktadır. Diğer yandan hayvansal yağ yerine organojellerin kullanımına yönelik de gittikçe artan bir ilgi söz konusudur.

Et ürünlerinde hayvansal yağın azaltılmasına yönelik çalışmalarda farklı yöntem ve formülasyonlar üzerinden çeşitli ikame maddelerinin kullanıldığı stratejiler geliştirilmiş ve bu stratejilerin kendine ait avantaj ve dezavantajları bildirilmiştir. Bu çalışmalarda hayvansal yağın ikame edilebileceğine dair umut veren sonuçlar elde edilmiş olmasına rağmen, birçoğu endüstriyel düzeyde uygulama alanı bulamamıştır. Mevcut bu çalışmada, et ürünlerinde hayvansal yağ ikame maddelerinin kullanımına odaklanılmış ve bu alandaki güncel çalışmalar hakkında bilgiler verilmiştir.

ET ÜRÜNLERİNDE KULLANILAN YAĞ İKAME MADDELERİ

Diyet Lifleri

Nişasta yapısında olmayan polisakkarit türevleri olarak tanımlanan diyet lifleri, ince bağırsakta sindirime ve emilime dirençli olan, kalın bağırsakta kısmen veya tamamen probiyotik mikroorganizmalar tarafından fermentasyona uğrayan yenilebilir bitkilerin temel unsurlarındandır (LaCourse, 2008). Et ürünlerinde diyet liflerinin kullanımı, emülsiyon stabilitesi ile su ve yağ tutma kapasitesini artırması, pişirme kayıplarını azaltması, son ürünün dokusunu ve sululuğunu iyileştirerek tekstürü modifiye etmesi, depolama stabilitesini iyileştirmesi ve nötr bir tada sahip olması nedeniyle tercih edilebilmektedir (Madane vd., 2019). Ayrıca, bir yağ ikame maddesi olarak diyet liflerinin kullanımı, tüketimden sonra tokluk hissini etkilemeden et ürünlerinin enerji değerini azaltan bir strateji olarak da uygulanmaktadır (Carvalho vd., 2019). Bu stratejinin, et ürünlerinde hayvansal yağı azaltmanın neden olduğu teknolojik ve duyusal kusurları engellemek için etkili olabileceği ve ayrıca et ürünlerinin diyet lifleri ile zenginleştirilmesinin obezite, diyabet ve hassas bağırsak sendromu gibi çeşitli hastalıkların

başlamasını önlemede yardımcı olabileceği bildirilmiştir (Sofi vd., 2017).

Diyet liflerinin farklı et ürünlerinde hayvansal yağ ikamesi olarak kullanıldığı birçok çalışma bulunmaktadır (Çizelge 1). Yağı azaltılmış burger köfterlerinde diyet lifi olarak inülin, buğday ve yulaf lifi ile fruktooligosakkarit ilavesinin etkilerinin araştırıldığı bir çalışmada, teknolojik ve duyusal özelliklerde kontrole göre istatistiki açıdan bir değişiklik olmadığı, bununla birlikte %6 inülin kullanılan örneklerde daha yüksek duyusal puanların elde edildiği ancak pişirme sonrasında bu burgerlerde daha düşük verim ve sertlik değerlerinin belirlendiği bildirilmiştir (Bis-Souza vd., 2018). Diğer yandan yüksek oranda diyet lifi içeren hindiba kökü tozu ile üretilen az yağlı burgerlerde ise daha yüksek verim ve daha düşük pişirme kaybının bildirildiği bir çalışmada burgerlerin besinsel kalitesinin ve duyusal profilinin geliştiği ifade edilmiştir (El Zeny vd., 2019). Aslinah vd. (2018), yüksek diyet lifi içeriğine sahip adzuki fasulyesi ununu köfte üretiminde hayvansal yağ ikamesi olarak kullanmışlardır. Araştırmada, adzuki fasülyesi unu kullanım oranı arttıkça köftelerde pişirme verimi ve nem içeriği ile sertlik ve çiğnenebilirliğin arttığı, bununla birlikte %25 ve %50 oranında adzuki fasulyesi unu kullanılan köftelerde kontrol grubuna kıyasla daha yüksek duyusal kabul edilebilirliğin tespit edildiği bildirilmiştir. Yağı azaltılmış burgerlerde diyet lifi olarak bezelye lifinin kullanıldığı başka bir çalışmada ise fizikokimyasal, tekstürel ve duyusal özelliklerde kontrole göre önemli seviyede bir değişimin meydana gelmediği belirlenmiştir. Araştırma sonucunda bezelye lifinin, sığır eti burgerlerinde bir hayvansal yağ ikame maddesi olarak kullanımının umut verici bir strateji olabileceği ifade edilmiştir (Polizer-Rocha vd., 2019).

Çizelge 1. Yağ ikamesi olarak diyet lifleri, hayvansal proteinler ve tahılların kullanımı

Yağ İkame Maddesi	İkame Oranı	Et Ürünü	Sonuçlar			Kaynak
			Fizikokimyasal	Duyusal-Tekstürel	Besinsel	
Adzuki fasulyesi unu	%25 %50 %75 %100	Köfte	• Artan pişirme verimi • Yüksek nem içeriği • Su tutma kapasitesinde artış	• %50 oranında ikame kontrole kıyasla iyi kabul edilebilirlik • Sertlik ve çiğnenebilirlikte artış	• Yağ içeriğinde azalma • Protein içeriğinde değişim yok	Aslinah vd. (2018)
Kinoa tohumu	%2,5 %5 %7,5 %10	Burger	• Pişirme veriminde artış	• Duyusal kalitede değişim yok	• Protein ve lif içeriğinde artış	Baioumy vd. (2018)
Buğday, inülin, fruktooligosakkarit ve yulaf lifi	%3 %6	Burger	• İnülin, fruktooligosakkarit ile verimde değişim yok • TBARS değişim yok	• Buğday ve yulaf lifi ilavesi ile duyusal kabulde farklılık yok	-	Bis-Souza vd. (2018)
Buğday filizi-kollajen emülsiyonu	%5 %10 %15 %20 %25	Köfte	• Pişirme kaybında azalma • b* artış • pH artış	• Genel kabul edilebilirlik kontrol, %5 ve %10 emülsiyon grubunda yüksek	• Protein çözünürlüğünde artış	Kim vd. (2018)
Kinoa	%5 %10	Ciğer ezme	• Lipid oksidasyonunda azalma	• Sertlikte artış	• Yağda %8 oranında azalma	Pellegrini vd. (2018)

İnülin	%20 %30 %40 %50 %60	Sosis	• Nem içeriğinde artış • b* değerinde azalma • Pişirme verimi değişim yok	• Sertlik ve çiğnebilirlikte azalma • Duyusal özelliklerde gelişme	• Yağ içeriğinde azalma • Lif içeriğinde artış	Prapasuwannakul (2018)
Moringa oleifera tohum unu	%1 %3 %5	Salam	• Lipid oksidasyonda azalma	• Duyusal kalitede olumlu etki	• Besleyici özelliklerde artış	Aurıema vd. (2019)
Yulaf lifi, inülin	%1	Sosis	• Nem içeriğinde artış • TBARS değişim yok	• %6'ya kadar inülin ve %0.85'e kadar yulaf lifi ilavesi ile duyusal kabulde değişim yok	• Yağ içeriğinde azalma	Bis-Souza vd. (2019)
Hindiba kökü tozu	%25 %50 %75	Burger	• Pişirme kaybında azalma • Pişirme veriminde artış	• Duyusal profilde gelişim	• Besin profilinde gelişim	El Zeny vd. (2019)
Buğday lifi	%5 %10 %15 %20	Sosis	• Nem içeriğinde artış • Pişirme kaybında değişim yok	• Sertlikte artış • Duyusal kabulde farklılık yok	• Yağ içeriğinde azalma	Juhui ve Hack-Youn (2019)
Discorea alata L. - hidrolize kolajen	%20 %40 %60 %80 %100	Sosis	• Emülsiyon stabilitesinde gelişme • Pişirme veriminde gelişme	• %40 yağ değişimine kadar genel kabulde değişim yok	• Yağ ve enerji içeriğinde azalma • Protein miktarında artış	Olanwanit ve Rojanakorn (2019)
Bezelye lifi	%1	Burger	• Pişirme kaybı ve büzülmede değişim yok	• Duyusal kabulde farklılık yok • Sertlik ve çiğnebilirlikte değişim yok	• Yağ içeriğinde azalma • Protein içeriğinde değişim yok	Polizer-Rocha vd. (2019)
Frukto-oligosakkaritler ve probiyotik suşlar	%2	Fermente sosis	• Lipid oksidasyonunda değişim yok	• Sertlik ve çiğnenebilirlikte artış	• Aroma ve uçucu profilinde iyileşme	Bis-Souza vd. (2020)
Chia müsilajı	%2,5 %5	Et emülsiyonu	• Renkte artış • Emülsiyon stabilitesini iyileştirmiş	• Sertlikte artış	-	Câmara vd. (2020)
İnülin, β-glukan, üzüm kabuğu	%3 %6 inülin %0,5 %1 β-glukan %0,5 üzüm kabuğu	Sosis	• Parlaklıkta azalma	• Frankfurt sosislerde tekstürel değişim yok • İspanyol sosislerde sertlik ve çiğnenebilirlikte azalma	-	Egea vd. (2020)
Kinoa ve teff tohumu	%5	Sosis	• Pişirme verimi, su tutma kapasitesi, emülsiyon stabilitesinde artış	• Duyusal kalitede değişim yok • Yapışkanlık ve sertlikte azalma	• Yağ ve enerji içeriğinde azalma	Öztürk-Kerimoğlu vd. (2020)

Yulaf β-glukan	%15 %30	Burger	• Su tutma kapasitesi artış	• Doku parametrelerinde artış	• Kolesterol içeriği azalmış	Szpicer vd. (2020)
Peynir altı suyu protein izolatı	%15	Sosis	• Emülsiyon stabilitesinde gelişim • Pişirme kaybında iyileşme	• Sertlik ve çiğnenebilirlik gibi tekstürel profilde gelişim	-	Kwon vd. (2021)
İnülin bazlı emülsiyon jel	%50	Salam	• Lipid oksidasyonunda artış • Kırmızılıkta azalma	• Kohesivlikte artış • Tekstürde iyileşme • Tat ve aromada düşüş	• Lif içeriğinde artış	Paglarini vd. (2021)
Patlıcan tozu	%1 %2 %3	Sosis	• Su ve yağ bağlama özellikleri ile nem içeriğinde artış	• Duyusal özelliklerde gelişme	• Yağ içeriğinde azalma • Protein miktarı değişmemiş	Zhu vd. (2021)
Hurma tohumu tozu-jelatin jeli	%100	Burger	• b* değerinde artış	• Yumuşaklık ve çiğnenebilirlikte artış	• Besleyici profilde gelişim	Essa ve Elsebaie (2022)
Sığır jelatini	%25 %50 %75 %100	Köfte	• Pişirme kaybında azalma • L* ve a* değerlerinde artma • Nem içeriğinde artış	• %50 yağ ikameli grup en iyi kabul edilebilirlik göstermiş	• Yağ içeriğinde azalma • Protein içeriğinde artış • SFA'da azalma • PUFA'da artma	Gao vd. (2022)
Chia	%20 %40 %60 %80 %100	Köfte	• Nem içeriğinde artış • Yüksek lipit stabilitesi	• Duyusal kalitede değişim yok	• Yağ ve protein içeriğinde azalma	Liu vd. (2022)
İnülin ve/veya fruktooligosakkarit	%3,5	Köfte	• Nem içeriğinde artış	• Benzer duyusal kabul	• Yağ içeriğinde azalma • Protein içeriği değişim yok	Montoya vd. (2022)
Peynir altı suyu proteini	%5 %10	Sosis	• Emülsiyon stabilitesinde artış • Pişirme veriminde artış	• Sertlik ve çiğnenebilirlikte düşüş • Duyusal kalitede değişim yok	• Yağ ve enerji içeriğinde azalma • Protein miktarında artış	Öztürk-Kerimoğlu vd. (2022)
Chia	%12,5 %25 %37,5 %50	Burger	• Renkte koyulaşma • Verimde değişim yok	• Tekstür parametrelerinde değişim yok	• Lipid içeriği ve kalori değerinde azalma • Diyet lifi içeriğinde artış	Rampe vd. (2022)

Kenevir yağı-karabuğday tohumu emülsiyon jeli	%25 %50	Salam türü et ürünü Alheiras	• Nem içeriğinde artma • L* ve a* değerinde azalma • Pişirme kaybında değişim yok	-	• Protein içeriğinde artma • SFA'da azalma • PUFA'da artma	Botella-Martinez vd., (2023)
Chia	%50 %100	Burger	• Pişirme veriminde artış	• Sertlik ve çiğnenebilirlikte azalma	• Beslenme profilinde iyileşme	Badar vd. (2023)
Badem unu	%25 %50 %75 %100	Sığır eti köftesi	• b* değerinde azalma • Nem içeriğinde azalma • Pişirme veriminde artma • Büzülmede azalma	• %100 ikame en yüksek sertlik ve çiğnenebilirlik, en düşük kohezivlik ve esneklik göstermiş	• SFA'da azalma • Oleik ve linoleik asit içeriğinde artma	Kırkyol ve Akköse (2023)
Modifiye kinoa protein emülsiyonu	%25 %50 %75 %100	Sosis	• Nem içeriğinde artma • L* değerinde artma • a* ve b* değerinde azalma	• %50 yağ ikamesinde duyusal özelliklerde değişim yok • %100 yağ ikameli sosisler en düşük tekstürel parametreler göstermiş	• Protein içeriğinde artma	Zhao vd., (2023a).

Montoya vd. (2022) yaptıkları bir çalışmada domuz ve piliç köftelerinde yağ ikamesi olarak inülin ve/veya fruktooligosakkarit kullanmışlardır. Araştırmada domuz ve piliç köftelerinde inülin kullanımının duyusal olarak kabul edilebilir olduğu ve yağın teknolojik özelliklerini taklit edebildiği bildirilmiştir. Szpicer vd. (2020) ise sığır burger üretiminde yağ ikamesi olarak diyet lifi (yulaf β-glucan konsantresi) kullanımının fizikokimyasal ve duyusal özelliklere etkisini incelemişlerdir. Diyet lifi içeren burgerlerin, kontrol örneklerine göre daha düşük kolesterol içeriğine ve daha yüksek su tutma kapasitesi ile doku (sertlik, kohezivlik ve elastikiyet) parametrelerine sahip olduğu belirlenmiştir. Araştırmacılar, bu yaklaşım sayesinde duyusal olarak kabul edilebilir olan daha sağlıklı ve az yağlı burger üretiminin mümkün olduğunu ifade etmişlerdir.

Essa ve Elsebaie (2022) yaptıkları bir araştırmada hurma tohumu tozundan elde edilen diyet lifi ve jelatinden oluşturulan kompozit jelini sığır eti burgerinde yağ ikamesi olarak kullanmışlar ve bu jelin diyet lifi içeriği artırılmış, doymuş yağ içeriği ise azaltılmış fonksiyonel ürünler geliştirmek için uygun olduğunu ifade etmişlerdir. Sığır eti burgerinde bu kompozit jelin kullanımının pişirme özelliklerini geliştirdiği, yumuşaklık ve esnekliği artırdığı, daha yüksek parlaklık, kırmızılık ve sarılığa neden olduğu bildirilmiştir. Piliç köftesinde hayvansal yağın buğday filizi ile kolajenden oluşturulan bir emülsiyon ile değiştirildiği bir çalışmada ise pH, b* değeri, protein çözünürlüğü ve kesme kuvvetinin kullanılan emülsiyon seviyesine bağlı olarak arttığı tespit edilmiştir. Ayrıca yağı azaltılmış piliç köftelerinde L* ve a* değerleri ile pişirme kaybı ve çaptaki küçülmenin azaldığı bildirilmiştir (Kim vd., 2018).

Emülsiyon tipi piliç sosislerinde yağ ikame maddesi olarak buğday lifinin kullanıldığı bir çalışmada, yağ ikame oranı arttıkça daha yüksek nem içeriği ile daha düşük yağ içeriğinin belirlendiği ve daha sert ürünlerin elde edildiği tespit edilmiştir (Juhui ve Hack-Youn, 2019). Bis-Souza vd. (2019) düşük yağlı Brezilya sosisinde

yağ ikamesi olarak yulaf lifi ve inülin kullanmışlar ve nem içeriğinde artış ile yağ içeriğinde azalma tespit etmişlerdir. Aynı zamanda ürünlerde diyet lifi kullanımının TBARS değerlerini etkilemediği, %6'ya kadar inülin ile %0,85'e kadar yulaf lifi ilavesinin son üründe teknolojik parametrelerde ve duyusal kabul edilebilirlikte önemli bir değişime neden olmadığı belirlenmiştir. Aynı araştırmacılar tarafından yapılan bir başka çalışmada ise yağı azaltılmış fermente sosislerde fruktooligosakkaritlerin kullanımının kontrole göre lipid oksidasyonu üzerinde etkili olmadığı, ürünlerin aroma ve uçucu profilinde iyileşmeye yol açtığı, bununla birlikte sertlik ve çiğnenebilirlikte artışa neden olduğu bildirilmiştir (Bis-Souza vd., 2020).

Egea vd. (2020) domuz etinden üretilen Frankfurt ve İspanyol tipi sosislerde hayvansal yağ yerine inülin ve β-glucan kullanımının tekstürel özellikleri etkilemediğini belirlemişler ve bu tip ürünlerde hayvansal yağı azaltmak için diyet lifi kullanımının iyi bir strateji olabileceği sonucuna varmışlardır. Benzer bir diğer araştırmada da domuz etinden üretilen sosislerde yağ ikame maddesi olarak inülin kullanımının, ürünün duyusal kabulünü, teknolojik kalitesini ve besinsel özelliklerini iyileştirdiği rapor edilmiştir (Prapasuwannakul, 2018). Başka bir araştırmada ise domuz etinden yapılan hayvansal yağı azaltılmış sosislerde diyet lifi olarak farklı oranlarda patlıcan tozu kullanılmış, artan diyet lifi içeriğine bağlı olarak su ve yağ bağlama ile dokusal ve duyusal özelliklerinin arttığı bildirilmiştir (Zhu vd., 2021). Aurıema vd. (2019) tarafından yapılan bir araştırmada piliç etinden üretilen mortadellada yağ ikamesi için diyet lifi olarak *Moringa oleifera* tohumu unu kullanımının fizikokimyasal özellikler, kimyasal bileşim ve lipid oksidasyonu üzerindeki etkisi incelenmiştir. %3 veya %5 oranında *Moringa oleifera* tohumu unu kullanımının 90 günlük depolama süresince lipit oksidasyonunda azalma sağladığı belirlenmiş ve et ürünlerinde hayvansal yağın ikamesinde antioksidan aktivitesi nedeniyle *Moringa oleifera* tohumu ununun doğal fonksiyonel bir bileşen olarak kullanılma potansiyeline sahip olduğu ifade edilmiştir.

Paglarini vd. (2021) tarafından yapılan bir araştırmada Bologna tipi sosislerde hayvansal yağ yerine %50 ve %100 oranında soya yağı ve inülin bazlı emülsiyon jeli kullanımının kohesivliği artırdığı ve daha iyi bir tekstür profili sağladığı belirlenmiştir. Ancak, Bologna tipi sosislerde daha yüksek lipit oksidasyonu belirlenmiş, renk parametrelerinde değişiklikler meydana geldiği ve kontrole göre tat ve aromanın azaldığı ifade edilmiştir. Kırkyol ve Akköse (2023) sığır eti köftelerinde hayvansal yağ ikamesi olarak badem unu kullandıkları çalışmalarında, kontrol örneklerine göre ikameli köftelerde daha düşük nem içeriği ile TBARS değerlerinin elde edildiğini bildirmişlerdir. Ayrıca badem unu ikamesinin köftelerde pişirme verimini artırırken büzülmede azalmaya neden olduğu görülmüştür. Bununla birlikte badem unu kullanımıyla toplam doymuş yağ içeriğinde azalış, oleik ve linoleik asit içeriğinde ise artış meydana geldiği tespit edilmiştir.

Yukarıda verilen çalışmalar değerlendirildiğinde, et ürünlerinde hayvansal yağın diyet lifleri ile ikame edilmesinin fizikokimyasal, tekstürel ve duyusal özellikler üzerinde kullanılan diyet lifine, kullanım oranına ve kullanıldığı et ürününe bağlı olarak farklı etkiler oluşturabildiği görülmektedir. Bununla birlikte diyet lifi kullanımının genel itibariyle ürünlerin besleyici özelliklerini geliştiği ifade edilebilir. Et ürünlerinde diyet lifi olarak inülin, buğday ve yulaf lifi ile fruktooligosakkaritlerin kullanımı ön plana çıkmaktadır.

Tahıllar

Çeşitli tahıllar, fenolik maddeler ve vitaminler ile mineraller gibi sağlık açısından yararlı bileşikler içerdikleri için fonksiyonel gıdalar olarak kabul edilmektedir (Dominguez vd., 2022). Et ürünlerinde bazı tahılların tohum, un veya müsilaj gibi formlarda yağ ikame maddesi olarak kullanıldığı araştırmalar bulunmaktadır (Pintado vd., 2016; Ding vd., 2018; Rampe vd., 2022). Ayrıca son yıllarda, bu tahılların emülsiyon jellerinin fonksiyonel özelliklerinden dolayı et ürünlerinde hayvansal yağ ikamesi olarak kullanımına odaklanan araştırmaların sayısı da giderek artmaktadır (Liu vd., 2022).

Et ürünlerinde hayvansal yağın tahıllar ile ikame edilmesi üzerine yapılan çalışmalarda özellikle kinoa ve chia tohumlarının kullanımına odaklanılmıştır (Çizelge 1). Baioumy vd. (2018) yaptıkları bir araştırmada az yağlı burgerlerde kinoa unu kullanımının pişirme verimini artırdığını ve son ürünün duyusal kalitesini bozmadığını bildirmişlerdir. Burgerlerde kullanılan kinoa oranının artmasıyla protein ve lif içeriğinin arttığı, yağ ve enerji değerlerinin ise azaldığı bulunmuştur. Böylece hayvansal yağ yerine kinoa kullanılarak elde edilmiş burgerlerin besleyici özelliklerinin iyileştiği ifade edilmiştir. Başka bir çalışmada yağı azaltılmış emülsifiye sosislerde kinoa unu ve/veya teff tohumunun ilavesi hem duyusal kaliteyi etkilememiş hem de daha yüksek pişirme verimi, su tutma kapasitesi ve emülsiyon stabilitesi sağlamıştır. Bu sosislerde renkte değişim gözlenmezken, daha yumuşak bir tekstür belirlendiği bildirilmiştir. Ayrıca, kinoa unu ve/veya teff tohumunun formülasyona dahil edilmesiyle yağ içeriğinin %50'den fazla azaltılabildiği böylece genel olarak ürün kalitesinden ödün vermeden daha sağlıklı emülsifiye sosislerin elde edilebileceği ifade edilmiştir (Öztürk-Kerimoğlu vd., 2020). Pellegrini vd. (2018), emülsifiye bir et ürünü olan domuz ciğeri ezmesinde hayvansal yağ yerine kinoa kullanımının teknolojik, duyusal ve oksidatif özellikler üzerinde olumlu etkisi olduğunu bildirmişlerdir. Ayrıca, kinoa içeren domuz ciğeri ezmelerinde daha düşük lipid oksidasyonu ile daha sert bir tekstür belirlenmiştir.

Ding vd. (2018), hayvansal yağın chia tohumuyla değiştirilmesinin, jambon benzeri ürünlerde duyusal kaliteyi etkilemediğini, işleme özelliklerini ve oksidatif stabiliteyi iyileştirdiğini tespit etmişlerdir. Araştırmada, chia tohumu kullanımının duyusal, besinsel, teknolojik ve fizikokimyasal özellikleri iyileştirici etkilerinin olduğu bildirilmiştir. Pintado vd. (2016) ise chia unu kullanımının, emülsifiye sosislerde yağ azaltımının neden olduğu teknolojik ve duyusal kusurları ortadan kaldırmak için mükemmel bir strateji olduğunu ve yeniden formüle edilmiş sosislerde yağ ve enerji azaltımı ile lif zenginleştirmesi sağlandığını bildirmişlerdir. Diğer yandan chia ununun daha yüksek lipid oksidasyonuna neden olduğu, renkte, dokuda ve duyusal özelliklerde değişikliklerin gözlemlendiği de ifade edilmiştir. Yağı azaltılmış et emülsiyonunda hayvansal yağ yerine chia müsilajının kullanıldığı bir çalışmada, sertlik ve renk parametrelerinde belirgin bir artış olduğu, chia müsilajının su tutma ve jel oluşturma özelliklerinden dolayı emülsiyon stabilitesini iyileştirdiği bildirilmiştir (Câmara vd., 2020). Rampe vd. (2022) balık burgerde yağ ikamesi olarak chia müsilajı kullandıkları bir araştırmada, burgerlerin lipit içeriğinde ve kalori değerinde önemli bir azalma olduğunu ve bu örneklerin kontrole göre daha koyu bir renk yoğunluğuna sahip olduğunu bulmuşlardır. Başka bir çalışmada sığır köftelerinde yağ ikamesi olarak chia müsilajı ile hazırlanan emülsiyon jeller kullanılmıştır. Kontrol grubuyla kıyaslandığında ikameli köftelerin nem içeriğinin arttığı, yağ ve protein içeriğinin ise azaldığı bildirilmiştir. Araştırmada, emülsiyon jellerinin sığır köftesine pozitif özellikler kazandırdığı ve az yağlı sığır köftesi üretmek için uygun bir yaklaşım olduğu ifade edilmiştir (Liu vd., 2022).

Badar vd. (2023) tarafından yapılan bir çalışmada manda eti burgerlerinde yağ ikamesi olarak chia unu emülsiyon jelleri kullanılmıştır. Sertlik ve çiğnenebilirlik yağ ikame oranının artmasıyla azalmış ve %50 oranında yağın ikame edilmesinin teknolojik ve duyusal özelliklerden ödün vermeden manda burgerlerinin besleyici profilini iyileştirdiği belirlenmiştir. Başka bir çalışmada modifiye kinoa proteini emülsiyonunun sosislerde domuz sırt yağı ikamesi olarak kullanılmıştır. Araştırmada kinoa proteini emülsiyonu nem ve protein içeriğinde artışa, kırmızılıkta ise azalmaya neden olduğu tespit edilmiştir. Frankfurter tip sosislerde %50 oranında yağ ikamesinde diğer ikameli gruplara göre daha yüksek su tutma kapasitesi ile tekstürel özellikler elde edilmiş olup oksidatif stabiliteyi iyileştirmiş ancak duyusal özellikleri etkilememiştir (Zhao vd., 2023a). Botella-Martinez vd., (2023) yaptıkları bir çalışmada hayvansal yağ ikamesi olarak kenevir yağı ve karabuğday tohumu ile oluşturulan emülsiyon jelini geleneksel bir Portekiz et ürünü olan Alheiras üretiminde kullanmışlardır. Domuz sırt yağının %25 ve %50 oranında emülsiyon jeli

ile değiştirildiği bu çalışmada, nem ve protein içeriğinde artış olurken toplam doymuş yağ asitleri azalmış, çoklu doymamış yağ asidi miktarı ise artmıştır. Araştırmacılar, karabuğday unu kullanılarak üretilen emülsiyon jelinin hayvansal yağ ikamesi olarak kullanımının daha sağlıklı et ürünleri elde etmek için umut verici bir strateji olabileceğini ifade etmişlerdir.

Çeşitli tahılların yağ ikamesi olarak kullanımının, renk, doku ve oksidatif stabilitede değişikliklere yol açabildiği, bununla birlikte yeniden formüle edilmiş et ürünlerinde yağın azaltılmasının neden olduğu kusurları iyileştirmek için faydalı bir strateji olduğu görülmektedir. Bir kalite parametresi olarak yağın görünür nitelikte olmasının arzu edildiği sucuk gibi bazı et ürünlerinde, tahılların bir yağ ikamesi olarak kullanımının uygun olmayabileceği de dikkate alınmalıdır.

Hayvansal Proteinler

Peynir altı suyu proteini veya kolajenden elde edilen hayvansal proteinler, et ürünlerinin hayvansal yağ içeriğini azaltmak için etkili bir alternatif olarak görülmektedir. Bu proteinler, yüksek su ve yağ tutma kapasitesi ile emülsifiye etme ve jelleştirme gücü gibi teknolojik özelliklere sahip olduğu için et ürünlerinde hayvansal yağı azaltmanın neden olduğu kusurları önlemede etkili olabilmektedir. Farklı et ürünlerinde yağ ikamesi olarak hayvansal proteinlerin kullanıldığı bazı çalışmalar Çizelge 1'de verilmiştir.

Öztürk-Kerimoğlu vd. (2022) tarafından yapılan bir çalışmada, yağı azaltılmış emülsifiye sosislerde hayvansal yağ yerine mikro partiküllü peynir altı suyu proteini kullanılmış, hayvansal proteinin sosislerin teknolojik özelliklerini geliştirdiği ve lipit oksidasyonu ile toplam yağ ve enerji içeriğinde azalma ile protein miktarında artış sağladığı belirlenmiştir. Ayrıca sosislerde sertlik azalırken renk değişikliği oluşmadığı, duyusal kalitede ise değişim olmadığı tespit edilmiştir. Başka bir çalışmada sodyum dodesil sülfat (SDS) ilave edilmiş peynir altı suyu protein izolatı jeli ile üretilen emülsifiye sosislerin teknolojik özellikleri ile doku profilinin geliştiği bildirilmiştir (Kwon vd., 2021). Olanwanit ve Rojanakorn (2019) emülsifiye sosislerde yağ ikame maddesi olarak hidrolize kolajen kullandıkları bir çalışmada, emülsiyon stabilitesi ve pişirme verimi gibi teknolojik özellikler ile besin kalitesinde gelişme olduğunu tespit etmişler, yağ ve enerji içeriğinde azalma ile protein içeriğinde artış belirlemişlerdir. Ayrıca renk parametrelerinde bazı değişimler gözlenirken, %40 oranına kadar yağ ikamesinin genel kabul edilebilirlikte önemli bir farklılık göstermediği belirlenmiştir. Gao vd. (2022) düşük yağlı sığır eti köftelerinde hayvansal yağ yerine sığır derisi jelatini kullanmışlardır. Araştırmada jelatin ilavesinin sığır köftelerinin nem ve protein içeriğini artırdığı, toplam doymuş yağ asidi içeriğinde azalmaya toplam çoklu doymamış yağ asidi miktarında ise artışa neden olduğu tespit edilmiştir.

Çalışmalar değerlendirildiğinde, hayvansal proteinlerin bir yağ ikame maddesi olarak kullanımının az yağlı et ürünleri üretmek için etkili bir strateji olabileceği görülmektedir. Bununla birlikte, bu stratejinin duyusal kalite üzerindeki etkisinin büyük ölçüde değiştirilen yağ yüzdesine bağlı olduğu da dikkate alınmalıdır. Özellikle bazı çalışmalarda formülasyonda %50'den fazla yağ ikamesi söz konusu olduğunda duyusal özellikler üzerinde olumsuz bir etki olduğu bildirilmektedir (Alves vd., 2016; Olanwanit ve Rojanakorn, 2019; dos Santos vd., 2020). Sonuçta, bu stratejinin emülsiyon haline getirilmiş ürünler için uygun olabildiği ancak tüketicinin yağ parçalarını görmeyi beklediği ve bunu bir görünüm özelliği olarak olumlu bir şekilde değerlendirdiği ürünlerde kullanımının uygun olmadığı söylenebilir.

Hidrokolloidler

Hidrokolloidler, hidroksil gruplar (OH) açısından zengin hidrofilik kolloidler olarak adlandırılmaktadır (Mahmood vd., 2017). Et ürünlerinde yağ ikamesi olarak hidrokolloidlerin kullanıldığı birçok çalışma yapılmıştır (Çizelge 2). Kim vd. (2020) yaptıkları bir çalışmada yağı azaltılmış et emülsiyonlarında üzüm çekirdeği yağı, jelatin ve aljinat kullanmışlardır. Bu uygulamanın stabiliteyi, dokuyu ve pişirme kaybını iyileştirdiği, ayrıca TBARS değerini düşürdüğü belirlenmiştir. Diğer bir çalışmada emülsifiye sosislerde hayvansal yağ yerine kitre gamı ikame

maddesi olarak kullanılmıştır (Abbasi vd., 2019). Çalışmada ikame maddesi kullanılan örneklerde pişirme kaybının azaldığı, emülsiyon stabilitesi ve oksidatif stabilite ile nem içeriğinin arttığı, doku parametrelerinin değiştiği ancak renkte bir değişim görülmediği belirtilmiştir. Zhao vd. (2018) tarafından yapılan bir araştırmada ise hayvansal yağın rejenere selülozla ikamesinin emülsifiye edilmiş sosislerin duyusal kalitesini bozmadığı ve selülozun lipit oksidasyonunu inhibe ettiği ifade edilmiştir. Ayrıca, emülsiyon stabilitesini iyileştirdiği, su ve yağ salınımını önemli ölçüde azalttığı belirlenmiştir. Çalışmada artan rejenere selüloz miktarlarıyla sertlik önemli ölçüde artmış, renk parametrelerinde de değişim gözlenmiştir. Zhao vd. (2023b) tarafından yapılan bir başka çalışmada domuz yağı modifiye edilmiş çapraz bağlı nişasta ile ikame edilmiş ve nişasta kullanımının emülsiyonun toplam yağ ve doymuş yağ asidi (SFA) içeriğinde azalmaya neden olduğu, su adsorpsiyonu ve şişme kabiliyeti nedeniyle daha düzenli ve yoğun protein ağ yapısı oluşturduğu, ayrıca su tutma kapasitesini de artırdığı tespit edilmiştir.

Çizelge 2. Yağ ikamesi olarak hidrokolloidler, yenilebilir mantarlar ve organojellerin kullanımı

Yağ İkame Maddesi	İkame Oranı	Et Ürünü	Sonuçlar			Kaynak
			Fizikokimyasal	Duyusal	Besinsel	
Selüloz nanolif	%30 %50	Sosis	• Su tutma oranında artış • Pişirme kaybı ve kırmızılıkta azalma	• Tekstürün gelişimi	-	Wang vd. (2018)
Rejenere selüloz	%0,4 %0,8 %1,2	Sosis	• Su ve yağ salınımında azalma	• Sertlikte artış	• Toplam yağ içeriğinde azalma	Zhao vd. (2018)
Kitre gamı	%0,25 %0,5 %1	Sosis	• Pişirme kaybında azalma • Emülsiyon stabilitesinde artış • Nem içeriğinde artış	• Duyusal kalitede değişim yok	• Yağ içeriğinde azalma	Abbasi vd. (2019)
Flammulina velutipes yağ/ su emülsiyonu	%5 %10 %15 %20 %25 %30 %37	Sosis	• Nem içeriğinde artış	• %20 veya daha az bir seviyede duyusal özelliklerini olumsuz etkisi yok	• Yağ içeriğinde azalma • Protein içeriğinde artış	Yang vd. (2019)
İstiridye mantarı *(Pleurotus eryngii)*	%100	Sosis	• Su tutma kapasitesinde artış • Pişirme kaybında artış	• Duyusal kalitede azalma	• Yağ içeriği ve enerji değerinde azalma	Wang vd. (2019)
Etil selüloz ve adipik asit- soya fasulyesi yağı oleojelleri	%50	Burger	• Sarılıkta artış • Kırmızılıkta azalma	• Sertlikte artış • Genel kabulde azalma	-	Adili vd. (2020)
Karnauba mumu ve adipik asit-soya fasulyesi yağı oleojelleri	%50	Burger	• Sarılıkta artış • Kırmızılıkta azalma	• Sertlikte artış • Genel kabulde azalma	-	Aliasl khiabani vd. (2020)
Prosella®-kaplan cevizi yağı hidrojel	%50 %100	Burger	• Parlaklık ve sarılıkta artış • Nemde artış	• Benzer duyusal kabul	• Toplam yağ içeriği ve enerji değerinde azalma	Barros vd. (2020)

Agaricus bisporus ve *Pleurotus ostreatus*	%30 %50	Sosis	• Nem içeriğinde artış • Lipid oksidasyon stabilitesi	• Yumuşak tekstür	• Besin kalitesinde artış	Cerón-Guevara vd. (2020)
Keten tohumu yağı ve balmumu, γ-oryzanol, β-sitosterol oleojelleri	%20 %40	Fermente sosis	• Renk parametrelerinde değişim	• Sertlikte azalma • Genel kabulde artış	• Yağ içeriğinde değişim yok • PUFA'da artış	Franco vd. (2020)
Balmumu veya etil selüloz- yağ karışımı (zeytin, keten ve balık) oleojelleri	%100	Burger	• Lipid oksidasyonunda azalma	• Genel kabulde azalma	• SFA'da azalma • MUFA ve PUFA'da artış	Gómez-Estaca vd. (2020)
Jelatin ve aljinat, üzüm çekirdeği yağı	%1	Et emülsiyonu	• Emülsiyon stabilitesi ve pişirme kaybında iyileşme	• Tekstür profilinde gelişme	-	Kim vd. (2020)
Gellan sakızı, kestane unu- chia yağı hidrojeli	%5 %10	Burger	• Pişirme veriminde artış • Nem içeriği değişim yok	• Sertlik ve çiğnenebilirlikte azalma • Benzer duyusal kabul	• SFA'da azalma • MUFA ve/veya PUFA'da artış	Lucas-González vd. (2020)
Keten tohumu yağı -balmumu oleojeli	%30 %60	Ezme	• Nemde artış	• Sertlikte azalma • Duyusal kabulde azalma	• Protein ve yağ içeriklerinde azalma	Martins vd. (2020)
Zeytinyağı-aljinat ve karragenanla hidrojeli	%5 %10 %15	Burger	• Lipid oksidasyonunda azalma • Nemde artış	• Sertlikte azalma • Genel kabulde düşüş	• SFA'da azalma • MUFA ve PUFA'da artış	Özer ve Çelegen (2020)
Zeytinyağı-chia yağı karışımı-balmumu oleojel	%80	Fermente sosis	• Lipid oksidasyonda artış	• Sertlikte azalma • Genel kabulde azalma	• Toplam yağ içeriğinde azalma • PUFA'da artış	Pintado ve Cofrades (2020)
Aljinat bazlı, chia unu veya yulaf kepeği - zeytinyağı hidrojeli	%80	Sosis	• Lipid oksidasyonunda azalma • Kırmızılıkta azalma • Sarılıkta artış	• Benzer kesme kuvveti	• Yağ içeriği ve enerji değerinde azalma	Pintado vd. (2020)
Domuz derisi, bambu lifi ve inülin- kanola yağı ile hidrojeli	%50 %100	Sosis	• L* değerinde artış • a* değerinde azalma	• Tekstürde değişim yok • %50 yağ değişimi duyusal kabulü etkilememiş	• SFA'da azalma • PUFA'da artış	dos Santos vd. (2020)
Prosella®-zeytin, kanola ve soya fasulyesi yağı hidrojeli	%50	Fermente sosis	• Lipid oksidasyonunda değişim yok • Nemde azalma	• Genel kabul edilebilirlikte artış	• SFA'da azalma • MUFA veya PUFA'da artış	Vargas-Ramella vd. (2020b)
Prosella®- alg ve/veya buğday tohumu yağları hidrojel	%100	Burger	• Nemde artış	• Benzer duyusal kabul	• Toplam yağ içeriği ve enerji değerinde azalma	Barros vd. (2021)

Agaricus bisporus ve *Pleurotus ostreatus*	%50	Karaciğer ezme	• Emülsiyon stabilitesinde gelişim	• %50 yağ değişimi duyusal kabulü etkilememiş	• Diyet lifi ve proteinde artış • Yağda azalma	Cerón-Guevara vd., (2021)
Prosella®-avokado ve kabak çekirdeği yağları hidrojel	%100	Burger	• Parlaklık ve kırmızılıkta azalma • Nemde artış	• Benzer duyusal kabul	• Toplam yağ içeriği ve enerji değerinde azalma	Cittadini vd. (2021)
Chia müsilajı, aljinat bazlı ajan, peynir altı suyu proteini ve/veya kollajen karışımı-zeytinyağı hidrojeli	%100	Salam	• Lipid oksidasyonda artış	• Tam ikame ile kabulde değişim yok • Sertlik azalmış	• SFA'da azalma • MUFA ve/veya PUFA'da artış	Câmara vd. (2021)
Gliseril monostearat-ayçiçek yağı oleojeli	%25 %50 %75 %100	Salam	• Sarılıkta artış • Kırmızılıkta azalma	• Duyusal kalitede değişim yok	• SFA'da azalma • MUFA ve PUFA'da artış	Ferro vd. (2021)
Auricularia cornea	%25 %50 %75 %100	Sosis	• Pişirme kaybında artış • Nem içeriğinde artış	• Sertlik ve kohesivlikte azalma • Çiğnenebilirlik ve elastikiyette artma	• Yağ içeriği ve enerji değerinde azalma	Fu vd. (2021)
Balmumu- kolza yağı oleojeli	%100	Burger	• Parlaklık ve sarılıkta artış • Nem içeriğinde artış	• Sertlikte azalma	• Toplam yağ içeriğinde azalma	Gao vd. (2021)
Polisakkarit hidrokolloidler ve meyve kabuğu unları	%2 %3	Burger	• Nem içeriğinde artış • Büzülmede azalma • Pişirme veriminde artış	• Sertlik ve çiğnenebilirlikte artış	• Yağ içeriğinde azalma	Mousa vd. (2021)
İnülin, yumurta akı tozu ve jelatin karışımı- yer fıstığı ve keten tohumu yağı hidrojelleri	%50 %100	Sosis	• Oksidatif stabilitede azalma • Nem içeriğinde artış	• Duyusal kabulde artış • Sertlik artmış	• Toplam yağ ve enerji içeriği azalma	Nacak vd. (2021)
İnülin, jelatin, yumurta akı tozu ve mikrobiyal transglutaminaz - yer fıstığı ve keten tohumu yağı karışımı hidrojel	%50 %100	Isıl işlem görmüş sucuk	• Oksidatif bozulma, nem içeriğinde artış	• Benzer sertlik • Benzer duyusal kabul	• Protein ve PUFA'da artış • Yağ içeriği ve SFA'da azalma	Öztürk-Kerimoğlu vd. (2021)
Ayçiçeği/kimyon yağı karışımı-karnauba mumu oleojeli	%25 %50 %75	Köfte	• Lipid oksidasyonunda artış	• Sertlikte azalma • Genel kabulde artış	-	Palamutoğlu (2021)
Agaricus bisporus	%5 %10 %15	Burger	• Yüksek pişirme verimi • Çapta küçülmenin azalması	• Yumuşak tekstür • Duyusal kalitede gelişme	• Yağda azalma	Patinho vd. (2021)

Soya proteini izolatı ve aljinat-zeytinyağı hidrojeli	%100	Sosis	• L* ve b* değerinde düşüş • a* değerinde artış	• Tam ikame ile kabulde azalma • Sertlik ve çiğnenebilirlikte artış	• Toplam yağ içeriğinde azalma	Pintado vd. (2021)
Prosella®-zeytinyağı hidrojel	%100	Burger	• Sarılık ve kırmızılıkta artış • Nemde artış	• Benzer duyusal kabul	• Lipid profilinde gelişme	Teixeira vd. (2021)
Prosella®- fıstık ve hindistan cevizi yağları hidrojel	%100	Burger	• Parlaklık ve sarılıkta artış • Nemde artış	• Benzer duyusal kabul	• Toplam yağ içeriği ve enerji değerinde azalma	Foggiaro vd. (2022)
Etilselüloz oleojel ve nişasta hidrojeli ile farklı oranlarda bigelleri	%25 %50 %100	Burger	• Lipid oksidasyonunda artma • Büzülme değerinde azalma	• %50 oranına kadar kabul edilebilir duyusal özellikler • Sertlik ve çiğnenebilirlik artmış	-	Ghiasi ve Golmakani (2022)
Soya proteini ve inülin- soya fasulyesi yağı hidrojeli	%50 %100	Salam	• Lipid oksidasyonda artış • Parlaklıkta artış • Kırmızılıkta azalma	• Tam ikamede kabulde değişim yok • Sertlik azalmış	• SFA'da azalma • MUFA ve/veya PUFA'da artış	Paglarini vd. (2022)
Mantar polisakarit özü	%50 %100	Köfte	• Nem tutma iyileşmiş • Pişirme verimi korunmuş	• Sertlik ve çiğnenebilirlikte artış	• Yağ içeriğinde azalma	See Toh vd. (2023)
Tapyoka nişastası	%25 %50 %75 %100	Domuz eti emülsiyonu	• Su tutma kapasitesinde artış	• Sertlik ve çiğnenebilirlikte artış	• Toplam yağ içeriğinde azalma • SFA'da azalma	Zhao vd. (2023b)
κ-karragenan zeytinyağı oleojeli ve jelatin hidrojeli bigelleri	%50	Sosis	• Ağırlık kaybında artma • nem içeriği ve su aktivitesinde artma	• % 2 karragenan ve %4 jelatin içeren hidrojel bigellerinde en yüksek sertlik	• Yağ içeriği ve enerji değerinde azalma	Zampouni vd. (2024)

Mousa vd. (2021) tarafından yapılan bir çalışmada yağı azaltılmış sığır eti burgerlerinde yağ ikamesi olarak hidrokolloidlerden ve meyve kabuğu unlarından ikili ve üçlü kompozitler kullanılarak %95 yağsız burgerlerin geliştirilmesi amaçlanmıştır. Araştırmacılar arap zamkı, karboksimetil selüloz ve nar unu üçlü kompozitinin en yüksek nem ve lif içeriği ile en düşük enerji ve büzülme değerlerini sağladığını bildirmişlerdir. Bunun yanı sıra en yüksek sertlik, yapışkanlık ve esneklik bu kompozit grubunda belirlenmiş ve bu grubun kontrol örneklerine benzerlik gösterdiği ifade edilmiştir. Diğer bir etkili uygulama ise Wang vd. (2018) tarafından selüloz nanolifi kullanılarak %50 oranında yağı azaltılmış emülsifiye sosislerde belirlenmiştir. Sosislerin nem içeriğinde artış, pişirme kaybı ve kırmızılıkta azalma ile tüm tekstürel parametrelerde kontrol örneklerine kıyasla daha yüksek değerlerin elde edildiği tespit edilmiştir.

Sonuç olarak, az yağlı et ürünlerinin formülasyonunda hidrokolloidlerin kullanılması fizikokimyasal, tekstürel ve duyusal özelliklerde önemli değişimler meydana getirebilmektedir. Bunlar arasında renk değişimleri, azalan pişirme kayıpları ve artan emülsiyon stabilitesi ile reolojik ve tekstürel parametrelerdeki gelişim sayılabilir.

Duyusal kalite düşünüldüğünde ise kullanılan hidrokolloid ile kullanım oranı etkili olabilmektedir. Ayrıca, bu stratejinin uygulanması emülsifiye tip et ürünlerinde daha kolayken, tüketici kabulü için yağ görünümünün önemli olduğu diğer et ürünlerinde daha zor olabilmektedir.

Yenilebilir Mantarlar

Yenilebilir mantarların, protein ve diyet lifi bakımından zengin olduğu bildirilmektedir (Pérez-Montes vd., 2021). Bu nedenle mantarlar et ürünlerinde yağ ikamesinin neden olduğu kusurları azaltmak için büyük bir potansiyele sahiptir. Fakat et ürünlerinde kullanılacak olan yenilebilir mantarların enzimatik esmerleşmeyi önlemek için ağartma işleminden geçmesi gerektiği ifade edilmiştir (Kurt ve Gençcelep, 2018). Ayrıca, yenilebilir mantarlarda mikrobiyal bozulmayı engellemek için kullanım öncesinde nem içeriğinin azaltılması gerekli olmakla beraber, bu işlem diyet lifleri ve proteinlerin konsantrasyonu açısından da avantajlı olabilmektedir (Alnoumani vd., 2017). Yenilebilir mantarların bir diğer avantajı da yüksek oranda umami bileşikleri içermesi olup, bu durum et ürünlerindeki yağ ve sodyum içeriklerinin ikamesinde üründe meydana gelen lezzet değişimini kısmen azaltabilmektedir (Dominguez vd., 2022). Yenilebilir mantarların sağlığa olan faydaları bilinmesine rağmen, et ürünlerinde hayvansal yağ ikame maddesi olarak kullanılmaları oldukça yeni bir yaklaşımdır.

Farklı et ürünlerinde yenilebilir mantarların hayvansal yağ ikamesi olarak kullanıldığı çalışmalar Çizelge 2'de verilmiştir. Wang vd. (2019) tarafından yapılan araştırmada domuz sosislerindeki hayvansal yağın tamamını ikame etmek için çiğ ve farklı şekilde pişirilmiş istiridye mantarı (*Pleurotus eryngii*) kullanılmıştır. Mantar ikamesinin ürünlerin yağ içeriği ve enerji değerini düşürmesinin yanı sıra artan protein ve diyet lifi içerikleri gibi başka beslenme avantajları da sağladığı ifade edilmiştir. Ayrıca mantarların yağ ikame edici olarak kullanılması domuz eti sosislerinin rengini olumsuz yönde etkilememekle birlikte, sosislerin tekstürünün, pişirme kaybı ve su tutma kapasitesinin mantarlara uygulanan ön işleme bağlı olarak değişkenlik gösterdiği bildirilmiştir. Diğer yandan yağın mantar ile ikamesinin sosislerin duyusal kalitesinde azalmaya yol açtığı da gözlenmiştir. Yang vd. (2019) yaptıkları bir araştırmada yağ ikame maddesi olarak *Flammulina velutipes* mantarının emülsifiye sosiste kullanımı ile ürün üzerindeki etkilerini incelemişlerdir. Hayvansal yağ yerine mantar kullanılarak üretilen sosislerin nem ve protein içeriğinde artış, yağ içeriğinde azalma görüldüğü bildirilmiştir. Ayrıca artan ikame oranı ile pişirme kayıpları önemli ölçüde azalmış, ikame miktarı %20'den az olduğunda ise sosislerin sertliği ve çiğnenebilirliği azalmasına rağmen elastikiyet ve kohesivlik gelişmiştir. Araştırmacılar emülsifiye sosislerde duyusal özelliklerde olumsuz bir etki olmadan %20 veya daha az bir seviyede yağ ikamesi olarak *Flammulina velutipes* mantarının etkili bir şekilde kullanılabileceğini ifade etmişlerdir.

Patinho vd. (2021) dana burgerlerdeki yağ içeriğini %25, %50 ve %75 oranında pişirilmiş mantar (*Agaricus bisporus*) ile değiştirdikleri bir çalışmada, daha yüksek verim ve daha düşük çap küçülmesi belirlemişlerdir. Burgerlerin pişirme özelliklerinde iyileşme olduğu, bununla birlikte daha kırmızı ve koyu bir renk ile daha yumuşak bir tekstürün belirlendiği rapor edilmiştir. Araştırmacılar, yağın %50 veya %75 oranında *Agaricus bisporus* ile değiştirilmesinin daha sulu, yumuşak ve lezzetli olarak nitelendirilen burgerlere sebep olduğunu bildirmişlerdir. Başka bir araştırmada ise emülsifiye sosislerdeki yağ içeriğinin %30 veya %50 oranında ikame edilmesinde farklı iki mantar (*Agaricus bisporus* ve *Pleurotus ostreatus*) ununun etkisi incelenmiştir. Mantar ununun kullanımı sosislerin besin kalitesini artırmış, fakat daha koyu bir renk ile daha yumuşak bir tekstüre sebep olmuştur (Cerón-Guevara vd., 2020). Yine aynı araştırmacılar tarafından yapılan bir başka çalışmada ise ciğer ezmesinde yağ içeriği %50 oranında *Agaricus bisporus* ve *Pleurotus ostreatus* unları ile ikame edilmiştir. Mantar unlarının kullanımı emülsiyon stabilitesini iyileştirmesine rağmen rengi olumsuz yönde etkilemiş, nem, karbonhidrat, diyet lifi ve protein içeriklerini artırmıştır. Çalışmada %7,5 oranında mantar unu kullanımının aroma ve tat parametreleri için kabul

edilebilir puanlar aldığı bildirilmiştir (Cerón-Guevara vd., 2021).

Fu vd. (2021) yaptıkları bir çalışmada pişirilmiş sosislerde domuz yağı ikamesi olarak *Auricularia cornea* mantarının kullanımını değerlendirmişlerdir. *Auricularia cornea* kullanımı, pişirilmiş sosislerde protein ve nem içeriğinin yanı sıra pişirme kaybı, su tutma kapasitesi gibi teknolojik özellikler ile tekstürel özelliklerden elastikiyet ve çiğnenebilirlikte artışa neden olmuştur. Ayrıca mantar ikameli sosislerin tümü duyusal açıdan kabul edilebilir olarak değerlendirilmiş, bununla birlikte en yüksek duyusal puanların %75 *Auricularia cornea* kullanılan grupta elde edildiği ifade edilmiştir. See Toh vd. (2023) yaptıkları bir araştırmada gıda uygulamaları için yağ ikamesi olarak mantardan elde edilen β-glukan ve unların kullanılabilirliğini incelemişlerdir. β-glukan ve kurutulmuş mantar tozunun piliç köftelerinde kullanımının nem tutmayı iyileştirdiği, pişirme verimini ve gıda matrisinin genel yapısını koruduğu tespit edilmiştir. β-glukan ekstraktları ve kurutulmuş mantar tozu ile yağ ikameli piliç köftelerinin sertlik ve çiğnenebilirlik parametrelerindeki artışların, mantarın cinsine ve köfteye eklenen ekstrakt miktarına göre değiştiği ifade edilmiştir.

Araştırmalar incelendiğinde et ürünlerde bir yağ ikame maddesi olarak mantar kullanımının, genel itibariyle protein ve nem içerikleri ile diyet lifi miktarını artırırken yağ içeriğini azalttığı ve böylelikle de besinsel kalitede iyileşme sağladığı görülmektedir. Bununla birlikte mantar kullanımının oksidasyonu ve mikrobiyal gelişimi artırarak raf ömrünü azaltabileceği dikkate alınmalıdır. Duyusal açıdan ise kullanılan mantar çeşidine, kullanım oranına ve kullanıldığı ürüne bağlı olarak farklı etkiler bildirilmiştir. Sonuçta bu strateji, beslenme açısından ümit verici sonuçlar sağlamasına rağmen, fizikokimyasal ve duyusal özelliklerin değerlendirildiği daha fazla çalışmaya da ihtiyaç vardır.

Organojeller

Son yıllarda hayvansal yağın kıvam, görsellik, renk gibi özelliklerini taklit edebilecek yağ ikame maddelerinin üretilmesini ve kullanılmasını içeren stratejilere odaklanan birçok çalışma bulunmaktadır. Bu kapsamda, daha sağlıklı olarak nitelendirilen çeşitli sıvı yağların veya bunların karışımlarının bir jel ağı içerisinde immobilize edildiği iki farklı tip yağ ikame maddesi dikkat çekmektedir. Bunlardan birisi sıvı yağların oleojelatörler yardımıyla üç boyutlu bir ağ içerisinde tutulduğu oleojellerdir. Diğer bir jel tipi olan emülsiyon hidrojelleri ise jelleştirici maddeler kullanılarak su/yağ emülsiyonunun bir jel yapısında hapsedildiği maddelerdir (Domínguez vd., 2021a). Emülsiyon hidrojelinin oluşturulmasında kullanılan jelleştirici ajanlar, önceki bölümlerde adı geçen çeşitli proteinleri, polisakkaritleri, hidrokolloidleri ve bunların karışımlarını içermektedir. Önceden ele alınan bölümlerin aksine, bunların kullanımı emülsiyonu jelleştirmek amacıyladır. Ayrıca, her bir jelleştirme ajanının veya oleojelatörün türü, özellikleri, kombinasyonu ve oranları hem emülsiyon hidrojellerinde hem de oleojellerde farklı jel özelliklerinin elde edilmesiyle sonuçlanmaktadır. Oleojellerin yağ içeriği genellikle %90'dan fazla olduğu için kullanıldığı ürünlerde toplam yağ içeriğinde önemli bir azalma olmadığı, hidrojel emülsiyonlarında ise yağ içeriğinin yaklaşık %50 veya daha az olması sebebiyle nihai üründeki toplam yağ içeriğinde önemli bir azalma meydana geldiği bildirilmektedir (Domínguez vd., 2021a). Oleojellerin veya emülsiyon hidrojellerinin özelliklerini ve potansiyel kullanımlarını derinlemesine tartışan çok sayıda inceleme çalışması mevcuttur (López-Pedrouso vd., 2021; Domínguez vd., 2021b; Herrero ve Ruiz-Capillas, 2021; Serdaroğlu, 2021; Abdullah vd., 2022; Li vd., 2022).

Farklı et ürünlerinde organojellerin hayvansal yağ ikamesi olarak kullanıldığı çok sayıda çalışma bulunmaktadır (Çizelge 2). Sığır eti burgerlerinde hayvansal yağın %50'sinin soya fasulyesi yağı oleojelleri ile değiştirildiği çalışmalardan birinde jelleştirme ajanı olarak etil selüloz ve adipik asit karışımı (Adili vd., 2020) diğerinde ise karnauba mumu ve adipik asit kullanılmıştır (Aliasl khiabani vd., 2020). Her iki çalışmada da yağın ikame edilmesiyle et ürününün sertliği artarken, sarılıkta artış ve kırmızılıkta düşüş olduğu bildirilmiştir. Ayrıca burgerlerin genel kabul edilebilirliğinde

azalma olduğu tespit edilmiştir. Başka bir çalışmada domuz eti burgerlerinde yağ ikamesi için balmumu veya etil selüloz kullanılarak bir yağ karışımı (zeytin, keten ve balık) ile yapılan oleojel kullanılmıştır (Gómez-Estaca vd., 2020). Bu çalışmada, burgerlerde oleojellerin kullanımının SFA'yı önemli ölçüde azaltırken, tekli doymamış yağ asitleri (MUFA) ile çoklu doymamış yağ asitlerini (PUFA) artırdığı bildirilmiştir. Bununla birlikte oleojel içeren burgerlerde duyusal açıdan tat parametresinde düşük puanlar elde edilmesi nedeniyle, genel kabul edilebilirlikte bir azalma olduğu ifade edilmiştir. Gao vd. (2021) tarafından yapılan bir çalışmada ise balmumu ve kolza yağı ile oluşturulan oleojeller sığır kalp eti burgerlerinde yağ ikamesi olarak kullanılmıştır. Burgerlerde oleojel kullanımının parlaklık ve sarılık parametrelerinde artış meydana getirdiği belirlenmiştir. Diğer yandan yağın oleojelle ikame edilmesi nem içeriğinde artışa ve toplam yağ içeriğinde azalmaya neden olurken, protein içeriğinde bir değişim gözlenmemiştir. Burgerlerde yapılmış bu iki çalışmada da hayvansal yağ yerine oleojel kullanımıyla sertliğin azaldığı, lipid oksidasyonunun arttığı tespit edilmiştir. Bu stratejiyle Palamutoğlu (2021) tarafından yapılan bir diğer araştırmada ise ayçiçeği/kimyon yağı karışımı ve karnauba mumu ile üretilen oleojel, dana eti köftelerindeki hayvansal yağın ikamesi için kullanılmıştır. Çalışmada lipid oksidasyonunda önemli bir artış ve sertlikte azalma gözlenmişken, duyusal açıdan genel kabul edilebilirlikte artış sağlandığı tespit edilmiştir.

Ferro vd. (2021) yaptıkları bir araştırmada gliseril monostearat ile immobilize edilmiş ayçiçek yağı oleojelini salamlarda kısmi veya tam yağ ikamesi için kullanmışlardır. Oleojel kullanımının toplam yağ içeriğini değiştirmediği, ancak yağ profili açısından SFA'yı azaltarak, MUFA veya PUFA'yı artırdığı belirlenmiştir. Ayçiçek yağı oleojeli içeren salamlarda renk parametrelerinden sarılığın artış, kırmızılığın ise azalma gösterdiği fakat duyusal olarak kontrole kıyasla herhangi bir farklılık olmadığı bildirilmiştir. Kuru fermente sosislerde zeytinyağı-chia yağı karışımı ve balmumu ile oluşturulan oleojelin (Pintado ve Cofrades, 2020) ve keten tohumu yağı ve balmumu veya sterol bazlı oleojelatörler (γ-oryzanol & β-sitosterol) ile oluşturulan oleojellerin kullanıldığı (Franco vd., 2020) çalışmalar mevcuttur. Pintado ve Cofrades (2020), %80 oranında hayvansal yağ ikamesinin toplam yağ içeriğinde azalmaya ve lipid oksidasyonunda bir artışa neden olduğunu, Franco vd. (2020) ise hayvansal yağın %20 veya %40 oranında değiştirilmesinin yağ içeriğinde herhangi bir etki oluşturmadığını ifade etmişlerdir. Her iki çalışmada da yeniden formüle edilen sosislerde PUFA miktarının arttığı, SFA ve MUFA'da bir azalma görüldüğü bildirilmiştir. Bununla birlikte, oleojelatör olarak balmumunun kullanılması, kuru fermente sosislerde nem içeriğini ve sarılığı artırırken, sertliği azaltmış böylelikle genel kabul edilebilirlikte azalma meydana geldiği bildirilmiştir. Martins vd. (2020) ise keten tohumu yağı ve balmumu ile oluşturulan oleojelin domuz ciğeri ezmesinde kullanımının nemde artışa, sarılık ile protein ve yağ içeriklerinde azalmaya neden olduğunu bildirmişlerdir. Bu çalışmada da PUFA'da önemli bir artış, SFA ve MUFA'da ise azalma görülmüştür. Bununla birlikte ezmelerin sertliğinde ve duyusal kabulünde bir azalma olduğu tespit edilmiştir.

Sonuç olarak et ürünlerinde yağ ikame maddesi olarak oleojel kullanım stratejisinin, tekstürel ve fiziksel özelliklerde değişimlere neden olabildiği görülmektedir. Özellikle ürünlerin sertliğindeki azalma ve renk açısından sarılığındaki artış dikkat çekmektedir. Ayrıca ürünlerdeki lipid oksidasyonunda da artış bildirilmektedir. Yüksek yağ içeriği (yaklaşık >%90) nedeniyle oleojel kullanımı et ürünlerinde toplam yağ içeriğini önemli oranda değiştirmemekte fakat SFA'da net bir azalma, MUFA ve/veya PUFA'da artış sağlamaktadır. Bütün bunlarla beraber ürünün genel kabul edilebilirliğinde bir azalma olduğunu da vurgulamak gerekir.

Oleojeller kadar hidrojellerin de burger ve sosisler gibi birçok et ürününde yağ ikamesi olarak kullanıldığı çalışmalar bulunmaktadır. Burgerlerin hidrojeller ile yeniden formüle edilmesinde aljinat bazlı Prosella® adlı ticari bir jelleştirici maddenin yaygın olarak kullanıldığı görülmektedir. Bu jelleştirici madde ile farklı yağlar kullanılarak oluşturulan hidrojellerin bir hayvansal yağ ikamesi

olarak farklı formülasyonlara sahip burgerlerde kullanımına yönelik çok sayıda çalışma bulunmaktadır (Barros vd., 2020; 2021; Cittadini vd., 2021; Teixeira vd., 2021; Foggiaro vd., 2022). Bu çalışmaların birçoğunda lipid oksidasyonunun hidrojel kullanımından etkilenmediği bildirilmiştir. Diğer yandan renk açısından önemli değişikliklerin meydana geldiği tespit edilmiştir. Hidrojel kullanılarak yağın ikame edilmesi sığır eti ve domuz eti burgerlerinde parlaklığı ve sarılığı artırırken (Barros vd., 2020; Foggiaro vd., 2022), at etinden üretilen burgerlerde parlaklığı ve kırmızılığı azaltmıştır (Cittadini vd., 2021). Ayrıca keçi eti burgerlerinde sarılığı ve kırmızılığı artırdığı gözlenmiştir (Teixeira vd., 2021). Bu renk farklılıkları muhtemelen kullanılan et türünün yanı sıra hidrojel oluşturmada kullanılan yağların renk özelliklerinden kaynaklanmaktadır. Çalışmalarda belirlenen bir diğer dikkat çekici sonuç ise hayvansal yağın hidrojel kullanılarak ikame edilmesinin tekstürel özelliklerde önemli değişimlere neden olmamasıdır. Bununla birlikte genel olarak burgerlerin tümünde nem içeriğinde artış, toplam yağ içeriği ve enerji değerinde bir azalma meydana gelmiş, protein içeriği ya değişmemiş ya da azalma eğilimi göstermiştir. Ayrıca tüm çalışmalarda kullanılan hidrojele bağlı olarak SFA içeriğinde azalma, MUFA ve/veya PUFA içeriğinde artış ile lipit profilinde gelişme gözlendiği bildirilmiştir.

Benzer sonuçlar burgerlerde farklı jelleştirici maddeler kullanılarak oluşturulan hidrojellerin kullanıldığı diğer bazı araştırmalarda da tespit edilmiştir. Örneğin, jelleştirici ajanlar olarak gellan sakızı ve kestane unu kullanılarak chia yağından oluşturulan emülsiyon hidrojelinin domuz eti burgerlerinde hayvansal yağ ikamesi olarak kullanıldığı bir çalışmada parlaklık ve sarılıkta artış, kırmızılık ve sertlikte ise azalma tespit edilmiştir (Lucas-González vd., 2020). Özer ve Çelegen (2020) tarafından yapılan bir çalışmada ise dana eti burgerlerinde hayvansal yağın kısmen veya tamamen aljinat ve karragenanla oluşturulan zeytinyağı emülsiyon hidrojeli ile ikame edilmesinin, lipit oksidasyonunu etkilemediği, ürünün kırmızılığında ve parlaklığında değişikliklere neden olduğu ve sertliği azalttığı bildirilmiştir. Ayrıca yağ asidi profilinde iyileşmenin (SFA'da azalma, MUFA ve PUFA'da artış) yanı sıra nem içeriğinde artış, yağ içeriği ve enerji değerinde ise düşüş tespit edilmiştir. Diğer yandan araştırmada hidrojel kullanılarak üretilen burgerlerin tüketici tarafından kabul edilebilirlik puanlarının, kontrol numunelerine kıyasla daha düşük olduğu ifade edilmiştir.

Sosis üretiminde domuz derisi, bambu lifi ve inülin kullanılarak oluşturulan kanola yağı hidrojelinin hayvansal yağ ikamesi olarak kullanıldığı bir çalışmada, %50 oranında yapılan ikamenin genel kabul edilebilirlikte önemli farklılıklar oluşturmadığı, fakat %100 ikamenin azalmaya yol açtığı gözlenmiştir (dos Santos vd., 2020). Diğer bir çalışmada ise inülin, yumurta akı tozu ve jelatin karışımı kullanılarak yer fıstığı ve keten tohumu yağı ile hidrojel oluşturulmuş ve emülsifiye edilmiş sosislerde yağ ikamesi olarak kullanılmıştır (Nacak vd., 2021). Sosislerde hayvansal yağın kısmen veya tamamen hidrojel ile değiştirilmesinin ürünün genel kabul edilebilirliğini artırdığı tespit edilmiştir. Hayvansal yağın zeytinyağı hidrojeli ile ikamesinin araştırıldığı bazı çalışmalarda ise farklı jelleştirici ajanlar kullanılmıştır. Yapılan bir çalışmada soya proteini izolatı ve aljinat bazlı jelleştiriciler ile oluşturulan zeytinyağı hidrojeli frankfurter tipi sosislerde (Pintado vd., 2021), bir diğer çalışmada ise chia müsilajı, aljinat bazlı ajan, peynir altı suyu proteini ve/veya kollajen karışımı ile oluşturulan zeytinyağı hidrojeli salamlarda kullanılmıştır (Câmara vd., 2021). Frankfurter tip sosislerde tam ikamenin tüketici kabulünde azalmaya yol açtığı, bununla birlikte salamlarda duyusal kalitenin değişmediği bildirilmiştir.

Paglarini vd. (2022) yaptıkları bir araştırmada salamlarda besin profilini geliştirmek için soya proteini ve inülin kullanılarak soya fasulyesi yağı hidrojeli kullanımını incelemişlerdir. Araştırmacılar hem tam hem de kısmi yağ ikamesinin duyusal puanlamada düşüşlere sebep olmasına rağmen kontrol numunelerine göre anlamlı bir fark oluşturmadığını ifade etmişlerdir. İncelenen bu çalışmaların tümünde genel olarak hidrojel kullanımının ürünlerde lipid oksidasyonunda önemli bir artışa sebep olduğu, parlaklıkta artış ve kırmızılıkta ise azalmaya yol

açtığı bildirilmiştir. Ayrıca ürünlerin nem içeriğinde artış, toplam yağ içeriği ile enerji değerinde düşüş gözlenmiştir. SFA'da azalma, MUFA ve/veya PUFA'da bir artış olduğu, ancak lipit profilinin ürünlere göre farklılık gösterdiği, bunun da kullanılan yağa bağlı olduğu bildirilmiştir. Diğer yandan hidrojel kullanımı ile salamlarda sertlik azalırken, emülsifiye sosislerde artmış, Frankfurter tipi sosislerde ise farklılık gözlenmemiştir.

Hayvansal yağ yerine emülsiyon hidrojelleri kullanılarak yeniden formüle edilmiş taze veya fermente sosisler üzerine yapılmış çalışmalar da bulunmaktadır. Pintado vd. (2020) tarafından yapılan bir çalışmada jelleştirici ajanlar olarak aljinat, chia unu veya yulaf kepeği kullanılarak zeytinyağı hidrojelleri oluşturulmuş ve taze sosislerde (longaniza) %90 oranında hayvansal yağ yerine kullanılmıştır. Hidrojel kullanımı sosislerin tekstürü üzerinde herhangi bir etki meydana getirmezken, lipit oksidasyonunu ve kırmızılığı (chia unu kullanılan hidrojelde) azaltmış, sarılığı ise artırmıştır. Ayrıca nem içeriğinin arttığı, protein ve yağ içeriği ile enerji değerinin düştüğü, lipit profilinde ise doymuş yağ asitlerinin azaldığı bildirilmiştir. Öztürk-Kerimoğlu vd. (2021) ısıl işlem görmüş sucukta inülin, jelatin, yumurta akı tozu ve mikrobiyal transglutaminaz jelleştirici ajanlarında immobilize edilmiş yer fıstığı ve keten tohumu yağı karışımı ile oluşturulan hidrojelleri kullanmışlardır. Araştırmacılar oksidasyon, nem içeriği, protein ve PUFA'da önemli bir artış, yağ içeriği, enerji değeri ve SFA'da ise azalma bildirmişlerdir. Bununla birlikte yer fıstığı ve keten tohumu ile oluşturulan emülsiyon hidrojel kullanımının ısıl işlem görmüş sucukların genel kalitesini etkilemediğini tespit etmişlerdir. Benzer sonuçların elde edildiği kuru fermente sosislerde yapılan bir başka araştırmada soya proteini izolatı ve jelatin ile jelleştirilen zeytinyağı ve chia yağı emülsiyon hidrojel karışımı kullanılmıştır (Pintado ve Cofrades, 2020). Duyusal analiz sonucunda zeytin ve chia yağı emülsiyon hidrojel kullanımının kuru fermente sosislerde genel kabulde önemli bir düşüşe neden olduğu belirlenmiştir.

Vargas-Ramella vd. (2020b) ticari bir jelleştirici ajan olan Prosella® kullanarak zeytin, kanola veya soya fasulyesi yağları ile oluşturulan hidrojellerin hayvansal yağ ikame maddesi olarak kuru fermente sosislerde etkilerini incelemişlerdir. Çalışmada lipid oksidasyonunda herhangi bir değişiklik olmadığı bildirilmişken, yağ içeriği ve SFA'da azalma ve protein, MUFA ve/veya PUFA'da ise artış olduğu gözlenmiştir. Araştırmada ilgi çekici bir sonuç olarak hidrojel kullanımının kuru fermente sosislerin genel kabul edilebilirliğini artırdığı tespit edilmiştir. Zampouni vd. (2024) yaptıkları araştırmada zeytinyağı bijelini sosislerde domuz sırt yağı ikamesi olarak kullanmışlardır. Çalışmada bijel kullanılan sosislerin ağırlık kaybı, nem içeriği ve su aktivitesinin arttığı, fakat renk özelliklerinde kontrole göre önemli bir değişimin olmadığı ifade edilmiştir. Benzer bir çalışma Ghiasi ve Golmakani (2022) tarafından sığır eti burgerlerinde hayvansal yağ ikamesi olarak etilselüloz bazlı oleojel ile nişasta bazlı hidrojelden elde edilen ayçiçek yağı bijeli kullanılarak yapılmıştır. Araştırmada, burgerlerde hayvansal yağ ikamesi olarak %75 oleojel fraksiyonlu bijel kullanımının uygun olduğu, ayrıca %50'ye kadar hayvansal yağ ikamesinin kabul edilebilir duyusal özellikler sergilediği ifade edilmiştir.

Et ürünlerinde hayvansal yağ yerine hidrojel kullanımının, hidrojel oluşturmada kullanılan jelleştirici maddelere ve yağlara bağlı olarak ürünlerin tekstür ve renk parametrelerinde önemli değişimlere neden olduğu görülmektedir. Emülsiyon hidrojellerinin düşük yağ içeriği (%20-50) et ürününün toplam yağ içeriğinin azalmasını sağlarken, yüksek su içeriği (~%50) nem içeriğinde artışa neden olmaktadır. Konu ile ilgili yapılan bazı çalışmalarda duyusal açıdan genel kabul edilebilirlikte azalma olduğu bildirilmiş olmasına rağmen, genel olarak emülsiyon hidrojellerinin kullanımı ürünlerin duyusal kalitesini korumuş veya artırmıştır. Ayrıca, hidrojel kullanımının SFA'yı azaltması ve MUFA ve/veya PUFA'yı artırması ürünlerin lipid profilinde belirgin bir iyileşme sağlamıştır.

SONUÇ

Bu çalışmada, et ürünlerinde yağ ikame maddelerinin kullanımı üzerinde durulmuş ve hayvansal yağ miktarının azaltılması veya ikamesi üzerine yapılmış araştırmalar hakkında özet bilgiler sunulmuştur. Bu kapsamda bir hayvansal yağ ikame maddesi olarak diyet liflerinin kullanımı kalite özellikleri dikkate alındığında az yağlı et ürünleri üretmek için etkili bir strateji olarak ön plana çıkmaktadır. Kinoa ve chia gibi tahıllar, ürünlerde yağın azaltılmasına ilaveten besinsel avantajlar sağlaması nedeniyle bir başka etkili yaklaşım olarak göze çarpmaktadır. Hayvansal proteinler, hidrokolloidler ve yenilebilir mantarların et ürünlerinde yağ azaltımının neden olduğu teknolojik ve duyusal kusurları azaltabildiği belirlenmiştir. Et ürünlerinde hayvansal yağın ikame edilmesinde, lipit profilinin geliştirilmesi ve insan sağlığı için besleyici açıdan avantajlı ürünlerin elde edilmesi de istenebilmektedir. Bu amaca et ürünlerinde hayvansal yağların yerine ω-3 ve ω-9 yağ asitleri bakımından zengin sıvı yağların oleojel ve hidrojel formunda kullanılmasıyla da ulaşılabilir.

Yağ ikamesi olarak kullanılan maddelerin yüksek su ve yağ bağlama kapasiteleri emülsifiye et ürünlerinde emülsiyon kararlılığını artırarak hem görünümde hem de dokuda iyileşme sağlayabilmektedir. Ayrıca diyet lifi, tahıllar, vb. gibi ikame maddeleri, antioksidatif özellikleri nedeniyle, ürünlerin oksidatif stabilitesi üzerinde olumlu bir etkiye de sahiptir. Emülsifiye et ürünlerinde arzu edilen özellikler korunarak yağ ikame maddelerinin kullanılması nispeten daha kolay bir uygulama olup, gözle görülebilir yağların istenildiği et ürünlerinde, hayvansal yağa benzer görünüm özelliklerine sahip yağ ikame maddelerinin kullanılması gerekmektedir. Bu kapsamda hidrojellerin kullanımı, düşük üretim maliyetleri, katı benzeri özellikleri hem hidrofilik hem de lipofilik bileşikleri çözme olasılıkları ile hayvansal yağı taklit etme kapasiteleri nedeniyle öne çıkmaktadır. Et ürünlerinde yağ ikamesi olarak kullanılacak maddelerin seçiminde hem ürün özelliklerinin hem de tüketici tercihlerinin dikkate alınması gerekmektedir. Bu kapsamda çeşitli et ürünlerinde farklı yağ ikame stratejilerinin üretim ve depolama boyunca araştırıldığı yeni çalışmalara da ihtiyaç duyulmaktadır.

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PLANT-BASED MEAT: A SUSTAINABLE ALTERNATIVE TO MEAT

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ABSTRACT
Due to the increase in the world population, the amount of meat used in human consumption has also increased in recent years. On the other hand, it is clear that animal-based meat production cannot sustain this growth and results in more pollution, land and water use, greenhouse gas emissions and biodiversity loss than the pollution occurring in plant food production. For this reason, there has recently been a trend towards new protein sources that meet the protein requirements of the human diet and improve animal welfare without increasing the carbon footprint. To respond to this increase and to to mitigate the adverse effects associated with animal production, plant-based meat production (PBM) has recently received attention. Here we have tried to provide detailed information about the production methods, product features and consumer preferences of PBM alternatives.
Keywords: Plant based meat, product features, consumer preferences, production methods

BİTKİ BAZLI ET: ETE SÜRDÜRÜLEBİLİR BİR ALTERNATİF

ÖZ
Son yıllarda artan dünya nüfusuna bağlı olarak insan beslenmesinde kullanılan et miktarı da artış göstermektedir. Buna karşın hayvansal et üretiminin bu artışı karşılayamayacağı ve bitkisel gıda üretiminde meydana gelen kirlilikten daha fazla kirliliğe, arazi kullanımına, su kullanımına, sera gazı oluşumuna ve biyolojik çeşitlilik kaybına yol açmakta olduğu gerçeği ortadadır. Bu sebeplerle insan beslenmesinde protein ihtiyacını karşılayacak ve karbon ayak izinin artışına yol açmayacak ayrıca hayvan refahını iyileştirecek yeni protein kaynaklarına yönelim son zamanlarda artış göstermiştir. Bu artışı karşılayabilmek ve hayvansal üretimde meydana gelen olumsuzlukları bertaraf etmek için bitki bazlı et (BBE) üretimi son zamanlarda ilgi uyandırmaktadır. Tüketicinin et yerine bitki bazlı yapay et (BBE)'i tercih etme ya da etmemesinin sebeplerinin ve tercih ettiğinde kendisine nasıl fayda sağlayacağının bilinmesi gerekmektedir. Burada bitki bazlı et alternatiflerinin üretim yöntemleri ürün özellikleri ve tüketici tercihleri ile ilgili detaylı bilgiler vermeye çalıştık.
Anahtar kelimeler: Bitki bazlı et, ürün özellikleri, tüketici tercihleri, üretim yöntemleri

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INTRODUCTION

It is estimated that food production accounts for 20-25% of human greenhouse gas emissions, with most of the carbon footprint coming from animal production (Bhattacharyya et al., 2023; Ahmad et al., 2022). It is reported that the world population will reach 9 to 10 billion people by 2050, the global demand for meat will reach 455 million tons, and the demand for food will increase by 98% (Rubio et al., 2020; Bhattacharyya et al., 2023). In this sense, the biggest challenge for global food security is meeting the demand for protein in a healthy and environmentally friendly way. In livestock production, it is reported that 81,7% of protein is lost during the conversion of feed and grass protein into meat protein (Zhang et al., 2022). Moreover, without alterations to current food production and supply systems, the rapid increase in global demand for animal protein is anticipated to be inconsistently fulfilled (Huang et al., 2022). In particular, livestock farming causes more pollution, land and water use, greenhouse gas emissions, and biodiversity loss than plant-based food production (McClements & Grossmann, 2021). Furthermore, producing one unit of plant-based protein requires only one-twentieth of the land resources needed to produce an equivalent unit of conventional meat protein (Papies et al., 2020). For these reasons, there has recently been a trend towards new protein sources that meet the protein needs of human nutrition and improve animal welfare without increasing the carbon footprint. Reflecting this trend, plant-based meat alternatives and cell-based meat have recently been launched to meet consumer demands and shape the future of foods (Kumar et al., 2022; Wang et al., 2023).

Extensive land usage is often leading to deforestation through the process of clearing land for grazing purposes. Livestock production systems also disrupt nitrogen and phosphorus cycles, affecting air, water and soil quality. This means that a transition to sustainable production and a shift of consumers to sustainable foods can prevent future environmental crises. Although animal-based meat is a valuable source of nutrients, reducing meat consumption is necessary because of the negative impacts of meat consumption on the environment and human health (Bhattacharyya et al., 2023). Today, as the world's population grows, our food choices influence not only our health and well-being, but also climate change and the future of our planet. Global food and agricultural productions account for a third of all greenhouse gas emissions, and animal-based meat production causes twice as much pollution as plant-based food production (Bhattacharyya et al., 2023). Compared to traditional animal-based proteins, the production of alternative plant-based proteins results in significantly fewer greenhouse gas emissions (e.g. 1/8 CO_2 equivalent per kilogram for chicken, 1/12 for beef and 1/9 for pork). (Ye and Mattila 2022). Excessive meat consumption has also been associated with several health problems. Red meat contains high amounts of cholesterol and long-chain saturated fatty acids, so excessive consumption of red meat may increase the risk of chronic diseases (Godfray et al., 2018). More than 1.8 million people die from ischemic heart disease every year, and a quarter of them are linked to excessive consumption of certain meat products (Rubio et al., 2020). In addition, consuming fatty meat increases the risk of cardiovascular disease and cancer, and consuming wild meat increases the risk of transmission of the virus from wild animals to humans. The results of a clinical trial reported that participants who used plant-based meat instead of animal-based meat for eight weeks exhibited a lower risk of cardiovascular disease (for example, reduction in fasting serum trimethylamine-N-oxide levels) (Crimarco et al., 2020).

It is believed that the health and environmental impacts of animal meat production and consumption can be eliminated by consuming sustainable foods such as plant-based meat alternatives to animal protein sources.

PLANT BASED MEAT PRODUCTION

Plant-based meats (PBM) are made from plant-based ingredients (such as beans, legumes, lentils and grains) and provide a sustainable source of protein that is similar to animal meat in texture, flavor, color and nutrition profile (Santo ve ark.,

2020). Innovations in protein ingredients evolve with diverse portfolios through the use of new proteins such as fava bean protein and mung bean protein, as well as microalgae, seaweed and fungi, and sweet lupine (chosen for its lack of alkaloids) (Boukid 2021). Besides all these plant protein sources, insects and single-cell proteins have recently gained interest as alternative protein sources due to their high nutritional value and protein content, sustainability and affordability (Boukid 2021). Commonly used plant protein sources are cereal (rice, wheat, oat, barley, core), legumes (soy, peas, lentils, beans, edamame), oil seeds (sunflower, sesame, canola, coconut), green leaf (beet, alfalfa, algae, duckweed), nuts (peanut, almond, pistachio, macadamia), pseudo cereal (chia, foxtail, quinoa), and others (mushroom, potato) (Wang et al., 2023).

In a study measuring the effectiveness of consumer reactions to plant-based burgers, 368 consumers rated images of the same plant-based burger online, along with information about the type of plant protein (soy, pea, or wheat). Plant-based protein type (soy, pea and seitan (wheat)) had no effect on consumer response, but meat reduction attitudes had a significant and strong impact (Moussaoui et al., 2023).

Table 1 lists the most commonly used protein sources for plant-based meat production, their functional properties and their uses in plant-based meat production.

Table 1. Summary of already used protein ingredients for meat analogue applications (Kyriakopoulou et al., 2021).

Protein Ingredient	Composition (%*w*/*w*)	Functionality	Application in Meat Analogues
Soy isolate (alkaline/acid precipitation treatment)	~90 % protein	Gelling, Good solubility and emulsification	Structuring process: Extrusion, spinning, freeze structuring, shear cell Role: Protein source, binder, texture, base for fat substitutes, emulsifier Products: Burger patties, sausages, minced meat
Soy isolate (additional heat treatment/ toasted isolate)	~90 % protein, denatured due to heat treatment	Decreased solubility, good gelling and increased water holding capacity	Structuring process: Shear cell, Extrusion, Role: Texture, Protein source, binder, base for fat substitutes Products: Burger patties, minced, sausages, meat
Soy concentrate	~70 % protein	Good texturization properties	Process: Shear cell, Extrusion, Role: Protein source, binder, texture Products: Burger patties, sausages, muscle-type products, minced meat
Soy milk spray dried powder	>45% protein, ~30 % fat	Good emulsification properties, high solubility	Process: Freeze structuring Role: Texture, emulsifier Products: Production of yuba and tofu
Soy flour/meal defatted	~43–56% protein, ~0.5-9% fat, ~3–7% crude fibre, >30% total carbohydrate	Native protein, Water binding capacity and fat retention	Process: Extrusion Role: Binder, Texture Products: Burger patties, sausages, muscle-type products, minced meat
Wheat Gluten isolate	75–80% protein, 15–17% carbohydrates, 5–8% fat	Dough forming/ Cross-linking capacity via S-S bridges, binding, low solubility	Structuring process: Shear cell, Extrusion Role: Texture, Adhesion Products: Muscle type products, Burger patties,
Pea isolate	~85% protein	Water and fat binding, emulsification, and firm texture after thermal processing	Process: Spinning, extrusion, shear cell Role: Binder, emulsifier, texture Products: Burger patties, sausages, minced meat, muscle-type products

Yang et al. (2023) presented the contents of four different plant-based meat analogs purchased from the market in America and China (Table 2). In their study, the protein level of meat was higher than 25 g/100 g, and the protein content of selected plant-based meat analogs was between 14.1-19.8 g/100 g. The average sodium content of the meat analogs used in the study was roughly 7.9 times higher than that of meat. Compared with red meat, plant-based meat analogs presented lower protein digestibility and released less bioactive peptides after in vitro digestion. In this instance, the consumption of plant-based meats presents certain drawbacks when compared to the consumption of animal meat in the human diet. PBMs accessible in the market mimic meat in three forms: grounds (such as patties, burgers, and nuggets), emulsions (sausages), and loose "crumbles" (chili meats or taco) (Pingali et al., 2023).

Table 2. The ingredient of the four plant-based meat analog products (Yang et al., 2023).

Product	Country	Ingredient
P1	America	Water, Methyl cellulose, Rapeseed oil, Refined coconut oil, Natural flavors, Rice protein, Cocoa butter, Potato starch, Phospholipid, Pea protein, Concentrated apple, lemon and pomegranate juice, Concentrated beet juice, Minerals and salt etc.
P2	America	Water, Soy leghemoglobin, Sunflower oil, Coconut oil, Zinc gluconate, Natural flavors, Potato protein, Cultured glucose, Yeast extract, Modified starch, Salt, Soy protein, Thickener (E461), Antioxidant (E306), Isolated soy protein, Niacin, Vitamin B1, B2, B6, B12, etc.
P3	China	Water, Soy protein, Guar gum, Cheese, Protein powder, Starch acetate, Protein solution, Sunflower oil, Spices, Acid hydrolyzed vegetable protein, Methyl cellulose, Edible glucose, Coconut oil, Salt, Yeast extract, Arabic gum, Beet juice extract, 5'-disodium nucleotide.
P4	China	Water, Isolated soy protein, Beet powder, Vital wheat gluten, Methyl cellulose, Vegetable oil, Edible glucose, Yeast extract, Pea protein, Lohan-kuo extract, Arabic gum, Starch acetate, Spices, Salt, etc.

Plant and fungi-based meat products incorporate the flavor, texture, and/or nutritional properties of meat, but have different compositions. Plant-based meat products can be divided into two flexible categories based on development time and technological complexity: traditional meat analogues and new plant-based meats. Traditional meat analogs have existed for thousands of years in Asia and include relatively simple derivatives of soy (such as tofu and tempeh) or wheat (seitan). On the other hand, new PBMs are characterized by the design and marketing of products that are almost equivalent to animal meat in all aspects, including taste, texture and nutritional value (Rubio et al., 2020).

Typically, PBM production involves three stages.
-Protein Isolation: Target plant proteins are extracted from plants, some of which undergo hydrolysis to improve their functionality such as solubility and cross-linking capacity.
-Formulation: The texture of the meat is improved by mixing ingredients such as plant proteins, food adhesives, plant-based oil and flour. Nutrients are added to match or exceed the nutritional profile of the meat.
-Processing: Plant proteins and other ingredients are mixed to create a meat-like structure through a protein reshaping processes (including stretching, kneading, cutting, pressing, folding and extruding) (Rubio et al., 2020).

A sustainable substitute to intensive animal-based meat production ought to be the development of plant-based meat alternatives with a fibrous and meat-like texture, that could be produced by means of extrusion technology, shear cell technology, self-assembly, spinning (electrospinning and wet-spinning), freeze casting, and by culturing mycoproteins (Zhang et al., 2022).

Extrusion is one of the cheap and short-term methods often used in the production of meat analogues to imitate the structural and textural properties of meat. The extrusion process can be divided into two groups based on moisture content: low moisture extrusion (20-35%) and high moisture extrusion (50-70%). High moisture extrusion of plant proteins is suitable for the production of meat analogues due to the targeted fiber structure that the cooling zone of this extrusion can provide. The use of high temperature and pressure causes changes in protein structure, gelatinization of starch, and destruction of anti-nutrient compounds in the process (Aydar et al., 2023).

The machines currently used for the production of PBMs are mainly divided into single-screw extruders and twin-screw extruders, depending on the number of screws (Wang et al., 2023). For high moisture extrusion, an interlocking and co-rotating twin-screw extruder is used, which mainly consists of the screw in the extruder barrel and the cooling die installed at the end. Approximately five steps are required for protein texturing from raw materials to the final extruded product, including raw material supply, mixing with water, melting, mold forming (die) and cooling (Zhang et al., 2022).

Innovative technologies are used to improve the organoleptic properties of PBMs include mycelium cultivation, 3D printing, shear cell technology and recombinant proteins (Rubio et al., 2020).

Traditionally, plant-based meat alternatives have been developed based on recipes that have been around for decades. The quality characteristics of meat substitutes, such as consistency, taste and color, depend on the choice of ingredients. The average consumer's choice of alternative meats is heavily influenced by excellent taste and flavor. Flavors, spices and precursors are used together with iron complexes to mimic the taste of meat (Ahmad et al., 2022). The meat alternative formula contains approximately 50-80% water, 4-20% non-textured protein, 10-25% plant protein, 3-10% flavor enhancing additives, 0-15% fat, 0-5% coloring agents (beet juice, carrot juice extract, lactoferrin and red yeast rice) and 1-15% binding substances. When combined, these ingredients provide meat alternatives with the essential sensory and textural properties. The high water content not only reduces costs, but also provides the required hydration, works as a softener during the process and aids emulsification. Protein added for nutrition provides texture, taste and physical appearance. Textured proteins can be replaced by mixing proteins from non-meat sources with meat or by replacing meat entirely with textured proteins to produce vegan and vegetarian foods. Meat extenders do not have the appearance, texture or taste of meat when cooked, but when mixed with meat they improve the overall quality characteristics of the product. Meat alternatives, on the other hand, are designed to mimic the texture, appearence, taste and color of meat when cooked without meat-containing ingredients (Sha and Xiong, 2020). Studies have shown that the addition of red beet, monascus red, oleoresin paprika, sorghum, and cacao to PBM as a single pigment does not adequately mimic the target color values for the exterior and interior of cooked meat. It has been shown that the cooked color of PBM can be achieved by using an optimized mixing ratio of red beet and cacao pigments (0.4 to 1.5 mg/g red beet and 1.1 to 1.3 mg/g cacao pigments). Furthermore, sensory evaluation showed that the color of PBM with optimum pigments was most similar to a beef patty, increasing the general acceptability of the improved appearance properties (Bakhsh et al., 2022; Ryu et al., 2023).

PBMs are frequently labeled using vegan and/or meat-like names (for example vegan meatloaf or soy burger). There is no clear definition of what

the term "vegan" means and no regulation explaining whether PBM can be labeled using meat-like names (Domke, 2018). Lima et al., (2023) found that the labels of 59 plant-based products sold in ten supermarkets in Brazil frequently included the phrase 100% vegetable.

Whole-muscle meat, on the other hand, has a fine texture that is microscopically similar to myofilaments in terms of tenderness as well as juiciness, making it difficult, if not impossible, to make from plant proteins. As a result, product development research on plant-based substitutes has been generally limited to restructured or reconstructed products. These meat-free products can be divided into two main groups: coarse-grained products and fine-grained products. Coarse-grained products include meatless burgers, patties, sausages and chicken nuggets. Fine-grained products are frequently emulsified products such as sausages and alternative salami. (Sha & Xiong, 2020).

Plant-based meat analogs contain proteins, fats or oils, carbohydrate sources, flavourings, coloring and binding agents. All these factors can contribute to meat analogues that resemble animal meat in terms of nutritional, textural and organoleptic properties. For instance, protein sources such as soy, gluten and pea proteins have nutritional and texturizing properties. They are also used for their other functional properties such as water and oil binding, emulsification, foam stabilization and gel formation during processing. Fats increase the juiciness, tenderness, nutritional value and overall taste of emulsion-type meat analogues. They are also significant components as key determinants of storage stability (Chen et al., 2023).

Animal meat products are commonly accepted by consumers due to the chewy taste provided by their fibrous structure. Although commercially available plant-based protein meats can largely mimic the fibrous taste of different animal meat products, there are still some problems with the overall texture and quality. Although some reconstituted ground meat products have been produced commercially in imitation of animal meat, whole meat (e.g. steak) has a complex hierarchical structure of muscle tissue, adipose tissue and connective tissue surrounding the muscle fibers. The complexity of muscle fibers makes it very difficult to fully understand their physical, chemical and functional properties. How to convert plant globular proteins into meat-like fibers to meet tissue necessities is a vital area of future research (Liu et al., 2023).

Most meat substitutes are derived from soy protein because it has particularly desirable properties and are available at low prices. In addition to soy protein, other proteins from oilseeds and proteins obtained by fermentation by microorganisms on various substrates are used in the production of meat substitutes. Currently, meat substitutes are produced using proteins derived from cereals such as corn, rice, wheat, defatted oilseeds, bean flour and cereals, defatted derivatives of soy flour and wheat flour, soy protein concentrates and wheat flour. Fermentation technology is also used to create meat color (Ou et al., 2023).

When the raw materials are heated, chemical changes occur that change the spices and flavors added to the premix. In addition, depending on the nature of these compounds, complex chemical reactions can occur at high pressure and temperature, releasing volatile components and causing significant loss of taste. In addition, heat treatment such as extrusion causes flavor components such as salt, acid compounds and sugar to interact with the protein network, leading to changes in taste quality and, as a result, changes in structural and textural characteristics. It can also affect Maillard or other chemical reactions (Ahmad et al., 2022). For this reason, it is very important to optimize the flavor and taste quality of plant-based meat and to control the quality of raw materials and the appearance of flavor.

The main organoleptic (i.e. sensory) characteristics of meat are appearance, aroma, taste and texture. Depending on the product, PBMs aim to mimic the appearance of raw or precooked meat. Heat-stable fruit and vegetable extracts (e.g. apple pulp, beet juice) or

recombinant heme proteins (e.g. LegH) are used to both regenerate the color of fresh meat and turn it brown when cooked. Some newer PBM products display visible semisolid plant-based oils (e.g., coconut oil, cocoa butter) to mimic the appearance of oil. Engineering is essential to comprehensively express the taste, smell, flavor and aroma of meat. Meat analogues contain flavor additives to add, enhance or mask specific flavors and typically represent 3 to 10% of the product. Many plant proteins have a bitter and astringent taste and require post-processing to selectively remove these compounds. Soy products in particular have a strong grassy, beany and bitter flavor related to saponin, lipoxygenase, and isoflavone compounds, which can be reduced by heating or germination. Developed in the 1980s, synthetic meat flavors consist of sugars, amino acids, nucleotides, glycoproteins, monosodium glutamate, salt and fat and have been shown to be equal to or better than meat extracts via sensory panels. Recombinant protein additives such as LegH can affect both the taste and color of PBMs. PBM texture may be affected by high moisture extrusion mycelium cultivation, shear cell technology, and 3D printing. Shear cell technology, extrusion and 3D printing are based on applying thermal, mechanical, and shear stresses to protein mixtures to obtain semi solid fibrous structures. Although many strategies are existing to design and tune the structure of plant proteins, it can be challenging to balance processing techniques to achieve the preferred mechanical properties while maintaining nutritional value. In contrast, mycelium cultivation includes the growing filamentous fungi, some strains of which resemble the microstructure of meat. Quorn™, a fungal based meat analogue, has provided alternative to meatballs, chicken nuggets, and minced meat since the 1960s. New startups grow mycelium to produce high quality meats such as steaks (Rubio et al., 2020).

THE PLACE OF PLANT BASED MEAT IN NUTRITION

The main plant proteins used in PBM formulations (pea, soy, and wheat) provide the same level of total protein content as animal meat. However, complementing more than one plant-based protein is often necessary to provide a balanced amino acid profile. For example, legume proteins (low in sulfur containing amino acids and high in lysine) and grain proteins (low in lysine and high in sulfur containing amino acids) proteins are suitable complements. Factors that reduce nutrient bioavailability of plant proteins after ingestion include protein structure, proteolysis-resistant structures, and antinutrients (such as tannins, phytates, and lectins). Some processing techniques such as soaking, heating, sprouting have been shown to increase digestibility (Rubio et al., 2020).

Nutrition also varies between traditional and new PBM products. For instance, tofu (traditional PBM) and Impossible™ (new PBM) share several benefits over animal meats, such as containing dietary fiber and mineral contents and lack of cholesterol. However, tofu-specific benefits include fewer calories, less fat, and no sodium. Impossible™-specific benefits include higher protein and vitamin B12 content. Concerns about the inclusion of LegH in PBM have been expressed with reference to correlations between heme iron intake and increased risk of diabetes (Rubio et al., 2020). Yeo et al., (2023) reported that, compared to meat products commonly consumed in Singapore, plant-based meat analogues contained significantly higher calcium, manganese, iron, magnesium, sodium and copper than meat products. They also reported that meat products had significantly higher mean potassium concentration compared to PBM.

Besides appearance, texture and flavor, nutritional value is also a crucial factor in why consumers select plant-based meat analogs. In a study on the nutritional composition of meat products and traditional meat products, Bohrer (2019) found that meat-like products were lower in saturated fatty acids and cholesterol and higher in carbohydrates and dietary fiber than traditional meat products. Zhou et al. (2021) investigated the in vitro digestion properties of beef and beef analogs. The results showed that the beef analog protein was digested faster in the stomach, but the protein and fat digestibility of the beef analog was

lower. In a study by Xie et al. (2022), plant-based analogues of beef and pork were found to have lower digestibility and release fewer potentially bioactive peptides than beef and pork.

CONSUMER OPINIONS ABOUT PLANT BASED MEAT

Plant-based products contain a wider range of phytochemicals and nutrients than animal meat. PBM meat reduces greenhouse gas emissions by 78-96%, resulting in a lower carbon footprint than conventional meat. Additionally, PBM production is more sustainable because it causes less damage to biodiversity. Plant-based meat alternatives, as sustainable products, have received increasing attention in recent years due to their potential to decrease the environmental impacts during production and consumption. Yet, it is hard to convince consumers of sustainable consumption through PBM. Consumers believe that PBMs are better for the environment and their health, but only a minority choose to buy PBMs. Moreover, meat consumption can only be reduced if meat consumers are convinced that sustainable food consumption brings environmental and personal benefits. Therefore, understanding consumers' perceptions of PBM is crucial from both environmental and marketing perspectives (Bhattacharyya et al., 2023). In a study conducted in South Africa, Szejda et al. (2021) reported that knowing about PBM has a significant positive relationship with PBM purchase intention. A recent study using Nielsen Consumer Panel data (Cuffey et al., 2021) shows that the majority of consumers did not consume PBMs until 2019. It also found that PBM spending among consumers dropped 75% after the first purchase, indicating that most consumers are buying PBM to try it out rather than consuming it on a regular basis.

Based on home scanner data for nearly 39 000 households in the United States from 2018 to 2020, 80% of households never purchased PBM and instead purchased only ground beef. Additionally, 17% of households purchased both ground beef and PBM. Of the remaining households that bought PBMs during the survey period, 40% were novelty seeking and one time purchasers (Neuhofer ve Lusk, 2022). Zhao et al. (2022) analyzed market spending data from 2017 to 2020 to evaluate consumers' PBM demand in the United States. According to data, US consumer purchasing patterns indicate that PBM is a complementary product to beef and pork and a substitute for chicken, turkey and fish.

Motivators behind consumer purchase/ consumption of plant-based meat substitutes may relate to traditional factors (taste, cost and convenience) and/or emerging factors (health and fitness, environment, safety, animal welfare and familiarity). Demotivators behind consumer purchase/consumption of plant-based meat substitutes may relate to health, environmental awareness, familiarity, meat attachment, meat enjoyment, men food, and food nephobia (Boukid 2021).

To be successful, plant-based meat alternatives must taste like meat. Taste (including mouthfeel) is very important in motivating regular meat consumers to change their eating habits by reducing meat consumption (Tuorila & Hartmann, 2020). Ideally, it is significant to mimic the properties of meat products before, during and after cooking. For instance, a beef steak analogue ought to be shiny, pinkish-reddish and tough before cooking, while becoming dull, brownish, tender and juicy after cooking, and this transition should take place under the same time temperature conditions as seen in a real beef steak (McClements and Grossmann, 2021). According to an online survey study with participants from Germany (N= 1039), meat substitutes have the best chance of effectively replacing meat when they thoroughly resemble highly-processed meat products in texture and taste and are offered at a competitive price. It is therefore recommended that alternative meat producers focus on imitating processed meat products rather than imitating cuts of meat such as steak or escalope (Michel et al., 2020).

Despite increasing consumer awareness of environmental issues, the consumption of plant-based protein foods instead of meat appears to face several obstacles in Western countries.

Consumers are reluctant to make this dietary change because of the traditional pleasure of eating meat, its nutritional and sensory appeal, and the convenience it provides (Kyriakopoulou et al., 2021). Even though many companies and researchers have produced plant-based meat analogs, there are still alterations in color, texture, smell, taste, flavor, mouthfeel, and nutritional properties compared to animal-based meat (Wen et al., 2023). Schouteten et al. (2016), in a sensory panel study comparing animal, plant and insect-based burgers, stated that animal burgers were associated with the emotional terms `satisfied, happy and pleasant', whereas plant burgers were associated with 'disappointed, insecure and displeased'. However, beef-like products are produced today, and extensive research is needed on consumer acceptance of these products. Neff et al. (2018) reported that interest in purchasing plant-based meat varies by age, gender, income, education and region.

Bryant et al. (2019) in a cross-country (US, China, and India) study found that the attitudinal determinants of purchasing similar meat in the US were attractiveness, excitement, and low disgust, whereas in China, health, appeal, taste, and sustainability were the primary determinants; and in India, sustainability, excitement, necessity, and goodness were predictors of intention to purchase plant-based meat. In a hypothetical choice experiment, Slade (2018) offered consumers the option of purchasing burgers made from beef, plant-based protein, or cultured meat. Willingness to purchase plant-based and cultured meat burgers was reported to be linked to age, gender, views on other food technologies, and attitudes toward the environment and agriculture. Although consumers in this study were told that all burgers tasted the same, their preference for beef burgers was clear. A mixed logit model predicts that if prices were the same, 65% of consumers would not buy a beef burger, 21% would buy a plant based burger, 11% would buy a cultured meat burger, and 4% wouldn't buy one.

The best-selling category of plant-based meat alternatives is burgers and patties ($120 million). Are plant-based meat products really good for the world? How serious is the situation in real meat production? How does it taste and look, does it have the texture of meat and the experience of cooking or eating real meat? What ingredients does it consist of? Are allergen warnings correctly stated on the label? In what quantities are the various additives used to create meat-like texture, juiciness and taste? Make sure that plant based meat is not a laboratory mixture of chemicals and that its ingredients contain the same amount of protein as real meat burgers. Is the source of plant protein used correctly stated? It is emphasized that these questions of consumers regarding nutrition, food safety, clean labeling, and cost and consumer self-confidence need to be answered. (Ahmad et al., 2022).

Estell et al., (2021) a plant based diet was explained as ‘following a vegan diet’ (55.3%, n = 352), 38% (n = 244) of participants expressed a plant based diet as ‘following a flexitarian diet’ and 27.8% (n = 177) explained it as ‘a vegetarian diet’. In open-ended responses, the most common sources of plant protein were tempeh, legumes, tofu, nuts, soy, whole grains, vegetables, and meat substitutes.

Van Loo et al. (2020) in their results from random parameter logit models indicate that, constant prices and conditional on choosing only a food product, 72% preferred farm raised beef and 28% preferred one of the alternatives, 16% plant based (pea protein) meat substitute, 7% plant based (animal like protein) meat substitute and 5% lab grown meat. With the addition of brand names (Certified Angus Beef, Impossible Foods, Beyond Meat and Memphis Meats), the percentage of farm-raised beef selection has increased to 80%. Environmental and technical information had little influence on the market conditional potrtion, but reduced the proportion of individuals who did not buy the option, showing that the information appeals more individuals to the market. Even though plant-based and lab-grown alternatives have seen noteworthy price discounts (50%), farm-raised beef sustains the largest market portion. Vegetarians, men, and the young and well-educated individuals are relatively more likely to prefer plant-based and lab-grown alternatives

to farm-raised beef. Judge and Wilson (2019) reported that female consumers prefer plant-based foods more than male consumers.

Studying 526 consumers in Beijing, China, Wang et al., (2022) investigated how food characteristics and information influence consumers' food choices regarding plant-based meat products. A discrete choice experiment was conducted using burgers with five characteristics (meat patty, sodium content, energy, flavor and price) as primes. To help examine the role of information, consumers were randomly presented with individual messages about food safety, nutrition, and environmental issues related to eating plant-based meat. This study shows that consumers in Beijing have relatively low knowledge of plant-based meat and have a negative preference for eating plant-based meat compared to conventional meat. Nevertheless, although consumers' willingness to pay for plant-based meat increased significantly after receiving nutritional information, they did not respond to the provision of food safety or environmental information. These findings propose that to support plant-based meat consumption, at least in the context of Beijing, China, information should be presented that is closely related to consumers' personal interests rather than the "public interest."

Seo et al. (2023) conducted a study using behavioral evidence theory to understand how PBM alternatives and characteristics influence consumer decision-making processes in a foodservice context. The results of the study showed that reasons "for" and "against" the use of PBM were significantly related to attitudes. Additionally, when the responsibility attributed to environmental problems was evaluated to investigate whether it could lead to more positive attitudes towards PBMs, it was reported that as the attributed responsibility increases, the health benefits operate more strongly, and low product availability and its negative impact on attitudes decrease as the attributed responsibility increases. Cor van der et al., (2019) reported that, in addition to different levels of technological innovation, a variety of social and institutional changes are needed for meat alternatives to achieve greater success. In Western societies, meat is deeply institutionalized. Eggs, dairy products, and cultured meat also fit into current dietary patterns. Significant changes in insect and algae consumption are required, and while legumes and plant-based alternatives are now institutionalized options, niche products have existed or remain, although there are signs of increasing social value (Cor van der et al., 2019).

CONCLUSION

Plant-based meat has taken a significant place as a choice rather than meat not only for vegetarians and vegans, but also for all consumers, as consumers' awareness of the negative effects of meat consumption on health and the environment has increased in recent years. Yet, to sustain this increase in consumption, the basic taste and texture that determine consumer preference must be improved. Developing ingredients that provide the desired meat-like texture and flavor as well as selecting/optimizing processing may be suitable increase strategies. In PBM production, it is required to use additional ingredients other than protein to mimic other sensory properties of meat such as color, aroma and mouthfeel. The diversity in ingredients and functionality requirements across different types of plant-based meat analogues (sausage and burger) complicates product development. It should also be emphasized that high-tech and potentially disruptive new options in PBM require a high degree of social coordination to make them feasible. Considering that recent studies have focused on the analysis of nutritional composition differences between plant-based and animal-based meat, a detailed comparison of their nutritional and digestive properties would be more useful for optimizing plant-based meat formulations and developing healthy products. Additionally, future events and policies are needed to clarify regulatory uncertainties surrounding plant-based analogues. Food labeling, health and nutrition claims ensure that consumers' trust in this product is placed on a solid and transparent basis.

CONFLICT OF INTEREST

The author inform no conflict of interest.

ETHICAL STATEMENT

The authors state that no ethical approval was needed.

AUTHOR CONTRIBUTION

All authors contributed to the manuscript writing and approval of the final version.

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UTILIZATION OF HAZELNUT SKIN AND HAZELNUT FLOUR IN GLUTEN-FREE CAKES: CORRELATION OF BATTER RHEOLOGY WITH CAKE QUALITY

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ABSTRACT
The effects of rice flour substitution with hazelnut skin (HS), hazelnut flour (HF), and HS-HF blend (1:1, w/w) at percentages of 0%, 5%, 10%, 15% (w/w) on gluten-free cake batters were studied from a rheological standpoint. Replacement with 5% HS increased Power-Law consistency index (K), reduced specific gravity and resulted in the highest cake volume. Increasing levels of HS gradually increased specific gravity, water activity, and reduced tan δ, leading to cakes with lower volume, darker (lower L*) color, harder texture. HF addition increased tanδ (at >5%) and specific gravity, producing cakes with lower volume, but similar color and texture to those of control. HS-HF blend improved cake color and hardness compared to HS added alone. Strong correlations were found between G′(ω) slope and cake volume (r=0.9939 for added HS, r=-0.9408 for added HF), the exponent *a* and cake volume (r=0.9447 for added HS, r=-0.8668 for added HF).
Key words: Rice cake, hazelnut skin, fiber, rheology, baking

GLUTENSİZ KEKLERDE FINDIK UNU VE FINDIK ZARI KULLANIMI: HAMUR REOLOJİSİNİN KEK KALİTESİ İLE KORELASYONU

ÖZ
Pirinç ununun %0, %5, %10, %15 (g/g) oranlarında fındık zarı (FZ), fındık unu (FU) ve FZ-FU karışımı (1:1, g/g) ile değiştirilmesinin glutensiz kek hamurları üzerindeki etkileri reolojik açıdan değerlendirilmiştir. 5% oranında FZ ilavesi ile Power-Law konsistens indeksi artmış, özgül ağırlık azalmış ve en yüksek hacimli kek elde edilmiştir. Artan oranlarda FZ ilavesi hamurun özgül ağırlık ve su aktivitesinde kademeli bir artışa neden olurken, tanδ değerini azaltmıştır. Böylece daha düşük hacimli, koyu renkli (düşük L*), katı tekstüre sahip kekler elde edilmiştir. FU ilavesi tanδ (>5%) ve özgül ağırlığı artırarak daha düşük hacimli, fakat renk ve tekstür açısından kontrolle benzer özelliklerde kekler elde edilmesini sağlamıştır. FZ-FU karışımı ise, tek başına FZ ilavesine kıyasla, keklerin renk ve sertlik değerlerini geliştirmiştir. Ayrıca, G′(ω) eğimi ile kek hacmi (r=0.9939 FZ ilavesi için, r=-0.9408 FU ilavesi için), katsayı *a* ile kek hacmi (r=0.9447 FZ ilavesi için, r=-0.8668 FU ilavesi için) arasında kuvvetli korelasyonlar tespit edilmiştir.
Anahtar kelimeler: pirinç keki, fındık zarı, lif, reoloji, pişirme

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INTRODUCTION

Gluten-free baked products have been widely consumed due to increasing prevalence of celiac disease, non-celiac gluten sensitivity, and due to the growing trend towards following a healthier diet (Xu et al., 2020; Gazza and Nocente, 2022). Thus, these products constitute one of the most rapidly growing segments of food industry. Consumers demand gluten-free baked products with similar texture, color, and flavor to those of wheat flour-based baked products (Gasparre and Rosell, 2023). This demand triggers a technological challenge in food industry for the manufacturing of gluten-free baked products. Besides the technological issues encountered during the processing of gluten-free baked products due to the lack of gluten in dough/batter systems that result in poor physical characteristics in the product (Yazar et al., 2017; Yazar and Demirkesen, 2023), gluten-free baked products often tend to have reduced quantities of proteins, B vitamins, iron, and fiber compared with products containing gluten, as they are prepared with gluten-free cereals and commercial grain products (Gularte et al., 2012). Gluten-free diet is often characterized by an excessive consumption of energy, proteins, and fats, and a reduced intake of complex carbohydrates and dietary fiber (Sabanis et al., 2009). Therefore, it is an ongoing need to re-design gluten-free formulations to obtain gluten-free baked products with similar nutritional composition and physical quality characteristics to those of their wheat-based counterparts (Gularte et al., 2012; Özyiğit et al., 2020). For this purpose, replacing common gluten-free ingredients with nuts was suggested to be a valuable strategy to produce healthier gluten-free baked products (Tuna et al., 2023).

Hazelnut (*Corylus avellana* L.) is one of the most popular tree nuts consumed worldwide, ranking second in tree nut production after almond (Del Rio et al., 2011; Çıkrıkçı et al., 2016). Turkey is the major producer of hazelnuts with the production rate corresponding to 63.9% of the total production in the world in 2022 (FAO, 2024). Hazelnut consists of a green leafy cover, a hard shell with a smooth surface, dark brown pellicular pericarp (also known as skin or testa), and an edible kernel (Contini et al., 2008; Çıkrıkçı et al., 2016). Hazelnut kernel was reported to be a source of flavonoids including monomeric flavan-3-ols, B-type procyanidins, and prodelphinidins (Del Rio et al., 2011). Total dietary fiber content of hazelnut was found as 17.8%, the majority of which (>96%) was insoluble fibers. The dietary fibers hazelnut was suggested to have potential to improve large bowel function (Tunçil, 2020). Hazelnut skin constitutes around 2.5% of the whole hazelnut and it is a by-product obtained during the roasting process (Del Rio et al., 2011; Çıkrıkçı et al., 2016). Roasted hazelnut skin was found to be an excellent source of phenolics, and its dietary fiber content was reported as 69.8%, that was mainly composed of insoluble fibers including lignin (55%) and fiber polysaccharides such as cellulose, pectic polysaccharides, and xyloglucans (45%). Thus, hazelnut skin can be considered as a value-added co-product for use as a functional food ingredient with prebiotic properties and antioxidant activity (Pelvan et al., 2018; Tunçil, 2020).

The information discussed above have clearly showed that the addition of hazelnut flour and hazelnut skin could improve the nutritional value of gluten-free baked products. Besides their high nutritional value, hazelnuts were also suggested to contribute to the flavor and texture of bakery products (Dervişoğlu, 2006). For this purpose, hazelnut flour with skin were added in gluten-free bread (Tuna et al., 2023) and gluten-free cookie (Doğruer et al., 2023) formulations. These studies evaluated the impact of added hazelnut flour on the rheological properties of doughs using empirical methods and on the physical properties of the resulting baked products. On the other hand, different forms of fibers obtained from hazelnut skin was used in wheat flour-based cake formulations and the impact of these fibers were evaluated in terms of linear viscoelastic properties of batters, physical properties and staling rates of cakes (Çıkrıkçı et al., 2016). The impact of added hazelnut skin on the empirical rheological properties of wheat flour dough was studied (Anıl, 2007; Durmuş et al., 2021). These studies have revealed that hazelnut flour without skin and hazelnut skin were not used in gluten-free

formulations. Therefore, this study focused on the impact of hazelnut flour (without skin) and hazelnut skin on the physical quality characteristics of a model rice flour-based gluten-free cake. For this purpose, flow and linear viscoelastic properties of cake batters with added hazelnut flour and hazelnut skin were studied and the correlations of the rheological properties with the physical cake characteristics were discussed.

MATERIALS AND METHODS

Materials

Rice flour (*Kenton*, Ankara, Türkiye) [11.78±0.19% moisture determined using the IR-35 rapid moisture analyzer, 8.78±0.62% protein determined according to the Kjeldahl method with the conversion factor of 6.25, AACC approved method no. 46-10.01 (AACC, 2010), 1.41±0.26 % fat determined according to the AACC approved method no. 30-20.01 (AACC, 2010), and 0.49±0.05% ash determined according to the AACC approved method no. 08-01.01 (AACC, 2010)], sugar, eggs, milk, sunflower oil, baking powder, and hazelnuts were purchased from a local market. Hazelnut flour (3.31±0.04% moisture, 17.5±0.56% protein, 64.6±0.42% fat and 2.43±0.04% ash) was produced by roasting at 105°C for 10 minutes, grinding, and grading the hazelnuts through a 10-mesh sieve (2 mm opening size). Hazelnut skin (HS) was obtained from *Fiskobirlik*- Hazelnut Agriculture and Sales Cooperatives (Giresun, Turkey). Hazelnut skin with 8.29±0.05% moisture, 1.8±0.08% protein, 11.8±0.25% fat, 2.1±0.02% ash, and 68±1.11% dietary fiber [determined according to the AOAC method no. 991.43 (AOAC, 2000)] was also graded using a 10-mesh sieve prior to its use in gluten-free cake formulations.

Methods

Batter and cake preparation

A gluten-free cake formulation containing 100% rice flour, 87.5% sugar, 51.5% egg, 50% sunflower oil, 27.5% milk, and 2.5% baking powder was used in the experiments (all percentages are given on flour weight basis, w/w). Hazelnut skin (HS), hazelnut flour (HF), and a mixture of both [HS:HF, 1:1 (w/w)] were added in gluten-free cake formulations at different percentages (0%, 5%, 10%, and 15%). The recipes for control and other gluten-free cake samples with hazelnut skin and flour were given in Table 1. The basic recipe used for control was adapted from the rice flour-based gluten-free cake formulations used in previous studies (Gómez et al., 2010; Ronda et al., 2011; Gularte et al., 2012). For the preparation of gluten-free cake batters, eggs were mixed for 4 minutes at high-speed using a mixer (Kitchen Aid K45, St. Joseph, MI, USA). Then, sugar was added, and the mixture was mixed for 3 minutes again at high speed. Sunflower oil and milk were then added into this mixture as mixing continued at medium speed for 2 minutes. Finally, all other dry ingredients were added into the mixture and mixed at low speed for 1 minute.

Table 1. Formulations of Gluten-free Cakes with Hazelnut Skin and Hazelnut Flour (on rice flour basis, w/w)

Formulation	Control	HS (5%)	HS (10%)	HS (15%)	HF (5%)	HF (10%)	HF (15%)	HS-HF (5%)	HS-HF (10%)	HS-HF (15%)
Rice flour	200	190	180	170	190	180	170	190	180	170
Hazelnut skin	0	10	20	30	0	0	0	5	10	15
Hazelnut flour	0	0	0	0	10	20	30	5	10	15
Egg	103.05	103.05	103.05	103.05	103.05	103.05	103.05	103.05	103.05	103.05
Sugar	175	175	175	175	175	175	175	175	175	175
Sunflower oil	100	100	100	100	100	100	100	100	100	100
Milk	55	55	55	55	55	55	55	55	55	55
Baking powder	5	5	5	5	5	5	5	5	5	5
Total (g)	638.05	638.05	638.05	638.05	638.05	638.05	638.05	638.05	638.05	638.05

HS: hazelnut skin, HF: hazelnut flour

Cake batter samples of 300 g were baked in duplicates in a convection oven (Vestel- AFB 902E, Türkiye) using disposable aluminum cake pans with the diameter of 14 cm. The baking mode of top-bottom heating was chosen, and the temperature was set to 170°C for 35 minutes. After baking, cake samples were removed from the pans and set aside at room temperature for 1 hour to cool down. The cakes were then covered with plastic wrap to prevent them from drying.

Cake batter properties

Moisture content and water activity

The moisture contents of cake batters were determined using the IR-35 Moisture Analyzer (Denver Instrument, Denver, CO, USA). This method is based on drying the sample under infrared bulb and measuring the percent moisture on a gravimetric balance once the sample weight is stabilized (McCartney and Tingley, 1998). Water activity of cake batters was measured using a water activity meter (Testo AG 400, Lenzkirch, Germany) to evaluate how the free water in the batter systems changed when rice flour was replaced with HS and HF.

Flow behavior

The rheological testing to characterize the flow behavior of gluten-free cake batter samples with added HS and HF was conducted at 25°C with Brookfield RVDV III ultra-rheometer (Brookfield Engineering, MA, USA) using the small size sample adaptor with the spindle SC4-28. Shear stress and apparent viscosity were measured as a function of shear rate over the range of 0.1-10 s^{-1} (Christaki et al., 2017). The obtained data were fitted to the Power Law model (Eq. 1):

$$\tau = K.\dot{\gamma}^{n} \quad (1)$$

where K is the consistency index (Pa.s), n is the flow behavior index, τ is the shear stress (Pa) and $\dot{\gamma}$ is the shear rate (1/s).

Linear viscoelastic behavior

Small amplitude oscillatory shear tests were conducted at 25 °C using the Haake Mars Rheometer (Thermo-Fisher Scientific, Germany). Batter samples were prepared just before the rheological testing. A 40 mm parallel plate geometry with smooth surface and a gap of 1 mm were used. Strain sweep tests were conducted to within the strain range of 0.01% to 100% at 1 Hz frequency to determine the linear viscoelastic region for the gluten-free cake batter samples studied. Frequency sweep tests were conducted within the frequency range of 0.1 to 10 Hz at a constant strain amplitude (γ: 0.1%) determined in the linear viscoelastic region. Frequency sweep data [G′ (Eq. 2), G″ (Eq. 3), tan δ (Eq. 4)] were fitted to the Power Law model (Yazar and Demirkesen, 2023):

$$G'(\omega) = G'_{\omega 1} \,.\, \omega^{a} \quad (2)$$

$$G''(\omega) = G''_{\omega 1} \,.\, \omega^{b} \quad (3)$$

$$\tan\delta(\omega) = \frac{G''(\omega)}{G'(\omega)} = \left(\frac{G''}{G'}\right)_{\omega 1} . \omega^{c} = (\tan\delta)_{\omega 1} . \omega^{c} \quad (4)$$

where $G'_{\omega 1}$ is the elastic modulus (Pa), $G''_{\omega 1}$ is the viscous modulus (Pa), and $(\tan\delta)_{\omega 1}$ represents the loss tangent at a frequency of 1 Hz; while ω is the angular frequency (rad/s). The *a*, *b*, and *c* exponents quantify the dependence degree of the moduli and the loss tangent to the oscillation frequency (Ronda et al., 2017; Yazar and Demirkesen, 2023).

Specific gravity

Specific gravity values of gluten-free cake batter samples were measured by dividing the weight of a certain volume of cake batter by the weight of distilled water with the same volume (Turabi et al., 2008). The measurements were conducted in duplicates.

Cake quality evaluation

Volume and density

Cake volume was determined according to rapeseed replacement method [AACC method 10-05.01 (AACC, 2010)]. After the cake samples were baked and cooled down for 1 hour, their weights were measured. Cake density was calculated as the ratio between the weight of the cake and its volume (Gómez et al., 2010; Gularte et al., 2012).

Volume, symmetry, and uniformity indices

Volume, symmetry, and uniformity indices of the cake samples was measured according to the AACC method 10-91.01 (AACC, 2010). For the evaluation of these parameters, cake samples were cut vertically through the center and the heights of cake samples were measured at three different points (*B*, *C*, and *D*) along the cross-sectioned cakes using the template provided by the method. Volume index (Eq. 5), symmetry index (Eq. 6), and uniformity index (Eq. 7) were calculated as follows:

$$\text{Volume index} = B + C + D \quad (5)$$

$$\text{Symmetry index} = 2C - B - D \quad (6)$$

$$\text{Uniformity index} = B - D \quad (7)$$

where *C* is the height at center, *B* and *D* are the heights at three-fifths of distance from center to edge (Cloke et al., 1984).

Textural properties

Crumb texture was determined by TA-XT2 texture analyzer (Stable Microsystems, Surrey, UK), 24 h after baking. Center of cake samples were cut into cube shapes with the dimensions of 35 x 35 x 35 mm and were compressed to 50% of their original thickness at a speed of 2 mm/s, with a 30 s delay between first and second compressions. A 75-mm aluminum compression platen probe and a load cell of 50 N were used. Hardness (N), springiness, cohesiveness and resilience values for the gluten-free cake crumb samples were calculated on the "Texture Profile Analysis" test graph (Gulerta et al., 2012). Mean values of eight measurements (2 cubic slices from the central part of the cake halves were collected from the duplicates of each baking test) were presented along with the standard deviations.

Color analysis

The surface and the crumb color of the cake samples were determined using the Minolta CR-400 chromameter (Minolta, Osaka, Japan). Readings were obtained in quadruplicate for each sample to quantify the differences in color between the cake samples with the addition of HS and HF, and they were provided as L*, a*, b* parameters according to the CIELAB system of color measurement. The a* value is a measure of greenness (-100) to redness (+100), the b* value ranges from -100 (blueness) to +100 (yellowness), while the L* value indicates the lightness on a scale ranging from 0 (black) to 100 (white) (Sabanis et al., 2009).

Statistical analysis

OriginPro 8.6 (Northampton, MA, USA) was used for statistical analyses with 95% confidence level. One-way analysis of variance (ANOVA) and Tukey's comparison tests were applied ($P < 0.05$) to compare the data obtained for gluten-free batter and cake samples with HS and HF. Lettering system was used to show significant difference between samples.

RESULTS AND DISCUSSION

Cake batter flow behavior

The apparent viscosity curves of cake batters with added HS, HF, and HS-HF blend on an equal weight basis in comparison to control were shown in Figure 1. A decrease in apparent viscosity with increasing shear rate was observed for all cake batters (Figure 1a,b,c), suggesting a shear thinning behavior for gluten-free cake batters with and without HS and HF. Shear thinning behavior mainly arises from the alignment of microstructures in a material being deformed in the direction of flow (Duvarcı et al., 2019). As the shear rate is increased further, the alignment with the flow becomes more complete, and the shear viscosity decreases further (Hyun, 2002). Similar behavior was previously found for gluten-free cake batters (Şakıyan et al., 2004; Turabi et al., 2008; Ronda et al., 2011). Gluten-free cake batter with 15% added hazelnut skin showed the highest apparent viscosity values within the studied shear rate range (Figure 1a) when compared to control and other batter samples.

Flow curves of cake batters were given in Figure 2. Batters with HS, especially at 15% (Figure 2a), and control showed slightly higher shear stress values when compared to batters with HF (Figure 2b) and HS-HF blend (Figure 2c). The shear stress (σ) versus shear rate ($\dot{\gamma}$) data obtained for the gluten-free cake batters provided a good fit (r^2 = 0.9930-0.9998) for the Power Law model (Eq.

1). The consistency index (K) and flow behavior index (n) for different batter formulations were shown in Table 2. The flow behavior index of batters ranged from 0.43 to 0.66. Flow behavior index values less than 1.0 indicates shear thinning behavior (Şahin, 2008), suggesting all gluten-free batter samples showed shear thinning behavior as also revealed by the apparent viscosity curves (Figure 1). The gluten-free cake batter with 15% hazelnut skin showed the lowest flow behavior index (0.43) and the highest consistency index (61). Addition of HS resulted in higher K values in gluten-free cake batters (42.2- 61.0) compared to control (42.5), batters with HF (34.0- 40.3), and batters with HS-HF blend (36.6-40.6). However, a decrease was found in K with the addition of HS at 10%, which was indicative of a more fluid-like behavior (Table 2). Insoluble dietary fibers, especially of a coarse particle size, was suggested to create rupture points in the dough matrix (Föste et al., 2020). Thus, the decrease in K with 10% added HS could be attributed to the disruptive effect of insoluble fibers on the continuity of the batter system. Insoluble fibers have been characterized by their water binding capacities (Föste et al., 2020) and this capacity was reported to increase with the increasing particle size of the fiber (Gómez et al., 2010). As the added HS (particle size ≤ 2 mm) increased to 15%, the high water absorption capacity of fibers suppressed the disruptive effect on the batter matrix and thus, K significantly increased ($p<0.05$). Consequently, shear stress, apparent viscosity, and Power Law indexes collectively pointed out to a decrease in the flow behavior of rice flour-based gluten-free cake batters with 15% added hazelnut skin, while suggesting an increase in the flowability with the addition of hazelnut flour.

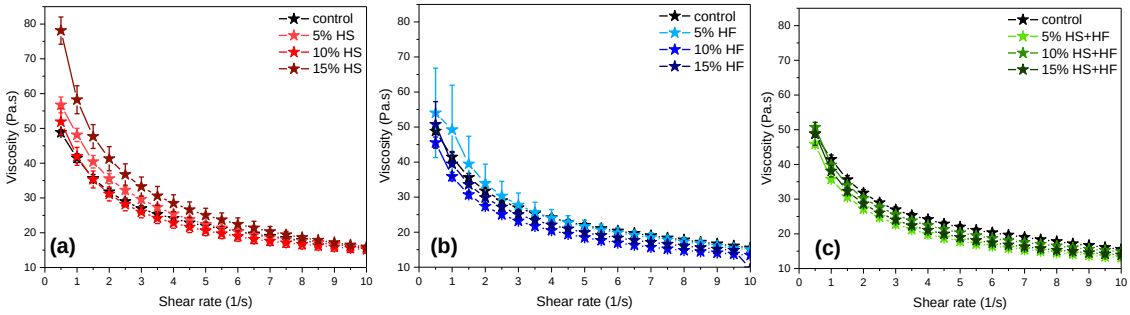

Figure 1. Apparent viscosity versus shear rate for the rice flour-based gluten-free cake batters with hazelnut skin (a), hazelnut flour (b), and blends of hazelnut skin and flour [1:1, w/w (c)] at different percentages (0%, 5%, 10%, 15%, w/w)

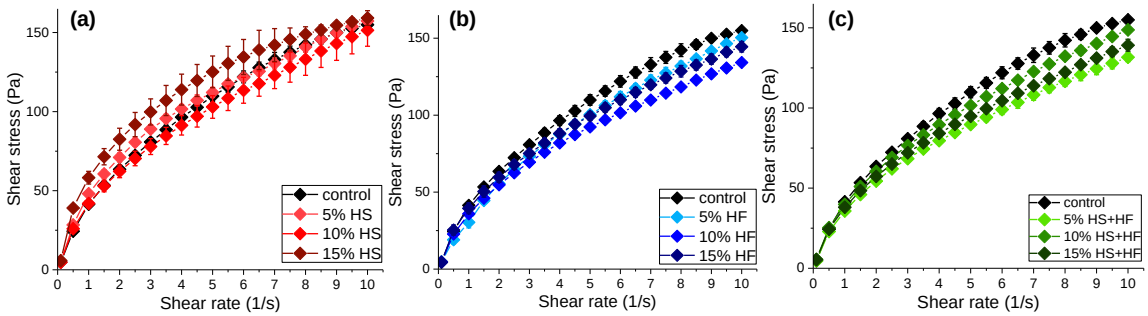

Figure 2. Flow curves of the rice flour-based gluten-free cake batters with hazelnut skin (a), hazelnut flour (b), and blends of hazelnut skin and flour [1:1, w/w (c)] at different percentages (0%, 5%, 10%, 15%, w/w)

Batter viscosity and flow behavior mainly depends on the water binding capacity of the dry ingredients (Doğan et al., 2005). Hazelnut skin was considered to increase batter viscosity by decreasing the amount of free water in the batter system due to its rich fiber content (≈68%), that was reported to range between 58.3%- 69.8% (Montella et al., 2013; Tunçil, 2020). Even though the moisture contents of cake batters were comparable to each other (Table 3), the significantly lower ($p<0.05$) water activity (a_w) found for the gluten-free cake batter with 15% HS (Table 3) supported the idea behind the higher viscosity observed for this cake batter. An increase in cake batter viscosity was also reported in other studies with the addition of insoluble fibers (Lee et al., 2004; Gómez et al., 2010) in cake formulations. On the other hand, the decrease in K values of gluten-free cake batters with added hazelnut flour was attributed to its high oil content. Hazelnuts contain around 57-69% of oil (Turan et al., 2015) and increasing the oil/fat content in cake formulations was previously shown to decrease batter viscosity (Prakash et al., 2001; Şakıyan et al., 2004).

Table 2. Properties of Cake Batters with Hazelnut Skin and Hazelnut Flour. The index and moduli presented correspond to the fitting of experimental measurements to Power Law Model [$\tau = K\dot{\gamma}^n$; $G'(\omega) = G'_{\omega 1}.\omega^a$; $G''(\omega) = G''_{\omega 1}.\omega^b$; $\tan\delta(\omega) = (\tan\delta)_{\omega 1}.\omega^c$]

Sample	*Flow behavior*			*Viscoelastic behavior*							
	K (Pa.s^n)	*n*	*r^2*	*$G'_{\omega 1}$*(Pa)	*a*	*r^2*	*$G''_{\omega 1}$*(Pa)	*b*	*r^2*	*$(\tan\delta)_{\omega 1}$*	*c*
Control	42.5	0.57	0.9981	98.01	0.64	0.9948	112.57	0.62	0.9998	1.15	-0.01
Hazelnut skin											
5%	49.9	0.50	0.9978	90.31	0.72	0.9763	111.88	0.64	0.9938	1.26	-0.07
10%	42.2	0.55	0.9998	75.27	0.71	0.9846	91.44	0.67	0.9961	1.21	-0.04
15%	61.0	0.43	0.9939	98.70	0.64	0.9842	111.65	0.62	0.9968	1.15	-0.02
Hazelnut flour											
5%	34.0	0.66	0.9930	99.32	0.61	0.9923	107.75	0.57	0.9997	1.08	-0.03
10%	36.8	0.56	0.9991	61.17	0.65	0.9960	74.67	0.61	0.9988	1.22	-0.03
15%	40.3	0.55	0.9995	64.98	0.70	0.9826	77.45	0.62	0.9971	1.19	-0.07
Hazelnut skin + hazelnut flour											
5%	36.6	0.55	0.9995	79.29	0.66	0.9854	85.85	0.62	0.9964	1.08	-0.03
10%	40.6	0.56	0.9996	69.05	0.69	0.9600	74.15	0.63	0.9884	1.10	-0.06
15%	38.7	0.55	0.9997	61.17	0.68	0.9912	79.01	0.64	0.9980	1.30	-0.04

Viscoelastic behavior of gluten-free cake batters

Viscoelastic parameters fitted to the Power-Law model along with the exponents were also provided in Table 2 to describe the viscoelastic responses of gluten-free cake batters with added HS and HF. Control batter had the highest $G'_{\omega 1}$ and $G''_{\omega 1}$ values, and the cake batter with 15% HS showed almost identical viscoelastic behavior to that of control. Increasing percentages of HF in gluten-free cake batters resulted in a decrease in both $G'_{\omega 1}$ and $G''_{\omega 1}$. For all batter formulations, viscous modulus $G''_{\omega 1}$ values were higher than elastic modulus $G'_{\omega 1}$, which resulted in $(\tan\delta)_{\omega 1}$ values greater than 1, ranging between 1.08 and 1.30 (Table 2). Ronda et al. (2011) also fitted the frequency sweep data obtained for rice flour-based glute-free cake batters with added proteins to the Power-Law model and found $(\tan\delta)_{\omega 1}$ values above 1. It should be noted that the tanδ obtained for gluten-free cake batters might vary depending on the cake formulation and the frequency sweep testing protocol, including the type of geometry and the gap used. Loss tangent (tanδ) has been described as the ratio of viscous to elastic components of a viscoelastic behavior (G''/G'). Since $0 \leq \delta \leq 90°$ for viscoelastic materials, tanδ can range from zero to infinity. A solid-like viscoelastic material exhibits phase angle smaller

than 45° (tanδ<1), while a liquid-like viscoelastic material exhibits phase angle greater than 45° (tanδ>1) (Duvarcı et al., 2019). The loss tangent data obtained for the gluten-free cake batters through the frequency sweeps [tanδ (ω)] were shown in Figure 3. Tanδ values ranged from 1 to 2 for all batter samples, suggesting liquid-like linear viscoelastic behavior (G″>G′). The lowest tanδ values were obtained for the batters with 5% HF, 5% HS+HF, and 10% HS+HF, while the highest tanδ was found for the batter with 15% HS+HF (Figure 3). These results showed that the highest degree of liquid-like viscoelastic behavior in gluten-free cake batters occurred with the addition of 15% HS+HF. On the other hand, tanδ values of control and batter with 15% HS overlapped throughout the whole frequency range (Figure 3), which was indicative of a similar viscoelastic response for these gluten-free cake batters.

The lowest exponent values, especially a and b, were observed for the gluten-free cake batter with 5% HF (Table 2), suggesting the least degree of frequency dependency for this sample. Considering the lowest $(\tan\delta)_{\omega 1}$ was also found for cake batter with 5% HF (Table 2), it can be concluded that replacing 5% of rice flour with hazelnut flour in the given gluten-free cake formulation contributed to the molecular interactions in the batter system.

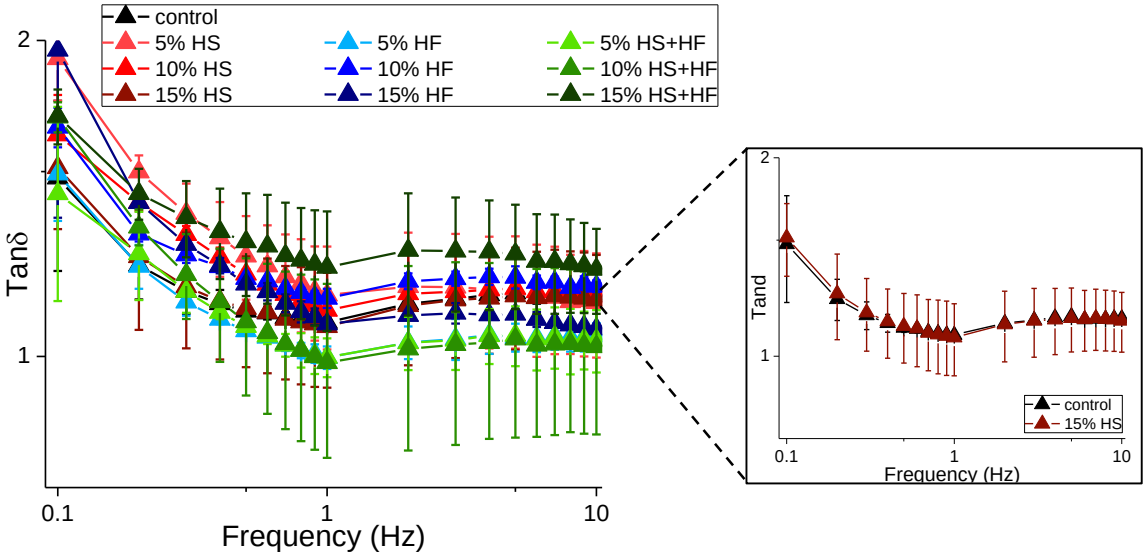

Figure 3. Tanδ values for gluten-free cake batters with added hazelnut skin (HS) and hazelnut flour (HF) versus frequency (ω: 0.1-10 Hz)

The magnitude of the slope of log G′ versus log ω in the frequency sweeps provides useful information about the structures of biopolymers. Strong gels (elastic gels or true gels) with a 3D network when log G′ versus log ω or log G″ versus log ω plots give nearly zero slopes, while for weak gels and highly concentrated solutions the plots have positive slopes approaching 2 (Georgopoulos et al., 2004). In other words, the increase in G′ slope versus frequency is indicative of an increase in viscous flow properties. The slope of G′ versus the applied frequency for the gluten-free cake batters analyzed in this study ranged from 0.62153±0.01 to 0.83181±0.02 (Figure 4). The lowest G′ slope was found for the batter with 5% hazelnut flour, which was even slightly lower than that of control. The slope of G′ remained the same with the added 10% hazelnut flour, but significantly increased when the hazelnut flour percentage increased to 15% (Figure 4). These findings revealed the slight thickening of the rice flour-based gluten-free cake batter when hazelnut flour was added up to 10%.

On the other hand, addition of hazelnut skin even at 5% resulted in a sharp increase in the slope of G′ versus frequency. However, as the percentage of added hazelnut skin increased to 10% and 15%, the slope of G′ gradually decreased, suggesting an increase in the elastic component of the cake batter that resulted in a viscoelastic response similar to that of control (Figure 4). Fiber macromolecules were suggested to impact the linear viscoelastic properties of dough/batter systems by competing for water due to their varying water binding and gelling capacities (Yazar and Demirkesen, 2023). Thus, the decrease in the G′ slope of the cake batter with 15% hazelnut skin was attributed to the significantly lower water activity when compared to batters with lower percentages of added HS (Table 3). The decrease observed in the G′ slope with respect to increasing percentages of added HS concurred with the decreasing tanδ values as the added HS percentage increased from 5% to 15% (Table 2 and Figure 3). Addition of fibers in different gluten-free dough/batter systems was also reported to cause a decrease in tanδ (Ronda et al., 2013; Djordjević et al., 2018).

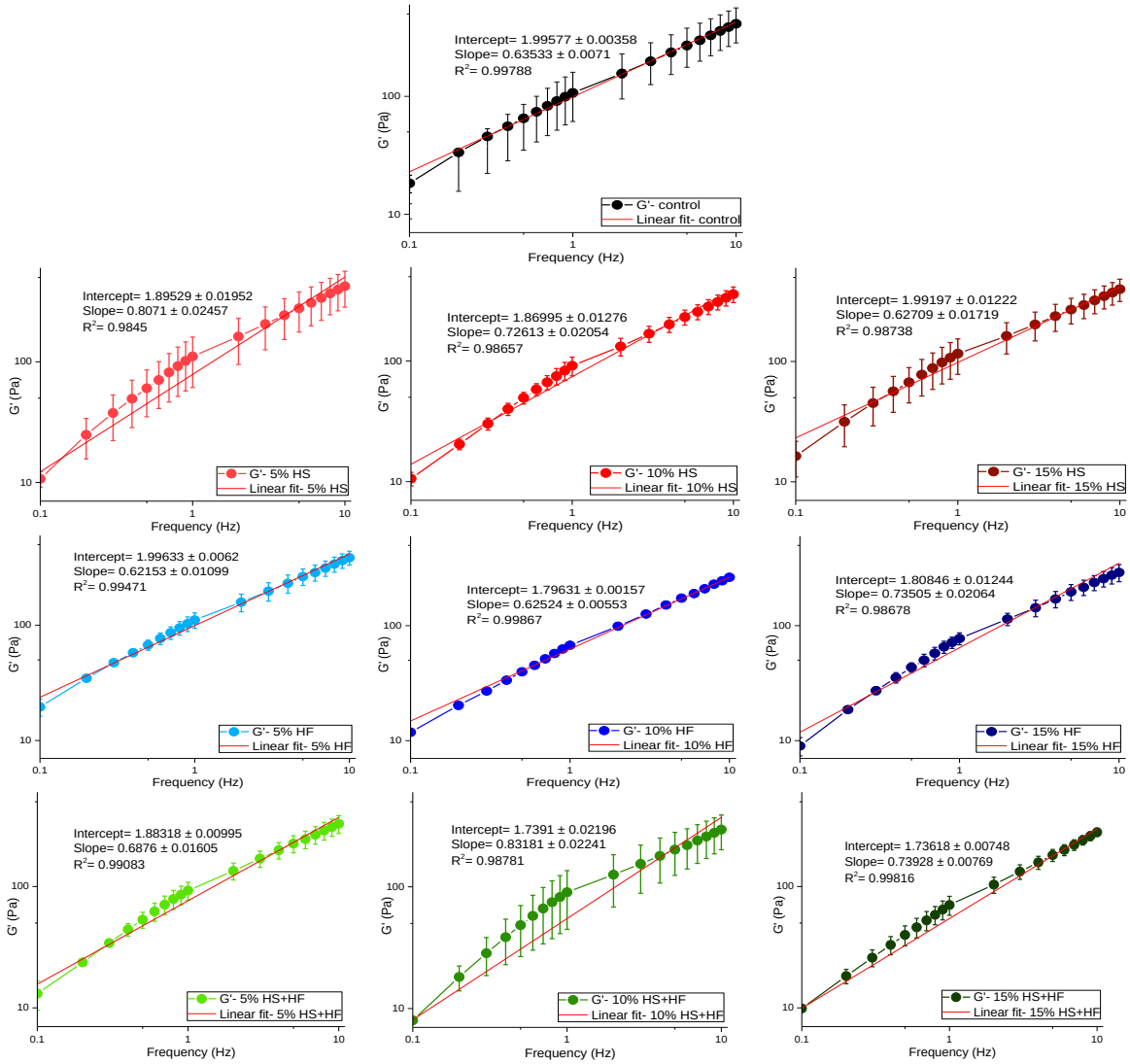

Figure 4. G′ slopes for rice-flour based gluten-free cake batters with added hazelnut skin (HS) and hazelnut flour (HF) versus frequency (ω: 0.1-10 Hz)

The highest G′ slope versus frequency was found for the gluten-free cake batter with 10% HS+HF (Figure 4), indicating the highest degree of fluid-like behavior occurred when the combination of HS+HF (1:1, w/w) was added at 10%. This finding revealed that the fluidity enhancing effect of 5% HS addition on the rheological properties of gluten-free cake batter became even more pronounced when 5% of rice flour was replaced with HF.

Specific gravity

The specific gravity values of the gluten-free cake batters were given in Table 3. Control batter had a specific gravity of 0.89. Replacing 5% of rice flour in cake batters with hazelnut skin resulted in a significant decrease (P <0.05) in the specific gravity of batters compared to that of control. Low specific gravity is desired in cake batters as it is associated with higher degree of air incorporation in the batter (Turabi et al., 2008). Thus, the results obtained in this study showed that the addition of hazelnut skin at 5% improved air incorporation in cake batters. The contribution of fibers to air incorporation into batter systems was attributed to the networks being formed upon the hydration of fibers, which might show emulsifying properties (Sabanis et al., 2009). However, specific gravity of batters started to increase gradually (P <0.05) as the percentage of added hazelnut skin increased above 5% up to 15% (Table 3), suggesting a decrease in the amount of air incorporated in batters.

Table 3. Quality parameters of cake batters with hazelnut skin and hazelnut flour

Sample		Moisture (%)	a_w	specific gravity
Control		24.19±0.04^{a,b}	0.89±0.0014^{e}	0.890±0.00^{b}
Hazelnut skin				
	5%	24.32±0.04^{b}	0.87±0.0028^{d}	0.850±0.00^{a}
	10%	23.97±0.13^{a,b}	0.86±0.0028^{c,d}	0.865±0.00^{a}
	15%	24.33±0.18^{b}	0.81±0.0007^{a}	0.895±0.00^{bc}
Hazelnut flour				
	5%	23.68±0.08^{a}	0.87±0.0021^{d}	0.910±0.00^{cd}
	10%	24.10±0.16^{a,b}	0.87±0.0042^{d}	0.915±0.00^{d}
	15%	23.74±0.20^{a,c}	0.85±0.0014^{b,c}	0.940±0.00^{e}
Hazelnut skin + hazelnut flour				
	5%	24.86±0.16^{d}	0.85±0.0000^{b}	0.860±0.00^{a}
	10%	24.20±0.06^{b,c}	0.85±0.0007^{b}	0.865±0.00^{a}
	15%	24.11±0.05^{a,b}	0.86±0.0084^{c,d}	0.885±0.00^{b}

Columns with different letters are significantly different (P <0.05).

On the other hand, replacing rice flour with hazelnut flour resulted in higher specific gravity values in gluten-free cake batters when compared to control batter (P <0.05). And the specific gravity values of gluten-free cake batters continued to increase gradually as the added hazelnut flour percentage increased (Table 3). These results pointed out to a reduction in air incorporation in batters with hazelnut flour when compared to control, in opposite to what was observed with the addition of hazelnut skin. Hazelnut flour particles were not able to aerate the batter system as they were dense due to their high oil contents.

Cake quality evaluation

Quality characteristics of cakes with HS, HF, and HS+HF blend (1:1, w/w), including density, volume index, crust and crumb color, were provided in Table 4.

Volume, symmetry, and uniformity indices

Fiber enriched gluten-free cake sample with 5% HS had significantly higher (P <0.05) volume index when compared to control (Table 4),

concurring with the lower specific gravity found for the batter with 5% HS (Table 3). When added at low percentages, HS was found to favor the air incorporation in the batter, leading to an increase in volume index. Besides, the increase observed in the consistency index, K (Table 2) and apparent viscosity (Figure 1a) with the addition of 5% HS seemed to favor the entrapment of air bubbles in the batter during baking, which also improved the volume index. The consistency of batters, like specific gravity, is a very important physical property affecting end-product quality since it controls retention of the small bubbles that have been initially incorporated into the batter during mixing (Turabi et al., 2008). Replacing the rice flour in cake formulations with HS at 10% and 15% resulted in volume index similar to that of control (P >0.05), pointing out to a gradual decrease in volume index as the percentage of added HS increased above 5%. Sudha et al. (2007) also reported a decrease in cake volume with respect to increasing levels of added apple pomace in the formulation due to strong water binding properties of this fiber. Besides, insoluble dietary fibers, especially those with a coarse particle size, were suggested to create rupture points in dough/batter matrix, favoring gaseous release caused by an impaired gas retention capacity (Föste et al., 2020). The findings in this study showed that replacing rice flour with HS at percentages above 5% gradually diminished gas retention in the batter.

Table 4. Quality Parameters of Cake Samples with Hazelnut Skin and Hazelnut Flour

Sample	Density (g/cm³)	*B* (cm)	*C* (cm)	*D* (cm)	Volume index (cm)	Color (crust)			Color (crumb)		
						L*	a*	b*	L*	a*	b*
Control	0.56±0.009^{b,c}	3.4±0.00^{a,b}	3.55±0.07^{a,b}	3.3±0.00^{a,b}	10.25±0.07^{a,b,c}	47.02±1.95^{d,e}	12.58±0.37^{d}	18.93±0.62^{g}	69.65±0.61^{h}	-2.52±0.39^{a}	19.91±0.48^{g}
Hazelnut skin											
5%	0.51±0.008^{a}	3.55±0.21^{a}	3.85±0.07^{a}	3.6±0.14^{a}	11±0.28^{d}	42.10±0.17^{b}	10.74±0.22^{c}	15.99±0.27^{e}	46.26±0.44^{e}	3.83±0.34^{d}	11.17±0.41^{e}
10%	0.54±0.006^{a,b}	3.5±0.00^{a}	3.7±0.14^{a,b}	3.45±0.07^{a,b}	10.65±0.21^{b,c,d}	41.09±1.21^{b}	9.58±0.30^{b}	15.25±0.42^{e}	36.72±0.98^{b}	5.10±0.15^{e}	8.08±0.43^{d}
15%	0.56±0.001^{b,c}	3.3±0.00^{a,b}	3.55±0.07^{a,b}	3.45±0.07^{a,b}	10.3±0.00^{b,c}	37.46±2.02^{a}	8.60±0.08^{a}	13.42±0.42^{d}	33.96±0.14^{a}	6.06±0.15^{g}	7.34±0.34^{c}
Hazelnut flour											
5%	0.57±0.004^{b,c}	3.4±0.00^{a,b}	3.45±0.07^{b}	3.3±0.00^{a,b}	10.15±0.07^{a,b,c}	48.17±0.40^{e}	15.44±0.27^{f}	23.43±0.19^{h}	71.03±0.33^{i}	0.06±0.24^{b}	20.15±0.17^{g}
10%	0.58±0.006^{c,d}	3.25±0.07^{a,b}	3.45±0.07^{b}	3.4±0.00^{a,b}	10.1±0.00^{a,b}	52.74±0.66^{f}	14.65±0.54^{e}	22.98±0.82^{h}	69.35±0.64^{h}	0.21±0.18^{b}	20.03±0.46^{g}
15%	0.61±0.009^{d}	3.1±0.00^{b}	3.4±0.00^{b}	3.2±0.00^{b}	9.7±0.00^{a}	51.94±0.63^{f}	14.38±0.17^{e}	17.75±0.36^{f}	65.63±0.09^{g}	2.55±0.26^{c}	15.53±0.29^{f}
Hazelnut skin + Hazelnut flour											
5%	0.54±0.007^{a,b}	3.4±0.00^{a,b}	3.7±0.14^{a,b}	3.6±0.14^{a}	10.7±0.00^{c,d}	45.61±0.33^{c,d}	11.37±0.33^{c}	12.13±0.23^{c}	52.14±1.29^{f}	3.46±0.24^{d}	8.21±0.29^{d}
10%	0.55±0.009^{b}	3.5±0.14^{a}	3.6±0.14^{a,b}	3.5±0.14^{a,b}	10.6±0.14^{b,c,d}	45.10±0.81^{c,d}	9.76±0.40^{b}	11.17±0.61^{b}	44.29±0.60^{d}	5.29±0.25^{e,f}	6.69±0.06^{b}
15%	0.56±0.012^{b,c}	3.35±0.07^{a,b}	3.65±0.07^{a,b}	3.35±0.07^{a,b}	10.3±0.21^{b,c}	44.57±0.91^{c}	8.39±0.67^{a}	9.8±0.37^{a}	40.41±0.72^{c}	5.62±0.30^{f,g}	4.96±0.22^{a}

Columns with different letters are significantly different (P <0.05).

The volume index values of gluten-free cakes decreased gradually with the increasing percentages of added HF ranging from 5% to 15%. However, this decrease was not significant (P >0.05), indicating the volume indices of cakes were not significantly affected by the replacement of rice flour with HF at percentages up to 15% (P >0.05). The increasing specific gravity of cake batters with added HF, which was indicative of reduced aeration in batter, was concurrent with the lower volume index values obtained with the addition of HF. However, it should be noted that starch gelatinization occurring during baking played a major role in determining cake quality (Wilderjans et al., 2008).The proximate analyses conducted for the rice flour used in this study suggested a carbohydrate content of 77.5% based on the "by difference" method introduced by Atwater and Woods (1896). Rice flour was reported to contain around 78% of starch on 14% moisture basis (Amagliani et al., 2017), concurring with the carbohydrate content of the rice flour used in this study. On the other hand, the proximate analyses revealed a much lower carbohydrate content for the hazelnut flour (without skin) used in this study, that was around 12%. Ultimately, denser hazelnut flour particles that were rich in fat and low in starch content could be the reason behind the decrease in the volume indices of cakes with added HF. Even though, hazelnut flour particles lacked the ability to aerate the batter (Table 3), the high amount of starch in rice flour might have compensated the detrimental effects of hazelnut flour on cake volume for the replacement ratios of up to 15%. Thus, addition of HF caused a significant increase

(P <0.05) in cake batter specific gravity, while resulting in no significant decrease (P >0.05) in cake volume index.

The volume index values suggested a significant difference (P <0.05) in comparison to control only for the cake sample with 5% added HS. Therefore, replacing the rice flour in cakes with the HS+HF blend on an equal weight basis (1:1, w/w) caused no significant change in the volume index of cakes at percentages up to 15% (P >0.05).

The AACC template method (10-91.01) parameters (B, C, D) used to determine the volume index values of cake samples were also provided separately in Table 4. These parameters indicated that none of the gluten-free cake samples with added HS, HF, and HS+HF blend collapsed after baking, as evidenced by the C values being higher than B and D values leading to positive symmetry index values for all cake samples. The uniformity index was 0.1 cm for control; while it ranged between -0.15- 0.05 cm, -0.15- 0.1 cm, and -0.2- 0 cm for the cakes with HS, HF, and HS+HF blend, respectively (Table 4). For the optimum cake, the uniformity index was suggested to be zero because positive or negative values occurred when one side of the cake was higher than the other one (Cloke et al., 1984). Thus, the optimum uniformity index values were obtained for the cakes with 10% and 15% of added HS+HF blend (Table 4).

Cake density

The decrease in cake density expresses that more air was incorporated into the batter, which results in higher cake volume, suggesting a negative correlation between cake density and volume index (Sabanis et al., 2009; Gómez et al., 2010). Cake sample with 5% HS, which had the highest volume index (11.0±0.28 cm), also had the lowest density with 0.51±0.008 g/cm^3 (Table 4). A gradual increase was recorded in cake density as the percentage of HS increased from 5% to 15% (Table 4). Increasing levels of fiber in cake formulations was suggested to increase batter density by disrupting the batter structure and thus leading to the release of the trapped air or CO_2 from the batter system (Föste et al., 2020; Kırbaş et al., 2019). Thus, cake volume decreases and results in an increase in cake density. Even though, increasing HS levels in cakes gradually increased cake density, the density and volume index of the cake with 15% HS were not significantly different (P >0.05) from those of control (Table 4). On the other hand, the cake sample prepared with 15% HF addition showed the highest density (0.61±0.009 g/cm^3), while having the lowest volume index (9.7±0.00 cm).

Crust and crumb color characteristics

Color is an important characteristic for baked products as, along with texture and aroma, it contributes to consumer preference. It depends on physicochemical characteristic of the dough (water content, pH, reducing sugars and amino acid content) and on the operating conditions applied during baking such as temperature, relative humidity, modes of heat transfer (Sabanis et al., 2009). The L*, a*, b* values for crust and crumb of the cakes with added HS and HF were given in Table 4. A significant decrease was observed in the L*, a*, b* values for crust as the percentage of added HS increased (P <0.05). These results showed that the cake crust became darker, as evidenced by the lower L*, with increasing percentages of added HS. The darkening of the crust for gluten-free breads (Sabanis et al., 2009; Gül and Şen, 2017) and layer cakes (Gómez et al., 2010) with increasing levels of added fiber was also reported in literature. The darker crust color obtained for cakes in the presence of added fibers was not reported to be due to the original color of the fiber; instead, it was mainly associated with the Maillard and caramelization reactions (Gómez et al., 2003). Fibers were suggested to change the pH of the batter by acting as a buffer or to change the available water in the batter, which might both affect the Maillard reactions and their resulting effects on crust color (Gómez et al., 2010). The decrease found in the a* values of the cake crust with increasing levels of HS pointed out to a decrease in the redness of crust color, that led to the formation of a brownish red. This was indicative of more brownish crust formation with added HS. The decrease in the b* values with

increasing levels of HS was indicative of a tendency towards the blue hue, suggesting a darker yellow color.

Crumb color depends to a high extent on raw materials since the increase in temperature is not high enough to give Maillard or caramelization reactions (Gómez et al., 2010). The L*, b* values for crumb decreased as the percentage of added HS increased (P <0.05), while the a* values for crumb showed an increasing trend (P <0.05). These parameters suggested a darker, less yellowish (brownish), and more reddish crumb in cakes due to the natural brown color of HS. Cıkrıkçı et al. (2016) also reported a decrease in L*, and an increase in a* values of cake crumbs when 10%-20% of wheat flour was replaced with different forms of HS. A decrease in b* values were only found with the addition of microfluidised HS. Anıl (2007) replaced 5% and 10% of wheat flour in bread with HS and found lower L* and b* values along with higher a* values for the crumb, concurring with the findings of this study.

Replacing the rice flour with HF in gluten-free cake formulations resulted in closer L*, a*, b* values of both crust and crumb to those of control when compared to cakes with added HS (Table 4). This was indicative of lighter crust and crumb colors for the cakes with HF than those of the cakes with HS (P <0.05). At higher levels of added HF (>10%), lower L* and b* values were obtained for the crumb when compared to those of control (P <0.05), suggesting a slightly darker creamy color formation in the cake crumb. When the color parameters for the cakes with the blend of HS and HF (1:1, w/w) were evaluated, a similar trend to that observed with the addition of HS was found for both crust and crumb, indicating the dominant effect of HS on the color characteristics of cakes.

Textural properties of cakes

Addition of increasing percentages of HS (5% to 15%) in rice flour-based gluten-free cakes resulted in a gradual increase (P <0.05) cake hardness (Table 5). A sharp increase was found in the resilience, cohesiveness, and springiness of cakes when 5% of rice flour in the formulation was replaced with HS. However, the values for these textural quality parameters decreased gradually as the amount of added HS in cakes increased up to 15% (Table 5). Nevertheless, the resilience, cohesiveness, and springiness values of cakes with HS even at 15% were higher than those of control (P <0.05). Gularte et al. (2012) also reported an increase in the hardness and cohesiveness, but a decrease in the resilience and springiness of rice flour-based gluten-free cakes when rice flour was replaced with fibers. The reason behind the decrease they found in the resilience and springiness of cakes with added fibers could be the higher replacement ratio [20% (w/w)] they used. Replacing flour with fiber (50, 80, 250 μm sized) at ratios up to 20% was suggested to improve the volume of wheat flour-based cakes (Gómez et al., 2010). The hazelnut skin used in this study was coarser with the particle size ≤ 2 mm and the best cake quality in terms of volume and texture was obtained with 5% added HS (Tables 4 and 5). Small sized fibers were found to better improve cake quality (Gómez et al., 2010). Thus, these findings revealed that the percentage of the insoluble fiber added in cake formulations should be lower (i.e., 5%, w/w, on flour weight basis) for an improved cake quality, if the fiber particles are coarse.

Replacing rice flour with HF up to 15% did not significantly affect the hardness of gluten-free cakes (P >0.05). However, an increase was found in the resilience, cohesiveness, and springiness of cakes with HF when compared to control (P <0.05). The increase in hardness observed for the cakes with HS was reduced (P <0.05) with the addition of HS-HF blend (1:1, w/w), but the hardness values were still higher than that of control (P <0.05).

A strong negative correlation was found between cake density and volume index (Table 4) with the addition of HS, HF, and HS-HF blend (r=-0.9908, r=-0.9902, and r=-0.9637, respectively). Cakes with relatively lower volume were found to be denser and had a packed crumb structure, which resulted in harder texture (Sabanis et al., 2009; Aydoğdu et al., 2018). However, cake

hardness and cake density obtained in this study for the cakes with added HS (r=-0.3094) and HF (r=-0.4630) provided weak correlations. This was due to the simultaneous contribution of HS to air incorporation in the batter and to cake hardness resulting from its water absorption ability. On the other hand, HF did not significantly alter cake hardness due to its high oil content; however, it showed detrimental effect on cake volume that led to an increase in cake density. Thus, cake density and hardness did not show a clear correlation for the cakes with added HS and HF.

Table 5. Textural quality parameters of gluten-free cakes with hazelnut skin and hazelnut flour

Sample		Hardness (N)	Resilience	Cohesiveness	Springiness
Control		1.017±156.62^{a}	0.151±0.00^{a}	0.410±0.21^{a}	0.542±0.03^{a}
Hazelnut skin					
	5%	1.69±118.12^{c,d}	0.214±0.00^{h}	0.521±0.00^{e}	0.726±0.01^{e}
	10%	1.85±57.98^{d}	0.193±0.00^{e,f}	0.479±0.01^{c}	0.682±0.02^{c,d}
	15%	1.88±158.34^{d}	0.176±0.00^{b}	0.450±0.01^{b}	0.673±0.01^{c,d}
Hazelnut flour					
	5%	1.067±86.41^{a}	0.189±0.00^{d}	0.478±0.01^{c}	0.627±0.02^{b}
	10%	1.021±171.87^{a}	0.181±0.00^{c}	0.469±0.01^{c}	0.614±0.02^{b}
	15%	1.007±49.87^{a}	0.198±0.00^{g}	0.504±0.00^{d}	0.662±0.01^{c}
Hazelnut skin + hazelnut flour					
	5%	1.366±51.35^{b}	0.232±0.00^{i}	0.548±0.00^{f}	0.732±0.01^{e}
	10%	1.471±108.81^{b}	0.197±0.00^{f,g}	0.502±0.01^{d}	0.704±0.01^{d,e}
	15%	1.522±63.92^{b,c}	0.194±0.00^{e,f,g}	0.474±0.00^{c}	0.696±0.03^{c,d,e}

Columns with different letters are significantly different (P <0.05).

Predicting cake quality through batter properties

Correlations of the linear viscoelastic properties of cake batters with cake quality

It has been well established that rheological properties of dough/batter systems are used to predict baked product quality (Ronda et al., 2017; Marchetti et al., 2020; Yazar and Demirkesen, 2023). In this section, the correlations of the Power-Law model parameters obtained in the linear viscoelastic region for cake batters with added HS, HF, and HS-HF blend with the volume and textural properties of the resulting cakes.

A positive correlation was found between the Power-Law exponent value "*a*" and volume indices (r = 0.9447) of cakes with the addition of HS, while addition of HF resulted in a negative correlation between these parameters (r=-0.8668). Ultimately, no strong correlation was found between *a* and cake volume (r=0.3481) when the rice flour in cake formulations was replaced with the HS-HF blend (1:1, w/w). When the correlations of the exponent *a* and $(\tan\delta)_{\omega 1}$ were evaluated, the Pearson coefficients were r=0.9552, r=0.7061, and r=0.1927 for cake batters with HS, HF, and HS-HF blend (1:1, w/w), respectively. High Pearson coefficient, as in the case for the batters with HS and HF, was suggested to allow the prediction of loss tangent from the exponent *a* and vice versa (Ronda et al., 2017). The positive correlations between *a* and $(\tan\delta)_{\omega 1}$ revealed that the more frequency-dependent the doughs were, the more fluid-like behavior they had. Thus, these correlations led us to the fact that the increase in the fluid-like behavior of the batter resulted in an increase in volume for the cakes with added HS at 0-10% (w/w), while causing a decrease in volume for those with added HF at 0-15% (w/w). This finding emphasized the contribution of fibers to the air incorporation in batter, and thus to cake volume, as previously reported by others (Gómez et al., 2010; Kırbaş et al., 2019; Özyiğit et al., 2019). This effect was more evident when HS was added at 5% and then at 10%, as evidenced by the specific gravities of these batters (Table 3). The

water binding ability of HS became more dominant when rice flour was replaced with HS at concentrations above 10%. And thus, the cake batter with 15% HS became less fluid-like and it had lower tanδ compared to those with 5% and 10% HS (Figure 3). Viscosity of cake batter is the controlling factor for the final cake volume. Higher cake batter viscosities help to retain more air bubbles in the batter and retard the rise of bubbles to the surface during baking. A highly viscous batter (less fluid-like) can hold the air bubbles inside; however, the expansion of this batter is restricted because of its high viscosity (Şahin, 2008). For this reason, cake batters became less fluid-like (tanδ decreased) as the percentage of HS increased from 5% to 15%, while the volumes of the resulting cakes decreased. The strong positive correlation between the loss tangent and cake volume (r=0.9978) for the cakes with HS (0%-15%, w/w) supported this finding. Among the cake batters with added HS, the highest tanδ was found for the batter with 5% (Table 2). And the resulting cake with 5% HS had the highest volume index (Table 4). Besides, tanδ values of the batter with 15% HS and control were the same as shown in Figure 3, whereas the cakes resulting from these batter samples had similar ($p>0.05$) volume indices (Table 4).

The volume of cakes with added HS showed strong positive correlation with the slope of G′ obtained versus frequency (r=0.9939), while a negative correlation was found between these parameters for the cakes with HF (r=-0.9408) and a weak correlation (r=0.4079) was found for the cakes with HS-HF blend. The correlations of cake volume index with the slope of G′ versus frequency were similar to its correlations with the Power-Law exponent *a*. And thus, strong positive correlations between *a* and the slope of G′ were found for the cakes with HS (r=0.9533), with HF (r=0.8985), and with HS-HF blend (r=0.9462). These correlations revealed that the Power-Law exponent *a* acted as a marker of elastic stability versus frequency.

The crumb hardness for the cakes with added HF (0%-15%, w/w) negatively correlated with the Power-Law exponent "*b*" (r=-0.9875). A negative correlation between crumb hardness and *b* (r=-0.68) was also provided by Ronda et al. (2015). The correlations between cake hardness and *b* were positive and weaker for the samples with added HS (r=0.4772) and HS-HF blend (r=0.7392). These correlations showed that the increase in the exponent *b*, indicative of viscous decay versus frequency, resulted in the reduction of cake hardness when the rice flour in cake formulation was replaced with HF. The correlation between these parameters shifted from negative to positive when rice flour was replaced with HS due to the water binding ability of HS. This means the viscous decay of cake batter might increase with added HS; however, it absorbs the available water in the batter system during baking, leading to an increase in cake hardness. Insoluble dietary fibers, such as HS, were reported to have high water absorption and high swelling properties without an increasing effect on viscosity (Föste et al., 2020). However, at percentages as high as 15% of rice flour replacement with HS, the binding of the available water in batter became evident even before baking (Table 3) and thus the exponent *b* showed a drop (Table 2), which explains the reason behind the weaker correlation obtained between *b* and cake hardness for the cakes with HS.

Correlation of specific gravity of cake batter with cake volume

Specific gravity has been regarded as an important batter property that helps predicting the resulting cake volume. Specific gravity values found in this study showed strong negative correlations with the volume indices of cakes with added HS (r=-0.9798), HF (r=-0.9563), and HS-HF blend (r=-0.9978) at ratios ranging from 0% to 15% (w/w) to replace rice flour in formulations. Low specific gravity in cake batters was associated with high volume in the resulting cakes, suggesting a negative correlation between the specific gravity of batter and the volume of the resulting cake (Turabi et al., 2008; Matos et al., 2014; Kırbaş et al., 2019). The highest specific gravity was obtained for the cake batter with 15% HF (Table

3) and thus the resulting cake with 15% HF showed the lowest volume index (Table 4). On the other hand, the cake batter with 5% HS having the lowest specific gravity (Table 3) resulted in the cake sample with the highest volume (Table 4).

CONCLUSION

Replacing rice flour with hazelnut skin (particle size ≤ 2 mm) in gluten-free cake formulations improved air incorporation into batter at 5%, as evidenced by the lower specific gravity of this batter compared to control. The specific gravity of batters with HS increased gradually as the percentage of HS increased. Increasing levels of HS, especially at 10%, disrupted the continuity of batter and imparted a more fluid-like behavior as evidenced by the decrease in K, G′, and G″. However, above 10% of rice flour replacement with HS, water activity increased, viscosity increased, tanδ dropped, and all these changes in batter collectively pointed out to the dominating effect of water binding ability of hazelnut skin. Thus, the highest volume in the resulting cakes was obtained with 5% HS. Even though, the cake with 5% HS had the highest volume, its hardness increased compared to control, which could be attributed to the high water binding and swelling properties of insoluble fibers during baking. The color parameters L*, b* were lower, while a* was higher for the crumb of the cakes with added HS, suggesting a darker and brownish crumb color in comparison to that of control.

Rice flour-based cakes became denser and had lower volumes compared to control when rice flour was replaced with HF at percentages up to 15%. However, the hardness of cakes was not affected by the addition of HF due to its high oil content. Besides, the color characteristics of the cakes with HF were similar to those of control. And therefore, to balance the conflicting effects of HS and HF on gluten-free cake volume and texture, this study showed that replacing 5% to 15% of rice flour with the blend of HS-HF on an equal weight basis (1:1, w/w) could be an alternative to improve the volume, texture, taste, while enhancing the nutritional profile of gluten-free cakes. The only drawback in terms of consumer acceptance has been considered to be the darker color imparted by the added hazelnut skin to the cakes, especially to the crumb, which can be easily eliminated by the addition of cocoa.

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CONFLICT OF INTEREST

The author has declared no conflict of interest.

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APTAMER TABANLI TANIMLAMA YÖNTEMİ VE GIDA GÜVENLİĞİNDEKİ UYGULAMALARI

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ÖZ
Aptamerler, tek sarmallı, kısa, sentetik nükleik asit dizileridir. Çeşitli hedef moleküllere karşı yüksek afinite gösteren ve spesifik olarak bağlanan aptamerler, gıdalarda bulunabilecek patojen mikroorganizmalar, biyotoksinler, alerjenler, pestisitler ve ağır metaller gibi çeşitli risk faktörlerinin hassas bir şekilde tespiti için kullanılmaktadır. Gıda güvenliği açısından risk oluşturan moleküllere spesifik olarak bağlanan aptamerlerin seçilmesi SELEX adı verilen bir süreçle gerçekleştirilir. Seçilen aptamer, hedef molekül ile özgün olarak etkileşime girer ve bu etkileşim elektrokimyasal, optik veya diğer biyosensör teknikler kullanılarak ölçülür. Gıda analizlerinde kullanılan geleneksel yöntemlere kıyasla daha hızlı sonuçlar veren, yüksek özgüllüğe ve duyarlılığa sahip aptamer tabanlı yöntemlere duyulan ilgi giderek artmaktadır. Bu derlemede aptamerlerin genel özellikleri ve SELEX prensibiyle üretimleri özetlenmiş ve gıda güvenliği alanındaki uygulamalarına örnekler verilmiştir.
Anahtar kelimeler: Aptamer, SELEX, biyobelirteç molekülü, biyosensör, kontaminasyon, gıda güvenliği

APTAMER BASED IDENTIFICATION METHOD AND ITS APPLICATIONS IN FOOD SAFETY

ABSTRACT
Aptamers are single-stranded, short, synthetic nucleic acid sequences. Aptamers, which show high affinity and specific binding to various target molecules, are used for the sensitive detection of various risk factors such as pathogenic microorganisms, biotoxins, allergens, pesticides and heavy metals that may be present in foods. The selection of aptamers specifically binding to molecules that pose a risk in terms of food safety is carried out through a process called SELEX. The selected aptamer interacts specifically with the target molecule and this interaction is measured using electrochemical, optical, or other biosensor techniques. There is a growing interest in aptamer-based methods with high specificity and sensitivity, which provide faster results compared to conventional methods used in food analysis. This review summarized the general properties of aptamers and their production by the SELEX principle and given examples of their applications in food safety.
Key words: Aptamer, SELEX, biosensor, biomarker molecule, contamination, food safety

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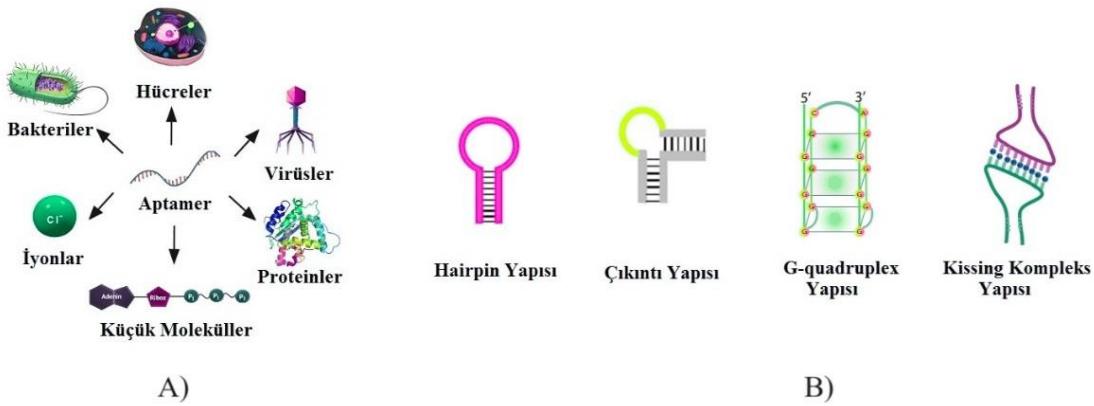
GİRİŞ

Küreselleşme, hızlı kentleşme ve nüfus artışına paralel olarak gıda kaynaklı hastalıklar dünya çapında giderek önemli bir halk sağlığı sorunu haline gelmiştir. Bu durum halk sağlığı açısından olduğu kadar ekonomik kayıpların önlenmesi bakımından da kritik bir konudur. Gıda kaynaklı hastalıklar yalnızca az gelişmiş veya gelişmekte olan ülkelerde değil aynı zamanda gelişmiş ülkelerde de sıklıkla rastlanılan bir problemdir (Kalita vd., 2023). Gıda üretim sürecinde, bitkisel ve hayvansal hammaddelerin üretimi, işlenmesi, depolanması ve taşınması sırasında, insan sağlığı için risk oluşturabilecek çeşitli etkenler gıdalara bulaşabilir. Bu risk faktörlerinden en önemlileri, pestisit ve veteriner ilaç kalıntıları, ağır metaller, patojen mikroorganizmalar ve mikrobiyel toksinlerdir. Bunların dışında bir kısım alerjenler de duyarlı kişiler üzerinde etkili olabilmektedir (Schmitz vd., 2020). Dolayısıyla akut veya kronik hastalıklara neden olan bu risk faktörlerinin hızlı ve çoklu analizini yapabilen güvenilir tespit yöntemlerine olan ihtiyaç giderek artmaktadır. Aptamer tabanlı sistemler, aptamerlerin enstrümental cihazlara entegre edildiği sistemlerdir. Diğer analiz yöntemlerine göre daha yüksek bir doğruluğa ve hızlı tespit yeteneğine sahip olan aptamer tabanlı sistemlerin, son yıllarda gıda kalite ve güvenliğinin kontrolünde kullanımına yönelik yoğun çalışmalar yapılmaktadır (Altalbawy vd., 2024).

APTAMER TANIMI VE APTAMERLERİN GENEL ÖZELLİKLERİ

Aptamerler belirli bir hedefe seçici olarak bağlanabilen kısa, sentetik, tek iplikli DNA (ssDNA) veya RNA moleküllerdir. Kelime manası "uygun parçacık" olan aptamer terimi, ilk kez 1990'lı yıllarda kullanılmaya başlanmıştır (Zhu vd., 2023). Aptamerler aminoasit, protein, küçük metal iyonu, organik molekül, bakteri, virüs, bitki ve hayvan hücresi gibi çeşitli hedeflere spesifik olarak bağlanabilmektedir (Şekil 1A). Bugüne kadar bahsedilen bu hedeflere spesifik binlerce aptamer üretilmiştir (Lee vd., 2023).



Şekil 1. A) Aptamerlerin bağlandığı hedefler, B) Aptamerlerin oluşturduğu yapı örnekleri

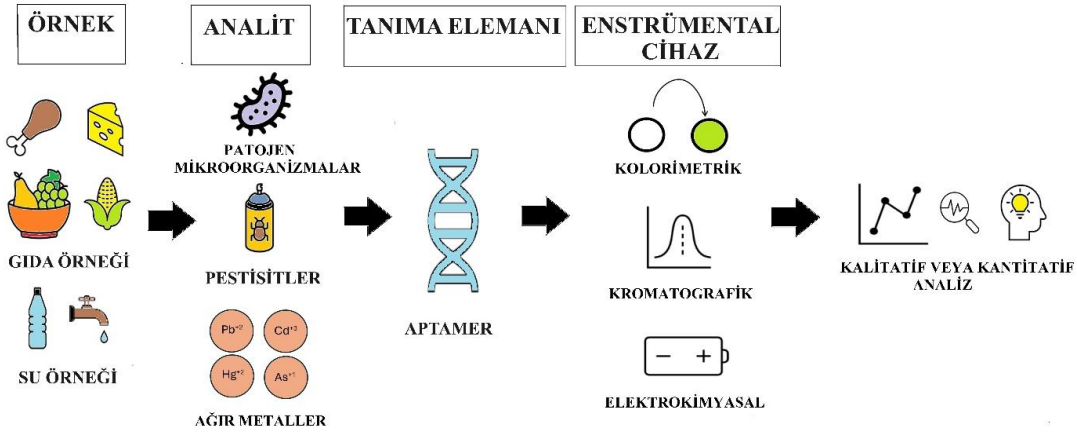
Aptamerler, hedef moleküle karşı konformasyonel adaptasyon sağlamak için yapısal değişim geçirerek hedefe uygun bir bağlanma alanı oluşturabilmektedir. Aptamerler bu bağlanma alanlarının oluşumu için; loop, pürin içeriği bakımından zengin çıkıntı, hairpin yapısı, dörtlü loop, pseudoknot, kissing kompleks ve G-quadrupleks olarak isimlendirilen 3 boyutlu yapısal motifleri içerirler (Şekil 1B) (Khan vd., 2022; Mahmoudian vd., 2024). Aptamerler, sahip oldukları bu moleküler yapılar sayesinde hedef molekül ile etkileşime girerek yüksek afinite ve özgüllükle hedeflerine bağlanabilmektedir (Zhang vd., 2021a).

Aptamerlerin hedeflerine yüksek afinite ve özgüllükle bağlanma özelliği, çoğu zaman antikorlara benzetilmekte ancak aptamerlerin antikorlara kıyasla bazı avantajlarının olduğu bildirilmektedir (Koerselman vd., 2023). Aptamerlerin başlıca avantajı, üretiminde hayvanların kullanılmaması ve in vivo koşullara

ihtiyaç duyulmamasıdır. Bu nedenle aptamerlerin üretimi antikorlara kıyasla daha az maliyetle ve kısa sürede gerçekleştirilir. Yalnızca immünojenik moleküllerle etkileşime girebilen antikorlara karşın aptamerler hem immünojenik ve hem de immünojenik olmayan hedef moleküllerle etkileşim gösterebilmektedir. Aptamerlerin diğer bir önemli avantajı da yüksek sıcaklık ve geniş bir pH aralığında (≈4–9) stabil olmalarıdır. Bunun aksine antikorlar, yüksek sıcaklığa duyarlı olduklarından geri dönüşümsüz denatürasyona maruz kalabilirler (Koerselman vd., 2023; Tang vd., 2023a; Musumeci ve Montesarchio 2023). Aptamerlerin antikorlara karşı en büyük dezavantajı ise, nükleazlara karşı dayanıksız olmalarıdır. Ancak bazı kimyasal modifikasyonlarla aptamerlerin nükleaz stabilitesi artırılabilmektedir (Koerselman vd., 2023).

APTAMER TABANLI TESPİT MEKANİZMASI

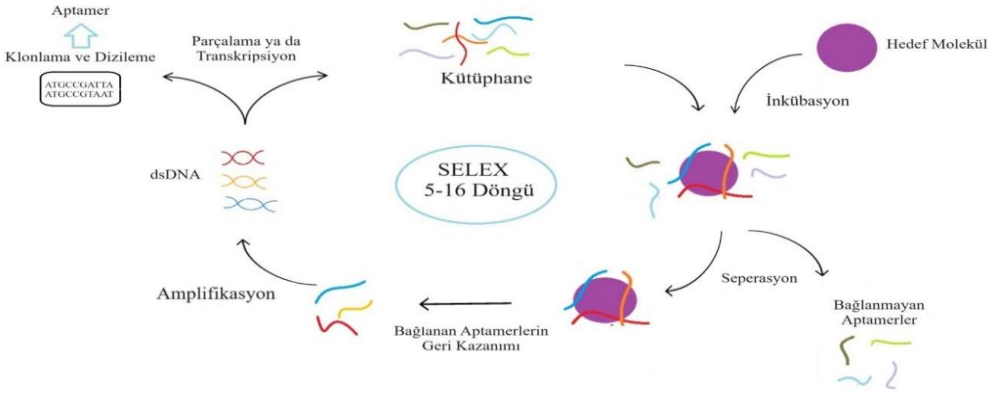
Aptamer tabanlı tespit için ilk aşama, hedef analite spesifik olarak bağlanacak aptamerin seçilimidir. Sonrasında seçilen aptamer bir enstrümental cihazla kombine edilmektedir. Bunun sebebi aptamer-hedef bağlanması sonucunda oluşan verilerin ancak bir enstrümental cihazla analiz edilebilmesidir (Şekil 2) (Verdian vd., 2019; Zhao vd., 2023).



Şekil 2. Aptamer Tabanlı Tespit (Verdian vd., 2019)

Aptamer seçilimi

Aptamerler, üssel zenginleştirme yoluyla ligandların sistematik evrimi (SELEX=**S**ystematic **E**volution of **L**igands by **EX**ponential enrichment) adı verilen bir in vitro yöntemle elde edilmektedir (Şekil 3) (Wolter ve Mayer, 2017; Qi vd., 2022).



Şekil 3. Aptamerlerin in vitro seçim döngüsü (Wolter ve Mayer, 2017)

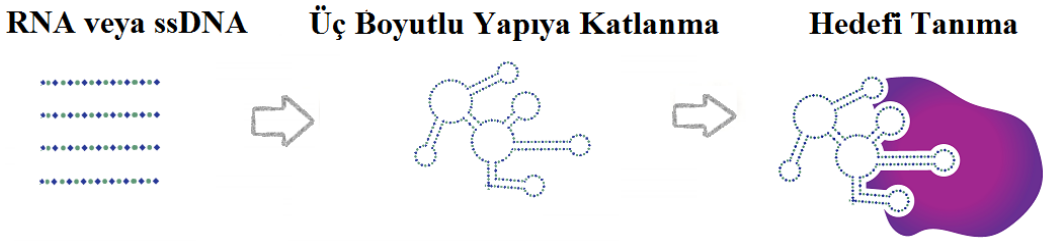
Aptamer seçilimini sağlayan her bir SELEX döngüsü, 5 temel aşamayı içermektedir. Bunlar;
-DNA veya RNA kütüphanesinin oluşturulması
-Oluşturulan kütüphanenin hedef molekül ile etkileşimi
-Hedefe bağlanan ve bağlanmayan aptamerlerin ayrılması
-Hedefe spesifik olarak bağlanan aptamerlerin PCR ile amplifikasyonu
-Hedefe spesifik bağlanma özelliği gösteren aptamerlerin klonlanması ve baz dizilerinin belirlenmesidir (Oliveira vd., 2022).

DNA veya RNA kütüphanesinin oluşturulması

SELEX'in ilk basamağı, DNA veya RNA kütüphanesinin oluşturulmasıdır. Bu aşamada, rastgele sıralanmış 10^{14}-10^{16} farklı oligonükleotid içeren bir kütüphane kimyasal olarak sentezlenir. Genellikle bu oligonükleotid dizilerinin merkezinde rastgele sıralanmış 30 ila 50 nükleotitten (nt) oluşan bir bölge bulunur (Manea vd., 2024). Dizilerin 5' ve 3' uçlarında ise PCR amplifikasyonunu sağlamak için 18 ila 21 nt'den oluşan primer bağlanma bölgeleri bulunur (Komarova ve Kuznetsov, 2019; Mili vd., 2024). DNA veya RNA aptamerlerinin seçiminde hemen hemen aynı protokol uygulanır, ancak DNA SELEX işlemine başlamadan önce çift zincirli DNA'ların, tek zincirli RNA'lara dönüştürülmesi gerekir. Bu amaçla, DNA'nın RNA'ya transkripsiyonu sağlayacak olan T7 RNA polimerazın tanıma bölgesi olan T7 promotörü, dizinin 5' ucuna eklenmektedir (Zou vd., 2019; DeRosa vd., 2023; Wang vd., 2024).

Oluşturulan kütüphanenin hedef ile inkübasyonu

Aptamer seçiliminde ikinci aşama, DNA veya RNA kütüphanesinin uygun tampon ve sıcaklık koşulları altında hedef molekül ile inkübe edilerek etkileşiminin sağlanmasıdır (Zhao vd., 2023). Bu aşamada inkübasyon ortamında sadece istenilen hedef molekülün bulunması ve yeterli miktar ve saflıkta olması zorunludur. Bu koşul sağlanamazsa aptamer birden fazla hedefle etkileşeceğinden spesifik aptamerin seçilimi mümkün olmayacaktır (Kohlberger ve Gadermaier, 2022). İnkübasyon ortamında bulunan aptamer, hedef molekülle etkileşime girdiğinde konformasyonel değişiklilik geçirir ve hedef için uygun bir bağlanma alanı oluşturur (Şekil 4) (Darmostuk vd., 2015; Onaş vd., 2022). Aptamerin oluşturduğu bu konformasyonel yapı, içinde bulunduğu çevresel koşullara (örneğin tampon çözeltinin bileşimi, sıcaklık ve pH değeri) bağlı olarak değişebilmektedir (DeRosa vd., 2023).



Şekil 4. Aptamer-Hedef İnteraksiyonu (Darmostuk vd., 2015)

Bu bağlanma alanın oluşmasında etkili olan birtakım faktörler söz konusudur. Bunlardan birincisi, aptamerlerin Watson-Crick eşleşmesi sergilemeyen baz çiftlerine (Örneğin; Wooble baz çiftleri ve Hoogsteen baz eşleşmesi) sahip olmasıdır. Bu baz çiftleri nedeniyle aptamerlerde oluşan geniş oluklar, hedef için uygun tanıma ve bağlanma alanları oluşturur (Seelam vd., 2019; Zhu vd., 2023). Ayrıca aptamerlerin hedeflerine bağlanmak için kullandığı bazı özel bağlar ve etkileşimler de bulunmaktadır. Oligonükleotid aptamerleri, baz eşleştirmesi, π–π istifleme, DNA/RNA şeker büzülmesi, hidrofobik, elektrostatik, kanonik olmayan intra moleküler etkileşimler, Van der Waals ve hidrojen bağları ile bir araya gelip 3 boyutlu yapılarını oluşturur. Bu üç boyutlu yapılar, aptamerin ilgilenilen hedef molekülle güçlü ve spesifik olarak etkileşime

girmesini sağlamakta ve hedefe en uygun aptamerin seçilme olasılığını artırmaktadır (Chen vd., 2023).

Hedefe bağlanan ve bağlanmayan aptamerlerin ayrılması

Aptamer seçiliminde bir sonraki aşama, hedef moleküle afinite göstererek bağlanan aptamerler ile bağlanmayan aptamerlerin birbirinden ayrılmasıdır. Hedef molekül ile spesifik aptamer arasında bağlanma gerçekleştikten sonra, önce hedefe bağlı olmayan aptamerler, sonrasında ise hedefe bağlı olan aptamerler ortamdan alınmaktadır. Bu işlemlerin gerçekleştirilmesinde membran filtrasyonu, afinite kromatografisi, jel filtrasyonu, manyetik ayırma, jel/kapiler elektroforez, akış sitometrisi veya yüzey plazmon rezonansı gibi çeşitli teknikler kullanılmaktadır (Liu vd., 2021a; Kohlberger ve Gadermaier, 2022).

Hedefe spesifik olarak bağlanan aptamerlerin PCR ile amplifikasyonu

SELEX'in bir sonraki aşaması hedefe bağlanma özelliği gösteren aptamer dizilerinin, polimeraz zincir reaksiyonu (PCR) ile amplifikasyonudur. Bu basamakta amaç, yüksek bağlanma afinitesi ve özgüllüğüne sahip aptamerleri çoğaltmaktır. Bu amaçla hedef molekül ile spesifik bağlanma özelliği gösteren DNA aptamerler, PCR ile RNA aptamerler ise ters transkripsiyon (RT)-PCR ile amplifiye edilirler. Elde edilen çift zincirli DNA dizileri yeniden tek iplikli RNA haline dönüştürülür ve hedef için yüksek bağlanma afinitesi ve özgüllük gösteren aptamerlerin seçilmesi için tekrardan seçim döngüsüne (SELEX) tabi tutulur. Bu işlem yaklaşık olarak 20 seçim döngüsüne kadar uzayabilir (Komarova ve Kuznetsov, 2019; Khan vd., 2022; Wei vd., 2023). Uygulanacak döngü sayısı oligonükleotid uzunluğu, ortamın pH'sı, iyonik kuvveti, oligonükleotid kütüphanesinin özellikleri ve hedef molekülün konsantrasyonu gibi bazı faktörlere bağlı olarak belirlenir. Aptamerler bir kez elde edildikten ve dizisi belirlendikten sonra, sınırsız miktarda aptamer kimyasal sentez ile kolayca elde edilebilmektedir (Bottari vd., 2020).

Hedefe spesifik olarak bağlanan aptamerlerin klonlanması ve baz dizilerinin belirlenmesi

Aptamer seçiliminde son aşama, hedefe kesin olarak bağlanma özelliği gösteren aptamerlerin klonlanması ve baz dizilerinin belirlenmesidir. Dizisi belirlenen aptamerler daha sonra stabiliteyi artırmak ve bağlanma özelliklerini iyileştirmek için çeşitli post SELEX modifikasyonlara tabi tutulmaktadır (Wang vd., 2019; Brown vd., 2024).

Hedefe bağlanan ve bağlanmayan aptamerlerin ayrımında kısa sürede ve yüksek verimde aptamer elde edilmesini kolaylaştıran yeni SELEX metotları geliştirilmiştir. Kapiler elektroforez-SELEX, manyetik boncuk bazlı SELEX, hücre SELEX yeni geliştirilen SELEX metotlarından sadece birkaçıdır. Örnek olarak hücre SELEX, hedef olarak canlı hücrelerin kullanıldığı bir SELEX stratejisi iken, kapiler elektroforez-SELEX tekrarlanan döngülerin süresini kısaltmak amacıyla kapiler elektroforezin kullanıldığı bir SELEX stratejisidir (Wei vd., 2023). Bunlara ek olarak in vitro üretimin ötesine de geçilerek in vivo SELEX yöntemleri geliştirilmeye çalışılmıştır. In vitro seçilim ile elde edilen aptamerlerin in vivo olarak da etkili olup olamayacağını belirlemek amacıyla aptamer seçilimi doğrudan canlı bir hayvanın vücudunda gerçekleştirilmiştir. Ancak in vivo olarak aptamerlerin seçiliminin maliyetli olması, güvenilir olmaması ve uygulanan organizmalara göre farklılıklar göstermesi, bu yöntemi büyük ölçüde sınırlandırmaktadır (Sola vd., 2020; Nasiri vd., 2024; Li vd., 2024).

Hedef molekülün özellikleri

Keşfedildiği 1990 yılından beri SELEX teknolojisi farklı hedef sınıflarına uygulanmıştır. Ancak belirli bir molekülü hedefleyip tanımlayacak olan aptamerin başarılı bir şekilde bağlanabilmesi için hedef molekülün de bazı özelliklere sahip olması gerekmektedir. Öncelikle aptamer ile hedef molekül arasında bir yük etkileşimi bulunmalıdır. Aptamerler, oligonükleotid karışımı olduğundan negatif bir yüke sahiptir. Bundan dolayı aptamerler, pozitif yüklü grupları (örneğin birincil amino grupları) veya atomları kolayca hedefleyebilirken, negatif yüke sahip gruplara

(örneğin, fosfat grupları) ve atomlara zayıf bir ilgi duymaktadır (Drees vd., 2023).

Hedef molekül seçiminde önemli olan bir diğer faktör ise, aptamerlerin hedeflerine bağlanmak için kullandığı kimyasal bağlar ve etkileşimlerdir. Aptamerler hidrojen bağı, Van der Waals bağı, kovalent olmayan kimyasal etkileşimler, hidrofobik ve istifleme etkileşimleri ile hedeflerine bağlanmaktadır. Bu nedenle seçilecek olan hedef molekülün hidrojen alıcı veya verici belirli fonksiyonel gruplara veya yapısal motiflere sahip olması istenir (Zhang vd., 2023a; Lee vd., 2023). Örneğin proteinlerde bulunan arjinin aminoasiti, aptamerin spesifik olarak hedefi tanımasında önemli bir rol oynar. Arjininde bulunan, katyonik guanidinyum grubu hem RNA hem DNA'daki bazlarla etkileşime girme yeteneğine sahiptir. Guanidinyum grubundaki pozitif yüklü azot, nükleotidlerin negatif yüklü fosfat grubuyla elektrostatik olarak etkileşime girebilir. Elektrostatik etkileşimlerin sonuncunda genellikle aptamerin bazları ile arjinin arasında güçlü hidrojen bağları oluşmaktadır (Fadeev vd., 2022). Örneğin; Ebola virüsüne spesifik RNA aptameri, virüsün glikoprotein yapısında bulunan arjinin 587 ve arjinin 596 rezidülerine bağlanmaktadır. Bağlanmanın, arjininde bulunan NH_2 grubu ile aptamerdeki guanin arasındaki hidrojen bağıyla sağlandığı tespit edilmiştir (Teng vd., 2019).

Aptamer tabanlı tespit

Hedef moleküllerin kalitatif ve kantitatif analizi için aptamerlerin enstrümental yöntemlerle kombine olarak kullanılması gerekmektedir. Bugüne kadar birçok aptamer-hedef molekül kompleksinin nicel ve nitel analizinde çeşitli enstrümental cihazların kullanılabileceği gösterilmiştir (Uğurlu vd., 2023).

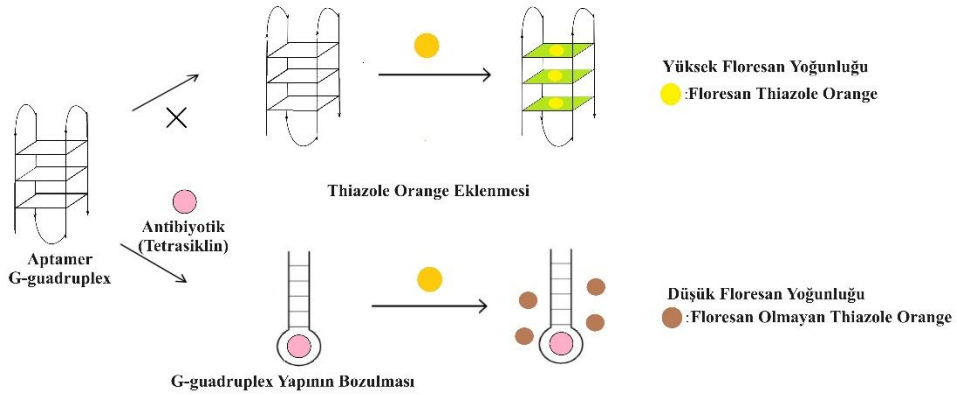
Hedef moleküllerin aptamerler ile tespitinde ön plana çıkan enstrümental sistem, aptamer tabanlı biyosensörler bir başka ifadeyle aptasensörlerdir. Son on yılda, farklı optik sensörler (floresans, lüminesans, elektrokemilüminesans, Floresans Rezonans Enerji Transferi (FRET), Yüzey Zenginleştirilmiş Raman Spektroskopisi (SERS), Yüzey Plazmon Rezonansı (SPR) gibi) kullanılarak birçok aptasensör tasarımı gerçekleştirilmiştir (Kara vd., 2023). Ayrıca optik sensörlerin dışında Elektrokimyasal Empedans Spektroskopisi (EIS), Döngüsel Voltametri (CV), Diferansiyel Puls Voltametri (DPV), Kare Dalga Voltametri (SWV) ve Alan Etkisi Transistör (FET) gibi birçok elektrokimyasal biyosensör de aptasensör tasarımında kullanılmıştır (Wang vd., 2023a; Huang vd., 2024).

Aptasensörlerde aptamer-hedef bağlanmasının uygun bir sinyal oluşturabilmesi için organik boyalar veya floroforlar kullanılabildiği gibi grafen oksit, kuantum noktaları, metal veya silika nanopartiküller gibi nanomateryaller de kullanılmaktadır (Ning vd., 2020). Floresans aptasensörlerde, aptamerlerin hedefiyle bağlanması sonucunda floresan sinyal oluşumunu veya söndürülmesini indükleyerek ölçülebilir bir veri oluşur. Tetrasiklin antibiyotiğinin tespiti için tasarlanan bir floresans aptasensörde, floresans boya ile aptamer etkileştiğinde floresans yoğunluğunun arttığı gözlemlenmiştir. Ancak ortamda hedef molekül (antibiyotik) varsa aptamere spesifik bir şekilde bağlanarak aptamer/boya kompleksinin oluşumunu engellemektedir. Bunun sonucunda floresan yoğunluğu azalmakta ve oluşan sinyal değişikliği ile hedefin varlığı tespit edilmektedir (Şekil 5) (Sun vd., 2018; Zhou vd., 2020).

Aptamerler kromatografi, kapiler elektroforez veya mikroakışkan sistemlerinin yanı sıra görüntüleme sistemleri ile de kombine edilebilmektedir. Aptamerler, floresans görüntüleme sistemi ile birleştirilerek; hücre içi iletişim, hücrelerin kimyasal bileşiklerle etkileşimi ve hücre dışına salgı gibi olaylar etkin bir şekilde gözlenebilmiştir (Lin vd., 2023).

APTAMERLERİN GIDA GÜVENLİĞİ ANALİZLERİNDEKİ UYGULAMALARI

Aptamerlerin çeşitli enstrümental cihazlar ile kombine edilmesiyle geliştirilen sistemler, gıdalarda bulunabilecek pestisit, herbisit gibi zirai ilaç kalıntıları, veteriner ilaç kalıntıları, patojen mikroorganizmalar, biyotoksinler, alerjenler ve organik kirleticiler gibi birçok risk etmeninin tespitinde kullanılmıştır. Bu uygulamalara ait örnekler Çizelge 1'de kısaca özetlenmiştir.



Şekil 5. Floresans aptasensörü ile antibiyotik algılama (Zhou vd., 2020)

Çizelge 1. Aptamer Tabanlı Tespit Yöntemlerinin Gıda Güvenliği Analizlerinde Kullanımı

Pestisit ve Veteriner İlaç Kalıntılarının Tespiti					
Örnek	Analit	Tespit Yöntemi	Doğrusal Aralık	Tespit Limiti	Kaynak
Musluk Suyu	Malatyon	FRET	0.01–1 µM	1.42 nM	(Chen vd., 2020)
Elma	İzokarbofos	Florometrik	5-50 µg/L	1.2 µg/L	(Gao vd., 2022)
Çay	Karbendazim	Florometrik	2.33–800 nM	2.33nM	(Su vd., 2020)
Süt	Diazinon	FRET	0.05–500 ng/mL	0.023 ng/mL	(Rong vd., 2020)
Süt	Tetrasiklin	Florometrik	20 ng/mL-10 g/mL	11.46 ng/mL	(Yang vd., 2023)
Süt	Streptomisin	Kolorimetrik	0.005-6 ng/mL	0.51 pg/mL	(Wang vd., 2020)
Bal	Kanamisin	Florometrik	5-600 nM	3.6 nM	(Li vd., 2023b)
Süt	Sülfadimetoksin	SERS	1.20-120.00 ng/mL	0.89 ng/mL	(Zhang vd., 2023b)
Balık	Kanamisin	CV	25–900 nM	13 nM	(Yao vd., 2020)
Patojen Mikroorganizmaların Tespiti					
Örnek	Hedef Patojen	Tespit Yöntemi (Tespit Süresi)	Doğrusal Aralık (KOB/ mL)	Tespit Limiti (KOB/mL)	Kaynak
Süt	*S. aureus*	Elektrokemilüminesans	3.0	$10\text{-}10^7$	(Liu vd., 2022)
Balık	*V.parahaemolyticus*	Elektrokemilüminesans	1	$1\text{-}10^6$	(Wei vd., 2021)
Süt	*E. coli* O157:H7	Florometrik	$10\text{-}10^6$	0.6031	(Zhang vd., 2022)
Süt	*S.* Typhimurium	Kolorimetrik (45 dk)	$10\text{-}10^7$	7	(Wei vd., 2022)
Tavuk	*C. jejuni*	Florometrik	$1\text{-}10^7$	1	(Liu vd., 2024)
Yağsız süt	*P. Aeruginosa*	Florometrik (1.5 sa)	$10\text{-}10^7$	1.0	(Zhong vd., 2020)
Biyotoksinlerin Tespiti					
Örnek	Analit	Tespit Yöntemi	Doğrusal Aralık	Tespit Limiti	Kaynak
Pirinç, Mısır	Aflatoksin B1	Florometrik	0-180 ng/mL	0.35 ng/mL	(Jia vd., 2020)
Süt	*S.aureus* Enterotoksin B	SERS	1–750 pg/mL	0.2 pg/mL	(Wang vd., 2022)
Buğday	Zearalenon	Florometrik	0.01–100 ng/mL	0.004 ng/mL	(Ma vd., 2023)
Mısır	Fumonisin B1	CV	10^{-11}- 10^{-4} g/mL	10 pg/mL	(Zheng vd., 2021)
Mısır unu	Okratoksin A	Florometrik	9 nM-500 nM	3.9 nM	(Yu ve Zhao, 2022)

Gıda Alerjenlerinin Tespiti					
Örnek	Analit	Tespit Yöntemi	Doğrusal Aralık	Tespit Limiti	Kaynak
Yumurta	Lizozim	FRET	2–70 nM	0.07 nM	(Ahmadi vd., 2021)
Soya unu	β-konglutin	ICP-MS	0.001–0.08 nM	2 pM	(Torregrosa vd.,2023)
Panna cotta	Gluten	Elektrokimyasal	1-100 µg/L	3.4 mg/kg	(Svigelj vd., 2020)
Kurabiye	Ara h1	Elektrokimyasal	25-800 ng/mL	11.8 ng/mL	(Pan vd., 2024)
Metal İyonlarının Tespiti					
Örnek	Analit	Tespit Yöntemi	Doğrusal Aralık	Tespit Limiti	Kaynak
Yeraltı suyu	Pb^{2+}	EIS	0.04×10^{-2} µg/L	0.8 µM	(Yadav vd., 2020)
Sebze	Hg^{2+}	Elektrokimyasal	1-10.000 nmol/L	0.045 nmol/L	(Zhou vd., 2024b)
Göl Suyu	Cd^{2+}	SWV	31.3 nM-1000 nM	90 pM	(Yu ve Zhao, 2024)

Pestisit ve veteriner ilaç kalıntılarının analizi

Tarımsal üretim ve hayvancılıkta yaygın olarak kullanılan pestisit ve veteriner ilaçlarının yoğun ve bilinçsiz kullanımı insan sağlığı açısından büyük bir tehdit oluşturmaktadır (Banerjee vd., 2023). Aptamer tabanlı biyosensörler pestisit ve veteriner ilaç kalıntılarının tespitinde, hızlı ve hassas olmasının yanında taşınabilir olması nedeniyle sahada tespit imkânı da sunmaktadır (Xie vd., 2022). Pestisit kalıntılarının tespitinde elektrokimyasal (Himanshu vd., 2024), floresan (Xu vd., 2022), kolorimetrik (Shen vd., 2022), kemilüminesans (Sun vd., 2024) ve SERS (Yan vd., 2023) aptasensörleri kullanılmıştır. Örnek olarak Mao vd. (2023) elektrokimyasal aptasensörü kullanarak, asetamiprid kalıntısını, yüksek bir seçicilik, tekrarlanabilirlik ve geniş bir doğrusal aralıkta belirleyebilmişlerdir. Asetamiprid tespit limiti Enzim-bağlı İmmünosorbent Analizi (ELISA), Diyot dizi Dedektörlü Yüksek Basınçlı Sıvı Kromatografisi (HPLC-DAD), Sıvı Kromatografi-Kütle Spektrometresi (LC-MS) yöntemleri ile $1.35x10^{-7}$-$4.49x10^{-12}$ M arasında belirlenirken Fei vd. (2015), elektrokimyasal aptasensörünün kullanıldığı bu yöntemde doğrusal aralık 10 pM-10 µM ve tespit limiti ise 1.3 pM olarak bulunmuştur. Sun vd. (2024) tarafından üç farklı sebze (kolza, ıspanak, marul) örneğindeki asetamiprid, aptamer tabanlı elektrokimyasal sensör ile başarılı bir şekilde tespit edilmiş ve sonuçlar LC-MS yöntemi ile elde edilen sonuçlarla kıyaslanmıştır. Elde edilen sonuçlar elektrokimyasal aptasensörde asetamipridin geri kazanımının LC-MS yöntemine göre daha yüksek olduğunu göstermiştir. Bu çalışma ayrıca elektrokimyasal aptasensörün iz miktarlardaki asetamipridi sahada tespit edebilme potansiyelini ortaya koymuştur. Veteriner ilaçlarının tespitinde de aptamer tabanlı yöntemlerin başarılı bir şekilde kullanabileceği gösterilmiştir. Örneğin; β-laktam grubu antibiyotiklerden biri olan azlosilinin tespiti için voltametrik bir aptasensör geliştirilmiş ve hedef antibiyotik için tespit limiti 1.2×10^{-9} mg/ml, analiz süresi ise, 30 ila 50 dakika olarak bildirilmiştir (Chinnappan vd., 2020).

Patojen mikroorganizmaların tespiti

Gıda kaynaklı patojen bakteriler dünya çapında önemli bir halk sağlığı problemidir. *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus, Clostridium perfringens* ve *Campylobacter jejuni* gıda kaynaklı salgınlardan sorumlu en yaygın patojen bakterilerdir. Gıda kaynaklı mikrobiyel hastalıkların önlenebilmesi amacıyla patojen mikroorganizmaların tespitinde hızlı sonuç veren, basit ve güvenilir yöntemlere ihtiyaç duyulmaktadır. Son zamanlarda aptamer tabanlı sistemlerin, klinik ve gıda kaynaklı bakteriyel patojenlerin tanımlanması için büyük bir potansiyele sahip olduğu gösterilmiştir. Aptamer tabanlı yöntemler, geleneksel yöntemlerden farklı

olarak bakteriyel hedefleri, kültüre alınmasına gerek duyulmadan kısa zamanda tespit edebilen sistemlerdir (Mishra vd., 2023). Bugüne kadar birbirinden farklı özelliğe sahip aptamer tabanlı birçok sistem gıdalarda bulunan patojenlerin tespitinde kullanılmıştır. Örneğin; gıda kaynaklı en yaygın patojenlerden *Salmonella* spp.'nın tespiti için EIS aptasensörü geliştirilmiş ve tavuk örneklerinde test edilmiştir. Bu aptasensör *Salmonella* spp.'nin geleneksel yöntemle yaklaşık 4-5 gün olan tespit süresini 2 saate kadar indirmiş ve tespit limiti 2 KOB/ml olarak belirlenmiştir (Qiao vd., 2023). Yapılan başka çalışmada ise, *L. monocytogenes*'in tespit edilebilmesi için bir floresans aptasensörü geliştirilmiştir. Empedans immünosensör sisteminde *L. monocytogenes*'in tespit limiti $4.7x10^2$ KOB/ml bulunurken, floresans aptasensör sisteminde tespit limiti 8 KOB/ml'ye düşürülmüştür (Liu vd., 2021b). Jiang vd. (2023) tarafından yapılan bir çalışmada ise, nanozim bazlı elektrokimyasal bir aptasensör geliştirilmiş ve *L. monocytogenes*'in tespit limiti 2 KOB/ml olarak bulunmuştur.

Biyotoksinlerin tespiti

Mikotoksin, fitotoksin, nörotoksin vb. toksinler, çeşitli organizmalar tarafından üretilen biyotoksin sınıfı toksik maddelerdir. Gıdalarla birlikte alınan biyotoksinler, kronik hastalıklara neden olmakta hatta yüksek miktarlarda alınması durumunda ölümle sonuçlanan akut hastalıklara yol açabilmektedir (El-Sayed vd., 2022). Bu nedenle, biyotoksinleri hızlı bir şekilde tespit eden hassas ve pratik yöntemlere ihtiyaç duyulmaktadır. Son yıllarda aptamerler, biyotoksinlerin tespitinde yaygın olarak kullanılan, pahalı ve kısa bir raf ömrüne sahip antikorların yerini alabilecek alternatif bir molekül sınıfı olarak öne çıkmaktadır (Kadam ve Hong, 2022). Zhang vd. (2021b) tarafından yapılan çalışmada, *Fusarium* türlerinin ürettiği bir mikotoksin olan T-2 toksininin (T-2) tespiti için kolorimetrik bir aptasensör tasarlamıştır. Altın nanopartiküller (AuNP'ler) ve T-2'ye spesifik aptamerden oluşan bu sensörde; AuNP-aptamer kompleksi, çözelti içinde kırmızı renkte görünürken, T-2 varlığında aptamerler, hedefe bağlanarak AuNP'lerin yüzeyinden desorbe olduğunda çözelti rengi kırmızıdan mora dönüşmektedir. Renk değişimine bağlı olarak T-2 toksinini analiz eden bu kolorimetrik aptasensörde tespit limitinin 0.124 nM olduğu bildirilmiştir (Zhang vd., 2021b; Tang vd., 2023b). Bunun dışında kontamine olmuş tahıllarda ve bunların ürünlerinde yaygın olarak bulunan aflatoksin B1 (AFB1), fumonisin B1 (FB1), okratoksin A (OTA), zearalenon (ZEN) gibi mikotoksinlerin tespiti için de aptasensör sistemleri geliştirilmiştir. Örneğin aflatoksin B1 için tasarlanan SERS aptasensörünün, 4.0×10^{-10} mg/ml seviyesine kadar ölçüm yapabildiği bildirilmiştir (Fan vd., 2023). Ek olarak aptamerler, alg toksinlerinin tespitinde ve dekontaminasyonunda da kullanılmıştır (Bilibana vd., 2022). Örnek olarak insan sağlığını tehdit eden ve tespit edilmesi oldukça zor olan kabuklu deniz ürünü toksini DTX-1, geliştirilen biyokatman interferometri aptasensör sistemi ile 614 pM seviyesine kadar ölçülebilmiştir (Li vd., 2020).

Gıda alerjeni tespiti

Alerjenler genellikle gıdalarda eser miktarda bulunmasına rağmen duyarlı olan bireylerin bağışıklık sistemini uyararak, cilt döküntüsü, ürtiker, ishal, karın ağrısı ve hatta anaflaktik şok gibi şiddetli akut reaksiyonları provoke edebilmektedir (Zhou vd., 2024a). Bu nedenle birçok ülkede, gıdaların etiketlerinde alerjen madde beyanı zorunlu kılınmaktadır. Gıda alerjeni olarak kabul edilen bileşenler arasında yumurta, süt, yer fıstığı, fındık, buğday, soya fasulyesi, kereviz, hardal, susam tohumu, balık, kabuklu hayvanlar ve yumuşakçalar bulunmaktadır. Gıda kaynaklı alerjik reaksiyonlar, alerjen gıdanın direkt kendisinin tüketimiyle ortaya çıkabileceği gibi, bu alerjenleri içeren gıda ürünlerinin tüketimi sonucu indirekt olarak da ortaya çıkabilmektedir. Bu nedenle gıda alerjenlerinin hassas bir şekilde tespiti büyük bir önem arz etmektedir (Calabria vd., 2022). Son yıllarda yapılan bir çalışmada, fıstık alerjeni olan Ara h1'e spesifik aptamerler içeren mikroakışkan kağıt bazlı analiz cihazı geliştirilmiş ve tespit limiti 11.8 ng/mL olarak bulunmuştur (Pan vd., 2024). Ara h1 dışında aptamer tabanlı sensör sistemleri, β-konglutin (acı bakla alerjeni), gluten ve lizozim (yumurta alerjeni) gibi gıda alerjenlerinin tespiti için de kullanılmıştır (Hong vd., 2021; Torregrosa vd., 2023).

Ağır metallerin tespiti

Endüstriyel atıklardan, kadmiyum, cıva, kurşun gibi ağır metal iyonları toprak ve suya geçerek bitkisel ve hayvansal hammaddelere bulaşmakta ve daha sonra gıda zincirine dahil olmaktadır. Ağır metaller ayrıca pestisitlerden, katkı ve ambalaj maddelerinden de gıdalara bulaşabilmektedir. Gıdalara bulaşan bu ağır metal iyonları insan sağlığını tehdit eden önemli risk faktörleri arasındadır (Scutaraşu ve Trincă, 2023). Gıda örneklerinde, bulaşan ağır metal iyonlarının tespit etmek için Atomik Absorpsiyon Spektrometrisi (AAS), Atomik Floresans Spektrometrisi (AFS), spektrofotometri, Eşleşmiş Plazma-kütle Spektrometrisi (ICP-MS), Endüktif Eşleşmiş Plazma-Atomik Emisyon Spektrometrisi (ICP-AES) yöntemleri yaygın olarak kullanılmaktadır (Sawan vd., 2022). Ancak son yıllarda aptamer tabanlı biyosensör sistemlerinin de ağır metal iyonlarının tespitinde başarılı bir şekilde kullanılabileceği bildirilmiştir (Çizelge 1). Örneğin Yalagandula vd. (2024) tarafından yapılan bir çalışmada göl suyundaki arseniğin tespitine yönelik bir EIS aptasensörü geliştirilmiş ve bu aptasensör ile arseniğin minimum tespit konsantrasyonu 0.076 µg/kg olarak belirlenmiştir. Ayrıca kurşun ve kadmiyumun yüksek seçicilik ve hassasiyetle tespiti için Qian vd. (2022) tarafından elektrokimyasal kâğıt bazlı çip sistemine dayalı bir aptasensör geliştirilmiş ve bu aptasensör ile kurşunun minimum tespit konsantrasyonu 46.23 pmol/L, kadminyumun minimum tespit konsantrasyonu ise 23.31 pmol/L olarak belirlenmiştir. Bunların dışında cıva ve talyum gibi diğer ağır metalllerin tespitine yönelik geliştirilmiş aptamer tabanlı yöntemler de bulunmaktadır. Örneğin Wang vd. (2023b), sularda bulunan cıva'nın belirlenebilmesi için FET aptasensör geliştirmiş ve tespit limitini 0.02 mg/kg olarak bildirmiştir. Srinivasan vd. (2023) ise nehir suyunda bulunan talyumun tespitine yönelik kolorimetrik bir aptasensör tasarlamış ve bu sensör ile 7.4 µM'a kadar düşük miktardaki talyumu tespit edebilmiştir.

SONUÇ

Aptamerler, belirli bir hedefi tanıma ve bu hedefe spesifik bir şekilde bağlanma kabiliyetine sahip olan moleküllerdir. Sahip oldukları eşsiz özellikler nedeniyle moleküler tanıma elemanı olarak kullanılan aptamerlerin floresans, elektrokimya, SERS, SPR, kolorimetri, afinite kromatografisi, kapiler elektroforez gibi çeşitli analiz teknikleri ile kombine edilmesi, geliştirilen aptasensörlerin birçok alanda kullanımının yolunu açmıştır (Xie vd., 2022; Li vd., 2023a). Gıda kalite kontrolü, aptamerlerin potansiyel olarak önemli kullanım alanlarından biridir. Çok sayıda patojen, pestisit ve veterinerlik ilacı, gıda alerjeni, biyotoksin ve metal iyonunun spesifik ve seçici bir şekilde aptamer ve aptasensörler ile başarılı bir şekilde tespit edilebileceği gösterilmiştir (Mohamad vd., 2023). Aptamer teknolojisi sunduğu birçok faydaya rağmen, bugüne kadar yalnızca birkaç aptamer tabanlı ürün (NeoVentures'in OTA okratoksin A ve aflatoksin tespit kiti ve afinite kolonu) ticari olarak piyasaya sürülmüştür. Hedefine karşı yüksek afiniteye sahip aptamerlerin üretiminin özel bir uzmanlık gerektirmesi, nükleaz degradasyonuna hassas olması ve yüksek maliyet, aptamerlerin ticari üretimi ve kullanımını sınırlandıran en önemli faktörlerdir (Kalita vd., 2023). Ancak aptamer teknolojisi oldukça hızlı gelişim göstermektedir. Gıda kalite ve güvenliğinin kontrolüne yönelik alternatif yöntemlere duyulan ihtiyaç, daha basit, uygun maliyetli ve pratik aptamer tabanlı tanımlama teknolojilerinin geliştirilmesini ve kullanılmasını teşvik etmektedir. Bu amaçla son yıllarda özellikle aptamer tabanlı taşınabilir el cihazlarının geliştirilmesine yönelik çalışmalar artmıştır. Diğer taraftan, yeni biyobelirteç adaylarının keşfinde makine öğrenimine dayalı yöntemlerin kullanımı, yüksek spesifiteye sahip aptamerlerin üretimini kolaylaştıracaktır. Bu ve benzeri teknolojik gelişmeler, gıda kalite ve güvenliğinin kontrolünde aptamerleri etkin bir araç haline getirerek sağlıklı ve güvenilir gıdaların tüketiciye ulaştırılmasına katkı sağlayacaktır.

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AN INVESTIGATION OF THE ACE INHIBITORY ACTIVITY, ANTIOXIDANT CAPACITY, AND PHYTOCHEMICAL CONSTITUENTS OF POLAR AND NON-POLAR EXTRACTS OF *ZIZIPHUS JUJUBA* FRUIT: STATISTICAL SCREENING THE MAIN COMPONENTS RESPONSIBLE FOR BIOACTIVITY

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ABSTRACT

Herein, the angiotensin I-converting enzyme (ACE) inhibitory activity, antioxidant capacity, total polyphenol contents (TPC), and phytochemical profiles of polar and non-polar extracts of dried *Ziziphus jujuba* fruits were investigated, along with the statistical determination of the main components responsible for ACE inhibitory activity. The non-polar extract expressed the strongest ACE inhibitory activity (99.81%) among the extracts. The non-polar extract also exhibited the highest DPPH scavenging activity (IC_{50} of 30.63), linoleic acid/β-carotene bleaching capacity (89.31%), and TPC (59.47 mg GAE/g). The phenolic profiles of the extracts were identified by LC-MS/MS, and the presence of seven triterpenoid species in the extracts was examined using GC-MS techniques. The principal constituents included 19 phenolics, 2 organic acids, and 4 triterpenoids. A Pearson correlation and principal component analysis were conducted to find the correlation between individual phenolic compounds and ACE inhibitory activity.

Keywords: *Ziziphus jujuba*, ACE inhibitory activity, antioxidant capacity, phytochemical profile, Pearson correlation, principal component analysis

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ZIZIPHUS JUJUBA (HÜNNAP) MEYVESİNİN POLAR VE APOLAR EKSTRAKLARININ ACE İNHİBİTÖR AKTİVİTESİ, ANTİOKSİDAN KAPASİTESİ VE FİTOKİMYASAL BİLEŞENLERİNİN İNCELENMESİ: BİYOAKTİVİTEDEN SORUMLU ANA BİLEŞENLERİN İSTATİSTİKSEL İNCELENMESİ

ÖZ

Bu çalışmada *Ziziphus jujuba*'nın kurutulmuş meyvelerinin polar ve apolar ekstraktlarının anjiyotensin I-dönüştürücü enzim (ACE) inhibitör aktivitesi, antioksidan kapasitesi, toplam polifenol içerikleri (TPC) ve fitokimyasal profilleri araştırılmıştır ve ACE inhibitör aktivitesinden sorumlu temel bileşenler istatistiksel olarak analiz edilmiştir. En yüksek ACE inhibitör aktivitesi (%99.81) meyvenin apolar ekstraktında tespit edilmiştir. Apolar ekstrakt ayrıca en yüksek DPPH radikal süpürme aktivitesi (IC_{50} : 30,63), linoleik asit/β-karoten ağartma kapasitesi (%89.31) ve TPC'yi (59.47 mg GAE/g) sergilemiştir. Ekstraktların fenolik profilleri LC-MS/MS ile tanımlanmış ve ekstraktlardaki yedi triterpenoid türünün varlığı GC-MS teknikleri kullanılarak incelenmiş ve 19 fenolik, 2 organik asit ve 4 triterpenoid tanımlanmıştır. ACE inhibisyon aktivitesinden sorumlu fenolik bileşenlerin belirlenmesi amacı ile Pearson korelasyonu ve temel bileşen analizi kullanılmıştır.

Anahtar kelimeler: *Ziziphus jujuba*, ACE inhibitör aktivitesi, antioksidan kapasitesi, fitokimyasal profil, Pearson korelasyon analizi, temel bileşen analizi

INTRODUCTION

Hypertension is the most prevalent risk factor for morbidity and mortality in cardiovascular disease, affecting one to three adults worldwide. This common disease leads to stroke, arteriosclerosis, myocardial infarction, renal disease, and numerous other health problems. Angiotensin I-converting enzyme (ACE), a key element in the renin-angiotensin aldosterone system (RAAS), is crucial in managing hypertension. ACE converts inactive angiotensin I to angiotensin II, an effector molecule that narrows blood vessels and inactivates bradykinin, the vasodilator, causing high blood pressure. A group of ACE-inhibiting drugs is considered in the management of hypertension, such as captopril, enalapril, lisinopril, etc. These ACE inhibitors reduce blood pressure by blocking the production of angiotensin II and preventing the constriction of blood vessels. However, these medications have side effects such as extremely low blood pressure, coughing, poor taste, and allergic reactions, while being prescribed frequently because of their well-known function and ease of availability. On the other hand, natural hypertension regulators work well in moderate settings and have few adverse effects, making them a respectable substitute for synthetic medications. Therefore, the search for natural ACE inhibitors has intensified because of these compounds' cost-effectiveness, safety record, and possible positive effects (Memarpoor-Yazdi et al., 2020; Paiva et al., 2023; Zheng et al., 2017).

Nature is a source of an almost limitless variety of molecular entities that can be used to produce new effective medications for a wide range of illnesses, and other valuable bioactive compounds. Although natural products have long been part of conventional medical systems since ancient times, the characterization of compounds extracted from plants and their usage in modern pharmaceuticals only began in the mid-nineteenth century. Since then, 30% of the secondary metabolites of plants have been isolated and their biological activities have been revealed (Wink, 2010). Natural product-derived compounds have emerged as crucial contributors to modern drug development, particularly in antioxidant, antibacterial, and antitumor agents (Hui et al., 2024; Oliveira et al., 2023). Also, it has been well documented that plant-based products act as enzyme inhibitors that bind to enzymes and decrease or block their bioactivity. Many natural products constitute dynamic fields of pharmacology and biochemistry due to the discovery and development of enzyme inhibitors that can be used in the treatment of metabolic disorders (Saleem et al., 2023).

Fruits, representing a wide and diverse spectrum of crops of plant origin, are considered a reservoir of natural bioactive substances with promising health benefits. Many fruits contain a high concentration of anthocyanins, flavonoids, phenolic acids, vitamins, saponins, carotenoids, terpenes, sugars, proteins, capsaicinoids, fatty acids, and alkaloids. Numerous studies have proven that fruit extracts and their active components have different bioactivities (Ruiz Rodríguez et al., 2021). The bioactivity of the plant extract depends on various factors, such as time, temperature, solvent concentration, and solvent polarity. Since no one solvent is likely to reliably extract every phytochemical contained in the plant material, using solvents with different polarities may result in extracting distinct phytochemicals depending on the chemical nature of the compounds. To assure the extraction of a wide range of compounds with varied polarities, the extraction method should involve different solvents of increasing polarity, from non-polar solvents (n-hexane) to more polar solvents (water) (Gil-Martín et al., 2022).

Ziziphus jujuba (*Z. Jujuba*), also called jujuba, is one of the 130–170 species belonging to the Rhamnaceae family, which is distributed throughout Asia and the Mediterranean. *Z. jujuba* is a small tree or shrub that grows in hot and subtropical regions globally and produces a bright red fruit that is utilized as food as well as traditional medicine. The fruit of *Z. jujuba* is rich in fiber, minerals, proteins, sugars, phenolic acids, carotenoids, vitamins (especially vitamin C), flavonoids, cerebroside organic acids, and volatile compounds that provide a pleasant characteristic aroma (Hernández et al., 2016). Various reports have been published on the biological activities of *Z. jujuba* such as the antioxidant, anticancer, antifungal, anti-inflammatory, immuno-stimulant, hepatoprotective, antiobesity, and gastrointestinal protective activities (Li et al., 2020; Zhu et al., 2024)

A limited number of studies have proven that extracts from various parts of the *Z. jujuba* plant exhibit ACE inhibitory activities, and these inhibitory activities are mainly attributed to the presence of phytochemicals in the extracts (Kamkar-Del et al., 2020; Memarpoor-Yazdi et al., 2020; Yücetepe et al., 2023). However, the components responsible for the actual activity have not yet been elucidated completely. Herein, as an initial step in identifying the compounds that may be responsible for the inhibitory activity of *Z. jujuba* for the treatment of hypertension, a chemoinformatic profile was produced employing solvents with varying polarity. The presence of 53 phenolic compounds in dried fruit extracts was comprehensively investigated qualitatively and quantitatively by LC-MS/MS techniques. The presence of seven triterpenoid species in the extracts was examined using GC-MS techniques. The inhibition activities of the extracts against the ACE were investigated. The antioxidant activity of the extracts was determined using DPPH radical-scavenging activity and linoleic acid/β-carotene bleaching assay. The TPC of extracts was also examined using the Folin-Ciocalteu method. A Pearson correlation analysis was conducted to find the correlation between individual phenolic compounds and ACE inhibitory activities. Principal component analysis (PCA) plots were created to show the variance among the different extracts.

MATERIAL AND METHODS

Material

The fresh wild fruits of *Z. jujuba* were purchased from a local market in Mersin, Türkiye. The surface contaminants of the fruits were sorted and washed with sterile distilled water. The seeded fruits were freeze-dried and stored without exposure to light at -80 °C until use.

Moisture content of fruits

The moisture content of fruits was measured using the gravimetric method (Ng et al., 2022). The results were given as percentages (%) as follows (1):

Moisture (%) = (fresh weight -dry weight) / fresh weight x 100 (1)

The final moisture content of the freeze-dried fruits was 13.72±2.59%.

Preparation of polar and non-polar extracts of dried *Z. jujuba*'s fruits

Lyophilized *Z. jujuba* fruits (25 g) were ground to powder in a laboratory blender (Waring Commercial Blender, USA). The yielded powder (3 g) was extracted using 20 mL of either deionized water or a mixture of ethanol and water (50:50, v:v) with the help of sonication in an ultrasonic water bath (SK06GT Kudos ultrasonic water bath, Korea) for 30 minutes. During sonication, the temperature of the ultrasonic bath was kept in the range of 30–40 °C with the addition of ice. The extract was centrifuged at 8,000xg (Hanil Science Industrial Combi 514R, Korea) for 15 minutes. The supernatant was taken into a different tube. 20 mL of ethanol-water (50:50, v:v) or deionized water mixture was added to the pellet and sonicated again. After repeating this process three times, the three supernatants were combined. The extracts were stored at -80 °C until analysis (Meng et al., 2011).

Determination of ACE inhibitory activity of the dried fruit extracts

The ACE inhibition assay was carried out by adapting the method reported by Kwon et al. (2006). 50 μL of the extracts (50 mg dry weight (DW)/mL) were added to 200 μL of NaCl-borate buffer (0.3 M, pH 8.3) containing 2.0 mU ACE-I solution and pre-incubated at 25 ºC for 10 minutes. 100 μL of hippuryl-histidyl-leucine (5.0 mM) solution was added to the reaction mixture and incubated at 37 ºC for one hour. 150 μL of 0.5 N HCl was used to stop the reaction. Lisinopril was used as the standard. The formation of hippuric acid was monitored using HPLC with a UV detector at 228 nm (Kwon et al., 2006). The inhibition percentage was calculated with the following equation:

$$\text{Inhibition\%} = [\text{Area}_{\text{control}} - (\text{Area}_{\text{sample}} - \text{Area}_{\text{blank}})]/(\text{Area}_{\text{control}} - \text{Area}_{\text{blank}}) \times 100 \quad (2)$$

Determination of the antioxidant activity of the dried fruit extracts

The DPPH free radical scavenging and the linoleic acid/β-carotene bleaching assay were used to determine the antioxidant properties of the extracts (Ciniviz & Yildiz, 2020). Each experiment was performed in triplicate. BHA (butylated hydroxyanisole) and BHT (Butylated Hydroxytoluene) were used as standard references.

Total polyphenols content (TPC) of the dried fruit extracts

The TPC of the fruit extracts was assessed by the Folin-Ciocalteu method (Ciniviz & Yildiz, 2020), using gallic acid as a reference compound. The results were expressed as micrograms of gallic acid equivalents per liter of the fruit extracts.

Qualitative and quantitative analyses of phytochemical content of the dried fruit extracts

Determination of the phenolic components using LC-MS/MS

The phenolic composition of the dried fruit extracts was analyzed according to the method developed and validated by Yilmaz et al. (2018) and (Yilmaz, 2020) using a Shimadzu-Neexera model UHPLC (ultra-high performance liquid chromatograph) combined with a Shimadzu-LCMS 8040 model triple quadrupole mass spectrometer. The liquid chromatography system included an analytical column (Inertsil ODS-4 model C18, 100 mm×2,1 mm, 2μm), an autosampler (SIL-30AC model), binary pumps (LC-30 AD model), a degasser (DGU-20A3R model), and a column oven (CTO-10ASvp model). The final concentrations of the extracts were 250 mg/L, and all samples were filtered before the injection. The solvent flow rate was 0.5 mL/min, and the injection volume was 5 mL. The eluent A contained water, 5 mM ammonium formate, and 0.1% formic acid; and the eluent B contained methanol, 5 mM ammonium formate, and 0.1% formic acid. The gradient elution profile was as follows: 20–100% B (0–25 min), 100% B (25–35 min), and 20% B (35–45 min) (Yilmaz et al., 2018).

Determination of the triterpenoid contents using GC–MS

The presence of seven triterpenoids in the dried fruit extracts was assessed using an Agilent 7890A model gas chromatography and an Agilent 5977B model mass spectroscopy system. A HP-5MS column (30 m × 0.25 mm × 0.25 μm film) was used for chromatographic separation. Fixed

helium gas (1 mL/min, 20 psi) was installed as the carrier gas. The GC oven was preheated to 80 °C for 2 minutes, then raised to 300 °C at a rate of 5 °C per minute, where it remained for 14 minutes. The volume of injection was set to 1.0 μL, and the split ratio was 1:10. The ionization energy of the electron ionization/mass spectrometer (EI/MS) was adjusted to 70 eV. MS data were collected by setting the complete scan mode and scan m/z to a density of 50-800 atomic mass units (amu). N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane was used for the derivatization of samples. The concentration of the samples was 1000 mg/L (Bakir et al., 2020).

Statistical analysis

The results were displayed as mean values from the three replications with standard errors (S.E). A one-way analysis of variance (ANOVA) was used to assess the statistical differences between the extracts. Both Levene's test for homogeneity of variances and the Shapiro-Wilk test for normality were conducted. These tests are essential to ensure the validity of our analysis. The results of these tests influenced our approach to the Duncan analysis by providing insights into the distribution and variability of the data among groups. The Duncan multiple comparison test was used to compare mean values, and variations between mean values were defined as significant when $P < 0.05$. SPSS statistics software, version 22.00 (SPSS Inc., Chicago, IL, USA), was used for the statistical analysis. The Pearson correlation coefficient was calculated for correlation analysis ($P < 0.05$) (IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp). Significance was based on a confidence level of 95% ($P < 0.05$). Principal component analysis (PCA) condenses or summarizes the interactions between a large number of variables that are assumed to be connected to a smaller number of fundamental dimensions to visualize and explain the data. PCA was used to correlate the data obtained from phenolic profiles and ACE inhibition. The software R package version 4.2.0 was used to define PCA plots and analyses with the libraries "factoextra", "FactoMineR", "ggcorrplot", <https://CRAN.R-project.org/package=here>.

RESULTS AND DISCUSSION

ACE inhibitory activities of the dried fruit extracts

The inhibition of ACE, a Zn-dependent peptidase that regulates blood pressure, is a crucial therapeutic strategy used in the treatment of hypertension, which is a serious disease that causes cardiovascular, brain, and kidney damage (Wu et al., 2022). The inhibition activities of polar and non-polar extracts against the ACE were determined as 71.84±1.04% and 99.81±2.01%, respectively (Fig.1). The inhibition activity of non-polar extract was as high as the standard, lisinopril, a commercial ACE inhibitor. At the same time, when compared with the literature, the ACE inhibition activity of the whole fruit (99.81±2.01%) was found to be higher than the ACE inhibition activity of methanol extracts obtained from the seeds (86.04±0.00%) and pulp (42.74±8.57%) of the fruit by ultrasound-assisted extraction method (Şensu et al., 2023). The results of the present study showed that the dried fruit extracts of *Z. jujuba* are a promising source to be used as a dietary supplement or a pharmaceutical reagent for the treatment of hypertension.

Antioxidant activity and total polyphenol contents of the dried fruit extracts

The DPPH radical scavenging activity and linoleic acid/β-carotene bleaching assay were used as tools to compare the antioxidative activities of the polar and non-polar extracts. BHA and BHT were used as standards. The antioxidant activity results of the extracts are presented in Fig.2A. The antioxidant activity of non-polar extracts was by far higher than that of polar extracts. This difference detected between extracts could be due to the type of polyphenols released into the non-polar solvent during extraction. Additionally, the activity of the non-polar extract may be attributed to other phenolic compounds that cannot be screened due to the lack of standards.

The results showed that the radical scavenging capacities of the polar and non-polar extracts were 170.30 ± 2.88 and 30.64 ± 1.37 μg/mL (DPPH, IC_{50}) and 12.08 ± 0.97% and 89.31 ± 1.59% (β-carotene). In a study, the antioxidant activities of the polar and non-polar extracts of *Z.*

jujube furit (without seed) were reported to be 400 µg/mL and 300 µg/mL (DPPH, IC_{50}) (Lin et al., 2020). Since the antioxidant activity of a fruit is affected by many factors such as variety, soil, water, altitude, extraction method and antioxidant assays, it is difficult to directly compare activities between studies (Zargoosh et al., 2019; Zhu et al., 2024).

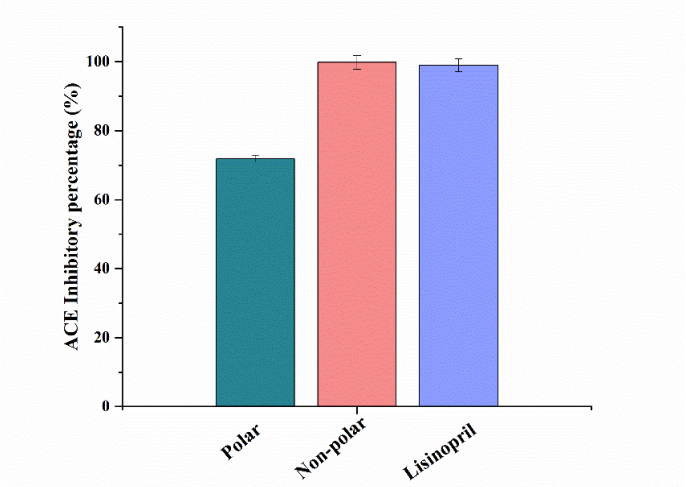

Figure 1. ACE inhibition activity of the dried fruit extracts of *Z. jujuba*. Results are expressed as mean ± standard deviation (n = 3)

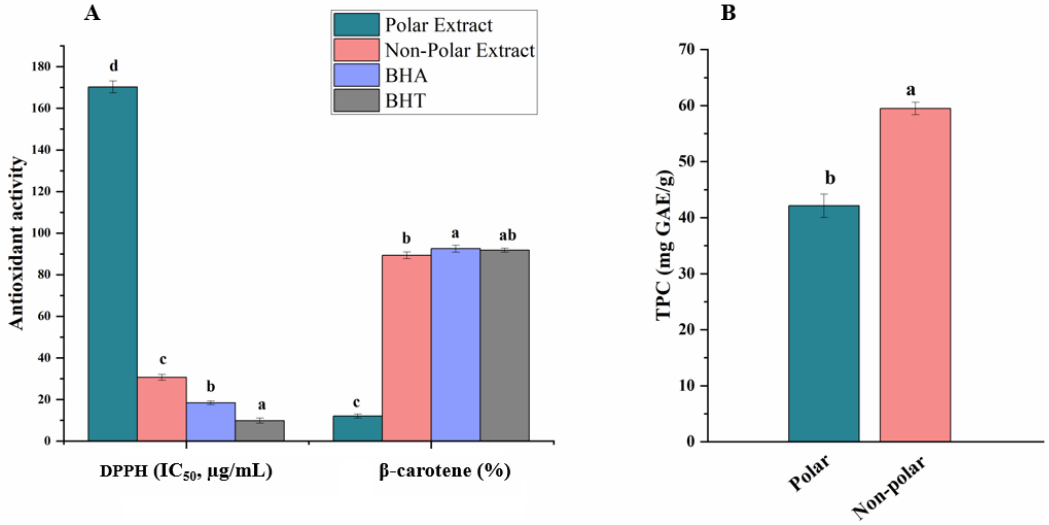

Figure 2. Antioxidant activity (A) and total phenolic content (B) of the dried fruit extracts of *Z. jujuba*. Results are expressed as mean ± standard deviation (n = 3). Different lowercase letters denote a significant difference between samples in the same group ($P < .001$)

The TPC of the extracts is shown in Fig. 2B. The TPC of the non-polar extract (59.47±1.12 mg GAE/g) was higher than that of the polar extract (42.13±2.08 mg GAE/g) ($P < 0.05$). The effects of solvent polarity on TPC showed results similar to those of this parameter's influence on the samples' antioxidant activity. Al-saeedi et al. (2016) reported that the TPC of fruit extracts obtained from *Z. jujuba* using different solvents (methanol, hexane, ethyl acetate, chloroform, and water) varied between 16.93 - 187.51 mg GAE/g and the TPC of water extract was 18.17 mg

GAE/g. Similar to our results, the amount of TPC was found to be higher in the nonpolar extracts than in the polar extract. Due to the different solvents used, a complete comparison could not be made, but the TPC of the water extract was reported to be much lower than in the present study. (Al-Saeedi et al., 2016).

Phenolic profiles of the dried fruit extracts

To obtain a more detailed phenolic profile of the fruit of *Z. jujuba*, two solvents with different polarities were used for the extraction process, and the phenolic profile of the extracts was analyzed by LC-MS/MS in comparison with 53 reference compounds. 21 of the phenolic species were discovered in at least one of the extracts at different levels. Phenolic compounds found in the extracts are listed in Table 1. The non-polar extract was found to have a higher diversity of phenolic compounds (eighteen distinct species) than the polar extract (thirteen distinct species). On the other hand, when the concentration of species detected in the two extracts was compared, the polar extract generally possessed amounts of phenolics that were far higher than the non-polar extract. The major components detected in the extracts are as follows: Polar extract: Quinic acid (84.16 mg/100 g DW), epicatechin (21.26 mg/100 g DW), fumaric acid (10.36 mg/100 g DW), catechin (5.90 mg/100 g DW), aconitic acid (4.04 mg/100 g DW), rutin (2.18 mg/100 g DW); Non-polar extract: Quinic acid (36.88 mg/100 g DW), protocatechuic acid (2.90 mg/100 g DW). Among the hydroxycinnamic acids investigated, quinic acid was the most abundant in the polar extract, with a level of 84.26 mg/100 g DW. In contrast, caffeic acid and p-coumaric acid were only detected in the non-polar extract. Three hydroxybenzoic acids (gallic acid, protocatechuic acid, and salicylic acid) are primarily found in non-polar extracts. Furthermore, epicatechin, catechin, and rutin were the most abundant flavonoids in the polar extract, with levels of 21.26, 5.90, and 2.18 mg/100 g DW, respectively. Remarkably, the lower values for all organic acids (fumaric acid and aconitic acid) were obtained in the non-polar extract. Wang et al. (2016) investigated the changes in phenolic compounds of *Z. jujube* fruits at three different edible maturity stages, using 12 standard phenolics. The results showed that the phenolic compounds varied with the ripening stage, and the most dominant flavonoid among the phenolics examined was rutin, and the most dominant phenolic acid was caffeic acid, followed by gallic acid, chlorogenic acid, and *p*-coumaric acid (Wang et al., 2016). In a study conducted by Yan et al. (2022), catechin, epicatechin and rutin were reported as the dominant species in *Z. jujube* fruit among 15 phenolic compounds (Yan et al., 2022). Quinic acid, which was found to be the most dominant species in the present study, was not used as a standard in either study.

Triterpenoid contents of the dried fruit extracts

Triterpenoids are a diverse group of secondary metabolites found in plants. They exhibit a broad spectrum of potential pharmacological effects combined with a low toxicity profile. Triterpenoids are thought to be the primary functional constituents in *Z. jujuba* fruit. However, the content of triterpenoids varies considerably from region to region and from cultivar to cultivar (Pan et al., 2023). In the present study, seven triterpenoid species were screened in the fruit extracts using GC/MS. The results are given in Table 2. Oleanonic acid, oleanolic acid, betulinic acid, and ursolic acid were detected in the non-polar extract. Oleanonic acid was the dominant triterpenoid acid in the fruit of *Z. jujuba,* with a level of 14.116 mg/100 g DW. A lower concentration of these acids in *Z. Jujuba* fruit was previously reported by Song et al. (2020) as follows: betulinic acid (516.41–4097.96 μg/g DW), oleanolic acid (36.70–837.46 μg/g DW), ursolic acid (5.27–685.33 μg/g DW), oleanonic acid + ursonic acid (9.83–244.80 μg/g DW) (Song et al., 2020). In the present study, none of the species were detected in the polar extract. Due to their low polarity, the detected triterpeneoids are practically insoluble in water and the extraction process requires the use of organic solvents (Castellano et al., 2022).

Table 1. Phenolic profiles of polar and non-polar extracts of the dried fruit of *Z. jujuba*

Reference Phenolic Compound	M.I. (m/z)[a]	F.I. (m/z)[b]	U[c]	Quantification (mg/100 g DW)	
Simple Phenols				Polar extract	Non-polar extract
Phenolic acids					
Hydroxycinnamic acids					
Caffeic acid	179.0	134.0	0.0354	ND	0.04
p-Coumaric acid	163.0	93.0	0.0516	ND	0.36
Quinic acid	190.8	93.0	0.0082	84.26	36.88
Hydroxybenzoic acids					
Gallic acid	168.8	79.0	0.0282	0.04	0.16
Protocatechuic acid	152.8	108.0	0.0411	0.28	2.90
Salicylic acid	137.2	65.0	0.0329	ND	0.04
Coumarins					
Coumarin	146.9	103.1	0.0237	ND	0.04
Polyphenols					
Flavonoids					
Flavones					
Luteolin	284.8	151.0/175.0	0.0174	ND	0.01
Flavonols					
Kaempferol	285.0	239.0	0.0209	ND	0.14
Nicotiflorin	592.9	255.0/284.0	0.0276	1.14	0.54
Rutin	608.9	301.0	0.0159	2.18	1.22
Quercetin	301.0	272.9	0.0543	0.04	0.64
Flavanones					
Hesperidin	611.2	449.0	0.0262	1.78	1.00
Hesperetin	301.0	136.0/286.0	0.0562	ND	0.04
Naringenin	270.9	119.0	0.0521	0.004	ND
Flavanols					
Catechin	288.8	203.1	0.0221	5.90	ND
Epicatechin	289.0	203.0	0.0221	21.26	ND
Non-Flavonoids					
Tannins					
Tannic acid	182.8	78.0	0.019	ND	0.36
Hydroxybenzaldehydes					
Protocatechuic aldehyde	137.2	92.0	0.0396	0.072	1.196
Organic acids					
Fumaric acid	115.2	40.9	0.0124	10.36	1.988
Aconitic acid	172.8	129.0	0.0247	4.03	0.222
Rutin-D3-IS[d]	612.2	304.1	ND	IS	IS
Ferulic acid-D3-IS[d]	196.2	152.1	ND	IS	IS
Quercetin-D3-IS[d]	304.0	275.9	ND	IS	IS

[a] MI (m/z): Molecular ions of the standard analytes (m/z ratio).

[b] FI (m/z): Fragment ions.

[c] U (%): percent relative uncertainty at 95 % confidence level (k = 2).

ND: Not determined.

Epigallocathechin, gentisic acid, chlorogenic acid, epigallocatechingallate, 1,5-dicaffeoylquinic acid, 4-hydroxybenzoic acid, vanilic acid, syringic acid, vanillin, syringic aldehyte, daidzin, epicatechingallate, piceid, ferulic acid, sinapic acid, cynaroside, miquelianin, isoquercitrin, *o*-Coumaric acid, genistin, rosmarinic acid, elagic acid, cosmosiin, quercitrin, astragalin, fisetin, daidzein, genistein, apigenin, amentoflavone, chrysin, acacetin were not detected either of the extracts.

Table 2. Triterpenoid contents of the fruit extracts by GC-MS

Compounds	R_t[a]	Molecularion-m/z (relative intensity %) (m/z)[b]	% RSD[c]	Three major fragment ions m/z (relative intensity %)			Polar extract (mg/100 g DW)	Non-polar extract (mg/100 g DW)
Alphaamyrin	17.99	498 (2.5)	0.025	218(100)	203(16.6)	189(18.3)	ND[d]	ND
Moronic acid	20.71	527 (21.1)	0.029	189(100)	203(40.3)	409(24.3)	ND	ND
Oleanonic acid	20.96	527 (12.3)	0.023	203(100)	408(64.5)	189(52.6)	ND	14.116
Oleanolic acid	21.55	601 (2.3)	0.026	203(100)	189(31.5)	320(28.6)	ND	8.158
Betulinic acid	21.90	601 (4.9)	0.019	189(100)	203(34.5)	320(21.8)	ND	6.778
Ursolic acid	22.55	601 (2.3)	0.015	203(100)	189(32.9)	320(79.6)	ND	9.082
Ursonic acid	22.91	527 (9.5)	0.028	203(100)	320(60.4)	189(24.9)	ND	ND

[a]Rt: Retention time.
[b]Mother ion(m/z): Molecular ions of the standard compounds (m/z ratio).
[c]RSD: Relative standard deviation
[d]ND: Not detected

Correlation analysis

Pearson's Correlation

The potential relationships between specific phenolic components and the enzyme-inhibitory properties of the polar and non-polar extracts of *Z. jujuba* fruit were highlighted using Pearson's correlation analysis (Fig.3). The direction of the correlation is either positive (acquiring a positive relationship) or negative (acquiring a negative relationship). 21 of the phenolic compounds identified in the fruit extracts showed a high correlation with the inhibition of ACE. Caffeic acid, coumarin, gallic acid, hesperetin, kaempferol, luteolin, *p*-coumaric acid, protocatechuic acid, protocatechuic aldehyde, quercetin, salicylic acid, and tannic acid showed a high positive correlation with ACE inhibitory activity. On the other hand, aconitic acid, catechin, epicatechin, fumaric acid, hesperidin, naringenin, nicotiflorin, quinic acid, and rutin showed a high negative correlation with ACE inhibitory activity.

Several studies were conducted on the anti-hypertensive properties of extracts obtained from different parts of plants. Also, several reports investigating the ACE inhibitory activity of individual phenolics revealed that caffeic acid (Agunloye & Oboh, 2018), coumarin (Ali et al., 2019), hesperetin (Yamamoto et al., 2008), protocatechuic acid (Safaeian et al., 2018), kaempferol, luteolin, and quercetin (Guerrero et al., 2012) have ACE inhibitory activity, consistent with our results. On the other hand, it has been reported that rutin, which was found to have a negative correlation in the present study, showed a dose-dependent ACE inhibitory activity; increasing the concentration from 100 μM to 500 μM increases the inhibition activity from 36% to 87% (Guerrero et al., 2012). The positive correlation of *p*-coumaric acid, protocatechuic aldehyde, salicylic acid, and tannic acid in ACE inhibitory activity has been shown for the first time in the literature with the presented study.

Principle Component Analysis

PCA analysis evaluated the relationship between individual phenols and ACE inhibitory activities. The first principle component (Dim 1) explained 99.8% of the total variance, while the second principle component (Dim 2) explained 0.2% of the variance. Together, PCs 1 and 2 accounted for 100% of the variance. As delineated in Fig.4, both positions of each phenolic in terms of the positive and negative sides of the axis are used to visualize which phenolics are positive contributors and which are not. A closer arrow denotes a high correlation, whereas the length of the arrows shows which variable contributes the most to the principle component. As shown in the PCA diagram, along axis 1 of the PCA analysis, 12 phenolics were grouped on the positive side and strongly contributed to ACE inhibitory activity. In the negative part of axis 1, nine phenolics formed an additional group, indicating a negative correlation with the ACE inhibitory activity. Upon examination of the biplot, it is apparent that ACE demonstrates a robust positive correlation with coumarin, protocatechuic acid, hesperetin, kaempferol, luteolin, protocatechuic aldehyde,

quercetin, *p*-coumaric acid, tannic acid, gallic acid, salicylic acid, and caffeic acid. Conversely, a negative correlation exists between ACE and aconitic acid, catechin, epicatechin, fumaric acid, hesperidin, naringenin, nicotiflorin, quinic acid, and rutin. These findings provide valuable insights into the associations between ACE inhibitory activities and specific compounds present in the polar and non-polar extracts of *Z. jujuba* fruit.

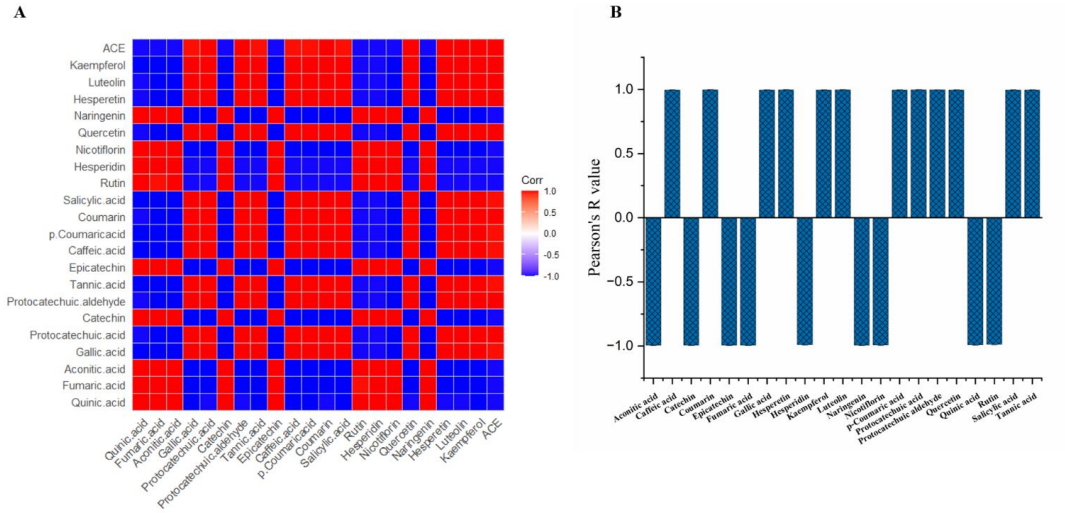

Figure 3. Pearson correlation of the phenolic compounds and ACE inhibitory activity in the dried fruit extracts of *Z. jujuba* (A) and bar graph of Pearson's correlation coefficient (B). The red and blue color's intensity represents higher to lower correlation levels. A correlation coefficient of +1 indicates a perfect positive correlation.

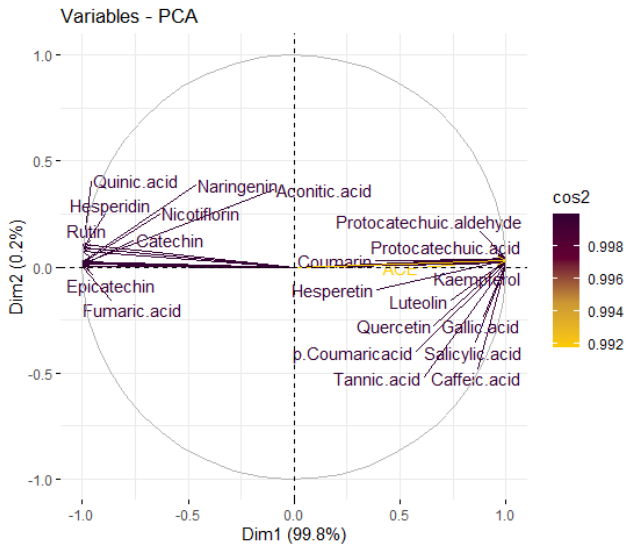

Figure 4. Principal component analysis of the phenolic compounds and ACE inhibitory activity in the dried fruit extracts of *Z. jujuba*. The figure depicts a biplot generated from data matrices representing the polar and non-polar extracts of *Z. jujuba* fruit in conjunction with ACE inhibitory activities. In a PCA biplot, the angle between the vectors representing the original variables serves as an estimate of the correlation between those variables. A slight angle signifies a positive correlation, while an angle near 180 degrees suggests a negative correlation

CONCLUSION

In the present study, ACE inhibitory activity, the phytochemical profile, radical scavenging capacity, and total phenolic content of polar and non-polar extracts obtained from the fruit of *Z. jujuba* were investigated, and the main components responsible for ACE inhibitory activity were revealed through statistical analysis. It was proven that the non-polar extract of the fruit of *Z. jujuba* exhibited excellent ACE inhibitory activity, radical scavenging capacity, and total phenolic content. The non-polar extract also contained high amounts of oleanonic acid, oleanolic acid, betulinic acid, and ursolic acid. The finding highlights the ACE inhibitory effects of individual phenolics, including caffeic acid, coumarin, gallic acid, hesperetin, kaempferol, luteolin, *p*-coumaric acid, protocatechuic acid, protocatechuic aldehyde, quercetin, salicylic acid, and tannic acid. Based on our results, the fruit of *Z. jujuba* could be a promising natural supplement for the treatment of hypertension. Both the fruit of *Z. Jujuba* and its by-products have the potential to be used in the pharmaceutical and food industries for future innovations.

CONFLICT OF INTEREST

The author(s) declares no conflict of interest.

AUTHORS' CONTRIBUTIONS

Bahar Tuba Findik: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. Hilal Yildiz: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. Esma Birisci: Methodology, Formal analysis, Data curation, Review and Editing. Serkan Yiğitkan: Methodology, Formal analysis. Pelin Köseoğlu Yılmaz: Methodology, Formal analysis. Abdulselam Ertaş: Methodology, Formal analysis, Data curation, Review and Editing. All authors approved the final manuscript and accepted to be held responsible for the content.

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ALTERNATİF PROTEİN KAYNAĞI OLARAK YENİLEBİLİR BÖCEKLER VE TÜKETİCİ KABULÜ

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ÖZ
Böcekler antik çağlardan beri birçok kültürde yerel mutfağın bir parçası olmuştur. Dünyanın birçok bölgesinde hali hazırda tüketilmekte olan böceklerin besin içeriği, onların alternatif protein kaynağı olarak da dikkat çekmesine sebep olmuştur. Dünya nüfusunun yaklaşık %10'u gıdaya erişim konusunda problem yaşamakta, neredeyse 1 milyar insan yetersiz beslenmeye bağlı hastalıklarla karşı karşıya gelmektedir. Artan nüfusun gıda talebini karşılamak için mevcut gıda üretim modeli yetersiz kaldığı gibi, nüfusa bağlı olarak artan tarımsal üretim de atmosfere daha fazla sera gazı salınımına sebep olarak küresel ısınmayı hızlandırmaktadır. Böcekler yüksek protein içerikleri sayesinde nüfusun protein ihtiyacını karşılamak için geleneksel hayvan proteinlerinin yerini alabilecek potansiyele sahiptir. Ancak bu hususta yetkili otoritelerin gıda güvenliği endişeleri olduğu gibi, tüketici kabulünde de zorluklar bulunmaktadır. Üretim modelleri ve ileri işleme teknikleri ile gıda güvenliği endişelerinin, farklı pazarlama ve market stratejileri ile de tüketici kabulünde yaşanan zorlukların üstesinden gelmek mümkündür. Bu derlemede alternatif protein kaynağı olarak yenilebilir böceklerin potansiyeli ve yenilebilir böceklere olan tüketici tutumu değerlendirilmiştir.
Anahtar kelimeler: Yenilebilir böcekler, alternatif protein kaynakları, tüketici kabulü

EDIBLE INSECTS AS ALTERNATIVE PROTEIN SOURCES AND CONSUMER ACCEPTANCE

ABSTRACT
Insects have been a part of local cuisine in many cultures since ancient times. The nutritional content of insects, which are currently consumed in many parts of the world, has attracted attention as an alternative protein source. Approximately 10% of the world's population has problems accessing food, and almost 1 billion people face diseases related to malnutrition. Just as the current food production model is insufficient to meet the food demand of the increasing population, increasing agricultural production in conjuction with population growth accelerates global warming by causing more greenhouse gas emissions into the atmosphere. Insects have the potential to replace traditional animal proteins to supply the protein requirements of the population because of their high protein content. However, in this regard, the competent authorities have food safety concerns, as well as

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difficulties in consumer acceptance. It is possible to overcome food safety concerns with production models and advanced processing techniques as well as the difficulties experienced in consumer acceptance with different marketing and market strategies. In this review, the potential of edible insects as an alternative protein source and consumer attitude towards edible insects were evaluated.
Keywords: Edible insects, alternative protein sources, consumer acceptance

GİRİŞ

Dünya insan gözünden, her ne kadar büyük bir gezegen gibi görünse de sınırlı kaynaklara sahiptir. Tarıma elverişli toprakların azlığı, gün geçtikçe artan kuraklık ve iklim değişikliği gibi sıkıntılar artan nüfusun gıda güvenliğini tehdit etmektedir (Rezvi vd., 2023). Günümüzde dünya 8 milyardan fazla insana ev sahipliği yapmakta ve nüfusun 2100 yılı itibari ile 10 milyarın üzerinde olacağı öngörülmektedir (Ritchie, 2023). Nüfusu beslemek için gıda üretim zincirinin büyümesi ve tarımsal faaliyetlerin artması sera gazı salınımında %31'lik bir oranda artışa neden olarak ekosisteme, biyolojik çeşitliliğe büyük zarar vermiş, asit yağmurları sel, kuraklık ve toprak kayması gibi çevresel felaketlere yol açmıştır (Kusmayadi vd., 2021). Artan üretime rağmen dünya nüfusunun %10'u yetersiz beslenme ve gıdaya erişim problemi ile karşı karşıyadır (Qu vd., 2023). Neredeyse 1 milyar insan yeterli protein ve ihtiyaç duyduğu kaloriye erişemediği için kas ve immün sistem zayıflığı, büyüme geriliği gibi yetersiz beslenmeye bağlı hastalıklarla yüz yüze gelmektedir (Doğan ve Özaltın, 2022). Artan popülasyon ile sürdürülebilir bir gıda üretim sistemi yaratmak gün geçtikçe daha da zorlaşırken, önümüzdeki on yıllarda gıda üretiminin nüfusa bağlı olarak dramatik bir şekilde artış göstermesi gerektiği düşünülmektedir (Berners-Lee vd., 2018).

Modern dünyada bitki ve hayvan kaynaklarını kullanarak var olan nüfusun ihtiyacı olan proteini üretmek sürdürülebilir olmadığı gibi, geleneksel yöntemlerle ihtiyacın karşılanması için verimli ve büyük tarım arazileri, temiz ve içilebilir su gibi uygulamalara ihtiyaç vardır. Giderek artan nüfus ve nüfusu etkileyen gıda kıtlığı düşünüldüğünde geleneksel kaynaklara alternatif protein kaynakları bulmak özellikle gelişmekte olan ülkeler için çözülmesi gereken bir problem haline gelmiştir (Cardoso Alves vd., 2023).

Ülkelerin gelişmişlik düzeylerinin ölçütlerinden biri de hayvansal protein tüketimidir. Gelişmiş ülkelerde hayvansal protein tüketimi daha fazla iken gelişmekte olan ülkelerde hayvansal protein tüketim miktarı halen istenen düzeylerde değildir (Terin ve Bilgic, 2018). Dünya genelinde kişi başına düşen günlük protein tüketimi ortalaması 83 gram iken, bunun yaklaşık %40'ını hayvansal proteinler oluşturmaktadır (Ergün Bayram, 2021). Ancak giderek artan akademik çalışmalar geleneksel hayvansal protein üretiminin çevreye ve iklim değişikliğine etkileri konusuna işaret etmektedir (Sanchez-Sabate ve Sabaté, 2019; González vd., 2020). Günümüzdeki gıda üretim modeli atmosfere salınan sera gazının 1/3'ünden sorumlu tutulmaktadır (Crippa vd., 2021). Nüfusun artışı ile birlikte gelen talebi karşılamak için üretimin de iki katına çıkacağı ve bu geleneksel üretim modelinin atmosfere saldığı sera gazında da üretime paralel olarak artış olacağı öngörülmektedir (van Zanten vd., 2016). Bu endişelere ek olarak et ve işlenmiş et ürünlerinin tüketiminin kanser, diyabet ve kardiyovasküler hastalıklar gibi kronik hastalık riskini artırdığını işaret eden çalışmalar vardır (Almeida vd., 2023).

Alternatif protein kaynağı olarak bitkiler, algler, fungiler, mikroorganizmalar ve böcekler kullanılabildiği gibi kültür etini de alternatif protein kaynağı olarak değerlendirmek mümkündür (Grossmann vd., 2021). Bu alternatif protein kaynakları birbirleri arasında içerdikleri protein miktarı, protein kalitesi ve proteinlerin aminoasit profili gibi faktörler ile karşılaştırılmaktadır. Besinsel içeriği haricinde aynı zamanda bu alternatif protein kaynaklarının çevreye olan etkisi, karbon ayak izi, su ve arazi kullanımı gibi faktörler de birbirlerine karşı avantajları ve dezavantajları bakımından irdelenmektedir.

GELENEKSEL HAYVANSAL PROTEİNLER İLE YENİLEBİLİR BÖCEKLERİN BESİN DEĞERLERİ

Geleneksel hayvansal protein üretiminin yarattığı bu sorunlara ve endişelere çözüm için; böceklerin alternatif protein kaynağı olarak kullanımı önerilen yaklaşımlardan biridir (McClements, 2020). Tüketilebilir bir çok böcek türü makro ve mikro besinler yönünden zengindir (Nowak vd., 2016). Çizelge 1'de yenilebilir bazı böcek türleri ile geleneksel hayvansal gıdaların karşılaştırılması bulunmaktadır.

Çizelge 1. Yenilebilir bazı böcek türleri ile bazı hayvan kaynaklarının besin içerikleri (Orkusz, 2021)

Besin Kaynağı	Enerji (kcal/100 g)	Protein (g/100 g)	Yağ (g/100 g)	Lif (g/100 g)
Acheta domesticus Y	153	20.5	5.06	4.6
Acheta domesticus L	137.5	15.4-17.5	4.4-7.9	2.3
Tenebrio molitor Y	178	24.13	6.14	7.4
Tenebrio molitor L	247	25	12.91	3.52
Gonimbrasia belina L	161	35.2	15.2	-
*Pyralida*e L	274.7	16.1	24.9	2.1-3.4
Koyun budu	196.56	15.12	15.12	-
Dana budu	85.32	15.72	2.45	-
Kaz eti	140.63	5.78	13.04	-
Ördek eti	199.04	8.64	18.30	-
Tavuk budu	125	17.8	6	-

Y: Yetişkin L: Larva

Yenilebilir 2000'in üzerinde bilinen böcek türü bulunmakta (Okyere, 2023) ve *Tenebrionidae* familyasının böcekleri (sarı un kurdu *Tenebrio molitor*, daha küçük un kurdu *Alphitobius Diainus* ve süper kurt *Zophobas morio*); cırcır böcekleri gibi bazı ortopteranlar (ev cırcır böceği *Acheta domesticus*; tropikal ev cırcır böceği *Gryllodes sigillatus*) ve iki benekli cırcır böceği *Gryllus bimaculatus* veya göçmen çekirge *Locusta migratoria* gibi çekirgeler ile büyük balmumu güvesi Galleria mellonella gibi bazı *Lepidopteran* türleri ve *Bombyx mori* gibi ipekböcekleri yenilebilir bu böcek türleri arasındadır (Huis vd., 2020). Yenilebilir böcekler protein, yağ, polisakkaritler ile vitamin ve mineralleri ihtiva etmektedir. Böcekler sadece besin değerleri yönünden değil ayrıca esansiyel aminoasitler ve doymamış yağ asitleri bakımından da zengin bir kaynaktır (Orkusz, 2021). Çizelge 2'de esansiyel aminoasitler bakımından geleneksel hayvansal protein kaynakları ile böcekler arasında karşılaştırma yapılmıştır. İçerdikleri yüksek B12 vitamini, demir, çinko gibi mineraller, lif, esansiyel aminoasitler, omega-3, omega-6 ve antioksidanlar sayesinde sağlık üzerinde de olumlu etkileri bulunmaktadır (Nowakowski vd., 2022).

Çizelge 2 Alternatif protein kaynaklarından elde edilen proteinlerin ve tavuk yumurtası proteininin aminoasit profili (g/100 g) (Sobczak vd., 2023)

Protein Kaynağı	Alg	Bakteri	Mantar	Kril	Böcekler	Tavuk Yumurtası Proteini
İzolösin	4.7	3.3	1.8	2.5	3.8	5.9
Lösin	8.6	5.4	2.9	4	6.5	8.41
Valin	6.2	4.2	2.2	2.6	5.2	7.25
Lizin	6.3	4.3	3	4.4	5.1	5.95
Fenilalanin+ trozin	9	5.8	3.1	5	9.7	9.97
Metiyonin+ sistein	3.1	2.2	1	2.4	3.5	6.16
Triptofan	0.9	0.8	0.3	0.7	1.2	1.48
Treonin	5.4	3.3	2	2.2	3.7	4.30

YENİLEBİLİR BÖCEKLERİN SAĞLIK ÜZERİNE ETKİLERİ

Yenilebilir böceklerin sağlık üzerine etkileri konusunda birçok çalışma bulunmaktadır. İçerdikleri kitin, yağ asitleri ve glikozaminoglikan gibi birçok besin maddesi potansiyel olarak insan sağlığı için yararlıdır. Yapılan çalışmalar yenilebilir böceklerin bağırsak florasında bulunan probiyotik bakterilerin gelişimini destekleyerek diyare, şişkinlik, antibiyotik yan etkileri gibi gastrointestinal rahatsızlıkları giderdiğini göstermiştir (Vangsoe vd., 2018). Gökkuşağı alabalıkları üzerinde yapılan bir çalışmada kara asker sineği larvaları ile beslenen alabalıkların bağırsak floralarındaki mikroorganizma çeşitliliğinin artığı gözlenmiş, floradaki bu çeşitlilik fırsatçı patojenlerin bakterilerle rekabetine yol açarak hastalıklara karşı dayanıklılığı artırdığı görülmüştür (Bruni vd., 2018). Cırcır böceklerinde bulunan glikozaminoglikan anti-inflamatuar etki göstererek kronik artrit hastalıklarına karşı etki göstermektedir (Ahn vd., 2014).

Diyabetik farelerde yapılan çalışmada glikozaminoglikan takviyesini içeren bir diyetle tedavi alan diyabetik farelerin, kan şekeri ve LDL-kolesterol seviyelerinde bir azalma ve antioksidan enzimlerin (katalaz, süperoksit dismutaz ve glutatyon peroksidaz) aktivitesinde bir artış gösterdiği bulunmuştur (Ahn vd., 2020). Yumurta tavukları üzerinde yapılan bir çalışmada kara asker sineği larvalarıyla beslenen tavukların daha düşük kolesterol ve trigliserit seviyesi gösterdikleri, kandaki kalsiyum değerlerinin de yükseldiği görülmüştür (Marono vd., 2017). Yenilebilir böcekler zengin vitamin ve mineral içeriğiyle hastalıkların önlenmesi konusunda potansiyel bir öneme de sahiptir. Cırcır böceğinin zengin B12 vitamini içeriği özellikle yaşlı bireylerde vitamin eksikliğine bağlı olarak gelişen bilişsel gerilemenin ve kemik kırılmalarının önlenmesi hususunda yardımcı olabilir, kardiyovasküler hastalıklarla ilişkilendirilen kan plazma proteini homosistein konsantrasyonunu azaltarak kardiyovasküler hastalık riskini de azaltabilir (D'Antonio vd., 2023).

İnsanlar üzerinde sağlık etkilerini inceleyen kapsamlı bir çalışma bulunmamakla birlikte yapılan bazı çalışmalardan olumlu sağlık sonuçları alınmıştır. Ji ve ark. (2022) ipek böceği (*Bombyx mori*) larvaları proteinlerinin kolon kanseri hücrelerinin kontrolsüz çoğalmasına etkisini inceledikleri çalışmada, ipek böceği larvaları proteininin kolon kanseri hücrelerinde oksidasyona sebebiyet verdiğini ve hücre apoptozunu artırdığını tespit etmişlerdir. 2008 yılında yine ipek böceği larvaları ile yapılan bir çalışma (Li vd., 2018) larvadan elde edilen protein izolatlarının hücre apoptozunu artırarak mide kanseri üzerinde etkili olabileceğini göstermiştir. Kim ve ark. (2010) kırlangıçkuyruklu kelebekler (*Papilio xuthus*) ile yaptıkları çalışmada izole ettikleri papain peptitlerinin mantarlara, Gram negatif ve Gram pozitif bakterilere karşı etki gösterdiğini ve insan kırmızı kan hücrelerine karşı ise hemolitik bir aktivite göstermediğini tespit etmişlerdir. Kan basıncını, kan şekerini ve kandaki lipit konsantrasyonunu düzenlediğini gösteren çeşitli çalışmalar da vardır (Wang vd., 2011; Wang vd., 2014; Aznar-Cervantes vd., 2021). Yenilebilir böceklerin insan diyetinin bir parçası olması yaygınlaştıkça alanda yapılan çalışmalar artacağı düşünülmektedir (Stull, 2021).

GIDA GÜVENİLİRLİĞİ

Biyolojik ve kimyasal riskler

Sağlık yararları ve besin değerleri düşünüldüğünde böceklerin tüketimi sağlıklı gibi gözükse de çevreden kaynaklı toksinler, böceklerin kendi zararlı metabolitleri ve pestisit kalıntılarının varlığı, ağır metal birikimleri ve patojen mikroorganizmaların varlığı gıda güvenilirliği endişelerinin başlıca kaynağıdır (Murefu vd., 2019; Henderson, 2022). Yapılan bir çalışmada un kurdu ve cırcır böceklerinde sporlu bakteriler ve *Enterobacter* spp. tanımlanmıştır (Klunder vd., 2012). Bir derlemede, incelenen bazı yenilebilir böceklerde tanımlanan bakterilerin çoğunluğunu *Bacillus* ve *Staphylococcus* cinslerinin oluşturduğu belirtilmiştir (Amedi vd., 2016). Poma ve ark. (2017) bazı yenilebilir böcek türleri ile yaptıkları çalışmada böceklerdeki ağır metaller ve dioksin, poliklorlu bifeniller (PCB), diklorodifeniltrikloroetan (DDT) ve pestisit kalıntılarının seviyesi ölçmüş, söz konusu kalıntı

miktarlarının yaygın olarak tüketilen hayvansal ürünlerden daha düşük seviyede olduğunu tespit etmişlerdir. Çizelge 3'de yenilebilir böceklerde gıda güvenilirliğini tehdit eden potansiyel tehlikeler listelenmiştir.

Çizelge 3 Yenilebilir böceklerde gıda güvenilirliğini tehdit eden potansiyel tehlikeler (Banach vd., 2022)

Tehlike		Tehlike	
Alerjenler	x	Polisiklik aromatik hidrokarbonlar (PAHs)	-
Mikroplastikler ve nanoplastikler	-	İşleme kontaminantları	x*
KİMYASAL TEHLİKE		Veteriner ilaç kalıntıları	x
Antinutrientler	x*	MİKROBİYEL TEHLİKE	
Bromlu alev geciktiriciler	-	Bakteriyel toksinler	-
Dioksinler ve poliklorlu bifeniller	x	GDO	-
Ağır metaller	x	Parazitler	x
Deniz biyotoksinleri	-	Prionlar	x
Mikotoksinler	x*	Sporlu bakteriler	x
Bitki koruma ürünleri ve biyositler	x	Vejetatif bakteriler	x
Bitki toksinleri	-	Virüsler	x

"-" Potansiyel risk bulunmamaktadır. "x" risk vardır. "x*" risk vardır ve bilgi eksikliği mevcuttur.

Avrupa Birliği sınırları içerinde insan tüketimine sunulacak yenilebilir böcekler için herhangi bir gıda güvenilirliği riskinin bulunmaması, doğru bilgilendirmenin ve etiketlemenin yapılması, beslenme açısından bir dezavantaj bulundurmaması koşulu aranmaktadır (Anonymous, 2015b). Yeni gıda ürünlerinin değerlendirilmesinde Avrupa Gıda Güvenliği Ajansı (EFSA) aktif rol almakta ve riskin değerlendirilmesinde söz konusu gıdanın tanımlanması, karakterize edilmesi, üretim ve işleme sırasında oluşabilecek risklerin göz önünde bulundurulması amaçlanmaktadır (Turck vd., 2021).

Kimyasal ve çevresel riskleri elemine etmek için böceklerin yaban hayattan toplanması yerine kapalı ve kontrollü bir ortamda tarımının yapılması çevresel bulaşanları engellemek için bir çözüm yolu oluştururken, böceklerin belirli bir diyetle beslenmeleri, kendi metabolitlerinden gelen toksisiteyi engellemeye yardımcı olacaktır. Uygun işleme metotları ile muamele edilmesi, mikrobiyolojik riskler elemine ederek, yenilebilir böcekler için yeterli gıda güvenilirliği sağlayacaktır (Murefu vd., 2019; Baiano, 2020; Imathiu, 2020).

Alerjenler

Gıda alerjisi vücudumuzun zararsız gıdalara ve gıda bileşenlerine gösterdiği savunma sisteminin bir yan etkisidir. Alerjik reaksiyon savunma sisteminin gıdada bulunan spesifik protein ve proteinlere vermiş olduğu anormal cevap olarak düşünülmektedir (de Gier ve Verhoeckx, 2018). Yenilebilir böceklerin gıda olarak kullanılması besin içerikleri ve sahip oldukları biyoaktif bileşenler nedeniyle avantajlı bulunmasına rağmen yenilebilir böcek tüketimine bağlı olarak potansiyel alerjik reaksiyonların ortaya çıkması da olasıdır (Jantzen vd., 2019).

Birçok böcek proteini alerjen olarak tanımlanmış ve gıda alerjisi, EFSA tarafından yenilebilir olarak kabul edilen ilk böcek türü olan *Tenebrio molitor* dahil birçok böcek türünde ortaya konmuştur (Cunha vd., 2023). Yang ve ark. (2023)'nın yaptığı derlemede yenilebilir böceklerde en yaygın gıda alerjeni olarak tropomyosin (TM) ve arjinin kinazdan (AK) bahsedilmektedir.

Yenilebilir böcek proteinleri tüketime bağlı olarak kendi başına gıda alerjisine sebep olduğu gibi, diğer gıda alerjenleri ile çapraz reaksiyon göstererek alerjiye de sebep olmaktadır. TM ve AK'nin farelerde yapılan çalışmada serumdaki histamin ve IgE seviyesini artırdığı görülmüştür (Han vd., 2018). Çizelge 4' de yenilebilir bazı böcek türleri ve raporlanan vaka sayıları verilmiştir.

Çizelge 4 Bazı böcek türleri ve tüketime bağlı olarak raporlanan vaka sayıları (de Gier Verhoeckx, 2018)

Böcek türü	Tüketilebilir kısmın % olarak ifadesi	Raporlanan vaka sayısı
Coleoptera (kın kanatlılar)	%31	3
Lepidoptera (pul kanatlılar)	%18	12
Hymenoptera (zar kanatlılar	%14	2
Orthoptera (düz kanatlılar)	%13	2
Hemiptera (yarım kanatlılar)	%10	11
İsoptera (termit)	%3	0
Odonata (kız böcekleri)	%3	0
Diptera (sinek)	%2	0
Diğer	%5	0

YASAL DÜZENLEMELER

Yenilebilir böceklerin gıda olarak insan tüketimine sunulması henüz dünya çapında tartışılan bir konu olduğu için birçok ülke mevzuatında bu konuda boşluklar bulunmaktadır. Literatür aramalarında böceklerin insan diyetinin bir parçası olması konusunda dünya çapında kabul görmüş bir yasal düzenleme olmamakla birlikte yenilebilir böceklerin işlenmesi ve pazarlanması ile ilgili bazı ülkelerde yasal düzenlemeler mevcuttur (Lähteenmäki-Uutela vd., 2021).

Asya ülkelerinde yenilebilir böceklerin tüketimi yaygın olmasına rağmen konuya özel bir yasal düzenleme bulunmamaktadır. Tayland'da festivallerde özellikle tercih edilen seyyar restoranlar yenilebilir birçok böcek türünü insan tüketime sunarken, Tayland Gıda ve İlaç İdaresi bu gıda ürünlerini Gıda ve Tarım Örgütü(FAO)'nün B.E.2522 (1979) yasasına dayandırarak diğer gıda olarak değerlendirmektedir (Halloran vd., 2015). Çin'de ise yenilebilir böceklerin tüketimi çok yaygın olsa da bu konuda özellikle bir yasal düzenleme bulunmamaktadır. Ancak zehirli ve nesli tükenmekte olan böceklerin tüketimi yasalarla engellenmektedir (Wang vd., 2020).

Kanada'da yenilebilir böceklerin insan tüketimine sunulabilmesi için böcek türüne spesifik başvurunun yerel otoritelerce onaylanması gerekmektedir. Amerika'da böcekler Gıda ve İlaç Dairesi tarafından gıda katkı maddesi sınıfında değerlendirilmekte, böcek bazlı gıda ürünlerinin satışına henüz izin verilmemektedir (Pressman vd., 2017). Yeni Zelanda ve Avustralya'da ise yenilebilir böceklere özel bir atıfta bulunan bir yönetmelik bulunmamaktadır. Ancak yeni gıdaların üretilmesi hususu genel bir yönetmelik ile ele alınarak üretilecek ürünlerin üretiminde yüksek hijyen standartlarına uyulması ve insan tüketimine uygunluğunun güvence altına alınması hususu bu yönetmelikte vurgulanmaktadır (Charlebois vd., 2014; Newsome vd., 2014).

Avrupa Parlamentosu ve Konseyinin yenilebilir böceklerle ilgili düzenlemelerinin tarihçesine bakıldığında: 2015/2283 sayılı tüzüğe göre yenilebilir böcekler ve bunların parçaları yeni bir gıda olarak kabul edilmekte ve 1 Ocak 2018 tarihinden itibaren yürürlüğe giren bu yeni yönetmelik, yenilebilir böcekler gibi yeni-yenilikçi gıdaların Avrupa Birliği pazarına sunulmasını kolaylaştırmaktadır (Anonymous, 2015a). Haziran 2021'de, *Tenebrio molitor* böceğinin larvaları olan sarı un kurdu, AB'de yeni bir gıda olarak onaylanan ilk böcek olurken göçmen çekirge *Locusta migratoria* Kasım 2021 tarihindeki bir düzenleme ile yenilebilir böcekler arasında izin verilen ikinci böcek olmuştur. Tropikal ev cırcır böceği (*Gryllodes sigillatus*), küçük un kurdu (*Alphitobius diaperinus*), kara asker sineği (*Hermetia illucens*) ve bal arısı (*Apis mellifera*) için hali hazırda başvurular bulunmaktadır (Kröger vd., 2022).

Ülkemizde yenilebilir böceklere özel herhangi bir yasal düzenleme bulunmamaktadır. Böceklerden elde edilen bazı gıda katkı maddelerinin kullanımı pratikte görülse de tüketici tepkileri nedeniyle alan çalışması kısıtlıdır.

TÜKETİCİ KABULÜ VE YENİLEBİLİR BÖCEKLER

Hali hazırda batılı ülkelerdeki bazı tüketiciler yenilebilir böcekleri ve bunlardan işlenmiş gıdaları tüketirken, dünya genelinde yenilebilir böcekleri tüketen 2 milyardan fazla insan bulunmaktadır (Tao ve Li, 2018). 2020 yılında böcek proteinlerine olan talep 120 bin ton iken bunun 2030 yılı itibari ile 500 bin tona ulaşacağı ve global market hacminin 8 milyar doları geçeceği tahmin edilmektedir (de Jong ve Nikolik, 2021; Liceaga vd., 2022).

Geleneksel hayvansal proteinlere alternatif olacak kaynakların araştırılması ve talebin bu yönde karşılanması ile ilgili en önemli sorunlardan bir tanesi tüketici kabulüdür (La Barbera vd., 2023). Tüketici kabulünde etkisi bulunan neofobi ve iğrenme faktörlerinin üstesinden gelmek için böceklerin nasıl gıda ürünlerine işleneceği de araştırmaların başka bir konusu olmuştur (Mancini vd., 2019; Patel vd., 2019).

Asya ülkelerinde özellikle Doğu Asya'da yenilebilir böcekler uzun yıllardır insanların diyetlerinde yer almaktadır. Asyalı insanların uzun yıllardır yenilebilir böcekleri diyetlerinin bir parçası haline getirmesinin sebebi, böceklerin yalnızca zengin protein kaynakları olması değil ayrıca elzem bir çok besini ihtiva etmesi ve diğer kaynaklara nazaran ucuz, uluşabilir olmasıdır (Raheem vd., 2019). Myanmar, Laos, Tayland, Endonezya, Kamboçya gibi bazı Güneydoğu Asya ülkelerinde yenilebilir böcekler yüz yıllardır geleneksel yerel mutfağın bir parçasıdır. Bu ülkelerde tarantula, ipek böceği larvası, arı larvası, çekirge, cırcır böceği gibi böcekler ve larvalar atıştırmalık olarak tüketilmektedir (Siddiqui vd., 2023).

Kuzey Amerika ülkelerinde son yıllarda yenilebilir böceklere ilgi giderek artarken, yapılan çalışmalar genç kuşağın yenilebilir böcekleri tüketmek konusunda daha açık fikirli olduğunu göstermektedir (Barton vd., 2020). Bu ülkelerde tüketici kabulü tüketilebilir böceklerin ne şekilde sunulduğuna bağlı olarak da değişmektedir. Örneğin cırcır böceklerinden elde edilen protein tozu ile üretilmiş protein barları hem yüksek protein içeriği hem de sürdürülebilirliği nedeniyle kabul görürken, böceklerin bütün halde sunulduğu işlenmiş diğer ürünler pek de kabul görmemektedir (Barton vd., 2020; Ardoin ve Prinyawiwatkul, 2021).

Güney Amerika ülkelerinde yüz yıllardır geleneksel protein kaynağı olarak böcekler tüketilmektedir. Ancak gıda üretimindeki modernizasyon ve batılılaşma ile yenilebilir böceklere olan ilgi azalmıştır. Çevre ve geleneksel hayvansal protein üretimi arasındaki ilişki anlaşıldıkça, yenilebilir böceklere olan ilgi de tüketici düzeyinde artmaya başlamıştır (Lucchese-Cheung vd., 2020).

Afrika ülkelerinde ise kültürün bir parçası olarak geleneksel lezzet olarak kabul gören böcekler yüksek bir tüketici kabul oranı göstermektedir. Özellikle besin kıtlığı nedeniyle yetersiz beslenmeyle karşı kaşıya kalan Afrika ülkelerinde yenilebilir böcekler geleneksel hayvansal protein kaynaklarına alternatif olarak yetersiz beslenme ile ortaya çıkan hastalıklarla mücadele konusunda umut olmaktadır (Hlongwane vd., 2021).

Yenilebilir böcekler geçmişten günümüze, dünyanın birçok yerinde örn. Afrika, Asya ve Latin Amerika'da tüketilmektedir**.** Ancak çoğu Avrupalı için yenilebilir böcekleri tüketmek yeni ve garip bir düşünce olarak görülmektedir. Bunun sonucu olarak Avrupa ülkelerinde yenilebilir böceklerin tüketimine karşı tüketicilerin kabul oranı oldukça düşük bulunmuştur (Raheem vd., 2019). Yapılan bir çalışmada İngiltere, Hollanda, İspanya, Polonya ve Finlandiya'nın da aralarında 5 Avrupa ülkesinde 1825 katılımcının yalnızca %9'u böcekleri tüketilebilir olarak kabul etmiştir (Grasso vd., 2019). Avrupalı tüketicilerin böceklere karşı olan tutumlarında en önemli etken tiksinti ve neofobi olmuştur. Böcekler Avrupalı tüketicilerce arzu edilmez ve tiksindirici olarak nitelendirilirken, yeni bir şeylerden korkmak olarak tanımlanan neofobi yaş, cinsiyet ve eğitim durumu gibi faktörlerden etkilenmektedir (van den Heuvel vd., 2019).

Yenilebilir böceklerin hammadde olarak kullanıldığı, atıştırmalıklardan et ürünlerine,

yenilebilir böcek bazlı kurabiyelerden çikolatalara ve cipslere kadar geniş bir ürün yelpazesi bulunmaktadır (Acosta-Estrada vd., 2021). Bu ürünlerin üretiminde güneş altında, dondurarak, tepsili kurutucu ve mikrodalga ile kurutma gibi prosesler olduğu gibi ultrason destekli ekstraksiyon ve soğuk atmosferik basınçlı plazma ekstraksiyonu gibi yeni işleme teknikleri de kullanılmaktadır (Melgar-Lalanne vd., 2019).

Tüketici kabulünü artırmak için yenilebilir böcekleri hali hazırda tüketilen ürünlere benzer üretmek başka bir strateji olarak düşünülmektedir (Pambo vb., 2018). Bu stratejiye uygun olarak Avrupa'da market raflarında yenilebilir böcek içerikli burger, ekmek, bisküvi, kraker, cips, şekerleme, içecek, pasta, pizza ve benzeri ürünleri görmek mümkündür (Dagevos, 2021).

SONUÇ VE TARTIŞMA

Yenilebilir böcekler zengin protein, vitamin ve mineral içerdikleri sayesinde alternatif protein kaynakları arasında yüksek bir potansiyele sahiptir. Geleneksel hayvansal protein üretiminin çevreye ve insan sağlığına etkileri düşünülecek olursa yenilebilir böceklere olan ilginin giderek artacağı söylenebilir. Ancak bu hususta en büyük engel tüketici kabulü olarak görülmektedir. Bir diğer engel ise yenilebilir böcekler üzerine yasal mevzuatın eksikliğidir. Yenilebilir böceklerin sağlık üzerine etkileri hususunda yeterli çalışma bulunmaması gıda güvenilirliği ve alerjisi hususlarında endişe yaratmakta, bazı böcek türlerinin yerel otoritelerce onaylanması sürecini yavaşlatmaktadır. Özellikle gıda alerjenleri hususunda yenilebilir böcekler üzerinde in vitro ve in vivo çalışmaların yapılması önem taşımaktadır.

Gıda güvenilirliği endişelerinin ve tüketici kabulü sorunlarının çözülmesi ile yenilebilir böcek içerikli ürünlerin piyasada görünürlüğünün artacağı ve alternatif protein kaynakları arasında önemli bir yer edineceği söylenebilir. Nüfusun hızla artması, iklim değişikliği, geleneksel gıda tedarik zincirinin çevreye olan etkisi, sera gazı salınımı ve temiz su kaynaklarının azalması gibi nedenler geleneksel gıda üretimine alternatif üretim modellerine yönelime sebep olmuştur. Artan nüfusa bağlı olarak gıda talebinin de artması, özellikle gelişmekte olan ülkeler için çözülmesi gereken bir sorun haline gelmiştir. Halihazırda sonlu kaynakların kullanıldığı mevcut gıda üretim sistemimiz daha sürdürülebilir, karbon ayak izi daha küçük, kaynaklara ve çevreye daha saygılı sistemler ile revize edilmesi gerekmektedir.

Yeni bir gıda olarak düşünüldüğünde yenilebilir böcekler ve bunlardan üretilmiş gıda ürünleri, mevzuatta var olan boşluklar, gıda güvenilirliği ve alerjisi hususundaki endişeler ve literatürdeki bilgi eksiklikleri nedeniyle gıda endüstrisinde yeni yeni yer bulmaktadır. Yeni olan çoğu şeye tüketicinin temkinli yaklaşımı, "yeni" sözcüğü ile nitelendirilen şey bir gıda ürünü olduğunda fobiye dönüşmektedir. Yenilebilir böceklerin tüketici kabulünü artırmak için ürünlerin insanların aşina olduğu ürünlere işlenmesi, toz haline getirilerek belli oranlarla ekmek, makarna vb. ürünlerin hamurunda kullanılması gibi pazar stratejileri mevcuttur.

Görece yeni olan bu fikir üzerine tüketici kabulünü artıracak ve gıda güvenilirliğini sağlayacak işleme yöntemlerini konu alan çalışmalar, yenilebilir böceklere olan kamuoyu ilgisi arttıkça artacaktır. Yine tüketici davranışları konusunda yapılacak çalışmalar ürün kabul edilebilirliği üzerinde yeni pazarlama stratejilerinin geliştirilmesi konusunda sektöre ışık tutacaktır.

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GREEN EXTRACTION OF CAROTENOIDS FROM LEMON PEELS

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ABSTRACT
Nowadays, there is a growing interest in fully utilizing agro-industrial wastes, with carotenoids gaining attention as valuable coloring agents. One of the potential sources for carotenoid extraction is lemon peel. The purpose of this study was to determine optimal extraction techniques for extracting as much carotenoids as possible from lemon peel. In this context, a comparison was conducted among extracts obtained via conventional, ultrasound-assisted (UAE), and ultrasound-assisted enzymatic (UAEE) extraction methods. The highest carotenoid content (0.792±0.01 mg/L) was achieved with UAEE, while the lowest (0.493±0.01 mg/L) was obtained conventionally. UAEE exhibited the highest antioxidant activity values among three methods: 753.80±5.79 mg TE/L (ABTS), 624.64±10.52 mg TE/L (DPPH), and 186.64±1.66 µmol TE/L (FRAP). In conclusion, UAEE showed promise in extracting carotenoids from lemon peel. Thus, by carotenoid extraction using green technology from waste lemon peels, with higher added value, richer in terms of phenolic composition and antioxidant properties, has been obtained.
Keywords: Ultrasound, enzyme, extraction, lemon peel

LİMON KABUKLARINDAN KAROTENOİDLERİN YEŞİL EKSTRAKSİYONU

ÖZ
Günümüzde, tarım endüstrisi atıklarının tam olarak kullanımına olan ilgi giderek artmaktadır ve karotenoidler, değerli bir renklendirici ajan olarak dikkat çekmektedir. Karotenoid ekstraksiyonu için potansiyel kaynaklardan biri limon kabuğudur. Bu çalışma, limon kabuğundan maksimum miktarda karotenoid elde etmek için optimal ekstraksiyon prosedürlerini belirlemeyi amaçlamıştır. Bu bağlamda geleneksel, ultrason destekli (UAE) ve ultrason destekli enzimatik ekstraksiyon (UAEE) yöntemleri ile elde edilen ekstraktlar arasında kıyaslama yapılmıştır. En yüksek karotenoid içeriği (0.792±0.01 mg/L) UAEE ile elde edilirken, en düşük içerik (0.493±0.01 mg/L) geleneksel yöntem ile elde edilmiştir. En yüksek toplam fenolik madde miktarı (TPC) UAEE ile elde edilmiştir. Benzer şekilde, UAEE, üç yöntem arasında en yüksek antioksidan aktivite değerlerini sergilemiştir: 753.80±5.79 mg TE/L (ABTS), 624.64±10.52 mg TE/L (DPPH) ve 186.64±1.66 µmol TE/L (FRAP). Sonuç olarak, UAEE, karotenoidlerin ekstraksiyonu için umut vaat etmektedir. Dolayısıyla, atık limon kabuklarından yeşil teknoloji kullanılarak karotenoid ekstraksiyonu ile daha yüksek katma değerli, fenolik bileşim ve antioksidan özellikler açısından daha zengin bir ürün elde edilmiştir.
Anahtar kelimeler: Ultrason, enzim, ekstraksiyon, limon kabuğu

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INTRODUCTION

Agriculture has been important to humanity in every age. The agricultural industry is responsible for generating a wide array of nutrients and enhancing their diversity through processing, fulfilling the nutritional requirements of individuals, and consequently playing a crucial role in the health and advancement of communities. Fresh fruits and vegetables constitute an integral part of agriculture. World fruit production recorded a growth of 63% from 2000 to 2022, with a total production volume in 2022 of 933 million tons. Turkey is the world's fourth-largest producer of fruits (FAO, 2022). In particular, increments became in the production of mandarin by 58.3%, 74.8% in oranges, 75.8% in lemons production in the citrus group of fruits (TUİK, 2023). Turkey is a major producer of citrus fruits: in the case of lemons (*Citrus limon* L.), more than 1.32 million tons were produced in 2023. Lemon (*Citrus limon* L.) is one of the important citrus plants among the fruit groups grown all over the world. Lemons include a variety of bioactive substances that have been linked to health benefits, including carotenoids, phenolic compounds, dietary fiber, essential oils, and vitamins (Benestante et al., 2023). Lemon fruit is used in many food and non-food products that are sold commercially because of its special qualities, which include increasing taste and flavor, supporting health, and having an appearance. Nonetheless, the leftover peel from these non-food and food industries is still a source of valuable bioactive components that can be used in related sectors such as the pharmaceutical, cosmetic, home care, and health care industries (Jagannath and Biradar, 2019). The peels from lemons make up between 50-70 % of the fresh fruit mass (John et al., 2017). It has been stated that the bioactive compounds in peels can help prevent diseases like diabetes, obesity, blood lipid reduction, cardiovascular disease, and some types of cancer (Li et al., 2023). These peels remaining bioactive components can be used as a source of vitamin C, carotenoids, and phenolic compounds, all of which have been shown to have a variety of health-promoting and antioxidant qualities (Magalhães et al., 2023). Lemon peels, which are waste from households, restaurants, and the processing industry, can be distributed free of charge, making them popular sources for extracting phenolic substances, essential oils, and producing natural colorants such as carotenoids (Güzel and Akpınar, 2017; Weldekidan et al., 2024).

Carotenoids are a group of pigments found in many plants, algae, and photosynthetic bacteria. They are responsible for the yellow, orange, and red colors in various fruits, vegetables, and other organisms. Carotenoids, which have an isoprene skeleton, are composed of 40 carbon atoms. In recent years, there has been a growing emphasis on research concerning plant pigments, particularly due to their provitamin A activity, and their recognition as natural antioxidants and bioactive compounds has elevated their significance (Ashokkumar et al., 2023). Fruits and vegetables high in carotenoid antioxidants are recognized to impact on human health. Citrus fruits are a staple in people's everyday diets and contain a considerable amount of carotenoids. This is why many of articles in recent years have focused on studying these fruits (González-Peña et al., 2023).

An important research area with potential implications for the chemical and pharmaceutical industries is the extraction of bioactive compounds from lemon peels. At this point, the conventional approach utilized in the pharmaceutical industry involves extracting bioactive constituents from peels through a solvent-based method, typically using Soxhlet extraction or maceration processes. Conventional extraction methods, which utilize diverse solvents to extract a range of bioactive compounds from natural sources, encounter several drawbacks including excessive solvent usage, extended extraction durations, and suboptimal extraction efficacy (Karne et al., 2023). Researchers have recently proposed several new extraction methods, like supercritical fluid extraction, microwave, enzymatic, and ultrasonic to assess the comparatively large amount of peels produced. One of these new techniques, ultrasound assisted extraction (UAE) is an inexpensive and simple in comparison with

traditional extraction methods. Ultrasound is identified as sound waves with frequencies above the threshold for human hearing (>16 kHz). It means to pressure waves with a frequency of 20 kHz and/or more and in food industry, ultrasound equipment is used in frequencies from 20 kHz to 10 MHz (Demirdoven et al., 2021). The primary mechanism of ultrasound extraction is associated with the cavitation phenomenon, where small bubbles form within a liquid solvent, rapidly expand to a critical size, and subsequently implode (Wang et al., 2015). The utilization of UAE has the potential to increase the value of these bioactive compounds, as it represents an efficient and environmentally friendly process. The method is characterized a more successful extraction and the use of moderate extraction temperatures, which are particularly advantageous for heat-labile chemicals (Junaid et al., 2023). Numerous variables are considered critical during UAE, such as applied ultrasonic power, frequency, extraction temperature, reactor properties, and solvent-sample interaction. It is believed that the majority of bioactive compounds are extracted during the first few minutes of the process (Siddiqui et al., 2023).

Enzymes are regarded as green chemicals and are a flexible type of biocatalyst because of their environmental beneficial. Enzymatic extraction of bioactive substances presents another viable alternative to conventional extraction techniques. Given that pectin constitutes a significant portion of the cell wall in many fruits, including lemon peel, pectinase is employed for this purpose. Pectinase is an enzyme that can be utilized to extract bioactive compounds from a variety of sources and to maximize fruit juice clarity (Chen et al., 2023; Radziejewska-Kubzdela, 2023). The hydrolysis of α-1,4-glycosidic linkages in polygalacturonic acid substance (Tapre and Jain, 2014) or pectic acid is catalyzed by the enzyme pectinase. Pectinase facilitates the breakdown of pectin in cell walls, leading to a more efficient release of β-carotene from chloroplasts. Recent studies have shown that, under specific ultrasonic intensity conditions, low-frequency ultrasonication can enhance the activity of enzyme preparations (Le and Nguyen, 2013; Wu et al., 2014; Wang et al., 2017; Osete-Alcaraz et al., 2019; Shahram et al., 2019; Larsen et al., 2021; Gamage and Choo, 2023). Therefore, ultrasound-assisted enzymatic extraction (UAEE), which employs the synergistic interaction of enzymatic hydrolysis and ultrasound, could fulfil the requirement for extracting carotenoids from lemon peels.

In the existing literature, numerous studies have focused on extracting essential oils and bioactive compounds, such as carotenoids and phenolics, from lemon peel, employing various methodologies. However, to date, no research has been conducted on UAE combined with enzymatic treatment for carotenoid extraction from lemon peel, nor on the synergistic extraction models between ultrasound and enzymatic treatment. Therefore, this study investigates the extraction of carotenoids as color pigments from lemon peel, a by-product of the food industry, utilizing three environmentally friendly methods: ethanol maceration (conventional), ethanol + UAE (combined), and UAEE. The aim is to determine the most effective method for carotenoid extraction, yielding higher quantities and antioxidant activity. As far as we know, no prior research has explored this specific domain, and the findings from this study regarding β-carotene extraction from lemon processing waste could potentially be applicable at an industrial level.

MATERIALS AND METHODS

Reagents and Chemicals

Pectinex Ultra Color (Novozymes-Denmark) enzyme preparate was used for enzymatic extraction. The enzyme preparate having pectin lyase and polygalacturanase activity (7700 PECTU/ml) was stored at +4 °C until the extraction. Folin–Ciocalteau reagent (PubChem CID 516996); sodium carbonate (Na_2CO_3) (PubChem CID 10340); potassium persulfate ($K_2S_2O_8$) (PubChem CID 24412); hexane (C_6H_{14}) (PubChem CID 8058); acetone (C_3H_6O) (PubChem CID 11434908), ethanol (CH_3CH_2OH) (PubChem CID 702) was provided from Merck Chemicals (Darmstadt, Germany). Gallic acid (PubChem CID 370); Iron (III)

chloride (PubChem CID 24380); 2,2- Diphenyl-1-picrylhydrazyl (DPPH) (PubChem CID 2735032); 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (PubChem CID 9570474); 2,4,6-Tripyridyl-*s*-triazine (TPTZ) (PubChem CID 77258); Trolox (PubChem CID 40634) was purchased from Sigma-Aldrich (Steinheim, Germany). All chemicals used in this study were of analytical grade.

Plant Materials

The lemon fruits were provided by a local supermarket (Tokat, Turkey). The lemons were washed with plenty of water to remove possible impurities on the surface. The skins were then peeled by hand, cut into smaller pieces, and then dried in a drying oven (Memmert 100-800, Germany) at 70°C for 8 hours. After being dried and ground into a powder using a laboratory grinder (Sinbo, SCM 2934, Turkey), the samples were stored at room temperature (20±5 °C) until the extraction step.

Extraction Processes

Conventional Extraction

In the extraction process, 1 g of lemon peels was mixed with 20 mL of the extraction solvent (ethanol 75% v/v) in a glass beaker and homogenized with ultra-turrax (IKA T18, Staufen, Germany) at third level speed (approximate 21.000 rpm/min) for three minutes. Then, extraction was carried out using a magnetic stirrer at 300 rpm and at a temperature of 40°C for 60 minutes. Following the extraction, the mixture was centrifuged at 6000 rpm for 10 minutes, and the clear liquid remaining on the sediment was kept at -18°C until it was further examined (Chatzimitakos et al., 2023).

Ultrasound-Assisted Extraction Processes (UAE)

The conventional extraction was combined with ultrasonication to demonstrate the effectiveness of ultrasound treatment. The ultrasound-assisted extraction (UAE) was performed with a laboratory scale ultrasonic bath (365x278x264 mm, WxDxH, Elmasonic S100H, 37 kHz, Singen, Germany). The tank of device (281x222x149 mm, WxDxH) is made of cavitation-resistant stainless steel the volume of 9.50 liters. Total power consumption of the device is 550W and maximum ultrasonic peak performance is 600 W. The temperature of bath can adjust by rotary switch from 30 to 80°C and the change of temperature was controlled continuously with water-resistant digital thermometer with a cable probe during analysis. The lemon peel was mixed with extraction solvent (ethanol solution, 75% v/v) at a solid-to-solvent ratio of 1:20 and homogenized using ultra-turrax (IKA T18, Staufen, Germany) at third level speed (approximate 21.000 rpm/min) for 3 minutes. It was subjected to UAE in the ultrasonic bath and sonicated with frequency of 37 kHz for 60 minutes at 40 °C. After UAE process, the mixture was centrifuged at 6000 rpm for 10 minutes, and the resulting supernatant was stored at −18°C until analysis. The extraction process was carried out by considering similar working conditions in the literature and supported by preliminary experiments (Boukroufa et al., 2017; Chatzimitakos et al., 2023).

Ultrasound-Assisted Enzymatic Extraction Processes (UAEE)

The interaction between enzyme macromolecules and ultrasonic has a major impact on the bioprocess's efficiency (Rokhina et al., 2009; Yun et al., 2023). Therefore, enzymolysis and ultrasonication synergistic models were investigated in this study. UAEE was carried out with a laboratory scale ultrasonic equipment (365x278x264 mm, WxDxH, Elmasonic S100H, 37 kHz, Singen, Germany). The sample (1 gram) was mixed with 7 mL of enzyme (pectinase solution, 10% v/v) and stirred with ultra-turrax (IKA T18, Staufen, Germany) at third level speed for 3 minutes. It was immersed in the ultrasonic bath and sonicated at a constant temperature of 40°C with frequency of 37 kHz for 60 min at constant power (550 W). At the end of extraction, all samples were kept in a water bath at 90°C for three minutes for enzyme inactivation, and then cooled approximately at 25°C. Finally, the samples were centrifuged (Universal 320 R, Tuttlingen, Germany) at 6000 rpm for 10 minutes. The carotenoid extract was obtained as

explained for UAE following the centrifugation and stored at −18°C until further analysis.

Analytical determinations

Total Carotenoid Content

The carotenoid content of the lemon peel extracts was determined by spectrophotometric method developed by Lee and Castle (2001). Firstly, 15 mL extract was mixed with 30 mL extraction solvent (hexane/acetone/ethanol; 50/25/25 v/v) and homogenized with ultra-turrax for 30 seconds. The mixture was centrifuged at 1968×*g* (4000 rpm having 12 × 15 mL tube vessels, EBA 21, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) for 10 min at 5°C. And then samples were measured at 450 nm wavelength. The carotenoid content was calculated in ppm β-carotene according to the formula Eq. (1), considering the molar absorption coefficient ($E^{1\%}$; E_{1cm}=2505).

$$c=\left({}^{a}/_{E}\times b\right)\times 1000 \quad (1)$$

c: unit concentration (w/v)
a: absorbance value
E: molar absorption coefficient, 2505
b: unit optical path length, 1 cm

Antioxidant Properties

Total Phenolic Compounds

The total phenolic compounds (TPC) of the samples were measured by the Folin–Ciocalteau method (Singleton and Rossi, 1965). Approximately 500 µL of sample was mixed with 2 mL of Folin–Ciocalteau reagent (10% v/v). The mixture was stirred with 1 mL of Na_2CO_3 solution (7% v/v) and stored in a light-free environment at 25°C for 30 minutes. Following this incubation period, the mixture was analyzed at a wavelength of 760 nm using a T80+ spectrophotometer (PG Instruments, Leicestershire, United Kingdom). Standard curves were generated based on the concentrations of gallic acid by correlating absorbance values measured at 760 nm. The concentration of samples was determined based on the absorbance values obtained from the standard curve created with different dilutions of gallic acid solutions, and expressed as milligrams of gallic acid equivalent (GAE) per liter of sample.

Antioxidant Activity

The antioxidant activity of the lemon peel extracts was conducted in accordance with 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-Diphenyi-l-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods. For ABTS method, ABTS radical stock solution was first prepared. Equal volumes of 2.45 mM $K_2S_2O_8$ solution and 7 mM ABTS stock solution were combined, and the mixture was then incubated for 16 hours at 25°C in darkness to prepare radical stock solution. Subsequently, the solution was adjusted to an absorbance of 0.700 at a wavelength of 734 nm by mixing 1 mL of this solution with 50 mL of sodium acetate buffer (20 mM sodium acetate, pH 4.5). Then, 100 µL of the extract was added to 2900 µL of the adjusted solution and allowed to stand at room temperature in darkness for 30 minutes. Following incubation, the absorbance of the extracts was measured at 734 nm. The obtained data were then calculated as milligrams of Trolox equivalents (TE) per liter using a standard curve (Pająk et al., 2019).

The DPPH radical is among the commonly used stable radical sources, widely employed for assessing the ability of antioxidants to donate electrons and scavenge free radicals. DPPH is commonly recognized as a method for assessing the free radical scavenging activities of natural compounds. The DPPH radical scavenging assay was evaluated using a previously employed methodology (Brand-Williams et al., 1995). In summary, 100 µL of carotenoid extract was mixed with 3900 µL DPPH solution (0.1 mM) and vortexed. The absorbance of the mixture was measured using a spectrophotometer at 517 nm after it was left at room temperature and in the dark for 30 minutes. The samples were analyzed for free radical scavenging activity (mg TE/L) using the calibration curves created for various concentrations of standard Trolox solutions.

The analysis of the FRAP was conducted according to Benzie and Strain (1996). In brief, TPTZ (10 mM), Iron (III) chloride (20 mM), and buffer solution (0.3 M sodium acetate; pH 3.6) were mixed in a 10:1:1 ratio to prepare the FRAP

reagent. Afterward, 2900 µL of FRAP reagent was blended with 100 µL of the extract and incubated at room temperature for 30 minutes in a dark environment. The absorbance was measured at 593 nm, and the results were calculated based on a standard curve as µmol TE/L.

Physicochemical Analyses

The pH, titratable acidity (TA) and total soluble solids (TSS) of carotenoid extracts were determined according to the AOAC methods (AOAC, 1995). The carotenoid extracts' pH values were measured with a pH meter (WTW Inolab, Germany). To calculate the TA, the titrimetric method was employed, and the obtained TA values were expressed as a percentage of citric acid (%). The TSS of extracts were recorded with a digital refractometer (RFM 330; Bellingham Stanley Limited, Atago-Palette, PR-101, Tokyo, Japan) at 20 °C, and the results were expressed in ºBrix.

Color

CIELAB color co-ordinates were used to determine the color parameters L* (darkness, brightness), a* (redness, greenness), and b* (blueness, yellowness) in the study. The color measurements were conducted using a Minolta colorimeter (CR-300, Osaka, Japan). The chroma (ΔC) values were determined using lemon peel as reference material for comparison. The ΔC and hue angle values of extracts were calculated according to Eq. (2) and Eq. (3).

$$\Delta C=[(a-a_{ref})^2+(b-b_{ref})^2]^{1/2} \quad (2)$$

$$\text{Hue angle}=\tan^{-1}\frac{b^*}{a^*} \quad (3)$$

Statistical Analysis

Statistical analysis was performed to assess the significance of differences among the obtained analysis results using ANOVA variance analysis followed by Duncan Tests. The mean ± standard deviation of three separate experiments was used to express all results. The statistical software package SPSS 17.0 for Windows (SPSS Inc., Chicago, USA) was employed for result evaluation. The coefficient of determination (R^2) indicated the fit of the polynomial model equation, and an F-test was used to determine its statistical significance.

RESULTS AND DISCUSSIONS

Extraction of Carotenoid from Lemon Peel

After extraction by conventional, ultrasound-assisted extraction (UAE) and ultrasound-assisted enzymatic extraction (UAEE) methods, 16.1 mL, 5.8 mL, and 17.1 mL of extract were obtained, respectively. The total carotenoid contents of these lemon peel extracts are presented in Table 1. The highest total carotenoid content was found to be 0.792±0.01 mg/L ß-carotene following ultrasound-assisted enzymatic extraction (UAEE). The lowest value of total carotenoids was determined as 0.493±0.01 ppm ß-carotene with conventional extraction. The results demonstrated statistically significant differences between the carotenoid contents of lemon peel extracts subjected to different extraction methods ($P < 0.05$). In our study, the total carotenoid content of the lemon peel extracts tended to increase as the ultrasonication process. This could be elucidated by the cavitation phenomenon, where microbubbles develop within a liquid solvent and rapidly enlarge until they reach a critical size, causing internal rupture. Under these conditions, the mixture environment experiences significant shear stress, physically decompose the cell walls (Lin et al., 2023). Consequently, they enhance the interface between the solvent and the targeted chemicals, facilitating the permeation of the lemon peels by the solvent. Throughout the extraction process, this occurrence leads to a more substantial transfer of pigment mass from within the cell to the solvent, resulting in β - carotene being more easily released from the matrix into the extraction medium (Xu et al., 2023). Sun et al. (2011) reported that extending the ultrasonication time from 20 to 120 minutes resulted in a significant ($P < 0.05$) enhancement in the efficiency of trans β-carotene extraction from the peel of Bendizao mandarin fruit. Similarly, Shahram and Dinani (2009) investigated the extraction of β-carotene pigment from orange processing waste by combining ultrasonic and enzymatic processes using ethanol solvent. They found that the increase in β-carotene content

extraction with the increase of ultrasonication can be attributed to the cavitation phenomenon induced by ultrasound. On the other hand, the use of enzymes in UAE has become widespread to perform the extraction process more effectively and quickly in recent studies (Ricarte et al., 2020; Umair et al., 2021; Gao et al., 2022; Suo et al., 2023). In this sense, the synergistic effect resulting from the using enzymes led to the highest amount of carotenoids being attained in the extracts obtained through the UAEE method. Studies on the increase of extraction efficiency with the use of enzymes also support the results obtained (Kumar et al., 2023; Singla et al., 2023). Pectin lyase and polygalacturonase, which are used as enzyme in this study, due to their pectin-degrading activity, increase the breakdown of the pectic substance. This study hypothesized that the degradation of pectic substances increases with the utilization of pectin lyase and polygalacturonase, enzymes known for their pectin-degrading activities. Consequently, the flow of extracts and the carotenoid content increased with the ultrasound process.

Table 1. Antioxidant Analysis Results of Extracts from Lemon Peel

	Carotenoid Extract		
	Conventional Extraction	UAE	UAEE
Total Carotenoid Content (TCC)	0.493±0.01^{c}	0.589±0.01^{b}	0.792±0.01^{a}
Total Phenolic Content (TPC)	504.01±5.11^{c}	559.10±3.53^{b}	1118.31±2.10^{a}
Antioxidant Capacity (ABTS)	418.41±9.40^{c}	473.28±6.41^{b}	753.80±5.79^{a}
Antioxidant Capacity (DPPH)	412.46±10.45^{c}	507.25±5.79^{b}	624.64±10.52^{a}
Antioxidant Capacity (FRAP)	138.01±1.36^{b}	130.31±0.96^{c}	186.64±1.66^{a}

UAE, ultrasound-assisted extraction; UAEE, ultrasound-assisted enzymatic extraction; TCC, mg/L ß-carotene; TPC, mg gallic acid/L; ABTS, mg Trolox/g; DPPH, mg Trolox/L; FRAP, μmol Trolox/L.
Results are given as mean ± standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$).

Antioxidant Properties

The total phenolic content (TPC) in the carotenoid extracts from lemon peels was statistically significantly influenced by the applied extraction techniques and parameters used, as shown in Table 1. The TPC of the extracts were ranged from 504.01±5.11 to 1118.31±2.10 mg GAE/L. UAEE-extracted carotenoids showed the highest TPC, followed by UAE and conventional-extracted carotenoids respectively. These findings are consistent with other studies utilizing UAEE, where authors generally concur that the enhanced polyphenol yields result from the complementary effects of cavitation and enzymatic hydrolysis (Athanasiadis et al., 2023; Lin et al., 2023; Mapholi and Goosen 2023). Three different methods were employed to evaluate the antioxidant capacities of the extracts obtained in this study, namely ABTS, DPPH, and FRAP assays. The antioxidant capacity (ABTS) content of lemon peel extracts using conventional, UAE, and UAEE methods were found to be 418.41±9.40, 473.28±6.41, and 753.80±5.79 mg TE/L, respectively. In the study, the carotenoid extracts obtained by UAEE extraction demonstrated the highest antioxidant capacity value. Similarly, the extract obtained with the UAEE also showed excellent DPPH radical scavenging ability (624.64±10.52 mg TE/L) compared with other extraction methods. The FRAP assay relies on antioxidants' capability to convert Fe^{3+} to Fe^{2+} in the presence of TPTZ, indicating of the potential antioxidant activity of natural products. As shown in Table 1, the FRAP values of the extracts obtained by conventional, UAE, and UAEE methods are 138.01±1.36, 130.31±0.96, and 186.64±1.66 μmol TE/L, respectively. All results in this section demonstrated statistically significant differences in the antioxidant properties of the carotenoid extracts subjected to different extraction procedures ($P < 0.05$). These results agree with those previously reported studies for carotenoid extraction with different extraction procedures

(Tchabo et al., 2015; Jagannath and Biradar, 2019; Karne et al., 2023; Suri et al., 2023). Dong et al (2019) similarly investigated phenolic compounds and antioxidant capacity of Eureka lemon fruits harvested at different months of the year. The TFC value in lemon peel was determined to range from 6.35 ± 0.24 to 7.96 ± 0.17 mg GAE/g, the ABTS value ranged from 25.21 ± 0.27 to 40.55 ± 0.32 µmol TE/g, and the DPPH value ranged from 8.28 ± 0.19 to 16.49 ± 0.56 µmol TE/g. In a study conducted by Chatzimitakos et al. (2023) aimed at defining optimal extraction procedures and parameters to obtain bioactive components from lemon peel by-products, antioxidant activity values were determined as 128.9 µmol TE/g (FRAP) and 30.3 µmol TE/g (DPPH). The results of these related studies and ours indicate that ultrasound plays a critical role in extracting the maximum amount of antioxidants, and enzyme utilization has a positive effect. In addition, ultrasonication enhances enzymatic hydrolysis by physically breaking down particles, increasing substrate-enzyme interaction, boosting the frequency of collisions between them, and overcoming the mass transfer limitations (Mercado-Mercado et al., 2018). In present study, this ensured better release and more efficient removal of bioactive compounds, resulting in an increase in TPC and antioxidant capacity values. Furthermore, our study revealed that carotenoid extracts obtained from fresh lemon fruit peels exhibited a higher concentration of antioxidants compared to fresh lemon fruit peels.

Physicochemical Analyses

The analysis results of physicochemical properties (pH, titratable acidity, total soluble solids) are shown in Table 2. The pH values were determined to be 4.45±0.02, 4.63±0.04, and 3.16±0.01 for the conventional, UAE, and UAEE extraction methods, respectively. As expected, the titratable acidity value (reported as % citric acid) of UAEE extracts with a low pH value was determined to be higher compared to the extracts obtained by other extraction methods, and the difference between them is statistically significant ($P < 0.05$). It is believed that the reason for this phenomenon is attributed to a synergistic effect resulting from the application of ultrasound and enzymes. This effect facilitates extraction through cell wall disruption and enhances mass transfer of organic acids by increasing cell membrane permeability (Dalagnol et al., 2017; Ladole et al., 2018; Ma et al., 2022). In this study, a statistically significant difference was observed in all physicochemical analysis results among the three extraction methods ($P < 0.05$). The pH, TA, and TSS values obtained in this study were consistent with the expected ranges based on similar investigations conducted on this subject (Bagde et al., 2017; Chatzimitakos et al., 2023).

Table 2. Physicochemical Analysis Results of Extracts from Lemon Peel

	Carotenoid Extract		
Analyses	Conventional Extraction	UAE	UAEE
pH	4.45±0.02[b]	4.63±0.04[a]	3.16±0.01[c]
Titratable Acidity (TA)	0.46±0.04[b]	0.28±0.03[c]	2.80±0.01[a]
Total Soluble Solids (TSS)	21.25±0.06[a]	19.96±0.00[b]	15.85±0.06[c]

TA, citric acid%; TSS, Brix; UAE, ultrasound-assisted extraction; UAEE, ultrasound-assisted enzymatic extraction. Results are given as mean ± standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$).

Color

The CIELAB color coordinates were used to define the color parameters L* (darkness, brightness), a* (redness, greenness), and b* (yellowness, blueness) (Meral et al., 2024). When examining the color values of the carotenoid extracts (Table 3), it was determined that the difference between the extraction methods was statistically significant for all color parameters ($P < 0.05$). The lightness factor, L*, generally decreased from the carotenoid extracts obtained via the UAE method to those obtained via the

UAEE method in the analysis of the color values. The decrease in the L* value reflects darkening caused by carotenoid accumulation in extracts obtained through various extraction methods (Ruiz et al., 2005; Sebdani and Abbasi, 2023). The a* value is associated with colors ranging from green to red, with negative values representing green tones and positive values representing red tones (Aghajanzadeh et al., 2023). In the present study, the positive a* values of the carotenoid extracts resulted in an increased red color of the extract. The b* color value took a positive value in all carotenoid extracts, indicating an increase in yellow color, with the highest b* value (32.83±0.09) being reached by the UAEE method, where the combination of ultrasound and enzyme was applied. Research on fruit color and quality has shown that the use of the color space, which includes hue and chroma values, is effective in characterizing fruit color (Garcia et al., 2016). For example, lemon peel gradually changes color from green to yellow. In this process, where the lemon peel color changes from green to yellow, the chroma value increases and the hue angle decreases with the accumulation of carotenoids (Baruah and Kotoky, 2018). In the extracts obtained from lemon peels there was an increase in chroma value due to the higher carotenoid content. While the Hue angle was lower in the extract obtained by UAE compared to that of the conventional method, the lowest value was determined in the UAEE method. In summary, the color value results determined within the scope of the study were found to be consistent with the total carotenoid composition of the extracts. While the L* and hue angle values of the extract obtained by UAEE, which had the highest carotenoid content, were found to be lower, the a*, b* and chroma values were found to be higher. Similarly, in a study on the effect of carotenoid extracts obtained from the flesh and peel of different apricot varieties on color components, it was found that there was a good correlation between carotenoid composition and L*, a*, b*, chroma value, and hue angle values (Ruiz et al., 2005).

Table 3. Color Analysis Results of Extracts from Lemon Peel

	Carotenoid Extract		
	Conventional Extraction	UAE	UAEE
*L**	45.06±0.04^{b}	52.65±0.10^{a}	33.02±0.10^{c}
*a**	7.89±0.13^{b}	0.72±0.10^{c}	13.87±0.07^{a}
*b**	14.02±0.12^{c}	29.75±0.17^{b}	32.83±0.07^{a}
Croma Value	16.09±0.14^{b}	29.76±0.12^{a}	35.64±0.14^{c}
Hue°	75.14±0.13^{b}	88.75±0.17^{a}	45.32±0.19^{c}

UAE, ultrasound-assisted extraction; UAEE, ultrasound-assisted enzymatic extraction.
Results are given as mean ± standard deviation. Different letters in the same row indicate significant differences ($P < 0.05$).

CONCLUSIONS

This study investigated, for the first time, the extraction of β-carotene from lemon processing waste powder using conventional extraction, ultrasound-assisted extraction, and a combination of ultrasound and enzymatic processes. To achieve this goal, the β-carotene content, antioxidant properties, physicochemical analysis and color parameters of L*, a*, and b*, chroma value, hue angle of the extracts were evaluated. The findings indicate that both UAE and UAEE are effective methods for extracting carotenoids from lemon peel. Comparative analysis of the extraction methods revealed notable differences in the obtained results. In UAEE, the β-carotene content was found to be approximately 61% higher than that of the conventional method. Furthermore, UAEE exhibited a remarkable 121% increase in TPC compared to the conventional method and a 100% increase compared to UAE. Regarding antioxidant capacity, UAEE outperformed conventional extraction across all assays: 80% higher in ABTS, 51% higher in DPPH and 35% higher in FRAP.

The increase in ABTS, DPPH and FRAP content observed with UAEE is consistent with the higher total phenol concentration estimated by UAEE, which confirms the typical correlation between antioxidant activity and TPC. The UAE, compared to conventional extraction, offers the advantage of promoting the rupture of the material's cells and the expansion of the cell walls' pores, thereby contributing to higher mass transfer and increased antioxidant capacity of the extract. These results demonstrate that the bioactive chemicals' extraction efficiency and yield can be significantly enhanced by carefully selecting the extraction methods and solvent composition, thus offering a more effective and energy-efficient approach. In conclusion, these findings underscore the superior efficiency of UAEE in extracting bioactive compounds from lemon peel, presenting UAEE as a promising approach for enhancing the value and quality of extracted compounds for various applications in the food and pharmaceutical industries. In particular, carotenoid extracts from lemon peel within the scope of study have a potential application as natural antioxidant and coloring agent for food formulations compatible with their acidic structure and it will contribute to the product protection and minimization of color losses. In the future, exploring the valorization of lemon by-products and devising an integrated and sustainable process for recovering lemon fractions would be of great interest. This research will serve as a reference study in the literature, contributing to the advancement of the 'zero waste' concept. It aims to reduce the environmental footprint and promote a circular economy, offering sustainable solutions applicable in the food industry.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

AUTHOR CONTRIBUTIONS

The authors declare that they have contributed equally to the article.

ETHICAL APPROVAL

Ethical approval is not required for this research.

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