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## Aims and Scope

### FOOD and HEALTH

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“Food and Health” journal will publish peer-reviewed (double-blind) articles covering all aspects of **food science and their health effect** in the form of original research articles (full papers and short communications), and review articles. Their team of experts provides editorial excellence, fast publication processes, and high visibility for your paper.

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## *In vitro* trypsin inhibitory activities of some plant and fruit extracts and chemical compounds

Ebru İLHAN LALE, İsmet Burcu TÜRKYILMAZ, Refiye YANARDAĞ

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### ABSTRACT

Trypsin, an enzyme from the serine protease class, is known to be involved in the degradation of proteins. Excessive activity of trypsin is strongly implicated in triggering many diseases, such as acute pancreatitis, inflammation, and tumour. Therefore, this enzyme's regular and balanced activity is necessary for normal physiological functions. Thus, there is a need to develop new trypsin inhibitors from natural sources and chemical compounds. In this study, the inhibitory effects of aqueous extracts prepared from 29 different plants and 10 different chemical compounds were investigated on the activity of trypsin due to its importance in the health sector. The present study's plant extracts and chemical compounds showed trypsin-inhibitory effects. The inhibitory activities of the extracts and chemical compounds increased in a dose-dependent manner. Several plant extracts and chemical compounds that showed high trypsin inhibitory activities may be appropriate for use as trypsin inhibitors to provide additional support to drug treatment in the health field.

**Keywords:** Trypsin, Plant extract, Enzyme inhibition, Chemical compounds

## Introduction

Proteases are important enzymes having proteolytic cleavage activities. Every protease enzyme hydrolyses peptide clusters from different points. They can control some physiologic processes (Cid-Gallegos et al., 2022). For example, the existence of proteases is strongly necessary for the regeneration and refreshing of lung tissue homeostasis (Greene & McElvaney, 2009). In addition, proteases regulate signalling functions (Gräwe et al., 2020). Although their biological necessity for maintaining regular biological activity in organisms is important (Zhang et al., 2021), their inhibitions have been studied for a long time due to specific reasons. Besides, there are many reports about inhibitions of proteases (Mancek-Keber, 2014; Elsässer & Goettig, 2021). The increased need for their inhibition studies has been raised due to their relations with tumour masses, inflammatory conditions, cardiovascular diseases, renal failures, etc. (Regulski et al., 2015; Srikanth & Chen, 2016; Park et al., 2020; Yi et al., 2020). Proteases can be classified according to their many properties. One of the well-known classifications is based on the functional group which exists at their active site. Metalloproteinases, aspartic proteases, cysteine proteases, and serine proteases are the basic sub-classes of this classification.

Trypsin (a digestive enzyme) is a serine protease initially synthesised as an inactive pre-form, called trypsinogen, that is then transformed to its active form, “trypsin”, by the proteolysis of eight amino acids from the N-terminal side of peptide structure (Hegyi & Sahin-Tóth, 2017). This enzyme is responsible for the digestion of proteins by cleaving the peptides from their lysine and arginine moieties. Nevertheless, its increased activity must be limited due to its role in acute pancreatitis, cancer, and inflammation (Li et al., 2014). “For this purpose, natural products have been preferred by many researchers due to their lesser side effects and productive inhibition (Navaei-Bonab et al., 2018; Geisslitz et al., 2022). The current study investigated the possible inhibitory effects of various plant extracts and chemical compounds on trypsin activity.

## Materials and Methods

### *Experimental Material and Chemical Compounds*

In this study, aqueous extracts of plants, fruits, vegetables, and leaves were used for enzyme inhibition studies. The experimental materials which were used in this study can be named as fruit parts of apple, blueberries, grapefruit, lemon, bitter melon, olive, and pomegranate; leaf parts of basil, black cabbage, green tea, fresh dock, lemon balm, mint, net-

tle, parsley, rocket, senna, smoke tree, thyme, and white cabbage; vegetable parts of faba bean, brussel sprouts, capers, garlic, onion, and radish, and plant parts of clove and fennel. All the plants, fruits and vegetables were purchased from a Local Market in Istanbul. Edible parts of the supplied samples were separated from their roots and stem, passed through distilled water, and dried in room conditions in the shade. Raw fruits were kept in a low-temperature oven for 2-3 days. The dried materials were kept at 4°C until their various extracts were prepared. Caffeine, catechin, epicatechin, gallic acid, glyoxylic acid, lansoprazole sodium, ranitidine, resorcinol, and vitamin U were chosen as chemical compounds. The chemical compounds were analytical grade and obtained from Merck, Sigma–Aldrich, Fluka, and BYK.

### *Preparation of the Plant, Fruit, and Vegetable Extracts*

The plant extracts were prepared by refluxing 20 g of the dried material with 200 ml of distilled water for eight hours, then cooled and filtered through a filter paper at room temperature. The filtrates were evaporated to dryness in a rotary evaporator. The recovered extract residue was placed in an initially tared crucible and then in an oven at 37°C. The extracts were kept at -20°C until the experiments were employed. All the extracts were dissolved in distilled water for experiments. Chemical compounds used were also dissolved in distilled water. Tannic acid was used as a standard and positive control.

### *In Vitro Trypsin Inhibitory Assay for Extracts and Chemical Compounds*

The inhibition of trypsin activity was determined by a spectrophotometric method (Ribeiro et al., 2010). A 10 µL of trypsin enzyme (0.3 mg / mL) dissolved in 1 mM HCl solution was placed into a test tube, and then 120 µL of 2.5 mM HCl solution was added to this enzyme solution. A 50 µL of plant and fruit extracts or chemical compounds (dissolved in distilled water) were added to this mixture, followed by 440 µL 50 mM Tris HCl (pH: 7.5) buffer solution and incubated at 37°C for 10 minutes. To this mixture, 500 µL 2.5 mM BAPNA (N- $\alpha$ -benzoyl-DL-arginine-p-nitroanilide) substrate solution dissolved in 1 mM HCl was added, followed by incubation at 37°C for 15 minutes. To terminate the reaction, a 120 µL of 30% acetic acid solution was added to each test tube, and the absorbance value was read in the spectrophotometer at 410 nm. As blank, 50 mM Tris HCl (pH: 7.5) buffer solution was used instead of sample, substrate, and enzyme solution. The 50 mM Tris HCl (pH: 7.5) buffer solution was used instead of plant extracts in the control solution.

The percent inhibition of trypsin activity was calculated as follows:

$$\text{Trypsin Inhibitory Activity (\%)} = (A-B)/(A) \times 100$$

A is the enzyme activity without an inhibitor, and B is the activity in the presence of an inhibitor.

The IC<sub>50</sub> was determined as the concentration of plant extracts and chemical compounds required to inhibit trypsin activity by 50%.

Percentage enzyme inhibition activities of the inhibitors were used to calculate half maximum inhibitors (IC<sub>50</sub>) for individual enzymes via regression analysis data.

## Results and Discussion

The effect of the aqueous extracts of plants on trypsin activity is presented in Table 1. The enzyme inhibitory activities of the extracts increased in a dose-dependent manner. The higher inhibitory activity is associated with a lower IC<sub>50</sub> value. Pomegranate was found to have the highest trypsin inhibitor activity with an IC<sub>50</sub> value of 14.21 ± 1.42 µg/mL. On the other hand, Basil, exhibited the lowest trypsin inhibition activity (775.59 ± 23.00 µg/mL) (Table 1). Tannic acid showed inhibitor activity with an IC<sub>50</sub> value of 36.47 ± 5.86 µg/mL. According to the results, the trypsin inhibitory activity decreased in order of pomegranate > mint > lemon balm > lemon > tannic acid > clove > bitter melon > fresh dock > senna > black cabbage > radius > thyme > green tea > nettle > brussels sprouts > smoke tree > arugula > faba bean > white cabbage > onion > garlic > grapefruit > parsley > blueberry > capers > fennel > olives > apple > basil (Table 1).

The effect of some chemical compounds on trypsin activity is given in Table 2. The inhibition % values of the chemical compounds on trypsin increased with increasing concentration. Tannic acid showed the highest trypsin inhibitory activity having an IC<sub>50</sub> value of 21.44 ± 3.15 µM, while lansoprazole sodium had the lowest trypsin inhibitor activity (2528.68 ± 228.15 µM) (Table 2). Trypsin inhibitory activity of chemical compounds decreased in the order of tannic acid > caffeine > glyoxylic acid > gallic acid > catechin > ranitidine > resorcinol > epicatechin > vitamin U > lansoprazole sodium (Table 2).

Trypsin (E.C. 3.4.21.4) is a serine protease that plays a vital role in the cleavage of peptides from the carboxyl moieties of lysine and arginine. It is secreted from the pancreas to the small intestine as zymogen (trypsinogen). In the small intestine, trypsinogen is converted to its active form, “trypsin”, via the activity of enterokinase and/or by a previously activated trypsinogen (as trypsin). Under certain pathological conditions, its activity increases; thus, inhibition is required to prevent deleterious outcomes such as acute pancreatitis. The most crucial issue is preventing the activation of pancreatic enzymes before entering the small intestine to prevent trypsin and other enzymes from digesting the pancreas. When these enzymes are activated within the pancreatic duct, the pancreas can be seriously damaged, or obstruction of the pancreatic duct can occur, leading to acute pancreatitis. This situation has been recognised by researchers (Zhan et al., 2019).

Trypsin contains histidine (His), serine (Ser) and aspartic acid (Asp) in its active site at position 57<sup>th</sup>, 195<sup>th</sup> and 102<sup>nd</sup>, respectively. Ser performs the first nucleophilic attack on the peptide to make the cleaving process of His easier, and then His does the same process for the final step of the cleavage mechanism. In general, the real inhibition process involves targeting the active site of the enzyme via two types of inhibition mechanisms; reversible and irreversible inhibitions (Sultana et al., 2022). In reversible processes, the inhibitor binds to the active site; then, the formed complex (proteinase-inhibitor complex) degrades the proteinases and frees the active inhibitor for new inhibitions. Contrary, in the irreversible mechanism, the inhibitor directly inhibits the activation of these enzymes (Clemente et al., 2019). This is the most popular process for inhibiting proteases (Bateman & James, 2011).

This study studied twenty-nine plant extracts and ten chemical compounds for trypsin inhibition. Tannic acid was used as a positive control for comparing the inhibitory activities of plant extract samples. The IC<sub>50</sub> value for tannic acid was 36.47 ± 5.86 µg/mL. According to obtained results, all the plant extracts showed trypsin-inhibitory activity. Among them, pomegranate was detected to have the best inhibitory activity corresponding to the lowest IC<sub>50</sub> value (14.21 ± 1.42 µg/mL). In addition to pomegranate, mint (IC<sub>50</sub> = 17.30 ± 1.75 µg/mL), lemon balm (IC<sub>50</sub> = 20.47 ± 2.36 µg/mL) and lemon (IC<sub>50</sub> = 21.87 ± 0.02 µg/mL) extracts had a higher inhibition effect and lower IC<sub>50</sub> values for trypsin inhibition as compared to tannic acid.

Table 1. Trypsin inhibitory activity of plant, fruit, and vegetable extracts

The name of the plant/Preferred parts of plants	Latin Names of Plants	Concentration (µg/mL)	Inhibition (%)*	IC <sub>50</sub> (µg/mL)*
Apple / Fruit	<i>Malus domestica</i>	10	8.01 ±2.09	251.50 ±15.92
		20	11.61 ±2.76	
		50	15.64 ±1.27	
		100	20.71 ±0.85	
		150	34.43 ±3.39	
Basil / Leaf	<i>Ocimum basilicum</i>	50	5.72 ±1.01	775.99 ±23.00
		100	20.53 ±4.29	
		150	22.71 ±2.31	
		250	27.72 ±1.05	
		500	33.52 ±2.27	
Black Cabbage / Leaf	<i>Brassica oleracea</i> var. <i>Acephala</i>	5	21.78 ±2.28	86.08 ±1.21
		7.5	28.53 ±4.14	
		25	35.29 ±0.57	
		50	40.52 ±0.00	
		75	45.06 ±1.85	
Bitter Melon / Fruit	<i>Momordica charantia</i>	5	3.59 ±0.72	67.42 ±3.08
		10	9.75 ±2.19	
		25	18.36 ±2.75	
		50	43.35 ±4.73	
		75	52.03 ±0.40	
Blueberries / Fruit	<i>Vaccinium myrtillus</i>	25	17.01 ±0.00	166.00 ±7.71
		50	23.36 ±2.01	
		75	28.66 ±0.42	
Brussel Sprouts / Vegetable	<i>Brassica oleracea gemmifera</i>	10	10.89 ±0.82	99.44 ±13.92
		25	22.61 ±3.59	
		50	32.01 ±6.64	
		75	38.70 ±0.76	
		100	50.46 ±7.16	
Capers / Vegetable	<i>Capparis spinosa</i>	10	10.95 ±1.89	177.18 ±14.30
		50	15.77 ±3.81	
		75	26.16 ±3.26	
		100	32.04 ±2.35	
Clove / Plant	<i>Syzygium aromaticum</i>	5	17.72 ±4.11	50.54 ±1.45
		10	26.18 ±1.13	
		25	40.48 ±1.87	
		50	50.56 ±2.96	
		75	57.29 ±3.28	
Faba Bean / Vegetable	<i>Vicia faba</i> L.	1	6.48 ±2.06	119.24 ±4.21
		5	10.82 ±0.07	
		25	22.64 ±3.73	
		50	28.79 ±0.62	
		75	34.95 ±1.86	
Fennel / Plant	<i>Foeniculum vulgare</i>	10	10.79 ±2.30	222.10 ±17.27
		50	16.27 ±4.95	



		75	21.51 ±0.96	
		100	27.98 ±3.59	
Fresh Dock / Leaf	<i>Rumex cristacus</i> DC	1	7.78 ±0.60	76.51 ±7.00
		5	13.87 ±1.36	
		10	17.72 ±1.96	
		50	39.02 ±2.10	
		75	49.22 ±4.50	
Garlic / Vegetable	<i>Allium sativum</i> L.	25	12.95 ±3.79	137.81 ±10.13
		50	19.77 ±1.72	
		75	30.81 ±4.43	
		100	41.13 ±1.34	
		125	43.47 ±1.44	
		150	53.67 ±5.49	
Grapefruit / Fruit	<i>Citrus paradisi</i>	10	12.91 ±1.36	140.35 ±49.92
		25	19.04 ±0.91	
		50	21.94 ±1.36	
		75	34.52 ±9.58	
Green Tea / Leaf	<i>Camellia sinensis</i>	10	20.09 ±1.85	94.15 ±2.36
		25	23.43 ±2.35	
		75	35.29 ±0.26	
		100	53.12 ±1.33	
		150	60.66 ±0.04	
Lemon / Fruit	<i>Citrus limon</i>	1	15.43 ±1.75	21.87 ±0.02
		2.5	21.34 ±3.68	
		5	22.89 ±5.72	
		10	31.21 ±0.46	
Lemon Balm / Leaf	<i>Melissa officinalis</i>	2.5	11.08 ±3.82	20.47 ±2.36
		5	23.21 ±3.04	
		7.5	28.11 ±1.65	
		10	30.82 ±1.91	
		15	37.40 ±5.52	
Mint / Leaf	<i>Mentha piperita</i> L.	2.5	12.52 ±4.70	17.30 ±1.75
		7.5	17.34 ±2.79	
		10	29.57 ±6.11	
		12.5	35.24 ±1.17	
		15	47.15 ±6.94	
Nettle / Leaf	<i>Urtica dioica</i>	10	18.27 ±0.57	94.71 ±8.94
		25	24.77 ±1.31	
		50	36.23 ±2.35	
		75	43.07 ±0.00	
		100	51.02 ±4.97	
Olive / Fruit	<i>Olea europaea</i> L.	5	11.61 ±4.47	241.90 ±51.77
		50	15.33 ±0.79	
		75	17.19 ±0.79	
		100	30.22 ±8.68	
Onion / Vegetable	<i>Allium cepa</i> L.	10	2.94 ±0.78	124.38 ±10.29
		25	9.25 ±2.71	
		50	22.44 ±5.42	
		75	29.31 ±0.48	
		100	39.80 ±4.46	

Parsley / Leaf	<i>Petroselinum crispum</i>	10	16.84 ±4.09	149.15 ±16.60
		50	17.73 ±1.41	
		75	33.67 ±0.28	
		100	44.02 ±0.85	
		150	50.60 ±5.64	
Pomegranate / Fruit	<i>Punica granatum</i> L.	1	4.18 ±4.28	14.21 ±1.42
		2.5	8.93 ±6.19	
		5	17.29 ±0.82	
		7.5	23.54 ±8.03	
		10	37.32 ±3.88	
Radiesh / Vegetable	<i>Trachystemon orientalis</i> (L.) G. Don	5	10.30 ±6.22	86.39 ±1.66
		25	24.15 ±4.07	
		50	30.68 ±1.03	
		75	47.58 ±2.39	
		100	54.74 ±0.21	
Rocket / Leaf	<i>Eruca vesicaria</i>	10	10.07 ±2.91	118.99 ±9.22
		25	13.23 ±0.78	
		50	24.68 ±1.83	
		75	31.82 ±2.14	
		100	44.00 ±3.96	
Senna / Leaf	<i>Cassia angustifolia</i>	10	5.92 ±3.83	81.06 ±3.76
		25	11.77 ±0.88	
		50	33.72 ±0.99	
		75	48.17 ±5.83	
		100	59.63 ±0.65	
Smoke Tree / Leaf	<i>Cotinus coggygia</i>	10	9.34 ±0.42	116.83 ±3.97
		25	16.50 ±0.47	
		75	31.57 ±0.46	
		100	48.27 ±4.63	
		150	60.71 ±0.01	
Tannic Acid (Positive Control)		17	32.16 ±2.76	36.47 ±5.86
		85	73.10 ±1.65	
		170	77.98 ±1.93	
		425	81.65 ±0.59	
Thyme / Leaf	<i>Thymus vulgaris</i> L.	10	8.04 ±4.00	90.32 ±0.65
		50	31.30 ±0.62	
		75	40.00 ±1.85	
		100	56.74 ±3.38	
		150	64.13 ±3.38	
White Cabbage / Leaf	<i>Brassica oleracea</i> var. capitata f. alba	10	10.56 ±1.41	121.62 ±0.79
		25	13.95 ±4.50	
		50	22.34 ±2.29	
		75	36.46 ±1.41	
		100	40.72 ±0.44	

\*Mean ± SD

Table 2. Trypsin inhibitory activity of chemical compounds

Chemical Compounds	Concentration ( $\mu\text{M}$ )	Inhibition (%) <sup>*</sup>	IC <sub>50</sub> ( $\mu\text{M}$ ) <sup>*</sup>
Caffeine	20	8.17 $\pm$ 3.85	122.15 $\pm$ 4.67
	25	24.93 $\pm$ 5.27	
	50	26.93 $\pm$ 3.65	
	75	36.68 $\pm$ 2.84	
	100	40.26 $\pm$ 2.23	
Catechin	10	6.16 $\pm$ 0.20	220.63 $\pm$ 92.44
	20	8.31 $\pm$ 2.02	
	25	13.61 $\pm$ 2.63	
	50	15.33 $\pm$ 4.26	
Epicatechin	1	6.64 $\pm$ 2.34	959.22 $\pm$ 27.86
	5	16.71 $\pm$ 2.85	
	100	18.31 $\pm$ 0.92	
	250	21.21 $\pm$ 1.83	
Gallic Acid	10	15.85 $\pm$ 0.21	185.17 $\pm$ 89.81
	20	18.03 $\pm$ 1.65	
	25	25.00 $\pm$ 2.05	
	50	30.09 $\pm$ 7.19	
	100	35.18 $\pm$ 9.04	
Glyoxylic Acid	10	7.66 $\pm$ 1.42	174.60 $\pm$ 17.88
	20	11.42 $\pm$ 0.21	
	25	13.01 $\pm$ 0.81	
	50	17.92 $\pm$ 2.45	
	100	31.36 $\pm$ 2.66	
Lansoprazole Sodium	10	11.15 $\pm$ 1.62	2528.68 $\pm$ 228.15
	100	15.44 $\pm$ 5.73	
	1000	27.39 $\pm$ 0.66	
Ranitidine	0.1	5.85 $\pm$ 0.00	385.00 $\pm$ 68.09
	5	12.14 $\pm$ 3.10	
	50	13.74 $\pm$ 2.06	
Resorcinol	10	10.54 $\pm$ 8.08	405.15 $\pm$ 45.28
	100	25.48 $\pm$ 4.35	
	250	34.55 $\pm$ 0.62	
Tannic Acid (Positive Control)	10	32.16 $\pm$ 2.76	21.44 $\pm$ 3.15
	50	73.10 $\pm$ 1.65	
	100	77.98 $\pm$ 1.93	
	250	81.65 $\pm$ 0.59	
Vitamin U	2.5	3.55 $\pm$ 1.20	1026.72 $\pm$ 307.67
	50	13.51 $\pm$ 0.07	
	100	18.15 $\pm$ 1.92	
	500	28.90 $\pm$ 6.41	

\*Mean  $\pm$  SD

The plants explored in the present study have been found to show many protective effects against various diseases. For instance, pomegranate and its bioactive compounds were proven to have a preventive effect on gastric cancer cells (Cheshomi et al., 2022) and a protective effect on different gastric disorders (Abd el-Rady et al., 2021). Mint oils, various extracts of lemon balm and lemon have been reported to protect the gastrointestinal system and the stomach (Saber et al., 2016; de Mashayekhi-Sardoo et al., 2020; de Oliveira Braga et al., 2022). Moreover, the common feature shared by these plants is their bioactive compounds since they all contain phenolic compounds and flavonoids (Abdellatif et al., 2014; Uçan et al., 2016; Tarantino et al., 2020; Fan et al., 2021). Phenolic compounds and flavonoids effectively inhibit many enzymes (Sacan & Yildiz Turhan, 2014; Dağsuyu & Yanardağ, 2021; Kan et al., 2021). These compounds may directly be related to blocking trypsin's active site by interacting with His, Ser and Asp amino acids. Ghosh et al. (Ghosh et al., 2008) revealed the interactions of some polyphenolic compounds and His-containing enzymes at their active site. They said this interaction changed the histidine stability and affected the carbon-hydrogen chemical shift. Moreover, the inhibitory capacities of polyphenolic compounds and their derivatives on serine proteases are related to the interaction between their polyphenolic skeleton and the hydrophobic region of these enzymes (Viskupicova et al., 2012). Furthermore, most legume plants have been reported to have serine protease inhibition effects (Haq et al., 2004). Based on this information and considering the bioactive components of the plants studied, it can be hypothesised that these plants can be used for effective trypsin inhibition.

The present study evaluated caffeine, catechin, epicatechin, glyoxylic acid, gallic acid, lansoprazole sodium, ranitidine, resorcinol, tannic acid, and vitamin U for trypsin inhibition assays. Tannic acid, considered a standard inhibitor, had the best trypsin inhibition effect due to its lowest  $IC_{50}$  value among the chemical substances. The compounds evaluated have phenolic and nonphenolic structures. Caffeine, glyoxylic acid and gallic acid exhibited lower  $IC_{50}$  values after tannic acid among the other compounds tested. The trypsin inhibition and inhibiting mechanisms of different synthesised compounds are explained in many research. According to the study of Hovhannisyan et al. (Hovhannisyan et al. 2009) nitrogen associated formyl groups could interact with proteins via hydrogen bonds with Ser residues located at the active site of the enzyme, as well as glutamine residues of substrate binding side. They also revealed that amino groups of different amino acid derivatives could interact with the carboxyl moiety of serine of the substrate binding pocket of trypsin.

Based on this approach, their active groups can explain the present findings for caffeine, glyoxylic acid and gallic acid.

It is well-known that polyphenolic compounds consist of hydroxyl groups, which are highly effective and determine the properties of these compounds. Due to this property, phenolic groups can interact with polar portions of this enzyme (Feng et al., 2018). Thus, catechin and epicatechin showed inhibitory activity on trypsin, with the inhibitory efficacy of catechin better than that of epicatechin. The difference between their inhibitory activities can be attributed to the position of the active moieties in their structure.

Ranitidine, resorcinol, and lansoprazole sodium had trypsin inhibitory activity, with the lowest  $IC_{50}$  value exhibited by Lansoprazole sodium ( $IC_{50} = 2528.68 \pm 228.15$  mM). Among these three compounds, ranitidine showed better inhibition activity on trypsin. Ranitidine, a proton pump inhibitor and a histamine blocker, has also been known to have a regulative effect on the gastrointestinal system (Satoh et al., 2014). Its active moieties may be suggested to be responsible for its trypsin inhibitory effect.

Vitamin U, also known as S-methyl methionine sulfonium chloride, is a methionine derivative that has a powerful antioxidant effect. It has protective effects on various toxicity models, regulative effects on antioxidant systems and enzymes, and gastroprotective effects (Turkyilmaz et al., 2019; Topaloglu et al., 2022). According to the study of Dağsuyu and Yanardağ (Dağsuyu & Yanardağ, 2021), sulfur compounds, including Vitamin U, had different trypsin inhibitory activities. They also mentioned that Vitamin U achieved this inhibition property via binding to the serine part of the enzyme's active site. In the present study, sulfur and/or Vitamin U-containing vegetables like Brussels sprouts, white cabbage, onion, etc., showed inhibitory activity on the enzyme trypsin. Therefore, Vitamin U and its food-based sources had trypsin inhibitory activity.

## Conclusion

The results of this study show that water extracts of pomegranate, mint and lemon balm exhibit good trypsin inhibitory activities. Phenolic compounds and flavonoids in the plant kingdom are responsible for the enzyme inhibitory activity. It is known that plant extracts with high inhibitory activity on trypsin contain phenolic and flavonoid compounds. Therefore, in our study, extracts of pomegranate, mint, and lemon balm, which inhibit the enzyme at a high rate, can be used in medicine as a source of natural trypsin inhibitor, in the form of raw extracts or as a source of bioactive compounds. There-

fore, these plant extracts can be used as phytochemical compounds in medicine, especially in gastrointestinal tract diseases, and for future therapeutic purposes.

#### Compliance with Ethical Standards

**Conflict of interests:** The author(s) declares that for this article, they have no actual, potential, or perceived conflict of interest.

**Ethics committee approval:** Authors declare that this study includes no experiments with human or animal subjects.

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**Disclosure:** -

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## Antioxidant and 3-hydroxy-3-methylglutaryl Coenzyme A reductase inhibitory activities of some plant samples

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### ABSTRACT

The antioxidant activity capacities of some plants, and their inhibitory effects on the HMG-CoA reductase enzyme, the rate-determining enzyme of cholesterol synthesis, were investigated in our study. Antioxidant activity capacity and inhibitory effect of the HMG-CoA reductase enzyme were detected in all plant extracts used in our research. From the results obtained, it was determined that both antioxidant activity and % inhibition values of HMG-CoA reductase enzyme increased as the plant extracts concentration increased. The strongest ABTS and DPPH radical scavenging activities were exhibited by pomegranate fruit extract ( $IC_{50} = 1.07 \pm 0.04$  mg/mL and  $IC_{50} = 0.39 \pm 0.01$  mg/mL, respectively). At the same time, lemon had the strongest DMPD radical scavenging activity ( $IC_{50} = 9 \times 10^{-4} \pm 6 \times 10^{-5}$  mg/mL). The best HMG-CoA reductase inhibitory activity was observed in persimmon fruit extract ( $IC_{50} = 0.71 \pm 0.18$  µg/mL). The inhibitory power of this extract was much higher than that of the enzyme's standard inhibitor, Atorvastatin ( $IC_{50} = 1.76 \pm 0.12$  µg/mL). The extracts' potent antioxidant and inhibitory properties can be attributed to the rich phytochemical composition of plant extracts. Thus, it may be a potential source of new bioactive compounds effective against oxidative stress, hypercholesterolemia and cardiovascular complications.

**Keywords:** Antioxidant activity, Enzyme, HMG-CoA reductase, Inhibition, Plants

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## Introduction

Free radicals are metabolic by-products continuously produced via normal metabolic processes of the biological system. These molecules are highly reactive and unstable, thus detrimental to organisms' general well-being. In humans, the excessive production of these molecules, coupled with compromised antioxidant-oxidant balance, present severe health challenges such as cardiovascular dysfunction, premature ageing, neurological disorders and mutagenesis (Aruoma, 1994; Bagchi and Puri, 1998; Floyd, 1999; Njie-Mbye et al., 2013) these lead modification and destruction of biologically important macromolecules such as lipids, proteins, DNA and carbohydrates. The biological system is armed with an antioxidant defence mechanism that helps eliminate the toxic effect of free radicals. This antioxidant defence system involves the activity of antioxidant enzymes (i.e., superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) that works synergistically with the non-enzymatic antioxidant systems that include vitamins (vitamin A, C and E), antioxidant minerals (i.e. copper, zinc and selenium), appropriate biological levels of uric acid and bilirubin, plant-based antioxidants (such as polyphenols) etc. Some of these antioxidant molecules can unilaterally capture or detoxify free radicals, thus essential for healthy living.

Cholesterol is a lipid synthesised by all animal cells via the mevalonate pathway (Brown and Goldstein, 1997; Espen-shade and Hughes, 2007). It is an indispensable component of the cell membrane and an important precursor of steroid hormones, bile and vitamin D (Yeagle, 1991; Hanukoglu, 1992; Haines, 2001; Payne and Hales, 2004). Despite the biological significance of cholesterol, its elevated level in human blood has health consequences such as the increased risk of atherosclerosis, heart attack, stroke, and peripheral artery disease (Lewington et al., 2007; Brunzell et al., 2008). Various studies have shown that lowering blood cholesterol levels can reduce the risk of coronary heart disease, regress existing atherosclerotic lesions in peripheral arteries, and reduce the risk of stroke risk and cardiovascular disease (Grundy et al., 2019). Thus, regulating both intake and biosynthesis of cholesterol is essential for normal well-being. The regulation of cholesterol levels in patients usually involves the use of statins. These group of compounds are known to be excellent inhibitors of hydroxy methylglutaryl-CoA (HMG-CoA) reductase (an important enzyme of the mevalonate pathway), thereby having the ability to slow down the biosynthesis of mevalonate and accumulation of cholesterol, and its accompanying defects (Nicholls, 2008; Kizer et al., 2010). Despite the proven effects of HMG-CoA reductase in reducing blood cholesterol levels and risk cardiovascular disease, they are

suggested to cause liver damage, neuropathy, pancreatic dysfunction, muscle pain/damage, bleeding stroke, an increased risk of diabetes mellitus and sexual dysfunction (Golomb and Evans, 2008; Bellostta and Corsini, 2012; Naci et al., 2013; Collins et al., 2016; Lehrer and Rheinstein, 2020). Therefore, finding alternative food-based sources capable of HMG-CoA reductase inhibition is of medical significance.

Plants are outstanding sources of phytoactive compounds, thus, exhibit remarkable antioxidant activities (Magaji et al., 2022). Reports have shown that flavonoids and polyphenols can prevent the oxidation of haemoglobin to methaemoglobin through the scavenging of nitrites, thereby preventing anaemia (Frei et al., 1988; Choi et al., 1988; Kang et al., 1996). Moreover, plant-based chemicals such as carotenoids, flavonoids, glycosides, polyphenols, saponins, and terpenes act as inhibitors of enzymes while also exhibiting regenerative, wound healing, anti-inflammatory, and antitumor activity. A diet rich in these phytochemicals positively correlates with lower degenerative and pathological diseases and improved well-being (Willcox et al., 2004; Mahato and Sharma, 2019). In the present study, the antioxidant activity and HMG-CoA reductase inhibitory effects of 80% ethyl alcohol extract of some plant extracts were investigated.

## Materials and Methods

### Plant Samples

Quince (*Cydenia vulgaris*), apple (*Malus domestica*), grapefruit (*Citrus paradise*), lemon (*Citrus limon*), pomegranate (*Punica granatum*), persimmon (*Diospyros kaki*), radish (*Raphanus sativus*) and onion (*Allium cepa*) were bought from local market, washed and rinsed with distilled water, then dried in the shade. Individual extracts were prepared with 80% ethyl alcohol using the dried plant parts.

### Preparation of Ethyl Alcohol Extracts

Five grams of the dried plant sample was placed in a 250 mL Erlenmeyer flask containing 50 mL of 80% ethyl alcohol; the flask was closed with a parafilm. The mixture was stirred continuously in a dark cupboard at room temperature for 7 days. The solvent used for extraction was removed from the resulting filtrate under reduced pressure using a rotary evaporator. The amount of extract obtained was weighed and recorded.

### ABTS Radical Scavenging Activity

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity was performed according to the method of Arnao et al. (2001). ABTS was dissolved in distilled water to make a 7.4 mM, adding 1 mL of 2.6 mM potassium persulfate. The solution was kept away from light at room temperature for 12 to 16 hours. To 1 mL of the resulting solution, 60 mL of methyl alcohol was added to obtain the freshly prepared ABTS radical cation solution required for each experiment. Plant extracts (150  $\mu$ L) were added to 2850  $\mu$ L of ABTS radical cation, then kept in the dark for 2 hours before taking absorbance reading at a wavelength of 734 nm. Butylated hydroxyanisole (BHA) was used as a standard ABTS radical scavenger. % ABTS radical scavenging activity was calculated as follows:

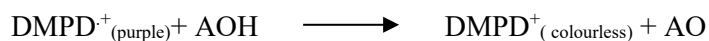
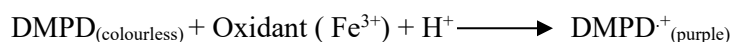
$$\text{ABTS radical scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

$A_0$  = Control absorbance value.

$A_1$  = Absorbance value of sample and standard.

### DMPD Radical Scavenging Activity

The basis of DMPD radical scavenging activity is that in acidic pH, DMPD forms a stable-coloured radical cation in a suitable oxidant solution. The DMPD extreme shows a maximum absorbance value of 505 nm. Antioxidants that transfer H atoms to the DMPD extreme lead to decolourising the radical solution.



DMPD radical scavenging activity was performed according to the method of Fogliano et al. (1999). A 209 mg DMPD radical was dissolved in 10 mL of bidistilled water. A 1 mL of this solution was taken and added to 100 mL of Na-acetate buffer (0.1 M, pH: 5.3). A coloured DMPD radical cation was obtained by adding 0.2 mL of 0.05 M  $\text{FeCl}_3$  to the solution. In the radical scavenging assay, 1 mL of the radical solution was added to 0.5 mL of extract solutions. After 10 minutes, absorbance was taken using a spectrophotometer at 505 nm against the buffer solution. Epicatechin was used as a standard. DMPD radical scavenging activity was calculated as follows:

$$\text{Percentage DMPD radical scavenging activity} = [(A_0 - A_1) / A_0] \times 100$$

$A_0$ : DMPD initial absorbance

$A_1$ : Absorbance of the sample

### DPPH Radical Scavenging Activity

1,1'-diphenyl-2-picrylhydrazil (DPPH) radical scavenging activity was determined according to the method developed by Brand-Williams et al. (1995). A 20 mg/L solution of DPPH in methyl alcohol was prepared daily. To 1.5 mL of the DPPH solution, 0.75 mL of various concentrations of plant extracts (0.001–5 mg/mL) were added. The absorbance value was read after 5 minutes against the blank at 517 nm using a spectrophotometer. A 0.75 mL methyl alcohol and 1.5 mL DPPH solution were used as control. Rutin (0.0001–1.0 mg/mL) was used as a standard percentage of DPPH radical scavenging activity and was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

$A_0$ : Absorbance of control

$A_1$ : Absorbance of sample and standard material

### HMG-CoA Reductase Inhibition Assay

The inhibitory activity of the HMG-CoA reductase was assayed using the procedure outlined in the CS1090 kit of Sigma Aldrich. Briefly, 910  $\mu$ L of buffer was placed into a test tube and added 5  $\mu$ L of the inhibitors (0.01–10.00  $\mu$ g/mL). The solutions were thoroughly mixed before adding 20  $\mu$ L of NADPH, and 60  $\mu$ L of HMG-CoA, then allowed to stand for 5 minutes at room temperature. After that, 5  $\mu$ L of HMG-CoA reductase was added to the mixture, gently mixed, and allowed to stand for another 5 minutes. The absorbance of samples was read spectrophotometrically at 340 nm against corresponding reagent blanks. Atorvastatin was used as the standard inhibitor of HMG-CoA reductase.

Percentage enzyme inhibition was calculated as follows:

$$\% \text{ Inhibition} = (A_0 - A_1 / A_0) \times 100$$

$A_0$ : Absorbance of control

$A_1$ : Absorbance of sample and standard material

For enzyme inhibitor activities, the results are given as half maximal inhibitory concentrations ( $\text{IC}_{50}$  values) calculated regression prepared from the concentrations of samples. Using regression analysis data, half maximum inhibition/scavenging concentration ( $\text{IC}_{50}$ ) was calculated from enzyme inhibition % activities and antioxidant activities % (for ABTS, DMPD, and DPPH radical scavenging). Lower  $\text{IC}_{50}$  values

indicate higher inhibitory potential and antioxidant activities of the tested plant extract.

## Results and Discussion

Plants are natural sources of biologically active compounds (such as vitamins and polyphenols) with diverse biochemical and therapeutic activities (Proteggente et al., 2002; Rufino et al., 2010; Rinaldo et al., 2014; Afam et al., 2021). Among the therapeutic benefit of fruits and vegetables is their strong antioxidant activities (Wang et al., 1996; Karadeniz et al., 2005; Almeida et al., 2011; Dandare et al., 2017; Magaji et al., 2022). Quince has been reported to contain high levels of ascorbic acid (Gheisari and Abhari, 2014; Mir et al., 2016). Phytochemical analysis reveals that apples contain the appreciable level of vitamin C, phenols and flavonoids (that include anthocyanins, catechin, chlorogenic acid, hydroxycinnamic acids, phloridzin, procyanidins and quercetin) (Siqueira et al. 2013; Djenidi et al., 2020; Asale et al., 2021; Vasile et al., 2021). Citrus, including grapefruit and lemon, were reported to be exclusively rich in vitamin C and  $\alpha$ -tocopherol, in addition to phytochemicals like flavonoids, condensed tannins, limonoids, gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, p-coumaric acid, salicylic acid, ferulic acid, anisic acid, triterpenes and sinapic acid (Garcia-Closas et al., 2004; Gorinstein et al., 2004; Uckoo et al., 2012; Agudelo et al., 2017; Makni et al., 2018; Alrasheid et al., 2019; Djenidi et al., 2020). In different varieties of pomegranate, phenolic compounds (ellagic acid, gallic acid, chlorogenic acid, caffeic acid, vanillic acid, ferulic acids trans-2- hydrocinnamic acid, quercetin) and flavonoids (catechin, rutin, quercetin and phloridzin) were identified (Hmid et al., 2017; Djenidi et al., 2020; Tekin and Kucukbay et al., 2020). Phytochemical analysis by Lee et al. (2012), Murathan (2020) and Aydin (2021) reported the presence of tartaric acid, malic acid, lactic acid, gallic acid, citric acid, catechins, phenols, flavonoids and ascorbic acid in persimmon fruit. Radish fruits are rich in flavonoids (such as myricetin, catechin, epicatechin, and quercetin), phenols (e.g. quercetin, o-coumaric acid, tyrosol, sinapic acid, vanillic acid, vinyl guaiacol), glycosides, terpenes and fatty acids (Beevi et al., 2010; Xiao et al., 2012; Baenas et al., 2014; Goyeneche et al., 2015; Park et al., 2016; Selyutina and Gapontseva, 2016; Chihoub et al., 2019). Onion, on the other hand, contains high levels of organosulfur and phenolic compounds such as onions, cycloalliin, isoalliin, methiin, quercetin and rutin (Alagarsamy et al., 2018; Zhao et al., 2021). These phytochemicals are responsible for fruits and vegetables' antioxidant activities and therapeutic benefits.

The antioxidant activities of plants investigated in the present study are presented in Table 1. Persimmon and quince exhibited minor ABTS radical scavenging activity (with  $IC_{50}$  values of  $17.80 \pm 1.12$  and  $9.47 \pm 0.83$  mg/mL, respectively), while apple  $IC_{50} = 6.32 \pm 0.09$  mg/mL, lemon ( $IC_{50} = 4.96 \pm 0.20$  mg/mL), onion ( $IC_{50} = 3.73 \pm 0.08$  mg/mL), grapefruit ( $IC_{50} = 3.63 \pm 0.11$  mg/mL) and radish ( $IC_{50} = 2.90 \pm 0.05$  mg/mL) had moderate ABTS radical scavenging activity. The strongest ABTS radical scavenging action was exhibited by pomegranate ( $IC_{50} = 1.07 \pm 0.04$  mg/mL). The ABTS radical scavenging activities of the plants above were far below that of BHA ( $IC_{50} = 0.07 \pm 3.0 \times 10^{-3}$ ), used as a standard. Analysis of DMPD radical scavenging activity revealed that lemon had the strongest DMPD radical scavenging activity ( $IC_{50} = 9 \times 10^{-4} \pm 6 \times 10^{-5}$  mg/mL), followed by grapefruit, onion and pomegranate respectively ( $IC_{50} = 0.04 \pm 1 \times 10^{-3}$ ,  $0.06 \pm 6 \times 10^{-3}$ ,  $0.08 \pm 6 \times 10^{-3}$  mg/mL). The minor scavenging activities were found in quince ( $IC_{50} = 1.15 \pm 0.03$  mg/mL), apple ( $IC_{50} = 1.20 \pm 0.06$  mg/mL), radish ( $IC_{50} = 1.25 \pm 0.04$  mg/mL) and persimmon ( $IC_{50} = 1.70 \pm 0.02$  mg/mL). Despite the excellent DMPD radical scavenging activity exhibited by lemon in the present study, its action was still below that of the standard used, i.e. epicatechin ( $IC_{50} = 6.6 \times 10^{-5} \pm 4.8 \times 10^{-5}$  mg/mL). As observed in the present study, pomegranate ( $IC_{50} = 0.39 \pm 0.01$  mg/mL) and grapefruit ( $IC_{50} = 0.99 \pm 0.05$  mg/mL) exhibited strong DPPH radical scavenging activities, though not beyond that of rutin ( $IC_{50} = 0.24 \pm 0.01$  mg/mL) which used as standard. Lemon, apple, onion, persimmon, and radish ( $IC_{50}$  values of  $1.97 \pm 0.08$ ,  $2.24 \pm 0.15$ ,  $2.45 \pm 0.17$ ,  $3.42 \pm 0.42$ ,  $4.74 \pm 0.04$  mg/mL, respectively) had moderate scavenging activities, while quince ( $IC_{50} = 9.02 \pm 0.17$  mg/mL) had the minor scavenging activity.

The outcomes of the present finding agree with previous reports that indicated antioxidant and free radical scavenging activity of reported quince, apple, grapefruit, lemon, pomegranate, persimmon, radish and onion. Quince has been shown to contain high levels of phytochemicals. The fruit exhibit promising antioxidant activity such as reducing power, ferric reducing antioxidant power (FRAP) and DPPH radical scavenging activity in the range of 70.9-89.5%, 1.40-1.68  $\mu$ M and 79.91-82.61%, respectively (Mir et al., 2016). Similarly, Muzykiewicz et al. (2018) reported that ripe and unripe quince fruit have DPPH, FRAP, and ABTS radical scavenging activity. At 60 minutes extraction time, 70% ethanol of the unripe fruit had DPPH and ABTS radical scavenging activity of  $29.43 \pm 0.31$  % and  $9.10 \pm 0.16$  %, respectively, as against  $15.78 \pm 0.59$  % and  $7.19 \pm 0.44$  % of the ripe fruit. DPPH radical scavenging activity of the unripe and ripe fruits are 1.51 and 0.81 mg Trolox/g, respectively. Asale et al. (2021) reported that apple juice had DPPH radical scavenging

activity and iron-reducing power of up to  $86.20 \pm 2.28 \mu\text{g/mL}$  and  $1.93 \pm 0.66 \text{ mg/mL}$ , respectively. The highest total antioxidant activity observed was  $0.46 \pm 0.08 \text{ mg BHT equivalent/g}$ . More so, a study by Siqueira et al. (2013) have shown that apple has both strong DPPH radical and ferric-reducing antioxidant power (FRAP), thus exhibiting excellent antiradical effects.

Citruses such as grapes and lemons are rich in ascorbic acid (Makni et al., 2018; Alrasheid et al., 2019) and thus are excellent antioxidant sources. Studies have shown that grapefruit had both DPPH and ABTS radical scavenging activity. Depending on the variety, DPPH scavenging activity ranged from  $35.25 \pm 0.15\%$  to  $46.08 \pm 0.10\%$ , while ABTS radical scavenging activity was between  $0.61 \pm 0.06\%$  to  $0.92 \pm 0.08$  (Sicari et al., 2018). In another study by Agudelo et al. (2017), grapes exhibited a DPPH scavenging activity ranging from  $5.61 \pm 0.07$  to  $8.61 \pm 0.10 \text{ mg/mL}$ , while the ferric reducing power of the fruit was between  $1.67 \pm 0.01$  to  $3.10 \pm 0.30 \text{ mg/mL}$ . The highest lipid peroxidation inhibition as  $\beta$ -carotene bleaching and TBARS formation inhibition are  $19.2 \pm 0.70 \text{ mg/mL}$  and  $4.17 \pm 0.06 \text{ mg/mL}$  (Agudelo et al., 2017). Conversely, Lemon had a DPPH radical quenching ability of  $\text{IC}_{50} = 434.50 \pm 5.9 \mu\text{g/mL}$  and a reducing power of around  $0.15 \mu\text{g/mL}$  (Makni et al., 2018). A report by Oikeh et al. (2015) showed that Grape and lemon juice have DPPH scavenging activity ( $24.0 \pm 0.10$  and  $5.25 \pm 0.40 \text{ mg/mL}$ , respectively) and FRAP  $364.2 \pm 10.25$  and  $122.75 \pm 3.25 \mu\text{mol/L Fe(II)/g}$  respectively). Ripe fruit of pomegranate has been shown to

have antioxidant activities by a testing method such as DPPH radical scavenging activity, ABTS scavenging activity and FRAP of  $319.2 \pm 4.2$ ,  $778.8 \pm 2.4$  and  $525.3 \pm 15.8 \mu\text{mol Trolox/g dry mass}$ , respectively (Magangana et al., 2021). Comparative antioxidant analysis between astringent, non-astringent and wild types of persimmons by Aydin (2021) indicated that the wild type had higher antioxidant potential than the other types. The ABTS, CUPRAC, DPPH, and FRAP activities were  $112.95 \pm 1.48$ ,  $550.24 \pm 1.30$ ,  $232.56 \pm 3.08$  and  $542.69 \pm 1.84 \mu\text{mol Trolox/g}$ , respectively. Research into the antioxidant potentials of radishes revealed that some varieties of the fruit could have superoxide radical scavenging activity of up to  $68.87\%$  at  $1000 \mu\text{g/mL}$  and a DPPH activity of about  $20.78\%$  (Park et al., 2016). Onion is an essential source of bioactive and antioxidant compounds as a vegetable. The antioxidant activities of this vegetable are reported not to be drastically influenced by heating or cooking (Moreno-Ortega et al., 2020). FRAP and DPPH radical scavenging activities were  $12.40 \pm 0.12 \text{ mg Trolox/g dry mass}$  and  $8.00 \pm 0.23 \text{ mg Trolox/g dry mass}$  before heating. After heating for 10 minutes at  $80^\circ\text{C}$ , the FRAP and DPPH radical scavenging activities were reduced to  $10.57 \pm 0.34$  and  $6.50 \pm 0.37 \text{ mg Trolox/g dry mass}$  (Ren et al., 2017).

In general, the antioxidant activities exhibited by fruits and the vegetable investigated in the present study can be attributed to the phytochemical component such as ascorbic acid, tocopherol, quercetin, and rutin, among other vitamins and polyphenols present in the plant extracts.

**Table 1:** Antioxidant activities of ethyl alcohol extracts from various plants.

Plant/Standard Inhibitor	ABTS ( $\text{IC}_{50}$ ; mg/mL)*	DMPD ( $\text{IC}_{50}$ ; mg/mL)*	DPPH ( $\text{IC}_{50}$ ; mg/mL)*
Quince	$9.47 \pm 0.83$	$1.15 \pm 0.03$	$9.02 \pm 0.17$
Apple	$6.32 \pm 0.09$	$1.20 \pm 0.06$	$2.24 \pm 0.15$
Grapefruit	$3.63 \pm 0.11$	$0.04 \pm 1 \times 10^{-3}$	$0.99 \pm 0.05$
Lemon	$4.96 \pm 0.20$	$9 \times 10^{-4} \pm 6 \times 10^{-5}$	$1.97 \pm 0.08$
Pomegranate	$1.07 \pm 0.04$	$0.08 \pm 6 \times 10^{-3}$	$0.39 \pm 0.01$
Persimmon	$17.80 \pm 1.12$	$1.70 \pm 0.02$	$3.42 \pm 0.42$
Radish	$2.90 \pm 0.05$	$1.25 \pm 0.04$	$4.74 \pm 0.04$
Onion	$3.73 \pm 0.08$	$0.06 \pm 6 \times 10^{-3}$	$2.45 \pm 0.17$
BHA	$0.07 \pm 3.0 \times 10^{-3}$	-	-
Epicatechin	-	$6.6 \times 10^{-5} \pm 4.8 \times 10^{-5}$	-
Rutin	-	-	$0.24 \pm 0.01$

\* Mean  $\pm$  SD of triplicate values

As seen in Table 2, the HMG-CoA reductase inhibitory activity of persimmon ( $IC_{50} = 0.71 \pm 0.18 \mu\text{g/mL}$ ) was higher than that of Atorvastatin ( $IC_{50} = 1.76 \pm 0.12 \mu\text{g/mL}$ ) which is a standard inhibitor of the enzyme. The inhibitory action of radish ( $IC_{50} = 1.81 \pm 0.56 \mu\text{g/mL}$ ) was close to that of the standard inhibitor. More so, lemon ( $IC_{50} = 5.31 \pm 0.80 \mu\text{g/mL}$ ) and apple ( $IC_{50} = 12.42 \pm 2.53 \mu\text{g/mL}$ ) also had intense inhibitory action on the enzyme. In contrast, onion, quince, pomegranate and grapefruit, respectively ( $IC_{50} = 62.92 \pm 2.94, 79.36 \pm 3.94, 329.98 \pm 163.14$  and  $13741.89 \pm 3485.70 \mu\text{g/mL}$ ) exhibited lower HMG-CoA reductase inhibitory activity. Statins are reported to be excellent inhibitors of HMG-CoA

reductase. They help down-regulate cholesterol biosynthesis (Collins et al., 2016). Plant-based statins (such as lovastatin, pravastatin, and simvastatin) derived from sterols during the fermentation process play a significant role in cholesterol biosynthesis via HMG-CoA reductase inhibition, thus reducing the risk of cardiovascular diseases and infarction (Furberg et al., 1994; Byington et al., 1995; Pedersen et al., 1998; Furberg, 1999). The HMG-CoA reductase inhibitory activity of fruits and vegetables observed in the present study is likely due to the rich phytochemical composition of the plant, which is in the form of polyphenols, sterols, vitamins, and fatty acids.

**Table 2:** HMG-CoA reductase inhibition activity of ethyl alcohol extracts prepared from various plants

Plant/Standard Inhibitor	Concentration ( $\mu\text{g/mL}$ )	Inhibition (%) <sup>*</sup>	$IC_{50}$ Values ( $\mu\text{g/mL}$ ) <sup>*</sup>
Quince	1.00	19.85 $\pm$ 4.9	79.36 $\pm$ 3.94
	10.00	35.95 $\pm$ 0.78	
	100.00	55.60 $\pm$ 1.17	
Apple	0.10	20.94 $\pm$ 4.87	12.42 $\pm$ 2.53
	1.00	30.41 $\pm$ 8.03	
	10.00	45.91 $\pm$ 4.00	
Grapefruit	1000.00	12.35 $\pm$ 4.64	13741.89 $\pm$ 3485.70
	5000.00	17.82 $\pm$ 0.48	
	10000.00	42.73 $\pm$ 7.58	
Lemon	0.10	6.35 $\pm$ 1.69	5.31 $\pm$ 0.80
	1.00	29.68 $\pm$ 3.54	
	10.00	76.84 $\pm$ 6.83	
Pomegranate	10.00	34.30 $\pm$ 1.18	329.98 $\pm$ 163.14
	100.00	47.56 $\pm$ 11.85	
	1000.00	70.17 $\pm$ 0.58	
Persimmon	0.01	36.87 $\pm$ 1.65	0.71 $\pm$ 0.18
	0.10	42.64 $\pm$ 1.02	
	1.00	54.60 $\pm$ 3.27	
Radish	0.10	37.95 $\pm$ 2.55	1.81 $\pm$ 0.56
	1.00	47.57 $\pm$ 6.74	
	5.00	82.95 $\pm$ 4.10	
Onion	50.00	45.50 $\pm$ 1.44	62.92 $\pm$ 2.94
	75.00	51.67 $\pm$ 3.45	
	100.00	72.93 $\pm$ 4.81	
Atorvastatin	0.50	4.81 $\pm$ 0.64	1.76 $\pm$ 0.12
	1.00	30.37 $\pm$ 8.91	
	2.50	72.59 $\pm$ 2.79	

\* Mean  $\pm$  SD of triplicate values

## Conclusion

In conclusion, the fruits and vegetable extracts examined in the present study showed strong antioxidant activity. The plants also exhibited promising HMG-CoA reductase inhibition activity, especially persimmon and radish fruit. These potent antioxidant and inhibitory properties can be attributed to the rich phytochemical composition of plant extracts. Thus, it can be a potential source of new bioactive compounds effective against oxidative stress, hypercholesterolemia and cardiovascular complications.

## Compliance with Ethical Standards

**Conflict of interests:** The author(s) declares that for this article, they have no actual, potential, or perceived conflict of interest.

**Ethics committee approval:** Authors declare that this study includes no experiments with human or animal subjects.

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**Disclosure:** -

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## Effect of melon seed powder on the quality characteristics of cupcakes

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### ABSTRACT

Enriching cereal-containing functional foods in the changing food industry is highly attractive. Examining the use of nutrient-rich melon seed powder (MSP), a waste, in bakery products is valuable in sustainability. Within the scope of the study, the use of MSP as an alternative raw material to whole wheat flour (WWF) in cupcake formulation was investigated. Four formulations containing varying proportions of MSP (0%, 10%, 20%, 30%) were evaluated. Depending on the increase of MSP substitution, an increase was observed in the protein, fat and total ash ratios of the cupcakes. The addition of MSP to the product caused browning in the colour of the cupcakes and an increase in hardness values while causing a decrease in their specific volumes. According to the sensory analysis results, while the control cake was the most liked group, it was determined that MSP addition could be used up to 20% in terms of general acceptability and odour in cake production.

**Keywords:** Functional Food, Melon Seed, Cupcake, Nutritive Value

## Introduction

Cereal-based foods are among the most consumed from the earliest times of humanity. For this reason, it is aimed to improve public health by enriching cereal-based foods with some components with functional properties and presenting them as "functional food" (Aksoylu et al., 2012). While these substitutions affect the nutritional values of the products, they also directly affect the functional properties such as increased gel-forming, stabilising, and water and oil-holding capacities (Khan et al., 2016). In this direction, waste products such as fruit peel, seed and pulp are becoming widespread in the enrichment of bakery products (Mirabella et al., 2013). Melon seeds are among the products that have attracted attention in evaluating food waste in recent years.

Melon seed is a precious product due to its rich composition. The chemical composition of melon seed contains 15-25% protein, 15% fibre, B and C vitamins, iron, magnesium, potassium, calcium, phosphorus minerals and 20-40% fat. The fat content of melon seeds is also precious. 59.0% linoleic acid and 26.4% oleic acid in it is highly effective on heart and brain health (Ok, 2020).

When the literature is examined, some studies have been found in which melon seed flour is used for enrichment in the production of gluten-free lokma, noodles and muffins. Dabral and Sharma (2021), in which the effect of melon seed flour on muffins was evaluated, it was seen that only the sensory properties of the cakes were examined. In this direction, the study aimed to evaluate the effects of using melon seed powder in different proportions on the cakes' physical, chemical, textural and sensorial properties. The study is considered to be valuable in terms of waste assessment and sustainability.

## Materials and Methods

Melon fruits, whole wheat flour, sugar, fresh whole eggs (approximately 65% of egg content is white and 35% is yolk), full-fat milk powder, shortening, baking powder, and vanilla were purchased from the local market in Kütahya, Türkiye.

### Preparation of Melon Seed Powder

Melon Seeds Powder (MSP) was prepared according to the method described by Kılmanoğlu et al. (2022). Melon seeds were separated from the melon fruits using a knife and pre-frozen at -40°C for 2 h. Then, the seeds were dried in a freeze-dryer (CoolerMed, Türkiye) for 24 h. Lyophilization was carried out at a vacuum of 0.04 mbar and at -50°C. Dry samples were ground in a mill (Ozlem, Türkiye). Melon seed powder is kept in sealed glass containers at ambient temperature (22°C ±2) until analysis.

### Preparation of Cupcakes

Cupcakes were prepared according to the method described by Gadallah et al. (2022). The formulation of cupcakes is given in Table 1. Sugar and shortening were mixed in a mixer (KitchenAid, USA) for 3 min at slow speed and then at medium speed for 2 min. Then, eggs were added and mixed at medium speed for 2 min. Milk powder and baking powder were added and mixed for 4 min. The prepared dough weighed 40 g, was put into cake moulds, and baked in an electric oven (Venarro, Türkiye) at 180°C for 30 minutes. The baked cupcakes were cooled for 1 hour and kept in sealed bags until the analysis.

**Table 1.** Formulation of Cupcakes

Samples	WWF (g)	MSP (g)	Fresh Whole Egg (g)	Sugar (g)	Shortening (g)	Baking Powder (g)	Milk Powder (g)
Control	100	0	85	60	50	4	3
10% MSP	90	10	85	60	50	4	3
20% MSP	80	20	85	60	50	4	3
30% MSP	70	30	85	60	50	4	3

WWF: Whole Wheat Flour, MSP: Melon Seeds Powder

### ***Determination of Chemical Properties***

The total protein content of WWF, MSP and cupcakes was determined by AOAC Method 979.09 (used as Conversion factor 5.70). Fat analysis was performed according to AACC Method 30-25.01. Total ash content was determined using the AOAC Method 923.03. The cupcakes' moisture content was determined using an infrared moisture analyser (RADWAG MAG 50, Poland). Oil holding capacity (OHC) and water holding capacity (WHC) were determined according to the methods described by Bin Ramli et al. (2010).

### ***Determination of Physical Properties***

The weight (g) of the samples was measured using a precision balance after 1h of cooling. The rapeseed displacement method was used to determine the volume of the cupcakes (Lee and Hosney, 1982). The specific volume of cupcakes was calculated from the volume divided by weight. The baking loss was calculated according to Equation 1 (Altunakar, 2003).

$$\text{Baking Loss} = (\text{Wd} - \text{Wc}) / \text{Wd} * 100 \quad (\text{Equation 1})$$

Wd: Dough weight before baking

Wc: Post-baking weight of the cake

### ***Determination of Colour Properties***

L, a, b values of cupcakes, MSP and WWF were determined with a colourimeter (PCE-CSM 4 Colorimeter, Türkiye). The colour properties of the flour and powder were determined using a rig belonging to the device. After the cupcakes were baked and cooled, measurements were obtained on the crust and crumb of the cupcakes (Su et al., 2005; Akbaş and Kılmanoğlu, 2022).

### ***Determination of Sensory Characteristics***

The sensory evaluation was conducted with 15 semi-trained panellists (9 females, 6 males) selected from Kutahya Dumlupınar University Pazarlar Vocational School staff and students. The cakes were coded after baking and presented to the panellists randomly. Panellists were asked to evaluate the samples for colour, hardness, taste, odour and overall acceptability. A scoring scale 5 was used (1: the minimum likes, 5: the maximum likes) (Bozdoğan et al., 2019).

### ***Texture Analysis***

The method specified by Tuna Ağırbaş et al. (2022) was used to carry out the texture analysis of the cupcakes. The hardness values of cupcakes were determined using TA, XT Plus C Texture Analyzer (Stable Micro Systems, England). The

samples were cut in 2.5 cm diameter and 2 cm height dimensions and analysed using an aluminium cylindrical probe with a diameter of 36 mm. Cupcakes were compressed to 50% of their original thickness with a load cell of 5 kg and a test speed of 2 mm/s. The time between two compressions was determined as 30 sec.

### ***Statistical Analysis***

SPSS Statistic 22 (SPSS INC., Chicago, IL, USA) software package was used for statistical analysis. The significance of the difference ( $p < 0.05$ ) between the obtained data was determined using the Tukey test and revealed by one-way analysis of variance (ANOVA). All analyses were repeated three times and are given with their standard deviations.

## **Results and Discussion**

The results obtained from the physicochemical, functional and colour measurements of WWF and MSP are shown in Table 2. The moisture content of MSP was lower than WWF, while the fat, protein and ash content were higher. Another study reported that fruit kernel flour has higher protein, fat and total ash content than wheat flour (Çelik and Pozan, 2020). For this reason, cupcakes produced with MSP are expected to contain more ash and protein than those prepared with whole wheat flour.

In the production of bakery products, the water-holding capacity of flour plays a critical role in forming texture in foodstuffs (Zayas, 1997). In addition to flour's protein and starch components, lipids in the product also affect the water-holding capacity (Sissons, 2008). Because MSP contains more fat than WWF, the water-holding capacity of MSP has been calculated to be lower than that of WWF. Fişek (2021) reported that oil components reduce the water-holding capacity.

Components with high oil-holding capacity act as emulsifiers and improve the viscosity and texture of foods (Aydın, 2020). When the oil holding capacities of flours were examined, the oil holding capacity of MSP was lower than that of WWF. This result can be interpreted as a reduction in the water and oil binding capacity due to the binding of lipids in melon seeds to hydrophobic groups and amylose units (Fişek, 2021).

The L value, which means the degree of white to black colour, recorded the lowest value in MSP ( $71.74 \pm 4.44$ ) compared to WWF ( $90.07 \pm 0.43$ ), while the redness (a) and yellowness (b) values of MSP were significantly higher than WWF.

**Table 2.** Physicochemical, functional, and colour properties of WWF and MSP

Sample	WWF	MSP
Moisture (%)	9.26 ±0.04	4.94 ±0.03
Total Ash (%)	1.12 ±0.07	6.44 ±0.06
Fat (%)	4.01 ±0.08	20.06 ±1.14
Protein (%)	13.64 ±0.10	17.22 ±0.07
WHC (g/g)	3.01 ±0.02	2.41 ±0.01
OHC(g/g)	2.05 ±0.01	1.60 ±0.00
L	90.07 ±0.43	71.74 ±4.44
a	1.47 ±0.36	7.28 ±0.05
b	9.84 ±0.58	29.13 ±0.49

**WWF:** Whole Wheat Flour, **MSP:** Melon Seed Powder, **WHC:** Water Holding Capacity, **OHC:** Oil Holding Capacity

Chemical analysis results of cupcakes are presented in Table 3. The cupcake content of fat significantly increases with the addition of MSP. This is because the fat content of MSP is more than WWF. It is stated that fruit and vegetable seeds have a high-fat content and enrich the product in terms of fat (Bialek et al., 2016).

It revealed that the moisture of cupcakes decreased proportionally to the addition of MSP. However, this decrease was not statistically significant ( $p > 0.05$ ). The decrease in moisture contents was interpreted as MSP containing less hydrophilic components due to its high-fat content (Manthey et al., 2004). Some studies show that seed powder-added cupcakes have low water holding capacity, which is coherent

with our results (Khan et al., 2016; SZidan and Suliman, 2022).

When the total ash content of the cupcakes is compared, it can be said that it increases as the MSP addition rate increases, but only the cupcake with 30% MSP significantly differs when compared to the control. Generally, the increase in the ash content of the products is important because it is associated with increased functionality. Ambigaipalan and Shahidi (2015) reported that the ash content of cakes prepared with palm kernel flour (2.5% and 5%) increased significantly.

The water-holding capacity is related to the amount and structure of the hydrophilic components in the molecular structure and their position to interact with water at the surface in the tertiary structure (Zayes, 1997). In food with high-fat content, the lipid component can easily form complex structures with starch through weak bonds or remove the hydrophilic groups of starch from water due to its hydrophobic structure (Fişek, 2021). It can be said that the WHC of cupcakes significantly decreases depending on the increase in MSP addition. This is related to the fact that the water-holding capacity of MSP is less than WWF (Fişek, 2021).

It was seen that the increase in MSP addition caused a decrease in the oil-holding values of the cupcakes. The change in oil holding capacity was statistically significant ( $p < 0.05$ ). It can be interpreted that the cupcakes' oil holding capacity decreases as the cupcakes' oil content increases. Joshi et al. (2015) reported that increased water and oil holding capacity was observed in samples with reduced fat content.

**Table 3.** Chemical analysis results of powder and cupcakes

Sample	Moisture (%)	Total Ash (%)	Fat (%)	Protein (%)	WHC (g/g)	OHC (g/g)
Control	25.71±1.62 <sup>a</sup>	1.74±0.01 <sup>bc</sup>	15.25±0.07 <sup>a</sup>	9.04±0.72 <sup>a</sup>	2.51±0.01 <sup>d</sup>	3.29±0.03 <sup>d</sup>
10% MSP	24.85±1.14 <sup>a</sup>	1.72±0.02 <sup>b</sup>	15.37±0.06 <sup>a</sup>	10.02±1.22 <sup>a</sup>	2.34±0.004 <sup>c</sup>	3.2±0.01 <sup>c</sup>
20% MSP	24.76±1.48 <sup>a</sup>	1.85±0.02 <sup>bc</sup>	16.33±0.06 <sup>b</sup>	14.36±0.49 <sup>b</sup>	2.21±0.01 <sup>b</sup>	2.74±0.01 <sup>b</sup>
30% MSP	24.58±0.34 <sup>a</sup>	1.88±0.00 <sup>c</sup>	17.87±0.02 <sup>c</sup>	15.14±0.69 <sup>b</sup>	2.11±0.02 <sup>a</sup>	2.41±0.01 <sup>a</sup>

**WWF:** Whole Wheat Flour, **MSP:** Melon Seed Powder, **Control:** cupcake without melon seed powder, **10% MSP:** cupcake with 10% melon seed powder, **20% MSP:** cupcake with 20% melon seed powder, **30% MSP:** cupcake with 30% melon seed powder, **WHC:** water holding capacity, **OHC:** oil holding capacity, Different letters within a column indicate significant differences at  $p < 0.05$

The raw materials used in bakery products directly affect the product's colour. Product colour is also one of the most critical parameters affecting the acceptability of the product (Aljobair, 2022). The crumb and crust colour analysis results of cupcakes are shown in Table 4. While L and a values were not statistically significant for the crumb colour of cupcakes, b values were different. It was stated that the increase in b value was due to the carotenoids that give the melon

seed powder its unique colour (Çelik and Pozan, 2020). Regarding the crust colour, the highest L and b values were observed in the control cupcake, while the lowest values were recorded in the cupcakes with 30% MSP. Ayoubi et al. (2022) reported that adding pomegranate peel powder caused a decrease in the brightness (L), b and a value of the cakes. Other studies stated that there are decreases in the L, b and a value of the products depending on the substitution rate (Sung et al., 2020; Gül and Şen, 2017).

**Table 4.** Crumb and crust colour analysis results of cupcakes

Samples/ Parameters	Crumb Colour			Crust Colour		
	L	a	b	L	a	b
Control	54.35±2.01 <sup>a</sup>	6.16±0.73 <sup>a</sup>	21.14±0.45 <sup>a</sup>	46.65±0.28 <sup>c</sup>	16.51±0.55 <sup>ab</sup>	31.16±0.43 <sup>c</sup>
10% MSP	51.28±2.47 <sup>a</sup>	6.69±1.13 <sup>a</sup>	22.97±1.76 <sup>ab</sup>	42.43±2.39 <sup>bc</sup>	17.38±0.66 <sup>b</sup>	29.69±2.60 <sup>bc</sup>
20% MSP	51.53±1.93 <sup>a</sup>	8.01±0.35 <sup>a</sup>	25.19±1.24 <sup>b</sup>	39.90±2.64 <sup>b</sup>	15.53±2.15 <sup>ab</sup>	24.48±3.22 <sup>ab</sup>
30% MSP	51.27±3.01 <sup>a</sup>	7.32±1.00 <sup>a</sup>	24.61±0.97 <sup>b</sup>	33.17±1.00 <sup>a</sup>	13.85±0.41 <sup>a</sup>	22.93±2.45 <sup>a</sup>

MSP: Melon Seed Powder, Different letters within a column indicate significant differences at  $p < 0.05$



**Figure 1.** Images of Cupcake Samples control, 10% MSP, 20% MSP, and 30% MSP (from left to right, respectively)

**Table 5.** Some characteristics of cupcake and dough samples

Sample	pH	Specific Volume (mL/g)	Baking Loss (%)	Hardness (g)
Control	6.82 ±0.01 <sup>b</sup>	2.63 ±0.05 <sup>c</sup>	16.67 ±2.21 <sup>a</sup>	2198.18 ±140.61 <sup>a</sup>
10% MSP	6.72 ±0.01 <sup>a</sup>	2.16 ±0.03 <sup>b</sup>	15.45 ±1.63 <sup>a</sup>	2237.73 ±105.36 <sup>ab</sup>
20% MSP	6.71 ±0.04 <sup>a</sup>	1.72 ±0.00 <sup>a</sup>	14.94 ±0.09 <sup>a</sup>	2596 ±22.96 <sup>ab</sup>
30% MSP	6.69 ±0.01 <sup>a</sup>	1.59 ±0.01 <sup>a</sup>	15.67 ±0.73 <sup>a</sup>	2854.23 ±189.33 <sup>b</sup>

MSP: Melon Seed Powder, Different letters within a column indicate significant differences at  $p < 0.05$

The pH of the dough and specific volume, baking loss and hardness values of cupcakes were presented in Table 5. When the pH values were examined, it was seen that the pH decreases as the addition of MSP increases. This pH decrease was significant compared to the control. In a study on cupcakes with bitter melon powder addition (3%, 6%, 9%, 12%), the cupcakes' pH decreases as the addition rate increases (An, 2014). MSP contains about 5 times higher lipids than whole wheat flour and, therefore, contains a high percentage of fatty acids. The pH of cupcakes with MSP is thought to decrease due to their fatty acids.

The addition of MSP significantly affected the specific volumes of the cupcakes. Specific volume was lower in formulations with reduced whole wheat flour. This situation showed parallelism with the texture data. The increase in the substitution ratio caused a decrease in the specific volume and increased the hardness values of the cupcakes. The data obtained by Lotfy and Barakat (2018) was found to be parallel with the study. Adding MSP causes dilution in the amount of gluten in the cupcake mix and reduces the viscoelastic network formed by gluten (Batista et al., 2018; Akbaş and Kılmanoğlu, 2022). Gluten protein contributes to the increase in the volume of the product by providing gas retention during cooking (Akubor and Ishiwu, 2013; Bustos et al., 2015).

Baking loss is reported as damage caused by gas leakage during the cooking process. It is essential for the cake's structural return and shelf life (Kim et al., 2012). In our study, the effect of MSP addition to cakes on baking loss was not statistically significant. Tuna Ağırbaş et al. (2022) found that different fruit seed flours (apricot, sour cherry, pomegranate, and pumpkin seed flour) did not cause a notable change in the baking loss of the cake. Similar results have also been reported in adding other raw materials with high-fat content to the cake (Bozdoğan et al., 2019; Grasso et al., 2020). Marchetti et al. (2018) emphasised that baking loss is essential for the food industry, and the lower the cooking loss, the higher the yield.

The hardness of a food product is one of the most significant factors determining its consumer acceptability. Therefore, the determined hardness is not desired to be very different from the values the consumers know (Batista et al., 2018). Adding MSP in cupcakes affected textural properties significantly ( $p < 0.05$ ). As the substitution ratio increased, the hardness values of the cupcakes also increased. The softest cupcake was controlled. This situation was interpreted as a decrease in the gas holding capacity with the weakening of the gluten network structure after the reduction of wheat flour. The decrease in volume has also led to the formation of products

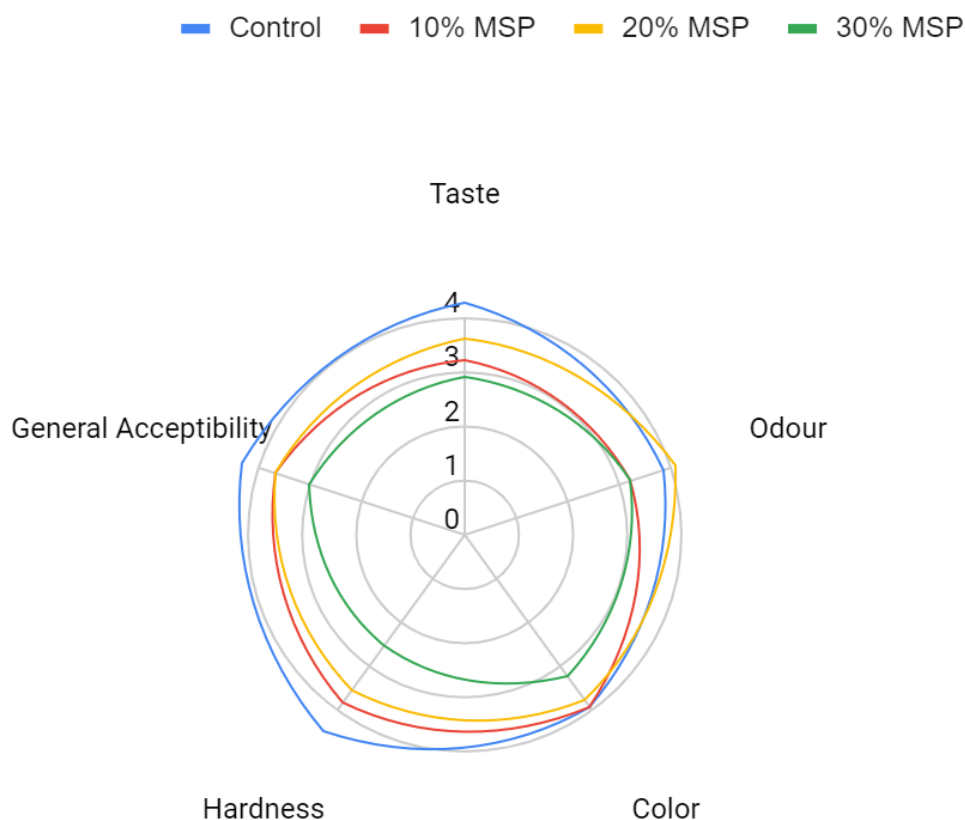
with a more complex structure (Ayoubi et al., 2022). The results were determined to be similar to other studies (Chang et al., 2015; Baltacıoğlu and Uyar, 2017; Gül and Şen, 2017). Polak et al. (2019) reported positive correlations between hardness and moisture data in cake production. In our study, a decrease in the moisture values of the cupcakes was recorded after the addition of MSP.

The sensory analysis results of the cupcakes produced with the addition of MSP at different rates are given in Figure 2. The most liked cake in terms of smell is the cake with a 20% MSP addition. In other scales, it was determined that the control cake got the highest score, and the addition of MSP negatively affected the results. When the colours of the cakes were examined, browning was observed on the cake surfaces as the MSP ratio increased (Figure 1). Gadallah et al. (2022) reported that adding pomegranate peel flour caused differences in the crust colours of the cakes. In another study, Baltacıoğlu and Uyar (2017) stated that the increase in the addition of pumpkin powder causes browning on the cake surfaces. Regarding taste, the most popular cake after the control cake is the 20% MSP-added cake. The least preferred cupcakes were the cupcakes with 30% MSP. Dabral and Sharma (2021) stated that adding melon seed powder and oat flour improved the taste characteristics of the cake samples and increased the overall taste. Consequently, the control cupcake was the most liked with a general acceptability score of 4.31, while the cupcakes with 10%-20% MSP were equally appreciated. Çelik and Pozan (2020) reported that the overall acceptability of noodle samples was adversely affected due to the increased addition of melon seed powder. These results are similar to our study.

## Conclusion

Our study results show that melon seed powder's functional properties are higher than wheat flour. With increased MSP substitution in all cupcakes, higher protein, fat and total ash content was noted compared to the control cupcakes. MSP is a waste today. However, due to the approaching climate crisis, it has been understood how valuable sustainability is in the food industry and how important it is to evaluate waste. With the study, it has been shown that this waste product can be evaluated in improving the functional properties of bakery products and can be added to the product at a rate of 20% without affecting consumer demands. For future studies, it is recommended to evaluate the functionality of melon seed powder by determining its properties, such as dietary fibre content, fatty acid composition, and phenolic and antioxidant contents.





**Figure 2.** General acceptability index values of sensory evaluation of cupcake samples (MSP: Melon Seed Powder)

### Compliance with Ethical Standards

**Conflict of interests:** The author(s) declares that for this article, they have no actual, potential, or perceived conflict of interest.

**Ethics committee approval:** Authors declare that this study includes no experiments with human or animal subjects.

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## Diyetisyene başvuran Türk kadınlarında tıknırcasına yeme davranışının klinik önemi: Hedonik açlık, beslenme durumu ve diyet alışkanlıkları üzerine bir araştırma

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### ÖZ

Diyetisyene başvuran kadınlarda tıknırcasına yeme bozukluğunun saptanması ve bu durumun hedonik açlık ve obezite ile ilişkisinin incelenmesi amacıyla bu çalışma planlanmıştır. 19-50 yaş aralığındaki 400 yetişkin kadın değerlendirilmiştir. Tıknırcasına yeme davranışı bozukluğunu incelemek için Edinburgh Bulimiya Araştırma Testi, hedonik açlık durumunu belirlemek için ise Besin Gücü Ölçeği kullanılmıştır. Katılımcıların %25.2'sinde tıknırcasına yeme davranışı bozukluğu, %74.7'sinde ise hedonik açlık mevcuttur. Tıknırcasına yeme davranışı bozukluğu olan kadınların büyük bir çoğunluğunda (%87.0) hedonik açlık durumu görüldüğü, tıknırcasına yeme davranışı bozukluğu olmayan kadınların beden kütle indeksi değeri olan kadınlara göre anlamlı derecede daha düşüktür ( $25.3 \pm 5.12 \text{ kg/m}^2$ 'ye karşı  $27.0 \pm 5.46 \text{ kg/m}^2$ ,  $p=0.005$ ). Tıknırcasına yeme davranışı bozukluğu olan kadınların olmayanlara göre daha sık diyetle başladıkları (%87.1'e karşı %41.8), daha iştahlı oldukları (%78.2'e karşı %29.8) ve daha düzensiz öğün alışkanlıklarına sahip oldukları (%68.3'e karşı %41.3) bulunmuştur ( $p=0.001$ ). Tıknırcasına yeme davranışı bozukluğu olmayan kadınların %64.3'ünün 15 günde 1 kereden daha seyrek şekilde hazır/işlenmiş/paketli ürünleri tükettiği, tıknırcasına yeme davranışı bozukluğu olan kadınların ise %57.5'inin haftada 1-4 kereden daha sık tükettikleri bulunmuştur ( $p=0.007$ ). Tıknırcasına yeme bozukluğu ile hedonik açlık ve beden kütle indeksi pozitif yönde anlamlı derecede ilişkilidir (sırasıyla  $r=0.522$ ,  $p=0.001$ ;  $r=0.234$ ,  $p=0.001$ ). Tıknırcasına yeme bozukluğu, diyetisyene başvuran kadınların beslenme durumu ve besin tercihinde önemli bir faktördür.

**Anahtar Kelimeler:** Hedonik açlık, Tıknırcasına yeme bozukluğu, Beslenme alışkanlıkları

### ABSTRACT

#### Clinical significance of binge eating behavior in Turkish women who applied to a dietitian: A research on hedonic hunger, nutritional status and dietary habits

It is conducted to detect binge eating disorder in women who apply to a dietitian and to examine its relationship with hedonic hunger and obesity. This study was conducted with 400 adult women aged 19-50 years. Bulimic Investigatory Test Edinburgh was used to examine binge eating disorder, and The Power of Food Scale was used to determine hedonic hunger status. 25.2% of the participants had binge eating disorder and 74.7% had hedonic hunger. While most women with binge eating disorder (87.0%) have hedonic hunger, women without binge eating disorder have a significantly lower body mass index ( $25.3 \pm 5.12 \text{ kg/m}^2$  vs  $27.0 \pm 5.46 \text{ kg/m}^2$ ,  $p=0.005$ ). It was found that women with binge eating disorder diet more frequently (87.1% vs. 41.8%), have more appetite (78.2% vs. 29.8%), and have more irregular meals than those without binge eating disorder (68.3% vs. 41.3%) ( $p=0.001$ ). While 64.3% of women without binge eating disorder consume processed/packaged products less than once in 15 days, 57.5% consume more than 1-4 times a week ( $p=0.007$ ). Binge eating disorder positively and significantly correlated with hedonic hunger and body mass index ( $r=0.522$ ,  $p=0.001$ ;  $r=0.234$ ,  $p=0.001$ , respectively). Binge eating disorder is an important factor in the nutritional status and food preference of women who apply to a dietitian.

**Keywords:** Hedonic hunger, Binge eating disorder, Dietary habits

## Giriş

Besin gereksinimini hızlıca karşılama isteği, fizyolojik olarak açlık hissedilmemesine rağmen haz amaçlı besin tüketimi ve sağlıklı beslenme alışkanlıklarının benimsenmesi optimal beslenmenin önünde büyük bir engeldir ve dengesiz beslenmeyi beraberinde getirmektedir. Yetersiz ve dengesiz beslenme, ağırlık kazanımıyla birlikte başta obezite olmak üzere diyabet, hipertansiyon, kalp damar hastalıkları, kanser gibi birçok sağlık sorunlarına yol açabilmektedir (Cena ve Calder, 2020). Obezitenin bir ortamın karakteristiği olan lezzetli, enerjisi yoğun besinlerin bolluğunun hedonik açlığı tetiklediği öne sürülmüştür (Mead ve ark., 2021). Lezzetli ama sağlıklı ve pratik besin seçeneklerine erişimin kolay ve ucuz olması bu besinlerin tüketimini artırmaktadır (Manasse ve ark., 2015). Bireyler fiziksel olarak aç olmamalarına rağmen, lezzetli besinleri tüketmeye yönelik arzu duymakta ve besin tüketimini homeostatik açlık olmaksızın gerçekleştirmektedir. Hedonik açlık, besine karşı metabolik bir gereksinim duyulmaksızın, zevk alma dürtüsü ile iştahta artışın olması olarak tanımlanmaktadır (Şarahman ve Akçil Ok, 2019). Hedonik açlığın obezite ve yeme davranışı bozukluğuna bağlı olarak görülen belirli beyin bölgelerindeki (oral somatosensör ile ilintili) sinirsel ve algısal yanıtla ilişkili olduğu ileri sürülmektedir (Mason ve ark., 2020). Fizyolojik açlık olmamasına rağmen motive edici ve ödüllendirici olmaları sebebiyle lezzetli besinlere karşı yoğun istek duyulması besin alımının kontrolden çıkmasına neden olabilmektedir (Horwath ve ark., 2020). Ağırlık denetiminde başarı sağlamak için bireye özgü beslenme programı düzenlemek ve bireylerin sağlıklı beslenme düzenini davranış haline dönüştürmek gerekir (Smetthers ve Rolls, 2018). İdeal vücut ağırlığını yakalamak/sürdürmek, beslenme ve sağlık sorunlarını en aza indirmek için beslenme stratejilerinin geliştirilmesi önemlidir (Smetthers ve Rolls, 2018). Beslenme sorunları ve ağırlık kazanımı sebebiyle diyetisyene başvuran bireylerin önemli bir kısmında yeme bozuklukları ve özellikle tıknırcasına yeme bozukluğu (TYB) görülebilmektedir (Compare ve Tasca, 2016; Yang ve ark., 2021). Tıknırcasına yeme bozukluğu, kısa bir zaman dilimi içinde tüketilebilir miktardan daha fazla miktardaki besinin tüketilmesi ve bu davranışı sergilerken özdenetimin sağlanamadığı, tekrarlayıcı yeme ataklarıyla karakterize bir yeme bozukluğudur (American Psychiatric Association, 2013). Beslenme alışkanlıklarının, hedonik açlığın ve obezitenin TYB ile ilişkili olabileceği düşünülmektedir (Ribeiro ve ark., 2018). Daha yüksek hedonik açlık, yetişkinler arasında yüksek enerjili besinlere yönelik artan bir dikkat eğilimi ile ilişkili iken daha düşük hedonik açlık ise aşırı kilolu ve obez yetişkinler arasında ağırlık kaybı ile ilişkilendirilmektedir

(Kaur ve Jensen, 2022). Hedonik açlıktaki bireysel farklılıklar ağırlık kaybı müdahalelerinin başarısını etkileyebilir (Mead ve ark., 2021). Tıknırcasına yeme bozukluğunun obezite, hedonik açlık ve diğer parametrelerle ilişkisini farklı örneklerde inceleyen az sayıda çalışma bulunmaktadır (Kaur ve Jensen, 2022; Ribeiro ve ark., 2018; Taş ve Gezer, 2022). Bununla birlikte, beslenme durumu ve beslenme alışkanlıklarını da dahil ederek bütüncül bir yaklaşımla TYB'yi değerlendiren çalışma bulunmamaktadır. Bu çalışma, diyetisyene başvuran kadınlarda TYB'nin saptanması ve TYB'nin hedonik açlık, beslenme durumu, beslenme alışkanlıkları ve obezite ile ilişkisinin incelenmesi amacıyla yürütülmektedir.

## Materyal ve Metot

### *Araştırmanın Yeri, Zamanı ve Örneklem Seçimi*

Diyetisyene başvuran bireylerde tıknırcasına yeme bozukluğunun saptanması ve hedonik açlık, beslenme durumu ve alışkanlıkları ile ilişkisinin değerlendirilmesi amacıyla yapılan bu çalışma; Türkiye geneli diyetisyene başvuran 19-50 yaş aralığındaki 400 yetişkin kadın ile yürütülmüştür. Çalışma verileri, online anket yöntemi ile elde edilmiş ve diyetisyenler aracılığıyla çeşitli illerde (n=20) yaşayan hedef kitleye ulaşılmıştır. Araştırmaya katılım için gönüllülük esas olup katılımcılara araştırmanın amacı ve kapsamına yönelik bilgi verilmiş ve onamları alınmıştır. Sosyo-demografik özellikleri, sağlık durumları, beslenme durumu ve alışkanlıkları ve fiziksel aktivite düzeyleri bir anket formu kullanılarak elde edilmiştir. Kanser, karaciğer, böbrek, nörolojik ve mental hastalığı olan bireyler, özel bir diyet programı uygulayanlar, 19 yaş altı ve 50 yaş üzeri olan bireyler, menopoz, hamilelik ve emzilik döneminde olan bireyler çalışmaya dahil edilmemiştir. Dahil etme ve hariç tutma kriterlerine göre katılımcı alım akış şeması Şekil 1'de verilmiştir.

### *Veri Toplama Araçları*

#### *Hedonik açlık durumunun değerlendirilmesi*

Bireylerin hedonik açlık durumunu belirlemek için Cappel-leri ve ark. (2009) tarafından geliştirilen Besin Gücü Ölçeği (BGÖ) uygulanmıştır. Besin tüketiminden bağımsız olarak, besin uyarılarının zengin olduğu bir ortamda, besinin birey üzerindeki psikolojik etkisi incelenerek hedonik açlığın değerlendirilmesi esasına dayanmaktadır. Ölçek, lezzetli besinlerin tüketiminden ziyade besinlere yakınlığın üç düzeyinde (besine bulunabilirliği, besin mevcudiyeti ve besin tadına bakılması) iştahı değerlendirmektedir. Ölçek 1 (hiç katılmıyorum) ile 5 (kesinlikle katılıyorum) arasında değişen 5'li likert

olarak sunulmakta ve değerlendirilmesi 5 puan üzerinden yapılmaktadır. Toplam puan, tüm maddelere verilen yanıtların toplanarak toplam madde sayısına bölünmesiyle elde edilir. Elde edilen puan arttıkça hedonik açlık yanıtı kuvvetlenmektedir. Buna göre BGÖ skoru 2.5 ve üzerinde ise “hedonik açlığın varlığına”, 2.5’un altında ise “hedonik açlık yokluğuna” işaret etmektedir (Cappelleri ve ark., 2009).

#### *Tıkınırcasına yeme durumunun değerlendirilmesi*

Edinburgh Bulimiya Araştırma Testi (BITE) testi, 33 sorudan oluşmakta olup bireylerde TYB’yi saptamak amacıyla kullanılmaktadır. Testin semptom skalası ve şiddet skalası olmak üzere 2 alt ölçeği bulunmaktadır. İlk 30 soru, semptom skalasını oluşturmaktadır ve evet/hayır olarak cevaplandırılmakta ve en yüksek 30 puan elde edilmektedir. 0-10 puan “yeme

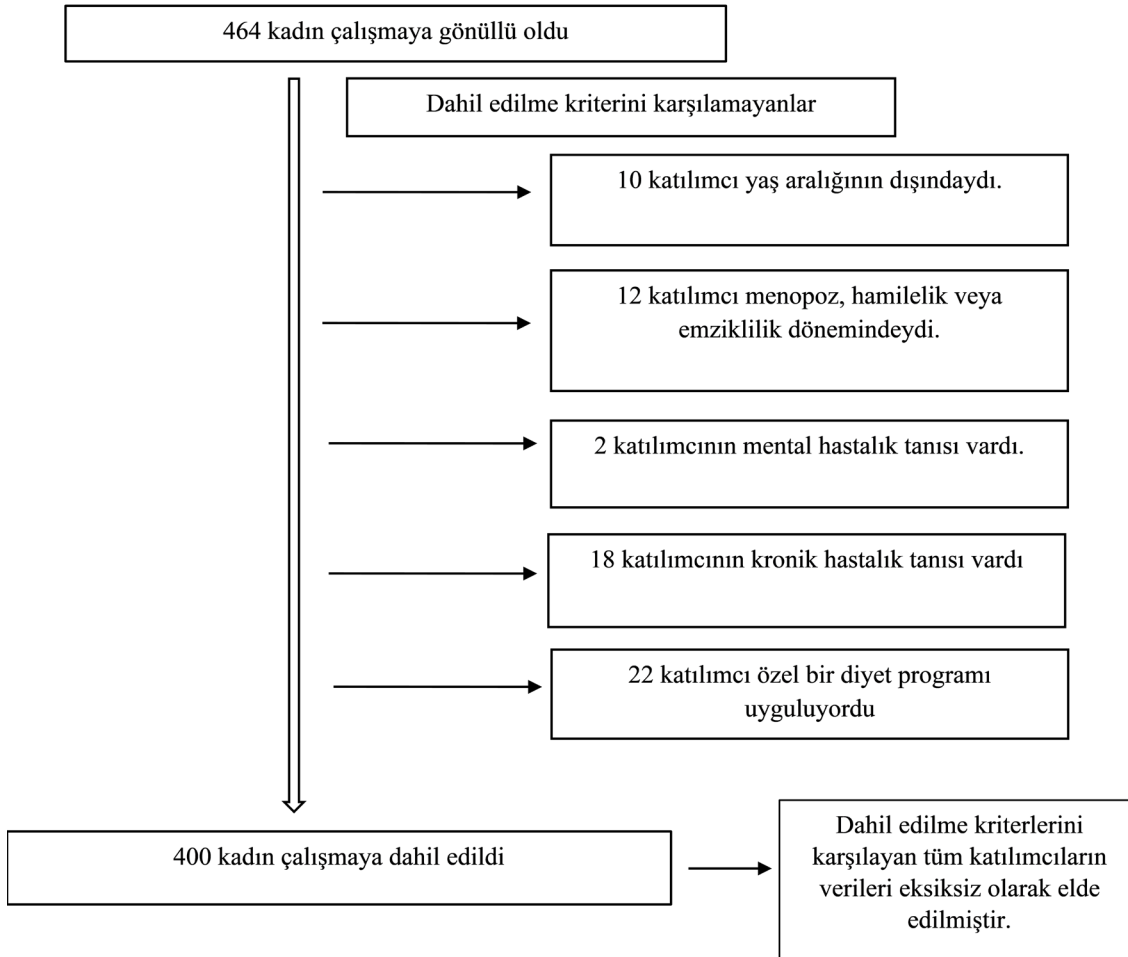
bozukluğu yoktur”; 11-19 puan “yeme bozukluğu yok, anormal yeme davranışı vardır”; 20 puan ve üzeri ise “tıkınırcasına yeme bozukluğu var” olarak değerlendirilmektedir. Şiddet skalası 3 sorudan oluşmaktadır. Yeme bozukluğunun ciddiyetini belirlemekte ve 5 puan ve üzeri yeme bozukluğunun varlığını belirtmektedir (Henderson ve Freeman, 1987).

#### *Fiziksel aktivite düzeylerinin değerlendirilmesi*

Katılımcıların fiziksel aktivite seviyelerinin değerlendirilmesi amacıyla iki soruluk kısa fiziksel aktivite değerlendirme aracı karşılıklı görüşme yolu ile uygulanmıştır. İki sorudan elde edilen toplam skor hesaplanarak 0-3 puan aralığında ise yetersiz aktif,  $\geq 4$  puan ise yeterli derecede aktif olarak değerlendirilmiştir (Marshall ve ark., 2005).

### Şekil 1. Katılımcı alımı akış şeması

Figure 1. Participant recruitment flow chart



*Beslenme durumunun ve alışkanlıklarının değerlendirilmesi*

Vücut ağırlığı kalibrasyonu yapılmış tartı aletiyle alınmış ve boy uzunluğu stadiometre ile diyetisyenler tarafından ölçülmüştür. Beden kütle indeksi (BKİ), vücut ağırlığının (kg) boy uzunluğunun karesine (m<sup>2</sup>) bölünmesi ile elde edilmiş ve Dünya Sağlık Örgütü (World Health Organization) referanslarına göre değerlendirilmiştir (World Health Organization (WHO) Global Database for Body Mass Index, 2023). Beslenme alışkanlıklarını saptamak amacıyla öğün tüketimi, iştah durumu ve bazı besinlerin tüketim sıklıklarına ilişkin bilgileri içeren soruların yanıtları online anket aracılığıyla elde edilmiştir.

*İstatistiksel Analiz*

Verilerin değerlendirilmesinde IBM SPSS versiyon 22 istatistik paket programı kullanılmıştır. Çalışma verileri değerlendirilirken tanımlayıcı istatistiksel metotlar kullanılmış olup verilerin dağılımı ise Shapiro-Wilk testi ile değerlendirilmiştir. Niceliksel verilerde normal dağılım göstermeyen iki grup karşılaştırmasında Mann-Whitney U testi, normal dağılım göstermeyen üç ve üzeri grubun karşılaştırılmasında Kruskal-Wallis testi kullanılmıştır. Nicel veriler arasındaki ilişkiyi belirlemek amacıyla Sperman's korelasyon analizi kullanılırken, nitel veriler arasındaki ilişkiyi belirlemek amacıyla Chi-square testi kullanılmıştır.

*Araştırmanın Etik İzni*

Çalışma protokolü, İstanbul Medipol Üniversitesi Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu tarafından E-10840098-772.02-1402 sayılı ve 189 karar numarası ile onaylanmıştır. Araştırma Helsinki İlkeler Deklarasyonu'na (<https://www.wma.net/what-wedo/medical-ethics/declaration-of-helsinki/>) uygun gerçekleştirilmiştir.

**Bulgular ve Tartışma**

Katılımcıların genel özellikleri Tablo 1'de verilmiştir. Diyetisyene başvuran kadınların büyük bir çoğunluğu bekar, üniversite mezunu ve gelir düzeyi yeterlidir. Kadınların yaş ortalaması 27.2 ±8.75 yıl iken BKİ ortalaması ise 25.4 ±4.99 kg/m<sup>2</sup>'dir. Katılımcıların tıknırcasına yeme davranışı ve hedonik açlık durumlarına yönelik bilgiler Tablo 2'de verilmiştir. Katılımcıların %25.2'sinde tıknırcasına yeme davranışı bozukluğu, %74.7'sinde ise hedonik açlık mevcuttur. Katılımcıların BGÖ toplam skoru yüksektir (3.2 ±1.06) ve bu durum hedonik açlığa işaret etmektedir.

Katılımcıların tıknırcasına yeme durumlarına göre hedonik açlık, BKİ ve fiziksel aktivite düzeyleri Tablo 3'de verilmiştir. Tıknırcasına yeme bozukluğu olan kadınların büyük bir çoğunluğunda (%87.0) hedonik açlık durumu görülürken, TYB bulunmayan kadınlarda %68.0 ile bu oran daha düşüktür (p=0,001). Tıknırcasına yeme bozukluğu olan kadınların %63.4'ü fazla kilolu veya obezken, TYB olmayan kadınların %55.2'si normal veya zayıftır (p=0.001). Beden kütle indeksi bakımından incelendiğinde, TYB olmayan kadınların BKİ değeri TYB olan kadınlara göre anlamlı derecede daha düşüktür (25.3 ±5.12 kg/m<sup>2</sup> ye karşı 27.0 ±5.46 kg/m<sup>2</sup>, p=0,005).

Katılımcıların tıknırcasına yeme durumlarına göre beslenme alışkanlıkları Tablo 4'de verilmiştir. Tıknırcasına yeme bozukluğu olan kadınların olmayanlara göre daha sık diyetle başladıkları (%87,1'e karşı %41,8), daha iştahlı oldukları (%78.2'e karşı %29.8) ve daha düzensiz öğün alışkanlıklarına sahip oldukları (%68.3'e karşı %41.3) bulunmuştur (p=0.001). Beslenme alışkanlıkları incelendiğinde, TYB olmayan kadınların %64.3'ünün hazır/işlenmiş/paketli ürünleri 15 günde 1 kereden daha seyrek şekilde tükettiği TYB olan kadınların %57.5'inin ise haftada 1-4 kereden daha sık tükettikleri bulunmuştur (p=0.007). Yağlı ve şekerli besinlerin tüketimine odaklanıldığında ise TYB olan kadınların %79.2'sinin haftada 1-4 kereden daha sık TYB olmayanların ise %40.8'inin 15 günde 1 kereden daha seyrek yağlı ve şekerli besinleri tükettiği saptanmıştır (p=0.008).

Tıknırcasına yeme durumu ile hedonik açlık, BKİ ve fiziksel aktivite arasındaki ilişki Tablo 5'de verilmiştir. Edinburgh Bulimiya Araştırma Testi semptom ve şiddet skoru ile BGÖ toplam skoru pozitif yönde anlamlı derecede ilişkili bulunmuştur (r= 0.522, r=0.176, p=0.001). Benzer şekilde, BITE semptom ve şiddet skoru ile BKİ değeri arasında pozitif yönlü anlamlı derecede ilişki saptanmıştır (r= 0.234, r=0.201, p=0.001).

Vücut ağırlığı sorunu ile diyetisyene başvuran bireylerde tıknırcasına yeme durumunun sıklıkla görülebileceği ve bu durumun bireyi ideal vücut ağırlığına getirmeyi amaçlayan beslenme programlarının başarısını etkileyebileceği düşünülmektedir. Bu çalışma, diyetisyene başvuran kadınlarda TYB'nin saptanması ve TYB'nin hedonik açlık, beslenme durumu, beslenme alışkanlıkları ve obezite ile ilişkisinin incelenmesi amacıyla yürütülmüştür.

**Tablo 1.** Katılımcıların genel özellikleri**Table 1.** General characteristics of the participants

	n	(%)
<b>Eğitim Durumu</b>		
İlkokul mezunu	9	2.2
Ortaokul mezunu	21	5.3
Lise mezunu	138	34.5
Üniversite mezunu	190	47.5
Lisansüstü	42	10.5
<b>Gelir Düzeyi</b>		
Gelirim giderimden az	90	22.5
Gelirim giderime eşit	212	53.0
Gelirim giderimden fazla	98	24.5
<b>Medeni Durum</b>		
Bekar	269	67.3
Evli	131	32.7
<b>Alkol Kullanımı</b>		
Evet	46	11.5
Hayır	354	88.5
<b>Sigara Kullanımı</b>		
Evet	66	16.5
Hayır	334	83.5
<b>Yaş</b> (Ort ± SS)		27.26 ±8.75
<b>Beden Kütle İndeksi</b> (kg/m <sup>2</sup> ) (Ort ± SS)		25.45 ±4.99
<b>Ağırlık</b> (kg) (Ort ± SS)		68.64 ±13.6

**Tablo 2.** Katılımcıların tıknırcasına yeme davranışı ve hedonik açlık durumları**Table 2.** Participants' binge eating behaviors and hedonic hunger status

	n	(%)
<b>Tıknırcasına Yeme Değerlendirmesi</b>		
Tıknırcasına yeme davranışı yok	146	36,5
Tıknırcasına yeme davranışı yok. anormal yeme davranışı var	153	38,3
Tıknırcasına yeme davranışı var	101	25,2
<b>BITE Toplam Skor</b> (Ort ± SS)		14.0 ±6.76
<b>BITE Şiddet Skor</b> (Ort ± SS)		3.7 ±3.73
<b>Hedonik Açlık Değerlendirmesi</b>		
Hedonik açlık yok	101	25,3
Hedonik açlık var	299	74,7
<b>BGÖ Toplam Skor</b> (Ort ± SS)		3.2 ±1.06
<b>BGÖ Alt Boyut Puanları</b> (Ort ± SS)		
Besin Bulunabilirliği		3.0 ±1.15
Besin Mevcudiyeti		3.3 ±1.20
Besinin Tadına Bakılması		3.2 ±1.10

Chi-Square Testi, Mann Whitney U Testi, p<0.05, BITE: Edinburgh Bulimiya Araştırma Testi, BGÖ: Besin Gücü Ölçeği



**Tablo 3.** Katılımcıların tıknırcasına yeme durumlarına göre hedonik açlık, BKİ ve fiziksel aktivite düzeyleri**Table 3.** Participants' hedonic hunger, BMI and physical activity levels according to their binge eating status

	TYB Var n (%)	TYB Yok n (%)	P
<b>Hedonik Açlık Durumu</b>			
Yok	14 (13.9)	94 (31.4)	<b>0.001</b>
Var	87 (86.1)	205 (68.6)	
<b>BITE Skor</b> (Ort ± SS)	3.83 ±1.1	2.95 ±0.96	<b>0.002</b>
<b>Fiziksel Aktivite Sınıflama</b>			
Yetersiz aktif	67 (66.3)	207 (69.2)	0.613
Yeterli derecede aktif	34 (33.7)	92 (30.8)	
<b>Fiziksel Aktivite Skor</b> (Ort ± SS)	3.83 ±1.1	2.96 ±0.97	
<b>BKİ Sınıflama</b>			
Zayıf	1 (1.0)	17 (5.7)	<b>0.001</b>
Normal	36 (35.6)	148 (49.5)	
Hafif Şişman	35 (34.7)	84 (28.1)	
Obez	29 (28.7)	50 (16.7)	
<b>BKİ</b> (Ort ± SS)	27.07 ±5.46	25.39 ±5.12	<b>0.005</b>

Chi-Square Testi, Mann Whitney U Testi,  $p < 0.05$ , BITE: Edinburgh Bulimiya Araştırma Testi, TYB: Tıknırcasına Yeme Bozukluğu, BKİ: Beden Kütle İndeksi

Hedonik açlık, fizyolojik olarak açlık durumu gelişmeden besin alımına yönelik çeldirici ve uyarıcı bir dürtü olarak tanımlanmaktadır (Santoso ve ark., 2019). Hedonik açlık zevkli bir duyum oluşturan herhangi bir besine yanıt olarak nöronal ödül sisteminin aktivasyonu ile ilişkili bir süreçtir (Lutter ve Nestler, 2009). Hedonistik davranış sağlıklı besin seçimi ile sonuçlanabilmektedir (Santoso ve ark., 2019). Grelinerjik sistemin mezolimbik dopaminerjik devre yoluyla hedonik ödülendirici ve motivasyonel yönlere aracılık ettiği düşünülmektedir (Singh, 2014). Homeostatik sistem, tokluk ve açlık mekanizması ve adipozite süreçlerinin (leptin, grelin ve insülin) bir düzenleyicisi olarak enerji dengesini düzenlemektedir. Leptin ve ghrelin salınımdaki değişimler ve hedonik sistem tarafından kontrol edilen ödülün aracılık ettiği durumlar yeme davranışları ile ilişkilendirmektedir (Gissoni ve dos Santos Quaresma, 2020). Bununla birlikte, anoreksijenik hormon olan beyin-türevli nörotrofik faktörün (BDNF) hedonik açlığı engelleyerek besin alımının azalması ile ilişkili olduğu ileri sürülmektedir (Mancuso ve ark., 2020). Bununla birlikte, ANKK1'in (rs1800497) ve DRD2 geninin (rs1799732) polimorfizminin BKİ ve hedonik açlık ile önemli derecede ilişkili olduğu bulunmuştur (Aliasghari ve ark., 2021). Yetişkin bireylerin hedonik açlık durumlarının ve yeme davranışının değerlendirildiği bir çalışmada (n=1276); hedonik açlık toplam ve alt skorlarının Hollanda Yeme Davranışı Anketi alt ölçek-

lerinden duygusal yeme ve dışsal yeme ile pozitif ilişkisi olduğu ( $p < 0.001$ ) ve hedonik açlık ile yeme davranış bozuklukları arasında ilişki olduğu ve obez bireylerin hedonik açlık ve yeme davranış bozuklukları açısından normal vücut ağırlığına sahip bireylere göre daha riskli olduğu görülmüştür (Ayıldız ve ark., 2021). Bu çalışmada tıknırcasına yeme davranışı bozukluğu ile BKİ arasında pozitif yönlü anlamlı derecede bir ilişki saptanmıştır ( $r = 0.234$ ,  $p = 0.001$ ). Tıknırcasına yeme bozukluğu olan kadınların büyük bir çoğunluğunun (%87.0) hedonik açlık deneyimledikleri, fazla kilolu/obez olduğu (%63.4'ü), TYB olmayanlara göre daha sık diyeteye başladıkları (%87.1'e karşı %41.8,  $p = 0.001$ ), daha iştahlı oldukları (%78.2'e karşı %29.8,  $p = 0.001$ ), daha düzensiz öğün alışkanlıklarına sahip oldukları (%68.3'e karşı %41.3,  $p = 0.001$ ), hazır/işlenmiş/paketli besinleri ( $p = 0.007$ ) ve şekerli/yağlı besinleri daha sık tükettikleri bulunmuştur ( $p = 0.008$ ). Obezite ile TYB'nin sık sık birlikte görülmesi ve her iki durumda da aşırı yeme davranışlarının bazı ortak davranışsal özellikleri olduğu vurgulanmaktadır (Boswell ve ark., 2021). Diyet paternlerinin incelendiği bir çalışmada (n=400); karıştırıcılar kontrol edildikten sonra hiçbir değişiklik olmaksızın tıknırcasına yeme bozukluğunun sağlıklı beslenme kalıpları ile pozitif, sağlıklı beslenme kalıpları ile negatif yönlü ilişkili olduğu bulunmuştur (Yousefi ve ark., 2022). Bununla birlikte, TYB'nin farklı nörobiyolojik özellikleri olduğu ve obeziteye kıyasla daha yüksek dürtüsellik ile karakterize olduğu bildirilmiştir (Boswell ve ark., 2021).

**Tablo 4.** Katılımcıların tıknırcasına yeme durumlarına göre beslenme alışkanlıkları**Table 4.** Eating habits of the participants according to their binge eating status

	TYB Var		TYB Yok		P
	n (%)		n (%)		
<b>Sık Sık Diyete Başlama</b>					
Evet	88 (87.1)		125 (41.8)		<b>0.001</b>
Hayır	13 (12.9)		174 (58.2)		
<b>İştah Durumu</b>					
Kötü/ pek iştahım yok	1 (1.0)		10 (3.3)		<b>0.001</b>
Fena değil	2 (2.0)		24 (8.0)		
İyi/ normal	19 (18.8)		176 (58.9)		
Fazla iştahlıyım	79 (78.2)		89 (29.8)		
<b>Düzenli Öğün Tüketimi</b>					
Evet	32 (31.7)		176 (58.7)		<b>0.001</b>
Hayır	69 (68.3)		123 (41.3)		
<b>Öğün atlama durumu</b>					
Öğün atlamıyorum	24 (23.8)		105 (35.1)		<b>0.001</b>
Sabah	26 (25.7)		52 (17.4)		
Öğle	46 (45.5)		142 (47.5)		
Akşam	5 (5.0)		-		
<b>Hazır, İşlenmiş, Paketli Besinler</b>					
Hiç	8 (7.9)		57 (19.1)		<b>0.007</b>
Ayda 1	19 (18.8)		70 (23.5)		
15 Günde 1 Kez	16 (15.8)		65 (21.7)		
Haftada 1-4 Kez	44 (43.6)		88 (29.4)		
Günde 1 Kez	5 (4.9)		12 (4.0)		
Günde 2-4 Kez	9 (9.0)		7 (2.3)		
<b>Şekerli ve Yağlı Besinler</b>					
Hiç	4 (4.0)		18 (6.0)		<b>0.008</b>
Ayda 1	6 (5.9)		58 (19.4)		
15 Günde 1 Kez	11 (10.9)		46 (15.4)		
Haftada 1-4 Kez	60 (59.4)		137 (45.8)		
Günde 1 Kez	7 (6.9)		25 (8.4)		
Günde 2-4 Kez	13 (12.9)		15 (5.0)		

Chi-Square Testi, Mann Whitney U Testi,  $p < 0.05$ , TYB: Tıknırcasına Yeme Bozukluğu

**Tablo 5.** Katılımcıların BITE Ölçeği ile BGÖ, BKİ ve fiziksel aktivite skoru arasındaki ilişki**Table 5.** The relationship between BITE and Power of Food Scale, BMI and physical activity score

BITE	BGÖ Toplam Skor				BGÖ Alt Boyut Skor				BKİ		Fiziksel aktivite skoru	
	Toplam skor		Besin bulunabilirliği		Besin mevcudiyeti		Besinin tadına bakılması		r	p	r	p
	r	p	r	p	r	p	r	p				
<b>Semptom Skor</b>	0.522	0.001	0.592	0.001	0.436	0.001	0.373	0.001	0.234	0.001	0.022	0.659
<b>Şiddet Skor</b>	0.176	0.001	0.237	0.001	0.190	0.001	0.055	0.277	0.201	0.001	0.059	0.240

Sperman's korelasyon testi,  $p < 0.05$ , BITE: Edinburgh Bulimiya Araştırma Testi, BGÖ: Besin Gücü Ölçeği; BKİ: Beden Kütle İndeksi

Yüksek enerjili besinlere ve yüksek ödül duyarlılığına yönelik eğilimin, periferik dokulardan beyin bölgelerine giden nöroendokrin sinyallemeyle değişirmesiyle aşırı besin tüketimini tetikleyebileceği düşünülmektedir (Baenas ve ark., 2023). Lezzetli besinlere yönelik artan nöral ve algısal yanıtlardan (oral somatosensöriyel işlemede yer alan bölgelerde) kaynaklı hedonik açlığın lezzetli besinlerin tüketimine yönelik motivasyonu ve tıknırcasına yeme olasılığının artırması muhtemeldir (Burger ve ark., 2016). Tıknırcasına yeme davranış bozukluğu ghrelin sinyalindeki ve melanocortin 4 receptor (MC4R) sinyalindeki disfonksiyon ile ilişkilendirilmektedir (Geliebter ve ark., 2008). Ayrıca, tıknırcasına yeme davranışı sergileyen bireylerde kısa sürede aşırı yemeye bağlı olarak mide kapasitesi/hacmi genişlemektedir. Genişleyen mide hacmi tokluk sinyallerinin azalması ile sonuçlanarak aşırı yemeye katkıda bulunabilmektedir (Geliebter ve ark., 2008). Bu çalışmanın bulguları tıknırcasına yeme semptom ve şiddet skoru ile hedonik açlık toplam skorunun pozitif yönde anlamlı derecede ilişkili olduğunu doğrulamaktadır ( $r=0.522$ ,  $r=0.176$ ,  $p=0.001$ ). Ödüle öncelik verme eğilimi (aşırı yemek yemenin hoş tadı), besinin hedonik açıdan ödüllendirici özelliklerine duyarlılığı daha da şiddetlendirerek, ödüllendirici uyarıların varlığında yeme kontrolünü kaybetmeye yol açabilir. Bununla birlikte hedonik açlığın tıknırcasına yemenin önemli bağımsız yordayıcısı olabileceği düşünülmektedir (Manasse ve ark., 2018). Literatür sonuçları hedonik süreçlerin tıknırcasına yemeyi teşvik etmede önemli olabileceğini düşündürse de (Burger ve ark., 2016; Manasse ve ark., 2018; Witt ve Lowe, 2014), güncel bir çalışmada tıknırcasına yeme atakları bildirmeyen bireylerle karşılaştırıldığında, atak bildiren bireylerin, yemenin olumsuz duygulanımı ( $t=-2.54$ ,  $p=0.013$ ) ve can sıkıntısını ( $U=503.50$ ,  $p=0.006$ ) azaltacağına dair daha fazla beklentisi olduğu bulunurken, hedonik açlık seviyeleri açısından bir farklılık göstermediği ( $t=-1.68$ ,  $p=.096$ ) saptanmıştır (Parker ve ark., 2022).

Hedonik açlık ve tıknırcasına yeme davranışı etkileşiminin birçok faktörden kaynaklanabileceği düşünüldüğünden bu çalışma bazı sınırlamalara sahiptir. Çok sayıda psikolojik ve demografik faktörü dikkate alan gelecek araştırmalara ihtiyaç vardır. Hedonik açlık ve tıknırcasına yeme davranışlarının değerlendirilmesi, bireylerin algısına bağlı olduğundan, tıknırcasına yeme durumlarının değerlendirilmesi farklı sonuçlanabilir. Ayrıca, mahcubiyet veya utanma duygusu, yeme davranışlarının ve alışkanlıklarının bildirimlerinde yanıltıcı olabilir. Sonuç olarak, hedonik açlık ve tıknırcasına yeme atakları arasındaki zamansal ilişkileri belirlemek için daha fazla veriye ihtiyaç olduğu görülmektedir.

## Sonuç

Çalışmanın sonuçlarına göre, tıknırcasına yeme davranışı sergileyenlerin %86.1'inin hedonik açlık deneyimlediği, %63.4'ünün fazla kilolu veya obez olduğu görülmüştür. Tıknırcasına yeme semptom ve şiddet skoru ile hedonik açlık skoru ve BKİ pozitif yönde anlamlı derecede ilişkili bulunmuştur. Tıknırcasına yeme davranışı bozukluğu, hedonik açlık, beslenme örüntüsü arasındaki ilişkiyi bütüncül olarak değerlendiren bir çalışma yoktur. Bu çalışmanın bütüncül olarak değerlendirmeye olanak sağlaması nedeniyle literatüre katkı sağlayacağı ve bu yönde yapılacak daha sonraki araştırmalar için bir yol gösterici olabileceği düşünülmektedir. Tıknırcasına yeme davranışı bozukluğu ve hedonik açlık kavramlarına yönelik daha geniş örneklemli ve detaylı araştırmalara ihtiyaç duyulmaktadır. Bununla birlikte, klinisyenlerin sadece yeme bozukluğuna odaklanmasının dışında bireylerin hedonik açlık, beslenme durumları ve davranışlarını bütüncül olarak değerlendirmesinin beslenme tedavisinin olumlu sonuçlanmasına katkısı olacaktır.

## Etik Standartlar ile Uyumluluk

**Çıkar çatışması:** Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

**Etik izin:** Çalışma protokolü, İstanbul Medipol Üniversitesi Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu tarafından E-10840098-772.02-1402 sayılı ve 189 karar numarası ile onaylanmıştır.

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## Introduction

Macrofungi are used as food as well as medicine in many countries. Macrofungi can be an essential food source for the rapidly increasing world population (Dülger et al., 1999). Besides having a role as food, macrofungi are also a source of income. They are collected, consumed and sold in over 85 countries (Boa, 2004).

Macrofungi can be divided into three groups: edible, inedible and poisonous. Edible mushrooms are collected from nature by people. People have learned which mushrooms are edible from their ancestors or by asking each other (Karasüleymanoğlu, 2014).

Türkiye has a rich edible mushroom flora due to its phytogeographic location. The richness of the mushrooms, which usually appear in spring and autumn, is undoubtedly due to the suitability of ecological conditions. Although Türkiye is rich in edible mushroom species, our people do not know enough about them. It is reported that only 3-5 mushroom species are recognised and consumed as food by the local people (Karasüleymanoğlu, 2014; Altuntaş et al., 2016; Çınar Yılmaz and Işıloğlu, 2016). Some mushroom species, which are well known and eaten in some regions of our country, are not known in other regions and are sometimes considered poisonous. Many types of mushrooms are exported from Türkiye as fresh, chilled, frozen, in brine or dried. *Lactarius deliciosus* (L.) Gray, *Craterellus cornucopioides* (L.) Pers, *Amanita caesarea* (Scop.) Pers, *Hydnum repandum* L., and *Cantharellus cibarius* Fr. are some exported species (Erdoğan et al., 2017). *Lactarius* species are known as çıntar, melki, kanlıca, and termit consumed in many regions of Türkiye (Adanacıoğlu et al., 2017). They have a very high market share. They are known as "Çıntar" in the Muğla region and are consumed by the locals. These mushrooms usually grow naturally in autumn and are common in pine and oak forests. Local people are collecting these mushrooms and sell them in public markets in Türkiye (Allı et al., 2006; Pekşen et al., 2007; Yılmaz and Bengü, 2018; Allı, 2022).

Mushrooms are distributed worldwide, and their consumption has increased in recent years due to their nutritional value and organoleptic attributes (Ergönül et al., 2018; Avcı and Avcı, 2019). Mushrooms are characterised as valuable nutrients due to their content of fatty acids, vitamins, fibres, carbohydrates, proteins and minerals (Yılmaz and Bengü, 2018). Because of these nutritional contents, mushrooms are essential to consumers. Other factors such as free sugars, free amino acids, organic acids, flavour 5'-nucleotides, and volatile flavour compounds significantly affect taste and flavour.

For these reasons, mushrooms have been a popular food ingredient and flavour for centuries. Today, several cultivated mushroom types can be found easily in the markets. However, wild mushrooms are preferred by consumers for their unique flavour, even if it is difficult to collect them (Xu et al., 2019).

Although it varies according to the place of growth and the type of mushroom, on average, 90% of the mushroom is water, 16-85% of dry weight is carbohydrates, 0.2-8.7% is lipids, 14-44% is proteins, 1-29% is ash. The amount of protein contained in mushrooms, which is classified as the most important protein source in developing world countries, is higher than most vegetables (Karasüleymanoğlu, 2014; Yılmaz and Bengü, 2018).

From a nutritional point of view, fresh mushrooms have high water content and neutral pH values. So microorganisms can quickly grow and thereby decrease the shelf life of fresh mushrooms to a few days. Similarly, the way of harvesting, handling and storage can affect microbiological growth. Ultimately, quality parameters like texture, colour, etc. are affected. These factors promote the growth of microorganisms, which leads to quality degradation and spoilage of fresh mushrooms (Chikthimmah and Beelman, 2006; Ergönül et al., 2018).

To our knowledge, limited scientific information is reported on the microbiological properties of wild edible mushrooms. This study aimed to assess the microbial hygienic quality of fresh wild edible mushrooms sold in Muğla province and evaluate the results in the context of public health. This data can contribute to evaluating the microbiological qualities of fresh mushrooms as highly appreciated food.

## Materials and Methods

### Materials

Fresh wild edible mushroom samples were used as test material in this study. Samples were purchased from ten different sellers in the Fethiye region of Muğla/Türkiye local market. All the samples were transferred to the laboratory in an ice-box within the original packages. The local people don't wash these fresh mushrooms with tap water before cooking. So, the wild edible mushrooms weren't washed to represent the consumer habit in this study. The samples were analysed a few hours after purchasing, and all analyses were performed in two replications with two parallels. This study analysed

twenty samples to detect some physicochemical and microbiological properties.

## Methods

### Physicochemical Analyses

Mushroom samples were tested for pH and titratable acidity (anhydrous citric acid, %) to detect the physicochemical properties. These analyses were used to support the results of microbiological values.

**Titratable Acidity (TA):** A 10 g sample was weighed and mixed with 100 mL distilled water for homogenisation. Then, it was filtered with Whatman No. 4. 25 mL of filtered samples were taken into Erlenmeyer and phenolphthalein (Tekkim, TK.930094.00102, Türkiye) was added as an indicator. This filtrate was titrated with 0.01N sodium hydroxide (NaOH, Tekkim, TK.170511.01002, Türkiye). Accordingly, titratable acidity was calculated as anhydrous citric acid % (AOAC, 1995).

**pH:** pH meter (Mettler Toledo, Five easy plus FP20, China) was calibrated with two buffer solutions (pH:4.01, Mettler Toledo, 51302069, Switzerland; pH:7, Mettler Toledo, 51302047, Switzerland) before reading. Then, the pH meter detected the pH values of homogenised samples as described for titratable acidity (AOAC, 1995).

### Microbiological Analyses

The microbiological analyses were designed to determine the microbiological quality of mushroom samples. A 25 g sample was weighed into the stomacher bag and then homogenised with 225 mL peptone water (0.1%) (Oxoid, LP0037, UK) by a stomacher (Isolab, 608.01.002 Germany) for 3 min. Decimal dilutions were prepared (1:10) with sterile peptone water for the enumeration of total mesophilic aerobic bacteria (TMAB), lactic acid bacteria (LAB), total yeast, total mould, *Staphylococcus aureus*, total coliform, and faecal coliform. In addition, the presence of *Escherichia coli* was investigated in all samples.

**Total Count of Mesophilic Aerobic Bacteria (TMAB):** Nutrient agar (NA, Biolife, 4018102, Italia) was used as a medium. The aliquots of 0.1 mL dilution were transferred into Petri dishes, and the spread plate method was performed. The Petri dishes were incubated at 30°C (Daihan Scientific, Thermo-Stable IG-105, Korea) for 24–48 h (FDA-BAM online, 2020a).

**Enumeration of Yeasts and Molds:** Serially diluted samples were surface plated on Potato Dextrose Agar (PDA, Biolife, 4019352, Italia). After the autoclave, PDA was aseptically

acidified with 10% tartaric acid (Carlo Erba, 41127, France). The Petri dishes were incubated at 25 °C for 5 days (FDA-BAM online, 2017).

**Enumeration of Lactic Acid Bacteria (LAB):** The viable cell number of lactic acid bacteria was performed on de Man, Rogosa and Sharpe Agar (MRSA, Biolife, 401728S2, Italia) at 30°C for 24–48 h (ISO 15214, 1998).

**Enumeration of *Staphylococcus aureus*:** Baird Parker Agar (BPA, Biolife, 4011162, Italia) was used. After autoclave, 50% egg yolk (Merck, 1.03784.0001, UK) was added into the cooling medium at 50 °C. 3.5% tellurite (Aldrich, P0677-25G, Japan) was sterilised by a membrane filter and put in the medium. The Petri dishes were incubated at 37 °C for 24–48 hours (FDA-BAM online, 2019).

**Enumeration of Total Coliform and Faecal Coliform, Detection of *E. coli*:** Total coliform and faecal coliform were detected by the most probable number (MPN) technique. 1 mL of aliquots of serial dilution were inoculated into three tubes containing Lauryl Sulphate Tryptose Broth (LSTB, Merck, 1.10266.0500, Germany). LSTB tubes were incubated at 37 °C for 24–48 h. After incubation of gas positive tubes were determined. A loopful of suspension from each gassing LSTB tube was transferred to Brilliant Green Bile Broth (BGLB, Merck, 1.05454.0500, Germany) and *Escherichia coli* Broth (ECB, Biolife, 4014252, Italia). BGLB and ECB were incubated at 37 °C for 24–48 h and 45 °C for 24–48 h, respectively. Then, BGLB and ECB tubes were controlled for gas production. Confirmed coliform and faecal coliform were indicated in BGLB and ECB medium. The detection of *E. coli* was examined on Eosin Methylene Blue Agar (EMBA, Merck, 1.01347.0500, Germany) from the gas-positive ECB tubes. Typical colonies were confirmed by IMViC tests (FDA-BAM online, 2020b).

### Statistical Analyses

All experiments had two replicates and two parallels (n=4). All statistical analyses were performed with the SPSS statistical package program (IBM SPSS Statistics Version 22; USA) by ANOVA variance analysis. The significance levels of  $P < 0.05$  were used for statistical differences. Duncan Tests established the significant difference between the means.

## Results and Discussion

Physico-chemical properties such as titratable acidity and pH were determined to evaluate the results of microbiological tests, as shown in Table 1. The results of titratable acidity analyses ranged from 0.005-0.020% (anhydrous citric acid %). The highest titratable acidity was shown in sample 9.



Sample 9 was significantly different ( $P < 0.05$ ). The samples' lowest and highest pH values were 6.82 and 7.54. The pH values of the mushroom were similar ( $P > 0.05$ ). The pH values correlated with the titratable acidity values. The food has been classified depending on the pH value. Low-acid foods have a pH greater than 5.7, meaning they contain low amounts of acid (Temiz, 1999). The average value of the pH was 7.18 for mushroom samples. So, the mushroom samples can be considered as low-acid foods.

**Table 1.** Physico-chemical properties of wild edible mushroom\*

Sample	pH	Titratable acidity (TA) (anhydrous citric acid %)
1	7.08 ± 0.01 <sup>abcde</sup>	0.008 ± 0.001 <sup>ab</sup>
2	6.96 ± 0.28 <sup>ab</sup>	0.006 ± 0.003 <sup>a</sup>
3	6.97 ± 0.20 <sup>abc</sup>	0.009 ± 0.000 <sup>ab</sup>
4	7.28 ± 0.06 <sup>defgh</sup>	0.009 ± 0.000 <sup>ab</sup>
5	7.33 ± 0.01 <sup>efgh</sup>	0.009 ± 0.001 <sup>ab</sup>
6	7.23 ± 0.08 <sup>cdefg</sup>	0.008 ± 0.002 <sup>ab</sup>
7	7.34 ± 0.01 <sup>efgh</sup>	0.005 ± 0.001 <sup>a</sup>
8	7.39 ± 0.13 <sup>fgh</sup>	0.007 ± 0.001 <sup>a</sup>
9	6.82 ± 0.24 <sup>a</sup>	0.020 ± 0.006 <sup>d</sup>
10	7.19 ± 0.11 <sup>bcddefg</sup>	0.009 ± 0.002 <sup>ab</sup>
11	7.02 ± 0.03 <sup>abc</sup>	0.007 ± 0.001 <sup>a</sup>
12	7.15 ± 0.00 <sup>bcddef</sup>	0.008 ± 0.000 <sup>ab</sup>
13	7.01 ± 0.04 <sup>abc</sup>	0.012 ± 0.001 <sup>cb</sup>
14	6.97 ± 0.02 <sup>abc</sup>	0.013 ± 0.001 <sup>c</sup>
15	7.54 ± 0.01 <sup>h</sup>	0.005 ± 0.001 <sup>a</sup>
16	7.12 ± 0.10 <sup>bcdde</sup>	0.008 ± 0.000 <sup>ab</sup>
17	7.44 ± 0.04 <sup>gh</sup>	0.005 ± 0.000 <sup>a</sup>
18	7.44 ± 0.08 <sup>gh</sup>	0.006 ± 0.000 <sup>a</sup>
19	7.11 ± 0.01 <sup>bcdde</sup>	0.014 ± 0.001 <sup>c</sup>
20	7.19 ± 0.09 <sup>bcddefg</sup>	0.008 ± 0.001 <sup>ab</sup>

\*n=4, (± standard deviation); Significant difference was detected between the values in the column ( $p < 0.05$ ).

It has been established that most microorganisms grow best at pH values around 7.0 (6.6-7.5) (Jay, 2000). The results of titratable acidity and pH of the samples are very favourable for the growth of numerous microbial species can be expected (Roberts et al., 2005). The analyses of TMAB, LAB, total yeast, total mould, *S. aureus*, total coliform, faecal coliform, and *E. coli* were performed to determine the initial microflora of mushroom samples.

Ordinary healthy mushrooms have high bacterial populations. The results of enumeration of TMAB ranged between 4.13 and 8.83 log CFU/g (Table 2). Similarly, total bacterial numbers ranged from 6.3 to 7.2 log CFU/g of fresh mushroom tissue, as demonstrated by Chikthimmah and Beelman, 2006. According to Venturini et al. (2011), TMAB counts of wild edible mushrooms from Spain were between 4.4 log CFU/g and 9.4 log CFU/g. TMAB values were presented between 6.8 and 9.2 log CFU/g for eight wild edible mushrooms from Türkiye (Ergönül et al., 2018). Three cultivated mushroom species were analysed, and their mesophilic aerobic counts were expressed as 4.87, 5.00, and 7.92 log CFU/g (Reyes et al., 2004).

LAB was present in relatively low numbers compared to the TMAB, with <1.00- 5.57 log CFU/g range results. The highest enumeration of LAB was determined in sample 3, and the lowest one was in sample 2 and sample 12 (Table 2). LAB counts of mushroom samples were investigated between 1.4 and 2.4 log CFU/g (Ergönül et al., 2018). The mean value of LAB was reported as 2.1 log CFU/g by Venturini et al. (2011). The average result of this study was higher. It came out as 3.70 log CFU/g. In another study, *Lactobacillus* sp. and *Pediococcus* sp. were isolated from fresh mushrooms. However, the enumeration was not declared (Halami et al., 1999).

*S. aureus* produces a staphylococcal enterotoxin that is extremely thermotolerant, which means it can survive the cooking process (Grispoldi et al., 2021). Detectable enumeration of *S. aureus* cells was not observed in most mushroom samples except sample 11 (Table 2). No significant differences ( $P > 0.05$ ) were detected in the samples (except sample 11). Venturini et al. (2011) and Ergönül et al. (2018) stated that *S. aureus* was not isolated in any of the samples examined.

Mushrooms also contain significant levels of yeasts and moulds. The count of yeast was established to be between 3.10 log CFU/g and 7.76 log CFU/g. The mould values of fresh mushrooms were determined as <1.00 and 3.93 log CFU/g (Table 2). Chikthimmah and Beelman (2006) showed that freshly harvested mushroom samples had approximately 3 log CFU of moulds and 6 log CFU of yeast per gram. Although the yeast population increased to 6.9 log CFU/g, mould counts were stable after 6 days of storage at 4°C. The total count of yeast and mould samples was higher than the previous study of Ergönül et al. (2018), who determined it to be between 1.9 and 3.3 log CFU/g. The yeast and mould results of mushroom samples determined by Venturini et al. (2011) were also lower than those indicated in the present study.

Table 2. Microbiological properties of wild edible mushroom

Sample	TMAB log CFU/g	LAB log CFU/g	<i>S. aureus</i> log CFU/g	Total Count of Yeast log CFU/g	Total Count of Mold log CFU/g	Total Coliform log MPN/g	Faecal Coliform log MPN/g
1	8.55 ± 0.17 <sup>ijk</sup>	3.31 ± 0.05 <sup>b</sup>	<1.00 ± 0.00 <sup>a</sup>	5.17 ± 0.19 <sup>fg</sup>	3.93 ± 0.13 <sup>h</sup>	3.90 ± 0.39 <sup>f</sup>	3.90 ± 0.39 <sup>c</sup>
2	7.50 ± 0.09 <sup>bcd</sup>	<1.00 ± 0.00 <sup>a</sup>	<1.00 ± 0.00 <sup>a</sup>	3.40 ± 0.22 <sup>ab</sup>	3.52 ± 0.11 <sup>efg</sup>	<0.30 ± 0.00 <sup>a</sup>	<0.30 ± 0.00 <sup>a</sup>
3	6.10 ± 0.06 <sup>a</sup>	5.57 ± 0.11 <sup>l</sup>	<1.00 ± 0.00 <sup>a</sup>	3.10 ± 0.14 <sup>a</sup>	3.18 ± 0.10 <sup>bc</sup>	2.80 ± 0.24 <sup>d</sup>	<0.30 ± 0.00 <sup>a</sup>
4	7.96 ± 0.04 <sup>efg</sup>	4.67 ± 0.22 <sup>efg</sup>	<1.00 ± 0.00 <sup>a</sup>	4.60 ± 0.24 <sup>def</sup>	3.36 ± 0.12 <sup>cdef</sup>	2.20 ± 0.23 <sup>bc</sup>	1.76 ± 0.29 <sup>b</sup>
5	8.01 ± 0.24 <sup>efg</sup>	4.44 ± 0.15 <sup>ef</sup>	<1.00 ± 0.00 <sup>a</sup>	3.61 ± 0.42 <sup>abc</sup>	3.15 ± 0.14 <sup>bc</sup>	<0.30 ± 0.00 <sup>a</sup>	<0.30 ± 0.00 <sup>a</sup>
6	7.22 ± 0.27 <sup>b</sup>	5.22 ± 0.05 <sup>h</sup>	<1.00 ± 0.00 <sup>a</sup>	4.02 ± 0.60 <sup>bcd</sup>	<1.00 ± 0.00 <sup>a</sup>	3.07 ± 0.15 <sup>de</sup>	1.56 ± 0.00 <sup>b</sup>
7	7.29 ± 0.28 <sup>bc</sup>	3.49 ± 0.07 <sup>b</sup>	<1.00 ± 0.00 <sup>a</sup>	3.40 ± 0.17 <sup>ab</sup>	3.29 ± 0.14 <sup>cde</sup>	<0.30 ± 0.00 <sup>a</sup>	<0.30 ± 0.00 <sup>a</sup>
8	8.83 ± 0.32 <sup>k</sup>	4.75 ± 0.24 <sup>fg</sup>	<1.00 ± 0.00 <sup>a</sup>	5.45 ± 0.04 <sup>g</sup>	3.23 ± 0.11 <sup>bc</sup>	1.96 ± 0.00 <sup>bd</sup>	1.76 ± 0.29 <sup>b</sup>
9	8.15 ± 0.16 <sup>fgh</sup>	3.91 ± 0.11 <sup>c</sup>	<1.00 ± 0.00 <sup>a</sup>	4.75 ± 0.18 <sup>ef</sup>	3.73 ± 0.10 <sup>hg</sup>	2.80 ± 0.24 <sup>d</sup>	<0.30 ± 0.00 <sup>a</sup>
10	7.89 ± 0.13 <sup>ef</sup>	<1.00 ± 0.00 <sup>a</sup>	<1.00 ± 0.00 <sup>a</sup>	7.23 ± 0.17 <sup>h</sup>	3.33 ± 0.17 <sup>cde</sup>	2.20 ± 0.23 <sup>bc</sup>	1.76 ± 0.29 <sup>b</sup>
11	8.31 ± 0.02 <sup>ghi</sup>	4.97 ± 0.43 <sup>gh</sup>	4.74 ± 0.35 <sup>b</sup>	4.57 ± 0.38 <sup>def</sup>	3.60 ± 0.15 <sup>fg</sup>	5.07 ± 1.26 <sup>g</sup>	5.07 ± 1.26 <sup>d</sup>
12	8.78 ± 0.01 <sup>jk</sup>	<1.00 ± 0.00 <sup>a</sup>	<1.00 ± 0.00 <sup>a</sup>	3.52 ± 0.19 <sup>abc</sup>	3.35 ± 0.08 <sup>cdef</sup>	<0.30 ± 0.00 <sup>a</sup>	<0.30 ± 0.00 <sup>a</sup>
13	7.38 ± 0.01 <sup>bcd</sup>	3.41 ± 0.09 <sup>b</sup>	<1.00 ± 0.00 <sup>a</sup>	3.12 ± 0.06 <sup>a</sup>	3.02 ± 0.08 <sup>b</sup>	2.20 ± 0.23 <sup>bc</sup>	<0.30 ± 0.00 <sup>a</sup>
14	8.30 ± 0.13 <sup>ghi</sup>	4.43 ± 0.13 <sup>ef</sup>	<1.00 ± 0.00 <sup>a</sup>	4.19 ± 0.41 <sup>cde</sup>	3.22 ± 0.06 <sup>bc</sup>	2.20 ± 0.23 <sup>bc</sup>	1.76 ± 0.29 <sup>b</sup>
15	7.69 ± 0.15 <sup>de</sup>	4.10 ± 0.09 <sup>cd</sup>	<1.00 ± 0.00 <sup>a</sup>	4.10 ± 0.53 <sup>cde</sup>	<1.00 ± 0.00 <sup>a</sup>	3.07 ± 0.15 <sup>de</sup>	<0.30 ± 0.00 <sup>a</sup>
16	4.13 ± 0.22 <sup>cde</sup>	4.73 ± 0.05 <sup>efg</sup>	<1.00 ± 0.00 <sup>a</sup>	4.54 ± 0.41 <sup>def</sup>	3.26 ± 0.15 <sup>bcd</sup>	2.50 ± 0.19 <sup>bcd</sup>	<0.30 ± 0.00 <sup>a</sup>
17	7.37 ± 0.20 <sup>bcd</sup>	4.07 ± 0.11 <sup>c</sup>	<1.00 ± 0.00 <sup>a</sup>	4.32 ± 0.21 <sup>de</sup>	3.34 ± 0.02 <sup>cde</sup>	2.20 ± 0.23 <sup>bc</sup>	1.56 ± 0.00 <sup>b</sup>
18	8.07 ± 0.07 <sup>fgh</sup>	4.48 ± 0.04 <sup>ef</sup>	<1.00 ± 0.00 <sup>a</sup>	5.58 ± 0.08 <sup>g</sup>	3.47 ± 0.07 <sup>def</sup>	3.07 ± 0.15 <sup>de</sup>	2.00 ± 0.06 <sup>b</sup>
19	8.25 ± 0.09 <sup>fghi</sup>	4.39 ± 0.08 <sup>de</sup>	<1.00 ± 0.00 <sup>a</sup>	4.36 ± 0.21 <sup>de</sup>	3.53 ± 0.05 <sup>efg</sup>	3.80 ± 0.24 <sup>ef</sup>	<0.30 ± 0.00 <sup>a</sup>
20	8.43 ± 0.09 <sup>hij</sup>	4.10 ± 0.07 <sup>cd</sup>	<1.00 ± 0.00 <sup>a</sup>	7.76 ± 0.34 <sup>h</sup>	<1.00 ± 0.00 <sup>a</sup>	2.00 ± 0.06 <sup>bc</sup>	<0.30 ± 0.00 <sup>a</sup>

$n=4$ , ( $\pm$  standard deviation); Significant difference was detected between the values in the column ( $p<0.05$ ).

Since coliform bacteria are common in both the intestines and nature (soil, plants, etc.), they are considered indicators of sanitation in the food industry. High levels of coliform microorganisms in food indicate that the required hygienic measures have not been taken during or after harvesting, storage, and sale (Frazier and Westhoff, 1988; Jay, 2000). Total coliform counts of the samples were obtained between 0.30 and 5.07 log MPN/g (Table 2). 80% of the mushroom samples were harbouring total coliform. "Faecal coliform" within the coliform group, located in the natural flora of the intestine system of humans and warm-blooded animals, is considered an indicator of faecal contamination. It is known that most bacteria identified as faecal coliforms in the coliform group are *E. coli*. The presence of *E. coli* and/or faecal coliform bacteria in any sample implies that the sample is directly or indirectly contaminated with faeces and may contain other intestinal pathogens. For this reason, *E. coli* and faecal coliforms are not allowed in any foodstuff, drinking, and/or utility water (Khan and Gupta, 2020; Schalli et al., 2022). The faecal coliform was found in 45% of the tested samples. Faecal coliforms were not detected in samples 2, 3, 5, 7, 9, 12, 13, 15,

16, 19 and 20 (Table 2). The highest results were in sample 11 for both total and faecal coliform. Similarly, eight wild edible mushrooms were tested for total coliform and mentioned 0.3-1.4 log CFU/g in four samples (50%) (Ergönül et al., 2018). In another study, coliform bacteria were detected in 23.4% of the tested mushroom samples (Venturini et al., 2011). Based on confirmation and identification tests, *E. coli* Biotype I was found in 35% of the tested samples (1, 4, 6, 8, 11, 17, and 18) and *Enterobacter aerogenes* in only one sample (sample 10). The environment of mushrooms, especially those grown in natural ecosystems, makes them vulnerable to pathogens since this can be excreted in the faeces of animals and insects and transmitted to the ascocarps.

## Conclusion

Unlike fruits and vegetables (whole or minimally processed), information on the microbial quality of fresh mushrooms is minimal, especially for commercial wild mushroom samples. As in other studies, the fresh mushroom species tested in this study were characterised by a high microbial load. Fresh mushrooms favour growing microorganisms due to their high

moisture character with carbohydrates, protein, vitamins, and minerals. From the standpoint of nutrients, fresh mushrooms are capable of causing quality degradation and spoilage.

Soil, water, air, insects, and animals impact the microbial flora of fresh mushrooms. The high count of bacteria diminishes microbial quality. The initial microbial load can affect the deterioration during postharvest. Picked fresh wild edible mushrooms may be placed into bags, boxes, or crates without washing. When harvesting, personal hygiene must be practised by pickers to prevent the transfer of human pathogens. Notably, pathogens such as faecal coliform and *E. coli* in mushrooms indicate that proper hygiene and sanitation rules weren't followed during picking, transportation, retailing and storage. Mushrooms can be contaminated with these microorganisms in nature, too. During storage, the fingers and shoes of people handling the mushrooms, their baskets, and knives can contaminate them. So, in general, the microbiological quality of the mushrooms sold in Muğla province is poor and threatens human health. The determination of yeast and mould, the presence of mycotoxin, and other existence of enteropathogens will be investigated in further studies.

#### Compliance with Ethical Standards

**Conflict of interests:** The author(s) declares that for this article, they have no actual, potential, or perceived conflict of interest.

**Ethics committee approval:** Authors declare that this study includes no experiments with human or animal subjects.

**Data availability:** Data will be made available on request.

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## Gıda güvenliği açısından kakaolu keklerde akrilamid tehlikesinin araştırılması

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### ÖZ

Akrilamid, özellikle ısıtım işlem uygulanan ürünlerde oluşan ve insan sağlığı üzerindeki olumsuz etkileri kanıtlanmış olan toksik bir Maillard reaksiyonu ürünüdür. Özellikle fırıncılık ürünleri ısıtım işlem uygulanarak üretildiklerinden akrilamid oluşumu yönünden risk teşkil etmektedir. Günlük diyetinde yaygın olarak tüketilen fırıncılık ürünlerinde akrilamid miktarının tespit edilmesi toplum sağlığı açısından büyük önem taşımaktadır. Çalışmanın amacı ülkemiz piyasasında satışa sunulmuş olan bazı kakaolu keklerde akrilamid miktarının incelenmesidir. Çalışmada Aydın ilinde yer alan zincir marketlerde ve pastanelerde ambalajlı ve ambalajsız şekilde satışa sunulmuş olan kakaolu kekler akrilamid miktarları yönünden incelenmişlerdir. Örneklerin ayrıca bazı temel bileşim özellikleri belirlenmiştir. Akrilamid miktarının kromatografik analizi, ekstraksiyon işleminin ardından, yüksek performanslı sıvı kromatografisi ile gerçekleştirilmiştir. Çalışmada kakaolu kek örneklerinin akrilamid miktarlarının 34.36-311.95 µg/kg arasında değiştiği belirlenmiştir. Kakaolu kek örneklerinin renk değerleri ile akrilamid içerikleri arasında ise herhangi bir korelasyon tespit edilmemiştir ( $p>0.05$ ). Genel olarak değerlendirildiğinde ise pastanelerden alınan ambalajsız kek örneklerinin marketlerde satışa sunulan paketlenmiş kakaolu keklerle göre daha yüksek nemli, dış yüzeylerinin daha sarı renkte ve daha yüksek akrilamid değerlerinin olduğu saptanmıştır ( $p<0.05$ ). Akrilamid maruziyetinin sağlık üzerine etkilerinin incelenmesi amacıyla çeşitli bilimsel topluluklar ve yasal kuruluşlar aracılığıyla risk değerlendirme çalışmaları yapılmaktadır. Bu değerlendirmeler sonucunda toplumda bulunan genel veya özel bazı gruplar için riskli gıdalar tespit edilmekte ve bu gıdalar için en uygun üretim modelleri oluşturulmakta ve toksik maddelerin düzeyleri için sınırlandırmalar getirilerek maruziyet minimuma indirilmeye çalışılmaktadır. Çalışma sonuçlarının kakaolu keklerde akrilamid maruziyetini azaltma konusunda yol gösterici olacağı düşünülmektedir.

**Anahtar Kelimeler:** Akrilamid, Gıda güvenliği, Kek, Kontaminant, Maillard reaksiyonu

### ABSTRACT

#### Investigation of acrylamide hazard in cocoa cakes in terms of food safety

Acrylamide is a toxic Maillard reaction product that occurs primarily in heat-treated products and has proven adverse effects on human health. Since bakery products are produced by applying heat treatment, they especially pose a risk regarding acrylamide formation. For public health, it is essential to determine the amount of acrylamide in bakery products that are widely consumed in the daily diet. The study aims to examine the amount of acrylamide in some cocoa cakes offered for sale in our country. The cocoa cakes, which were sold as packaged and unpackaged in chain markets and patisseries in Aydın province, were examined regarding acrylamide contents. Some significant compositional properties of the samples were also determined. Chromatographic analysis of acrylamide, after extraction, was carried out using high-performance liquid chromatography. The study determined that the acrylamide amounts of the cocoa cake samples varied between 34.36-311.95 µg/kg. No correlation was found between the colour values of the cocoa cake samples and their acrylamide content ( $p>0.05$ ). Various scientific societies and legal organisations conduct risk assessment studies to examine the health effects of acrylamide exposure. As a result of these evaluations, risky foods are determined for some general or particular groups in society. The most appropriate production models are created for these foods, and exposure is minimised by limiting the levels of toxic substances. The study results will be a guide for reducing acrylamide exposure in cocoa cakes.

**Keywords:** Acrylamide, Cake, Contaminant, Food safety, Maillard reaction

## Giriş

Akrilamid ( $C_3H_5NO$ , 2-propenamid), renksiz, uçucu olmayan, katı kristal bir yapıya sahip ve suda çözünebilen bir bileşendir. Molekül ağırlığı 71.08 kDa'dur (Ashoor vd., 1984; Eriksson, 2005). Akrilamid, ticari olarak nitrilhidraz kullanılarak akrilonitrilin hidrolizi ile üretilmektedir. Monomer ve polimer olmak üzere 2 ayrı formda bulunmaktadır. Akrilamid, 1994 yılında Uluslararası Kanser Araştırma Ajansı (IARC) tarafından insanlar üzerinde potansiyel karsinojen olarak nitelendirilmiş ve deney hayvanları üzerindeki karsinojen etkisinden dolayı Grup 2A olarak sınıflandırılmıştır. Bu sınıflandırma 2002 yılında Dünya Sağlık Örgütü tarafından da onaylanmıştır (Krishnakumar ve Visvanathan, 2014). İnsan diyetinde günlük akrilamid maruziyetinin kg vücut ağırlığı başına 0,3-0,8  $\mu g$  olduğu belirtilmektedir (WHO, 2002).

Gıdalarda akrilamid varlığı ilk kez 2002 yılında İsveç Ulusal Gıda Yönetimi (SNFA) birimi tarafından bildirilmiş ve genellikle patates, tahıl ve fırıncılık ürünlerinde ısı işlem etkisi ile oluşan, önemli bir ısı işlem kontaminantı olarak nitelendirilmiştir. Akrilamid en çok bitkisel kaynaklı gıdalar ile patates, tahıl ve fırıncılık ürünleri gibi nişasta içeriği yüksek ürünlerde ısı işlem etkisi ile oluşmaktadır. Akrilamidin, kızartma işlemi uygulanmamış ve pişirilmemiş ürünlerde tespit edilmediği bildirilmiştir. Ayrıca et ve balık gibi hayvansal kaynaklı ürünlerde de çok düşük miktarlarda bulunduğu rapor edilmiştir (Krishnakumar ve Visvanathan, 2014). Gıda kompozisyonu, yüksek sıcaklık (120°C'den yüksek), yüksek karbonhidrat miktarı, serbest asparajin miktarı, indirgen şeker miktarı, pH, su içeriği, amonyum bikarbonat miktarı ve rekabet eden aminoasitlerin varlığı gıdalarda akrilamid miktarının farklı olmasına neden olmaktadır (Matthäus vd., 2004; Taubert vd., 2004; Elmore vd., 2005; Vinci vd., 2012).

Gıdalarda akrilamid oluşumuna neden olan temel metabolik yol, Maillard reaksiyonunun bir parçası olan serbest amino asitlerle (asparajin vb.) indirgen şekerlerin (fruktoz vb.) reaksiyonudur (Krishnakumar ve Visvanathan, 2014). Gıdalarda potansiyel akrilamid oluşumu büyük ölçüde gıdanın glikoz ve fruktoz içeriğine bağlıdır (Biedermann, 2002; Pedreschi vd., 2006). Serbest asparajin miktarının çavdar çeşitlerinde (Curtis vd., 2010), çavdar ve buğday içeren pişirilmiş un ve hamurlarda akrilamid oluşumunu etkileyen temel etmen olduğu bildirilmiştir (Hamlet vd., 2008). Gıdalarda akrilamid oluşumunu sağlayan farklı mekanizmaların varlığı literatürde bildirilmiştir. Ortamda asparajin bulunmaması durumunda, lipitçe zengin gıdalarda akrolein ve amonyak akrilamid oluşumuna neden olabilmektedir. Akrolein ve akrilik asit, lipitlerin (trigliseritlerin) yüksek sıcaklıkta degradasyonu sonucu oluşan ürünlerdir (Gertz ve Klostermann, 2002). Diğer yandan

glutamin, sistein ve aspartik asit gibi aminoasitlerin de düşük miktarlarda akrilamid oluşturabildiği bildirilmiştir (Ezeji vd., 2003). Ancak bu mekanizma gıdalarda akrilamid oluşumu sırasında yaygın görülmemektedir (Krishnakumar ve Visvanathan, 2014).

Avrupa ülkelerinde akrilamid maruziyetinin %50' sinin patates kaynaklı, %20'sinin ise fırıncılık ürünleri kaynaklı olduğu belirtilmektedir (Keramat vd., 2011). Fırıncılık ürünlerinde; gıda matrisinin, ortamdaki prekürsörlerin (serbest asparajin, fruktoz, glikoz ve maltoz) ve uygulanan termal işlemin türü ve süresine göre akrilamid miktarı değişiklik göstermektedir. Ortamdaki indirgen şekerlerin varlığından çok, fırıncılık ürünlerinde akrilamid içeriğini etkileyen temel faktör asparajin miktarıdır (Mesias vd., 2022). Diğer yandan gıda matrisindeki düşük nem miktarı reaktantların çözünürlüğünü azaltmakta ve hareketlerini sınırlandırmakta, bu nedenle akrilamid oluşum reaksiyonunu da yavaşlatmaktadır. Bu nedenle formülasyon ve işlem koşullarının modifiye edilmesi son üründe akrilamid miktarını önemli ölçüde değiştirmektedir. Sıcaklık, uygulanan ısı yoğunluğu ve su aktivitesi gibi faktörler üründe akrilamid miktarını doğrudan etkilemektedir (Sarion vd., 2021).

Fırıncılık ürünleri yüksek miktarlarda akrilamid içerebildiklerinden, toplum sağlığı için risk oluşturma potansiyelleri bulunmaktadır. Özellikle kek, bisküvi, kraker gibi ürünler çocuklar tarafından tüketildiklerinden, bu ürünler çocuklar için daha büyük risk oluşturmaktadır. Yapılan çalışmalarda çocukların akrilamid maruziyetinin yetişkenlerden daha fazla olduğu belirtilmektedir (Mojska vd., 2010; Boon vd., 2005; Hilbig vd., 2004). Bu nedenle özellikle tüketimi yaygın ürünlerde akrilamid maruziyetinin tespit edilebilmesi ve gerekli önlemlerin alınabilmesi amacıyla, bu ürünlerde akrilamid miktarının hassas analiz teknikleriyle tespit edilmesi gerekmektedir. Çalışmanın amacı ülkemiz piyasasında satışa sunulmuş olan bazı kakaolu keklerin akrilamid miktarının incelenmesidir. Çalışmada Aydın ilinde yer alan zincir marketlerde ve pastanelerde ambalajlı ve ambalajsız şekilde satışa sunulmuş olan kakaolu kekler akrilamid miktarları yönünden incelenmişlerdir. Çalışmada analiz edilen ürün grubu özellikle çocuklar tarafından tüketildiğinden ve çocuklarda akrilamid maruziyeti yetişkenlere göre daha yüksek olduğundan, elde edilen bulguların akrilamid maruziyetini azaltma konusunda yapılacak yeni çalışmalar için yol gösterici olabileceği düşünülmektedir.

## Materyal ve Metot

### Materyal

Akrilamid standardı, asetik asit (%100) ve hidroklorik asit Merck'ten (Darmstadt, Almanya), sodyumhidroksit, tiyosalisilik asit, metanol, asetonitril ve demir (II) asetat trihidrat Sigma Aldrich'ten (Missouri, Amerika), etil asetat Isolab'tan (İstanbul, Türkiye) temin edilmiştir. Çalışmada kullanılan ultra saf su ise Milli-Q su arıtma sistemi (Millipore, Bedford, Amerika) kullanılarak elde edilmiştir.

### Yöntem

Kakaolu kek örnekleri ise Aydın ili Merkez ilçesinde (Efeler) faaliyet gösteren çeşitli zincir marketlerden elde edilen ambalajlı ve etiketli olarak satışa sunulan (endüstriyel olan; M) 16 farklı kakaolu kek örneği ve yine Efeler ilçesinde faaliyet gösteren pastane ve satış yerlerinde paketsiz olarak açıkta satışa sunulan (endüstriyel olmayan; P) 15 farklı kakaolu kek örneği toplanmıştır. Toplanan örnekler Efeler ilçesinin çeşitli bölgelerinden homojen dağılım olacak şekilde satın alınmıştır (Şekil 1).

Toplanan örnekler üzerinde akrilamid analizi tek paralelli; nem, kül, renk, pH analizleri 3 paralelli gerçekleştirilmiştir.



**Şekil 1.** Kakaolu kek örneklerinin Aydın-Efeler ilçesindeki dağılımı (n=31)

**Figure 1.** The distribution of cocoa cake samples in the Aydın-Efeler district (n=31)

### Standart çözeltilerinin hazırlanması

Kalibrasyon eğrisinin oluşturulmasında kullanılan akrilamid standart çözeltileri belirli konsantrasyonlarda ultra saf su içinde çözülerek hazırlanmıştır.

### Kek örneklerinin hazırlanması

Örneklerin hazırlanmasında Shi vd. (2009), Hauet vd. (2016), Pal vd. (2018) tarafından belirtilen yöntemler, bazı değişiklikler yapılarak kullanılmıştır. İlk olarak ince öğütülmüş bir örnek 50 mL'lik bir santrifüj tüpüne 0.5 g olacak şekilde tartılmış ve üzerine 10 ml ultra saf su eklenmiştir. Ardından karışım 10 dakika süreyle vortekslenmiştir. Daha sonra örnek 5000 rpm'de 10 dakika süreyle santrifüj edilmiştir. Ardından 5 mL supernatant 50 mL'lik bir tüpe aktarılmış ve üzerine 50 µl sodyum hidroksit (0.1 M) eklenmiştir. Daha sonra çözeltinin pH'ı  $8 \pm 0.3$ 'e ayarlanmıştır. Çözeltiye 0,5 mL 2-merkaptobenzoik asit ilave edilmiş ve karanlıkta 100 dakika boyunca su banyosunda bekletilmiştir. Daha sonra üzerine 1 mL doymuş kurşun (II) asetat trihidrat çözeltisi eklenmiş ve 8000 rpm'de 10 dakika süreyle santrifüj edilmiştir. Supernatant 50 mL'lik bir santrifüj tüpüne aktarılmış ve pH, HCl (3M) ile 1.5'e ayarlanmıştır. Çözelti 8000 rpm'de 10 dakika santrifüj edildikten sonra supernatant 20 mL'lik bir cam santrifüj tüpüne aktarılmış ve etil asetat kullanılarak ekstraksiyon yapılmıştır. Ardından organik faz 70°C'de buharlaştırılmış ve kalıntı 250 µl mobil fazda yeniden çözülmüştür. Akrilamid miktarının tespiti, vakumlu gaz giderici, kuaterner pompa, termostatlı kolon bölmesi ve diyet dizi dedektöründen (DAD) oluşan bir HPLC sistemi (Agilent 1260, DAD dedektörü G13115D 1260 DADVL) kullanılarak gerçekleştirilmiştir. Kromatografik ayırım 25°C'de termostatlı bir C18 RP kolonu (250x4,6 mm, Hydrochrome, Excel, UK) kullanılarak gerçekleştirilmiştir. Enjeksiyon hacmi 10 µL'dir. Örnekler 210 nm dalga boyunda analiz edilmiştir. Mobil faz olarak ultra saf su, asetonitril (%40, %60) ve saf su ile asetonitrilin %0,1'i kadar formik asit kullanılmıştır. Kalibrasyon eğrisi akrilamid standartları ile oluşturulmuştur ( $y=86.70615589x+89.13033$   $R^2=0,99$ )

### Renk analizi

Kakaolu kek örneklerinde renk analizi Hunterlab ColorFlex EZ renk tayin cihazı kullanılarak gerçekleştirilmiştir. Renk özellikleri olarak  $L^*$  (koyuluk, parlaklık),  $a^*$  (+kırmızı, -yeşil) ve  $b^*$  (+sarı, -mavi) değerleri saptanmıştır. Kek örnekleri tüketim biçimlerine göre (öğütülmeden) analiz edilmiştir. Örneklerin dış yüzeyleri ve dilimlenerek iç yüzeyleri analiz edilmiştir (Baltacıoğlu, 2017).



## Nem analizi

Nem miktarının belirlenmesi için 4 gr öğütülmüş kakaolu kek örneği 3 saat 105°C sıcaklıktaki etüvde kurutulmuştur. Kurutma ve soğutmadan sonra örneklerin kuru ağırlığı ölçülmüş ve nem içeriği hesaplanmıştır (Uçar, 2011).

## Kül analizi

Örneklerin kül analizi AOAC (1990)'a göre gerçekleştirilmiştir. Örnekler, önceden sabit tartıma getirilmiş porselen kroze içerisine tartılarak, kül fırınında (Elektro-mag M1813, Türkiye) 550±5°C'de kalıntı beyaza yakın renk alana kadar yakılmıştır. Yakma işlemi sonunda krozelerde kalan örnek kütlesi başlangıçtaki örnek kütlesine oranlanarak keklerin % kül miktarı hesaplanmıştır.

## pH analizi

Kek örneklerinde pH analizi için 130°C'de 55 dakika kurutulmuş keklerden 10 g alınmıştır 90 mL distile su ile 10 dakika laboratuvar tipi parçalayıcıda yüksek devirde (WiseTis HG15A, Kore) homojenize hale getirilmiştir ve bu süre sonunda pH metre (Phenomenal VWR, Almanya) kullanılarak pH belirlenmiştir (Baltacıoğlu ve Uyar, 2017).

## İstatistiksel analiz

Ölçülen değişkenler arasındaki ilişkinin gücünü ölçmek için Pearson korelasyon katsayısı (r) ve Spearman korelasyon katsayısı (ρ) hesaplanmıştır. Pearson korelasyon katsayısı doğrusal ilişkilerin gücünü yansıtırken, Spearman sıra korelasyonu monotonik ilişkinin gücünü yansıtmaktadır. Tüm istatistiksel hesaplamalar için SPSS 22.0 paket programı kullanılmıştır. İstatistiksel analizler sonucunda ortalamaların aralarındaki farklılıklar 0.05 güven aralığına göre gösterilmiştir.

## Bulgular ve Tartışma

Kakaolu kek örneklerine ait nem, kül ve pH değeri sonuçları Tablo 1' de sunulmuştur. Kek örneklerinin nem içerikleri %10.80-39.88 arasında değişen değerlerde yer almıştır. Diğer yandan örneklerin kül miktarları %1.55-6.54 arasında değişmiş ve pH değerleri ise 5,98-8,99 aralığında bulunmuştur. Örneklerin nem ve kül miktarları ile pH değerlerinin konuyla ilgili yapılmış benzer çalışmalarla büyük oranda uyum içinde olduğu belirlenmiştir. Moreno (2019) tarafından yapılan çalışmada kakao tozu içeren kekin nem miktarının %34.6 olduğu, kül miktarının ise %1.33 olarak belirlendiği bildirilmiştir.

Diğer yandan Wallace vd. (2010) tarafından yapılan çalışmada kakaolu keklerin kül miktarı kuru madde içinde %5.63 olarak belirtilmiştir. Rosa vd. (2015) tarafından yapılan çalışmada ise kakao tozuyla yapılan keklerde nem içeriğinin %36.04; kül içeriğinin ise %3.93 olduğu bildirilmiştir. Ayrıca Stahl vd. (2009) tarafından yapılan çalışmada kakao içeren kekin pH değerinin 8.8 olduğu rapor edilmiştir. Market ve pastanelerde satılan kakaolu kek örneklerinin nem içeriklerinde istatistiksel olarak önemli bir fark olduğu (p<0.05) pastanelerde satılan kek örneklerinin nem içeriğinin daha yüksek olduğu görülmüştür. Pastaneden alınan ambalajsız örneklerde her ne kadar daha yüksek kül ve pH değeri saptanmış olsa da bu farklılık istatistiksel olarak önemli bulunmamıştır (p>0.05).

Kakaolu kek örneklerine ait renk değerleri Tablo 2'de sunulmuştur. Örneklerin renklerini değerlendirme amacıyla CIELAB (Commission Internationale de l'éclairage, *L*, *a*, *b*) kolorimetrik sistem indeksleri kullanılarak *L*\* (açıklık; siyah için *L*\*=0, beyaz için *L*\*=100), *a*\* (yeşil için *a*\*<0, kırmızı için *a*\*>0) ve *b*\* (mavi için *b*\*<0, sarı için *b*\*>0) değerleri belirlenmiştir. Çizelge'de yer alan açıklık/koyuluk/parlaklık özellikleriyle ilişkilendirilen *L*\*değerleri incelendiğinde, *L*\* değeri 100'e yaklaştıkça rengin açıldığı bilindiğinden, kek örneklerinin dış yüzey renkleri değerlendirildiğinde, K5 kodlu örnek en koyu renge sahipken, K19 kodlu örnek en açık renge sahip olmuştur. Diğer yandan örneklerin *a* değerleri 0'dan büyük olup, kırmızılık değeri en yüksek örnek K20 kodlu örnek olmuştur. Sarılık değerini ifade eden *b*\* değeri ise en yüksek K24 kodlu örnekte tespit edilmiş, en düşük ise K5 kodlu örnekte saptanmıştır. Örneklerin iç yüzeylerine ait renk değerleri incelendiğinde ise K1 kodlu örnek en koyu renge sahipken, K20 kodlu örnek en açık renge sahip olmuştur. Diğer yandan kırmızılık değeri en yüksek örnek K11 kodlu örnek olup, kırmızılık değeri en düşük olan örnek ise K10 kodlu örnek olmuştur. Ayrıca örneklerin *b* değerleri incelendiğinde sarılık değeri en yüksek örnek K11 kodlu örnek, en düşük olan ise K10 kodlu örnek olarak belirlenmiştir. Örnek gruplarının ortalamaları üzerinden değerlendirme yapılacak olduğunda ise pastane örneklerinin dış yüzeylerinin daha sarı (daha yüksek *b* değeri) olduğu görülmüştür (p<0.05). Diğer ortalama veriler arasında istatistiksel olarak anlamlı bir fark bulunmamıştır.

**Tablo 1.** Kakaolu kek örneklerinin nem, kül miktarları ile pH değerleri(n=31)\***Table 1.** The moisture and ash contents, pH values of cocoa cakes samples (n=31)

Örnek Kodu	Nem (%)	Kül (%)	pH	Örnek Kodu	Nem (%)	Kül (%)	pH
M1 (K1)	14.65 ±0.26	1.94 ±0.15	6.16 ±0.43	P1(K2)	18.00 ±0.09	1.55 ±0.04	7.02 ±0.60
M2 (K3)	16.00 ±0.09	1.73 ±0.09	6.21 ±0.42	P2(K5)	11.78 ±0.59	2.35 ±0.03	6.79 ±0.35
M3 (K4)	10.80 ±0.54	2.28 ±0.09	5.98 ±0.54	P3(K9)	22.15 ±0.15	1.67 ±0.03	6.65 ±0.42
M4 (K6)	14.91 ±0.03	1.98 ±0.05	6.20 ±0.45	P4(K10)	26.87 ±0.07	2.62 ±0.02	7.54 ±0.30
M5 (K7)	15.84 ±1.59	2.15 ±0.12	6.02 ±0.43	P5(K13)	20.96 ±0.3	2.61 ±0.01	6.65 ±0.57
M6 (K8)	14.14 ±0.15	2.00 ±0.07	6.07 ±0.60	P6(K16)	14.87 ±0.03	2.77 ±0.03	7.51 ±0.06
M7(K11)	18.59 ±0.03	2.62 ±0.01	6.36 ±0.05	P7(K17)	33.75 ±0.03	1.83 ±0.01	7.24 ±0.02
M8 (K12)	14.25 ±0.55	2.21 ±0.01	6.13 ±0.00	P8(K18)	15.66 ±0.05	2.05 ±0.06	7.25 ±0.08
M9 (K14)	14.30 ±0.09	2.40 ±0.09	6.48 ±0.22	P9(K19)	39.88 ±7.15	1.84 ±0.17	7.62 ±0.12
M10 (K15)	14.95 ±0.03	2.04 ±0.03	6.13 ±0.04	P10(K20)	35.07 ±0.03	1.57 ±0.03	8.08 ±0.00
M11 (K25)	16.31 ±1.37	2.56 ±0.01	6.82 ±0.07	P11(K21)	25.65 ±0.02	1.56 ±0.02	7.97 ±0.07
M12 (K27)	15.64 ±0.24	1.97 ±0.01	6.52 ±0.02	P12(K22)	21.85 ±0.44	2.02 ±0.03	8.71 ±0.13
M13 (K28)	15.64 ±0.24	2.18 ±0.01	7.02 ±0.01	P13(K23)	19.61 ±0.02	1.75 ±0.09	7.29 ±0.02
M14 (K29)	18.37 ±1.09	2.10 ±0.10	6.89 ±0.02	P14(K24)	34.98 ±0.26	6.53 ±0.22	8.98 ±0.06
M1 (K30)	12.97 ±1.37	2.21 ±0.27	6.88 ±0.02	P15(K26)	14.83 ±0.02	2.45 ±0.04	6.60 ±0.14
M1 (K31)	17.73 ±0.02	2.43 ±0.10	6.31 ±0.13				
<b>Ortalama</b>	16.08 ±2.31 <sup>A</sup>	2.23 ±0.32 <sup>C</sup>	6.60 ±0.29 <sup>D</sup>	<b>Ortalama</b>	22.84 ±9.75 <sup>B</sup>	2.41 ±1.26 <sup>C</sup>	7.65 ±0.82 <sup>D</sup>

\* Kısaltmalar: M: Market; P: Pastane

Örneklerin renk değerleri ile akrilamid içerikleri arasındaki ilişki incelendiğinde ise, herhangi bir korelasyon tespit edilmemiştir ( $p>0.05$ ). Kakaolu kek örneklerinde saptanan akrilamid miktarı Tablo 3 ve Şekil 2'de verilmiştir. Örneklerdeki akrilamid miktarları 36.87-311.95  $\mu\text{g}/\text{kg}$  arasında değişen değerler almıştır (Tablo 3). Marketlerden alınan paketli kakaolu kek örneklerinde akrilamid değeri ortalaması 104.93  $\pm$ 41.04 iken ambalajsız kek örneklerinde ise bu değer (147.54  $\pm$ 64.29) daha yüksek olduğu saptanmıştır ( $p<0.05$ ). Konuyla ilgili Mousa (2021) tarafından yapılan çalışmada kinoa içeren keklerde akrilamid miktarının 356.8-2147.2  $\mu\text{g}/\text{kg}$  arasında değiştiği bildirilmiştir. Diğer yandan Markova vd. (2012) tarafından yapılan çalışmada karabuğday ve zencefil içeren keklerde akrilamid miktarlarının 208-346  $\mu\text{g}/\text{kg}$  kuru madde düzeyinde olduğu belirtilmiştir. Razia vd. (2016) tarafından yapılan çalışmada ise Pakistan'da satışa sunulmuş olan bazı kek örneklerinde akrilamid miktarının 23.5-35.5 düzeyinde olduğu rapor edilmiştir. Ayrıca Nematollahi vd. (2020) tarafından gerçekleştirilen çalışmada İran'da satışa sunulmuş olan keklerde akrilamid düzeyinin 177  $\mu\text{g}/\text{kg}$  düzeyinde olduğu bildirilmiştir.

Ek olarak Ölmez vd. (2008) tarafından yapılan çalışmada toplam 311 geleneksel üründe akrilamid miktarı araştırılmış ve kraker, bisküvi ve bebek bisküvilerinde ortalama akrilamid miktarlarının sırasıyla 247, 198 ve 152  $\mu\text{g}/\text{kg}$  düzeyinde olduğu belirtilmiştir. Diğer yandan Şenyuva ve Gökmen (2005) tarafından yapılan çalışmada ülkemiz marketlerinde yer alan 120 farklı üründe akrilamid miktarları incelenmiş ve en yüksek akrilamid miktarı krakerlerde (1072  $\mu\text{g}/\text{kg}$ ) tespit edilmiştir. Diğer yandan Pacetti vd. (2015) tarafından yapılan çalışmada Kolombiya'da satışa sunulan 112 farklı üründe akrilamid miktarı analiz edilmiş ve en yüksek akrilamid içeriği bisküvilerde (1104  $\mu\text{g}/\text{kg}$ ) tespit edilmiştir. Konuyla ilgili yapılan bir başka çalışmada (Rufian-Henares vd., 2007) İspanya'da marketlerde satışa sunulan bisküvi ve ekmek benzeri ürünlerde akrilamid miktarları araştırılmış ve bisküvilerde ortalama ve en yüksek akrilamid miktarı sırasıyla 423 ve 2085  $\mu\text{g}/\text{kg}$  olarak belirlenmiştir. Gertz (2004) ise keklerde akrilamid miktarının 36-40  $\mu\text{g}/\text{kg}$  arasında değiştiğini rapor etmiştir.

Genel olarak değerlendirildiğinde ise pastanelerden alınan kek örneklerinin daha yüksek nem değerine sahip, dış yüzeylerinin daha sarı (b) ve akrilamid değerlerinin marketlerde satışa sunulan paketli keklerle kıyasla daha yüksek olduğu saptanmıştır ( $p<0.05$ ). Elde edilen veriler genel olarak literatürle benzerlik göstermektedir.

Tablo 2. Kakaolu kek örneklerinin renk değerleri(n=31)\*

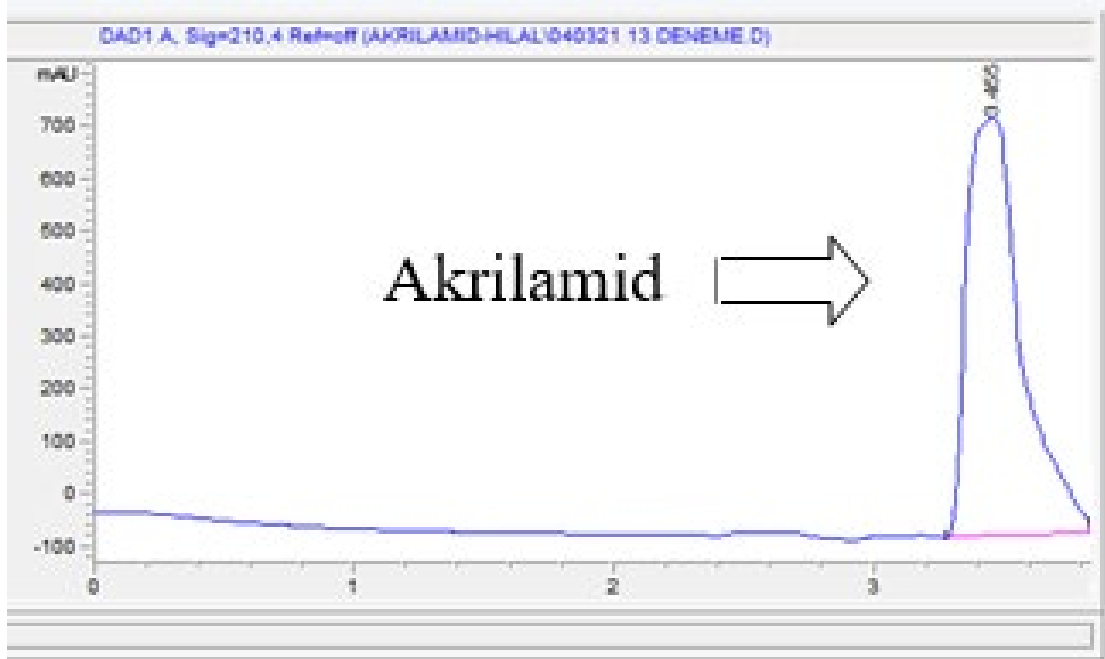
Table 2. Color values of cocoa cake samples (n=31)

DIŞ YÜZEY									
Örnek Kodu	L	a	b	WIE	Örnek Kodu	L	a	b	WIE
M1 (K1)	23.31±0.03	8.79±0.01	8.27±0.04	-95.13±0.04	P1(K2)	33.43±0.02	13.4±0.04	15.97±0.01	-140.37±0.07
M2 (K3)	22.43±0.07	9.16±0.02	9.85±0.03	-116.01±0.01	P2(K5)	8.5±0.11	5.55±0.21	3.72±0.03	-75.01±0.04
M3 (K4)	23.6±0.01	9.17±0.17	10.65±0.06	-121.68±0.06	P3(K9)	16.97±0.06	9.44±0.18	8.93±0.02	-122.17±0.02
M4 (K6)	29.52±0.09	9.14±0.13	12.01±0.04	-118.37±0.07	P4(K10)	12.93±0.02	7.13±0.04	5.21±0.05	-82.71±0.04
M5 (K7)	23.01±0.01	8.96±0.09	10.04±0.07	-116.61±0.02	P5(K13)	22.78±0.05	10.75±0.1	11.66±0.02	-134.27±0.01
M6 (K8)	30.9±0.05	13.25±0.06	17.71±0.13	-164.39±0.19	P6(K16)	32.36±0.03	13.36±0.01	19.28±0.11	-172.92±0.08
M7(K11)	36.01±0.18	14.06±0.08	19.87±0.11	-164.46±0.03	P7(K17)	25.44±0.57	10.73±0.78	10.59±0.03	-114±0.05
M8 (K12)	26.29±0.06	10.9±0.04	14.04±0.01	-147.82±0.04	P8(K18)	21.97±0.07	7.61±0.03	6.44±0.01	-77±0.08
M9 (K14)	19.43±0.09	11.04±0.41	11.65±0.19	-145.9±0.17	P9(K19)	39.95±0.41	12.6±0.07	15.96±0.43	-122.06±0.05
M10(K15)	14.88±0.04	10.94±0.18	11.93±0.60	-168.95±0.32	P10(K20)	38.7±0.02	14.95±0.01	22.82±0.06	-177.54±0.02
M11(K25)	23.29±0.02	7.64±0.22	7.3±0.06	-84.37±0.01	P11(K21)	24.83±0.04	10.33±0.11	10.83±0.56	-118.77±0.17
M12(K27)	23.44±0.23	7.68±0.01	7.28±0.04	-83.84±0.09	P12(K22)	38.66±0.03	11.72±0.07	19.81±0.11	-157.55±0.14
M13(K28)	12.29±0.26	9.63±0.52	8.18±0.22	-129.17±0.04	P13(K23)	17.01±0.09	8.09±0.05	5.49±0.01	-75.67±0.08
M14(K29)	17.43±0.02	9.78±0.08	9.7±0.21	-130.33±0.31	P14(K24)	38.08±0.04	14.56±0.02	25.07±0.01	-196.96±0.04
M1 (K30)	20.11±0.07	13.43±0.12	14.59±0.06	-174.05±0.12	P15(K26)	27.67±0.09	11.96±0.11	12.34±0.23	-124.79±0.15
M1 (K31)	20.46±0.19	7.16±0.17	4.61±0.1	-57.02±0.2					
Ortalama	22.9±5.92 <sup>A</sup>	10.05±2.08 <sup>B</sup>	11.11±3.97 <sup>C</sup>	-126±34.15 <sup>E</sup>	Ortalama	26.62±10.08 <sup>A</sup>	10.82±2.82 <sup>B</sup>	12.94±6.66 <sup>D</sup>	-126.12±38.5 <sup>E</sup>
İÇ YÜZEY									
Örnek Kodu	L	a	b	WIE	Örnek Kodu	L	a	b	WIE
M1 (K1)	12.11±0.08	8.61±0.01	7.15±0.08	-114.93±0.03	P1(K2)	30.53±0.04	10.87±0.01	13.45±0.09	-128.16±0.07
M2 (K3)	19.64±0.12	9.14±0.05	9.23±0.11	-117.27±0.03	P2(K5)	19.13±0.01	4.74±0.09	4.2±0.06	-55.14±0.03
M3 (K4)	21.25±0.04	5.59±0.01	4.27±0.08	-51.96±0.01	P3(K9)	15.17±0.03	3.36±0.01	2.16±0.07	-31.8±0.05
M4 (K6)	20.34±0.01	10.86±0.02	11.91±0.05	-145.89±0.06	P4(K10)	13.37±0.16	2.86±0.04	1.62±0.06	-25.23±0.01
M5 (K7)	19.97±0.08	10.06±0.11	11.8±0.09	-146.8±0.17	P5(K13)	39.83±0.09	11.3±0.14	22.08±0.09	-171.75±0.07
M6 (K8)	17.26±0.04	9.09±0.09	8.01±0.03	-108.99±0.01	P6(K16)	29.14±0.03	12.13±0.06	11.56±0.09	-112.56±0.05
M7(K11)	37.47±0.06	15.41±0.03	26.63±0.07	-209.54±0.01	P7(K17)	31.51±0.13	8.11±0.08	10.72±0.06	-100.88±0.02
M8 (K12)	24.5±0.11	10.34±0.09	12.9±0.06	-143.02±0.04	P8(K18)	20.97±0.08	9.7±0.07	9.08±0.02	-110.76±0.08
M9 (K14)	20.75±0.05	9.82±0.09	11.19±0.04	-136.95±0.03	P9(K19)	44.51±0.06	12.2±0.04	15.95±0.03	-110.06±0.07
M10(K15)	19.54±0.01	7.31±0.04	6.38±0.06	-82.16±0.02	P10(K20)	49.29±0.02	9.17±0.11	14.73±0.09	-90.84±0.08
M11(K25)	25.08±0.13	9.34±0.09	11.52±0.04	-126.51±0.05	P11(K21)	26.99±0.13	13.78±0.09	12.62±0.07	-128.08±0.05
M12(K27)	25.12±0.01	10.17±0.05	11.22±0.04	-122.34±0.05	P12(K22)	41.05±0.05	11.78±0.07	17.96±0.03	-135.39±0.09
M13(K28)	23.17±0.06	9.98±0.13	9.25±0.09	-106.06±0.07	P13(K23)	17.56±0.07	8.76±0.04	6.29±0.08	-84.9±0.07
M14(K29)	21.06±0.04	9.25±0.02	8.7±0.06	-106.15±0.02	P14(K24)	36.63±0.03	12.31±0.06	15.78±0.07	-130.11±0.11
M1 (K30)	31.73±0.11	11.97±0.06	15.27±0.03	-140.71±0.12	P15(K26)	28.71±0.14	10.87±0.09	13.07±0.08	-130.04±0.06
M1 (K31)	21.66±0.07	10.15±0.04	9.6±0.13	-114.53±0.09					
Ortalama	22.54±5.76 <sup>A</sup>	9.82±2.08 <sup>B</sup>	10.94±4.981 <sup>C</sup>	-123.36±33.81 <sup>D</sup>	Ortalama	29.63±11.05 <sup>A</sup>	9.46±3.37 <sup>B</sup>	11.41±5.86 <sup>C</sup>	-103.05±40.17 <sup>D</sup>

\* Kısaltmalar: M: Market ; P: Pastane

**Tablo 3.** Kakaolu kek örneklerinin akrilamid miktarları ( $\mu\text{g}/\text{kg}$ ) (n=31)**Table 3.** The acrylamide levels in cocoa cake samples (n=31)

Örnek (Market)	Akrilamid miktarları ( $\mu\text{g}/\text{kg}$ )	Örnek (Pastane)	Akrilamid miktarları ( $\mu\text{g}/\text{kg}$ )
1	187.9552	2	91.72209
3	99.7838	5	194.8636
4	90.22277	9	101.7675
6	60.90536	10	125.1684
7	80.22348	13	133.4031
8	74.16855	16	185.6716
11	129.7471	17	129.5971
12	123.7729	18	311.9487
14	162.905	19	154.7741
15	139.4119	20	34.35592
25	36.87016	21	194.2177
27	127.0829	22	107.5572
28	75.34493	23	129.4126
29	116.5185	24	202.1064
30	121.1894	26	116.5877
31	52.76291658		
<b>Ortalama</b>	$104.93 \pm 41.04^A$	<b>Ortalama</b>	$147.54 \pm 64.29^B$
<b>Minimum</b>	36.87016	<b>Minimum</b>	34.35592
<b>Maksimum</b>	187.9552	<b>Maksimum</b>	311.9487

**Şekil 2.** Kakaolu kek örneklerindeki akrilamidin yüksek performanslı sıvı kromatogramı. Koşullar: Agilent 1260, DAD de-  
dektörü; 210 nm dalga boyu; enjeksiyon hacmi 10  $\mu\text{L}$ ; mobil faz: asetonitril/formik asit**Figure 2.** High-performance liquid chromatography of acrylamide in cocoa cake samples. Conditions: Agilent 1260, DAD detector; wave-  
length: 210 nm; injection volume: 10  $\mu\text{L}$ ; mobile phase: acetonitrile/formic acid

## Sonuç

Akrilamid, insanlar üzerindeki potansiyel karsinojen etkileri kanıtlanmış olan toksik bir Maillard reaksiyon ürünüdür. Özellikle fırıncılık ürünleri yüksek sıcaklıklar kullanılarak üretildiklerinden, akrilamid oluşumu yönünden önemli bir risk grubunu oluşturmaktadır. Çalışmamızda ülkemiz piyasasında ambalajlı ve ambalajsız şekilde satışa sunulmuş olan ve sık tüketilen farklı kakaolu kek örneklerinde akrilamid miktarları analiz edilmiştir. İncelenen örneklerin ortalama değerleri arasında, marketlerde satılan ambalajlı kek örneklerinin nem içeriğinin daha düşük değerli ve daha az sarı olduğu saptanmıştır ( $p < 0.05$ ). Çalışmada 31 çeşit kakaolu kek örneklerinin akrilamid miktarlarının  $36.87-311.95 \mu\text{g}/\text{kg}$  arasında değiştiği belirlenmiştir. Pastanelerden temin edilen kek örneklerinin akrilamid içeriğinin diğer keklerle göre daha yüksek olduğu bulunmuştur ( $p < 0.05$ ). Örneklerin yüzey ve merkez renkleri birbirlerinden önemli derecede farklı olsa da akrilamid miktarı ile renk değerleri arasında herhangi bir korelasyon tespit edilmemiştir ( $p > 0.05$ ). Literatürde keklerde nem miktarının %5' in altına düşmesi durumunda akrilamid oluşumunun önemli derecede azaldığı rapor edilmiştir. Örneklerin nem miktarı ve akrilamid düzeyi arasında belirgin bir korelasyon tespit edilmese de akrilamid oluşumunu azaltmak amacıyla, yeni araştırmalarda keklerde nem miktarının azaltılması yoluna gidilebileceği düşünülmektedir. Çalışmada analiz edilen ürün grubu özellikle çocuklar tarafından tüketildiğinden ve çocuklarda akrilamid maruziyeti yetişkenlere göre daha yüksek olduğundan, yapılacak yeni çalışmalarda ve gıda endüstrisinde bu ürün grubunda akrilamid miktarını azaltmaya yönelik yeni yaklaşımların ortaya konulması önem arz etmektedir. Ayrıca akrilamidin azaltılması ve kontrolü için yasal otoritenin ve endüstrisinin diğer ülkelerde olduğu gibi ülkemizde de daha etkin ve öncü rol üstlenmesi gerekmektedir.

## Etik Standartlar ile Uyumluluk

**Çıkar çatışması:** Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

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**Açıklama:** -

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## Elma kabuğundan fenolik maddelerin ultrases destekli ekstraksiyonu

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### ÖZ

Bu çalışmada, elma kabuğundan fenolik bileşiklerin ultrases destekli ekstraksiyon (15, 30 ve 45 dakika) ve konvansiyonel ekstraksiyon (15, 30, 45, 60, 75 ve 90 dakika) yöntemleri ile ekstrakte edilmesi araştırıldı. Elma kabuğu ekstraktlarının antioksidan kapasitesi (DPPH), toplam fenolik madde, toplam flavanoid madde ve bireysel fenolik içeriği belirlendi. İki farklı ekstraksiyon yöntemi ile elde edilen elma kabuk ekstraktlarında klorojenik asit, rutin, kateşin, epikateşin, prosiyanidin B1, prosiyanidin B2 ve prosiyanidin C1 tespit edildi. Konvansiyonel yöntemde 90 dakikalık ekstraksiyon sonunda toplam fenolik, toplam flavonoid ve antioksidan aktivite sırasıyla 1848.50 ±0.40 mg GAE/kg, 571.01 ±2.88 mgKA/kg ve 9.70±0.00 mmol Trolox eq./g olarak bulunurken 45 dakikalık ultrases destekli ekstraksiyonda toplam fenolik, toplam flavonoid ve antioksidan aktivite sırasıyla 2021.83 ±65.69 mg GAE/kg, 532.66 ± 2.43 mgKA/kg ve 11.73 ±0.08 mmol Trolox eq./g olarak belirlendi. Konvansiyonel yöntemle karşılaştırıldığında, ultrases destekli ekstraksiyon uygulamasının elma kabuklarından fenolik madde ekstraksiyonunun süresini önemli ölçüde azalttığı ve ekstraksiyon verimliliğini arttırdığı tespit edildi.

**Anahtar Kelimeler:** Antioksidan, Ekstraksiyon, Elma kabuğu, Fenolik bileşikler, Ultrases

### ABSTRACT

#### Ultrasound-assisted extraction of phenolic compounds from apple peel

This study investigated the extraction of phenolic compounds from apple peel by ultrasound-assisted extraction (15, 30 and 45 min) and conventional extraction (15, 30, 45, 60, 75 and 90 min). The antioxidant capacity (DPPH), total phenolic content, total flavonoid content, and individual phenolic compounds of apple peel extracts were determined. HPLC analysis indicated that apple peel extracts had chlorogenic acid, rutin, catechin, epicatechin, procyanidin B1, procyanidin B2 and procyanidin C1. In the conventional method, the total phenolic content, total flavonoid content and antioxidant activity of apple peel extract in 90 min of conventional extraction was found as 1848.50 ±0.40 mg GAE/kg, 571.01 ±2.88 mgKA/kg and 9.70±0.00 mmol Trolox eq./g, respectively. In 45 min of the ultrasound-assisted extraction, total phenolic content, total flavonoid content and antioxidant activity of extracts were determined as 2021.83±65.69 mg GAE/kg, 532.66 ±2.43 mgKA/kg and 11.73 ±0.08 mmol Trolox eq./g respectively. The application of ultrasound-assisted extraction markedly decreased extraction time and improved the extraction efficiency compared with the conventional method.

**Keywords:** Antioxidant, Apple peel, Extraction, Phenolic compounds, Ultrasound



## Giriş

Meyve ve sebzeler insan beslenmesinde hayati öneme sahiptirler ve çeşitli mekanizmalar yoluyla kardiyovasküler hastalıklara ve kansere karşı koruma sağlayabilen birçok antioksidan bileşik içermektedir (Wolfe ve Liu, 2003). Elma (*Malus domestica* Borkh.), Rosaceae familyasının bir üyesi olup dünyada yaygın olarak yetiştirilen önemli bir meyvedir (Rana ve Bhushan, 2016). Ayrıca doğal antioksidanlar olarak işlev gördüğü bilinen C vitamini ve bazı fenolik bileşiklerin kaynağı olduğu için insan beslenmesinde önemli bir yere sahiptir (Wu ve ark., 2007). Elma fenolikleri, hücreyi serbest radikallerin zararlı etkilerine karşı koruyan ve düşük yoğunluklu lipoproteinlerin oksidasyonunu engelleyen güçlü antioksidan aktiviteye sahip bileşiklerdir (Rana ve Bhushan, 2016). Elma kabuğunun, soyulmuş elma dokusundan daha fazla toplam fenolik madde ve antioksidan aktivite (1.2-3.3 ve 1.5-9.2 kat) içerdiği bildirilmiştir (Drogoudi ve ark., 2008). Bir diğer çalışmada da dört çeşit elma örneğinde toplam fenolik ve flavonoid içeriğinin en yüksek kabuklarda bulunduğu, bunu soyulmuş elma dokusu + kabuk ve soyulmuş elma dokusu örneği izlemiştir (Wolfe ve ark., 2003).

Meyve ve sebzelerin işlenmesinde önemli miktarlarda atık/yan ürün ortaya çıkmaktadır. Atıkların bir polifenol kaynağı olarak kullanılması, gıda işlemcileri için önemli ekonomik fayda sağlayabilir. Ayrıca, bu atık veya yan ürünlerde bulunan polifenollerin güçlü antioksidan ve sitoprotektif aktiviteleri, bunları insan beslenmesi açısından da son derece önemli hale getirmektedir (Kabir ve ark., 2015). Çeşitli elma ürünleri (elmalı turta, elma sosu, elma cips vb.) üretiminin yan ürünlerinden biri olan elma kabuklarını insan beslenmesine sağlıklı bir gıda bileşeni olarak dahil etmenin yollarının belirlenmesi, sağlık açısından pek çok yarar sağlayabilir (Rupasinghe ve ark., 2008).

Bitkisel dokulardan elde edilen ekstraktlar gıda, ilaç ve kozmetik endüstrilerinde yaygın olarak kullanılmaktadır. Bitkilerden fonksiyonel özelliklere sahip doğal bileşikler elde etmek için farklı ekstraksiyon tekniklerinin etkileri geniş çapta araştırılmıştır (Wang ve Weller, 2006). Ekstraksiyon süresini kısaltmak, solvent tüketimini azaltmak, ekstraksiyon verimini ve ekstraktların kalitesini artırmak için konvansiyonel ekstraksiyona alternatif teknikler geliştirmek popülerite kazanan bir konudur. Son yıllarda bitkilerden nutrasötiklerin ekstraksiyonu için ultrases destekli ekstraksiyon, mikrodalga destekli ekstraksiyon, süperkritik akışkan ekstraksiyonu ve hızlandırılmış solvent ekstraksiyonu dahil olmak üzere çeşitli yeni ekstraksiyon teknikleri geliştirilmiştir (Wang ve Weller, 2006). Bunların arasında ultrases destekli ekstraksiyon, geleneksel ekstraksiyon tekniklerine kıyasla ucuz, basit ve etkili

bir alternatiftir. Ultrases kullanılarak elde edilen ekstraksiyon verimindeki artış, esas olarak, bir ultrasonik dalganın geçişiyle çözücüde üretilen akustik kaviteasyonların etkilerine atfedilir (Wang ve ark., 2008). Domates atıklarından likopenin ekstrakte edildiği bir çalışmada, ultrases destekli ekstraksiyonun hem süre hem de sıcaklık açısından geleneksel yöntemle kıyasla daha avantajlı olduğu bildirilmiştir (Kumcuoğlu ve ark., 2011). Bu çalışmada iki farklı ekstraksiyon yönteminin sağlık açısından önemli olan elma kabuk dokusundaki fenolik bileşiklerin ekstraksiyonu üzerine etkisi araştırılmıştır.

## Materyal ve Metot

### Materyal

Çalışmada ekstraksiyon için 2020 yılında Hakkari ilinde yerel bir üreticinin bahçesinden hasat edilen Starking Delicious elma çeşidine ait kabuklar kullanılmıştır. Hasattan hemen sonra laboratuvara getirilen elmalar hemen yıkanmış ve manuel bir kabuk soyucu ile kalınlığı 2 mm olacak şekilde elma kabukları soyulmuştur.

### Kullanılan Kimyasallar ve Reaktifler

Kateşin, epikateşin, klorojenik asit, rutin, DPPH, 6-hidroksi-2,5,7,8-tetrametilkroman-2-karboksilik asit (Trolox), asetik asit Sigma-Aldrich Co.'dan (St. Louis, MO, ABD) satın alınmıştır. Prosiyanidin B1 ve prosiyanidin B2 aber'den (Almanya), prosiyanidin C1 Chem Scene'den (ABD) temin edilmiştir.

### Metot

Çalışmada ultrases destekli ekstraksiyon (U) ve konvansiyonel ekstraksiyon (K) yöntemleri ile elma kabuğundan fenolik madde ekstraksiyonuna çalışılmıştır. Çalışmada meyve: su oranı 1:10 (W:V) olarak belirlenip her iki ekstraksiyon yönteminde de aynı şekilde kullanılmıştır.

Ultrases destekli ekstraksiyon için Bandelin (80/320 W, 35 kHz, Germany) marka ultrasonik su banyosu kullanıldı. Ekstraksiyon için elma kabukları oda sıcaklığında 15, 30 ve 45 dakika boyunca üç farklı sürede ultrases destekli ekstraksiyona tabi tutuldu.

Konvansiyonel yöntemle ekstraksiyon ise oda sıcaklığında ve karanlıkta 90 dk boyunca dairesel çalkalayıcı (150 rpm) üzerinde gerçekleştirilmiştir. Ekstraksiyon işlemi 6 farklı (15, 30, 45, 60, 75 ve 90 dk) sürede gerçekleştirildi.

Elde edilen ekstraktlar kaba filtre kağıdı ile süzülükten sonra bekletilmeden kimyasal analizlerde kullanılmıştır.

### Toplam Fenolik Madde Analizi

Elma kabuğu ekstraktlarının toplam fenolik madde analizi Singleton ve Rossi (1965) tarafından bildirilen metod kullanılarak belirlendi. 0.4 mL ekstrakta, 2 mL Folin-Ciocalteu ajanı (%10) ilave edildi. Daha sonra elde edilen karışıma 1.6 mL sodyum karbonat çözeltisi (%7.5'lik) eklenerek karıştırılıp oda sıcaklığında bir saat bekletildi. Süre sonunda spektrofotometrede 765 nm'de okuması yapıldı. Sonuçlar mg gallik asit eşdeğeri/kg (mg GAE/kg) olarak ifade edildi ( $y=0.0103x + 0.0237$ ,  $R^2=0.996$ ).

### Toplam Flavonoid Madde Analizi

Kabuk ekstraktlarının toplam flavonoid madde miktarı, Zhishe ve ark. (1999), tarafından bildirilen yöntemde bazı modifikasyonlar yapılarak belirlenmiştir. 1 mL ekstrakt üzerine 5 mL saf su ve 0.3 mL NaNO<sub>2</sub> (%5) çözeltisi eklenip 5 dk inkübe edilmiştir. Ardından 0.3 mL AlCl<sub>3</sub> (%10) ilave edilerek 5 dk daha inkübe edildikten sonra karışıma 2 mL 1 M NaOH eklendi ve saf suyla 10 mL'ye tamamlanarak 510 nm dalga boyunda spektrofotometrede okuması yapıldı. Sonuçlar mg kateşin eşdeğeri/kg (mg KA/kg) olarak ifade edildi ( $y=0.0035x + 0.0509$ ,  $R^2=0.9992$ ).

### DPPH

Örneklerin DPPH radikali sönmeme aktivitesi Pyo ve ark. (2004), tarafından bildirilen yöntem modifiye edilerek kullanılmıştır. 0.1 mL ekstrakt üzerine 3.9 mL DPPH çözeltisi (0.025 g/L metanol) eklenip karıştırıldıktan sonra oda koşullarında bir saat boyunca karanlıkta bekletilip 515 nm'de spektrofotometre ile absorbansı ölçüldü. Sonuçlar trolox eşdeğeri (mmol Trolox eq./g) olarak ifade edildi ( $y=0.1082x + 0.4997$ ,  $R^2=0.9997$ ).

### Bireysel Fenolik Madde İçeriği

Elma kabuğu ekstraktlarının fenolik madde içeriğinin tespiti Colaric ve ark. (2005)'e ait yöntemde bazı modifikasyonlar yapılarak kullanıldı. Ekstraktlar 0.45 µm PVDF filtreden geçirildikten sonra HPLC cihazı ile analiz edildi. Çalışmada Shimadzu marka LC-20 AD pompa, SPD-M20A model DAD dedektör ve CTO-10AS VP model kolon firmından oluşan yüksek basınç sıvı kromatografisi kullanıldı. Fenolik bileşenlerin tespiti, Waters Symetry C18 (250x4 mm ID, partikül büyüklüğü 5 µm) kolonu (Waters, ABD) ile 1 mL/ dk'lık akış hızında 25 °C'de gerçekleştirildi. Mobil faz olarak % 2 asetik asitli su (A) ve % 0.5 asetik asit: asetonitril (1: 1, v/v; B) kullanıldı. Gradyan programı: 0. dk % 90 A, 50. dk % 45 A, 60. dk % 0 A olarak belirlendi. Flavan-3-oller 280 nm'de, hidrokisisanamik asitler 320 nm'de ve flavonoller 360 nm'de belirlendi.

### İstatistiksel Analizler

Verilerin ortalama ve standart sapması SPSS istatistik programı (sürüm 20.0) kullanılarak verilmiştir. Örnekler arasındaki farklılıkların belirlenmesi için Duncan çoklu karşılaştırma testi uygulanmıştır.

## Bulgular ve Tartışma

### Elma Kabuklarının Toplam Fenolik Madde, Toplam Flavonoid Miktarı ve Antioksidan Aktivitesi

Çoğu meyvede kabuk, meyveye aromasını veren uçucu bileşiklerin renk maddelerinin ve fenolik bileşikler gibi diğer birçok besin ögesini en yüksek oranda içeren katı kısımdır (de Torres ve ark., 2010). Farklı yöntemler ve sürelerde elma kabuğundan ekstrakte edilen toplam fenolik madde miktarı Şekil 1'de verilmiştir. Her iki ekstraksiyon yönteminde artan ekstraksiyon süresi ile toplam fenolik madde miktarı artmıştır ( $p<0.05$ ). Uğurlu ve Bakkalbaşı (2023), ultrases destekli ve konvansiyonel yöntemle ekstraksiyonda yeşil cevizden ekstrakte edilen toplam fenolik madde miktarının artan ekstraksiyon süresi ile arttığını bildirmişlerdir. Çalışmamızda ultrases destekli ekstraksiyonla 45. dakikada elde edilen toplam fenolik madde miktarı (2021.83 mgGAE/kg) konvansiyonel yöntemle 90. dakikada elde edilen fenolik madde miktarlarından (1848.50 mgGAE/kg) daha yüksek bulunmuştur. Benzer durum elma posasından ultrases destekli ve konvansiyonel ekstraksiyon yöntemleri ile elde edilen ekstraktların toplam fenolik madde miktarında da tespit edilmiştir (Viroto ve ark., 2010). Bir diğer çalışmada da kırmızı üzümde klasik yöntemle kıyasla ultrases destekli ekstraksiyonla daha kısa sürede daha yüksek toplam fenolik madde miktarı elde edildiği bildirilmiştir (Carrera ve ark., 2012). Sonuç olarak çalışmamızda ultrases destekli ekstraksiyon ile 45. dakikada elde edilen toplam fenolik madde miktarlarının aynı süredeki konvansiyonel ekstraksiyona kıyasla daha fazla olduğu tespit edilmiştir.

Elma kabuğundan ekstrakte edilen toplam flavonoid madde miktarlarına ait sonuçlar Şekil 2'de verilmiştir. Ultrases destekli ekstraksiyonda artan ekstraksiyon süresi ile toplam flavonoid madde miktarı 30. dakikaya kadar artmış 45. dakikada ise azalmıştır. Ancak bu azalma istatistiksel olarak önemsiz bulunmuştur ( $p>0.05$ ). Benzer durum elma püresinden iki farklı ultrases genliği (% 50 ve 70) ile farklı sürelerde (5, 10, 15 ve 20 dk) toplam flavonoid madde ekstraksiyonunda da gözlenmiştir. Her iki ultrases genliğinde de ekstraksiyon süresinin 15. dakikasına kadar toplam flavonoid madde miktarının arttığı 20. dakikada ise azaldığı bildirilmiştir (Egüés ve ark., 2021). Benzer bir durum *Terminalia catappa* L yaprağında ultrases ile farklı sürelerde (20, 40 ve 60 dk) ekstraksiyonun yapıldığı çalışmada da tespit edilmiştir. 40. dakikaya

kadar toplam flavonoid madde miktarının arttığı 60. dakikada ise azaldığı bildirilmiştir (Annegowda ve ark., 2010). Bu durumun biyoaktif bileşenlerin uzun süreli sonikasyonda parçalanmasından kaynaklanabileceği bildirilmiştir (Annegowda ve ark., 2010). Çalışmamızda konvansiyonel yöntemde ise artan ekstraksiyon süresi ile toplam flavonoid madde miktarı sürekli bir artış göstermiştir ( $p < 0.05$ ), ultrases destekli ekstraksiyonda olduğu gibi azalma meydana gelmemiştir. Ultrases destekli ekstraksiyonda 30. dakikada elde edilen toplam flavonoid madde miktarı (569.58 mg KA/kg) konvansiyonel yöntemle 90. dakikada elde edilen flavonoid madde miktarı (571.03 mg KA/kg) ile benzer düzeyde bulundu. Kırmızı ejder meyvesi kabuğundan (*Hylocereus polyrhizus*) ultrases destekli ekstraksiyon ile elde edilen toplam flavonoid madde miktarının konvansiyonel yöntemle elde edilen toplam flavonoid madde miktarından daha yüksek bulunduğu bildirilmiştir (Ramli ve ark., 2014). Bir diğer çalışmada da red araçá meyve kabuğundan (*Psidium cattleianum* Sabine) fenolik maddelerin ekstraksiyonunda benzer durum gözlenmiş olup konvansiyonel yöntemle kıyasla ultrases destekli ekstraksiyon ile daha yüksek toplam fenolik madde ve toplam flavonoid madde elde edildiği bildirilmiştir (Meregalli ve ark., 2020).

Elma kabuğundan elde edilen ekstraktların antioksidan aktivite değerleri (DPPH) Şekil 3'te verilmiştir. Her iki ekstraksiyon yönteminde artan ekstraksiyon süresi ile DPPH değeri artmıştır ( $p < 0.05$ ). Farklı mandalina kabuklarının ultrases destekli ekstraksiyonunda artan ekstraksiyon süresi ile (5, 15 ve 30 dk) DPPH yöntemi ile belirlenen antioksidan aktivite değerlerinin arttığı bildirilmiştir (Anticono ve ark., 2021). Çalışmamızda ultrases destekli ekstraksiyonla 30. dakikada elde edilen DPPH değeri (10.51 mmol Trolox eq./g) bile konvansiyonel yöntemle 90. dakikada elde edilen DPPH miktarlarından (9.70 mmol Trolox eq./g) daha yüksek bulunmuştur ( $p < 0.05$ ). Ultrases destekli ekstraksiyonun 15. ve 30. dakikalarındaki antioksidan aktivite değerleri konvansiyonel yöntemle elde edilen antioksidan aktivite değerlerinin yaklaşık 60. ve 90. dakikalarına denk geldiği ya da daha yüksek olduğu tespit edilmiştir. Sonuç olarak, ekstraktta istenen antioksidan aktivite değerine ulaşabilmek için ultrases destekli ekstraksiyon konvansiyonel yöntemle kıyasla ekstraksiyon süresini yaklaşık olarak %50 azaltabildiği tespit edilmiştir. Ciriguela (*Spondias purpurea* L.) kabuğundan farklı ekstraksiyon yöntemleri (ultrases, mikrodalga ve konvansiyonel) kullanılarak biyoaktif bileşiklerin elde edildiği çalışmada ultrases destekli ekstraksiyon ile daha yüksek toplam fenolik madde ve antioksidan aktivite elde edildiği bildirilmiştir (Júnior ve ark., 2021). Benzer durum siyah akasya (*Robinia pseudoacaciae*) çiçeklerinden fenolik bileşiklerin farklı ekstraksiyon yöntemlerinin (ultrases, soxhlet ve maserasyon) kullanıldığı çalışmada da gözlenmiş olup, daha kısa ekstraksiyon

süresi ile ultrases destekli ekstraksiyonda toplam fenolik maddenin daha yüksek olduğu ve daha iyi antioksidan aktivite verdiği bildirilmiştir (Gajic ve ark., 2019).

### **Elma Kabuklarının Fenolik Madde İçeriği**

Fenolik asitler, flavan 3-oller ve flavonoidler gıdanın tadına ve rengine katkıda bulunan ve ayrıca sağlık üzerine olumlu etkileri olan bileşen gruplarıdır (McSweeney ve Seetharaman, 2015). Elma kabuğundan elde edilen ekstraktlarda kateşin, epikateşin, prosiyanidin B1, prosiyanidin B2, prosiyanidin C1, klorojenik asit ve rutin tespit edildi ve bunların ekstraktlardaki içerikleri Tablo 1'de verildi. Her iki ekstraksiyon yönteminde de tespit edilen tüm fenoliklerin ekstraktlardaki içerikleri artan ekstraksiyon süresi ile artarken ( $p < 0.05$ ), rutin miktarı ultrases destekli ekstraksiyonda 30. dakikadan sonra azalmıştır. Ultrases destekli ekstraksiyonda özellikle 30. ve 45. dakikada elde edilen fenolik maddelerin miktarı, kateşin ve rutin hariç, konvansiyonel yöntemle elde edilen fenolik maddelerden daha yüksek bulunmuştur. Bu da ultrases destekli ekstraksiyonun konvansiyonel yöntemle kıyasla daha yüksek bir ekstraksiyon etkisine sahip olduğunu göstermektedir. Çalışmamızda ultrases destekli ekstraksiyonla 45. dakikada elde edilen kateşin miktarı konvansiyonel yöntemle 75. ve 90. dakikalarda elde edilen kateşin miktarları ile benzer bulunmuştur ( $p > 0.05$ ). Ultrases ile 45. dakikada elde edilen ekstrakttaki klorojenik asit miktarı 30. dakikada elde edilen değere kıyasla az miktarda yüksektir ancak bu fark istatistik olarak önemsizdir ( $p > 0.05$ ). Ultrasesle 30 ve 45. dakikalarda elde edilen klorojenik asit miktarları ile konvansiyonel yöntemde 90. dk da elde edilen klorojenik asit miktarları da benzer düzeydedir ( $p > 0.05$ ). Ayrıca rutin miktarında da ultrases destekli ekstraksiyonla 45. dakikada azalma meydana gelmiş ve 30. dakikaya kıyasla bu azalma istatistiksel olarak önemsiz bulunmuştur ( $p > 0.05$ ). Benzer durum ultrases destekli ekstraksiyon ile yeşil cevizlerden fenolik maddelerin ekstraksiyonunun yapıldığı farklı bir çalışmada da tespit edilmiştir. Ultrases destekli ekstraksiyon ile juglon, gallik asit, neoklorojenik asit ve rutin miktarlarının 50. dakikaya kadar arttığı 60. dakikada ise azaldığı bildirilmiştir (Uğurlu ve Bakalbaşı, 2023). Çalışmamızda ultrases destekli ekstraksiyon uygulanarak elde edilen prosiyanidin C1, kateşin ve klorojenik asit bileşiklerinin sırasıyla 30, 45 ve 30-45. dakikalardaki miktarları konvansiyonel yöntemle elde edilen prosiyanidin C1, kateşin ve klorojenik asit miktarlarının yaklaşık olarak 90. dakikasına denk gelmiştir. Ayrıca ultrases destekli ekstraksiyon ile elde edilen epikateşin, prosiyanidin B1 ve prosiyanidin B2 bileşiklerinin 45. dakikadaki miktarlarının konvansiyonel ekstraksiyon ile elde edilen 90. dakikadaki miktarlarından daha yüksek bulunmuşken, rutin miktarı ise daha düşük bulunmuştur. Bioaktif bileşenlerin ekstraksiyonunda

ultrases destekli ekstraksiyonun konvansiyonel ekstraksiyona göre daha etkili olmasına ait benzer durum zerdeçaldan kurkumin ve sinnamik asit eldesinde de tespit edilmiştir (Yang ve ark., 2020). Portakal kabuklarından fenoliklerin ekstrakte edildiği çalışmada, ultrases destekli ekstraksiyon ile elde edi-

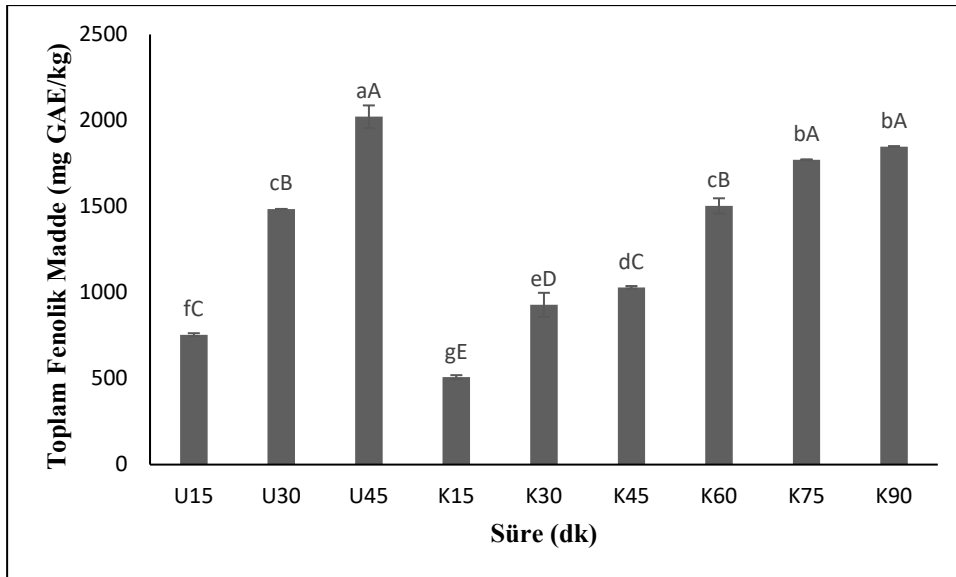
len naringin ve hesperidin miktarlarının, kovansiyonel yöntem ile elde edilen miktarlarından daha yüksek olduğu bildirilmiştir (Khan ve ark., 2010). Benzer sonuçlar patates kabuğundan da klorojenik asit ve kafeik asit eldesinde de bildirilmiştir (Kumari ve ark., 2017).

Tablo 1. Elma kabuğu ekstraktlarının fenolik madde içeriği (mg/kg)

Table 1. Phenolic substance content of apple peel extracts (mg/kg)

Ekstraksiyon Yöntemi	Süre (dk)	Kateşin	Epikateşin	Prosiyanidin B1	Prosiyanidin B2	Prosiyanidin C1	Klorojenik Asit	Rutin
Ultrases	15	7.44 ±0.34 <sup>cB</sup>	35.39 ±2.24 <sup>cC</sup>	9.03 ±0.02 <sup>hC</sup>	24.02 ±0.59 <sup>gC</sup>	11.08 ±0.14 <sup>cC</sup>	34.31 ±0.61 <sup>cB</sup>	14.31 ±0.14 <sup>hC</sup>
	30	14.70 ±2.25 <sup>aA</sup>	85.16 ±1.24 <sup>bB</sup>	29.91 ±0.34 <sup>bB</sup>	82.63 ±1.00 <sup>eB</sup>	18.99 ±0.33 <sup>bB</sup>	46.80 ±1.12 <sup>aA</sup>	113.50 ±0.88 <sup>bB</sup>
	45	15.23 ±2.10 <sup>aA</sup>	107.54 ±1.92 <sup>aA</sup>	34.52 ±0.44 <sup>aA</sup>	118.59 ±1.26 <sup>aA</sup>	42.95 ±2.52 <sup>aA</sup>	47.38 ±3.29 <sup>aA</sup>	110.71 ±0.58 <sup>bA</sup>
Konvansiyonel	15	7.06 ±0.63 <sup>eD</sup>	25.58 ±0.79 <sup>dD</sup>	8.15 ±0.14 <sup>fF</sup>	30.89 ±1.02 <sup>fE</sup>	5.08 ±0.03 <sup>dC</sup>	32.50 ±3.04 <sup>cC</sup>	46.10 ±5.13 <sup>gF</sup>
	30	8.96 ±0.18 <sup>cC</sup>	36.85 ±2.47 <sup>cC</sup>	11.06 ±0.03 <sup>gE</sup>	58.05 ±0.57 <sup>eD</sup>	9.85 ±0.17 <sup>cB</sup>	37.35 ±3.18 <sup>bcBC</sup>	54.11 ±0.41 <sup>fE</sup>
	45	9.14 ±0.14 <sup>bcC</sup>	37.83 ±2.53 <sup>cC</sup>	13.41 ±0.08 <sup>fD</sup>	68.29 ±0.61 <sup>dC</sup>	10.49 ±0.00 <sup>cB</sup>	39.35 ±1.72 <sup>bcABC</sup>	73.29 ±0.00 <sup>eD</sup>
	60	11.50 ±0.00 <sup>bB</sup>	60.26 ±0.45 <sup>dB</sup>	18.67 ±0.13 <sup>cC</sup>	83.65 ±2.31 <sup>cB</sup>	17.60 ±0.20 <sup>bA</sup>	42.80 ±3.44 <sup>abAB</sup>	84.27 ±0.97 <sup>dC</sup>
	75	15.51 ±0.39 <sup>aA</sup>	63.69 ±0.40 <sup>dB</sup>	20.33 ±0.21 <sup>dB</sup>	85.70 ±1.77 <sup>cB</sup>	18.52 ±1.11 <sup>bA</sup>	43.43 ±5.46 <sup>abAB</sup>	91.50 ±3.49 <sup>cB</sup>
	90	15.80 ±0.29 <sup>aA</sup>	72.58 ±0.58 <sup>cA</sup>	22.19 ±0.22 <sup>cA</sup>	90.57 ±1.49 <sup>bA</sup>	18.87 ±1.41 <sup>bA</sup>	46.75 ±0.36 <sup>aA</sup>	143.36 ±0.22 <sup>aA</sup>

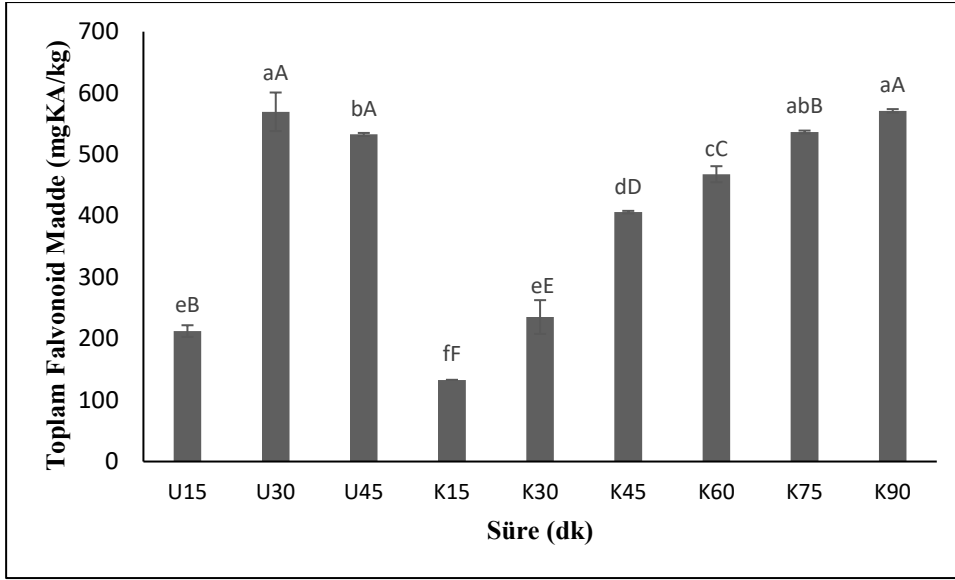
Değerler ortalama±standart sapma şeklinde verilmiştir. Küçük harfler aynı sütundaki tüm örnekler arasındaki farkı, büyük harfler aynı ekstraksiyon yöntemi uygulanan örneklerin ekstraksiyon süreleri arasındaki farkı göstermektedir (p<0.05).



Küçük harfler tüm örnekler arasındaki farkı, büyük harfler aynı ekstraksiyon yöntemi uygulanan örneklerin ekstraksiyon süreleri arasındaki farkı göstermektedir (U: Ultrases, K: Konvansiyonel).

**Şekil 1.** Ultrases destekli ekstraksiyon ve konvansiyonel ekstraksiyon ile farklı sürelerde elde edilen ekstraktların toplam fenolik miktarlarındaki değişimi.

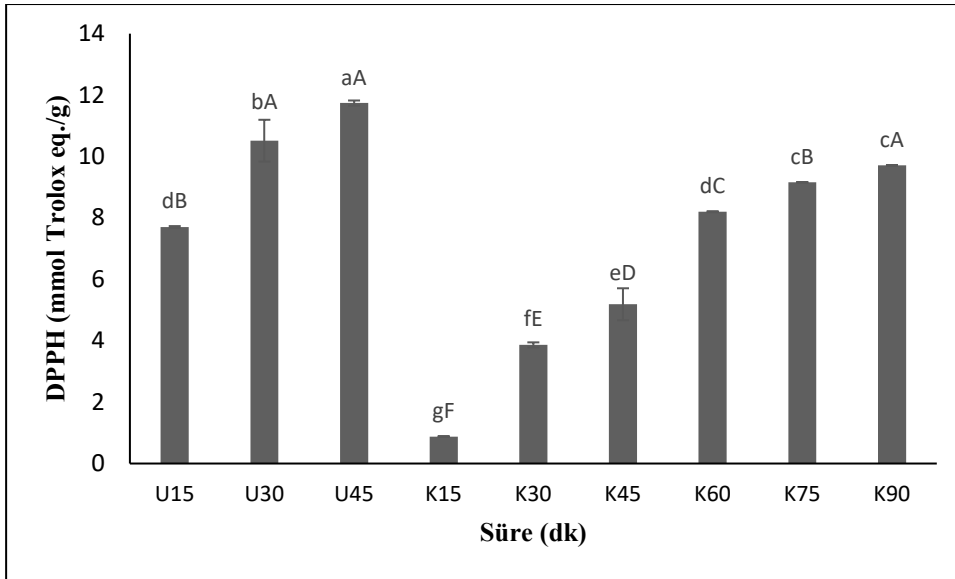
**Figure 1.** The change in the total phenolic content of extracts obtained by ultrasound assisted extraction and conventional extraction at different time intervals



Küçük harfler tüm örnekler arasındaki farkı, büyük harfler aynı ekstraksiyon yöntemi uygulanan örneklerin ekstraksiyon süreleri arasındaki farkı göstermektedir (U: Ultrases, K: Konvansiyonel).

**Şekil 2.** Ultrases destekli ekstraksiyon ve konvansiyonel ekstraksiyon ile farklı sürelerde elde edilen ekstraktların toplam flavonoid madde miktarlarındaki değişimi

**Figure 2.** The change in the total flavonoid content of extracts obtained by ultrasound assisted extraction and conventional extraction at different time intervals



Küçük harfler tüm örnekler arasındaki farkı, büyük harfler aynı ekstraksiyon yöntemi uygulanan örneklerin ekstraksiyon süreleri arasındaki farkı göstermektedir (U: Ultrases, K: Konvansiyonel).

**Şekil 3.** Ultrases destekli ekstraksiyon ve konvansiyonel ekstraksiyon ile farklı sürelerde elde edilen ekstraktların antioksidan değerlerindeki değişimi

**Figure 3.** The change in the antioxidant values of extracts obtained by ultrasound assisted extraction and conventional extraction at different time intervals

## Sonuç

Elma kabukları genellikle işlenmiş elma ürünlerinin üretiminde ortaya çıkmakta ve yüksek katma değerli ürünlere dönüştürülmeyip atılmaktadır. Buna karşın elma kabukları fazla miktarda biyoaktif bileşiklere ve yüksek düzeyde antioksidan aktiviteye sahiptirler. Bu çalışmada farklı ekstraksiyon yöntemlerinin (ultrases ve konvansiyonel) elma kabuğundan ekstrakte edilen biyoaktif bileşen miktarı ve antioksidan aktivite üzerine etkileri incelenmiştir. Her iki ekstraksiyon yönteminde artan ekstraksiyon süresi ile toplam fenolik madde ve antioksidan aktive değerlerinin arttığı tespit edilmiştir. Ultrases destekli ekstraksiyon ile ekstrakte edilen toplam fenolik madde, toplam flavonoid madde ve antioksidan aktive değerlerinin konvansiyonel yöntemle kıyasla daha yüksek olduğu bulunmuştur. Elmadan elde edilen ekstraktların kateşin, epikateşin, prosiyanidin B1, B2 ve C1 ile klorojenik asit ve rutin gibi sağlık açısından önemli biyoaktif fenolik bileşikler içerdiği ve bunların ekstrakttaki miktarlarının artan ekstraksiyon süresi ile arttığı tespit edilmiştir. Sadece toplam flavonoid madde ve rutin bileşiğinin miktarlarının ultrasonik ekstraksiyonda 30. dakikadan sonra bir miktar azaldığı tespit edilmiştir. Sonuçlar, elma kabuğunun insan sağlığı açısından faydalı bileşikler içerdiği ve fonksiyonel bir gıda veya katma değerli bir bileşen üretimi için potansiyel ve önemli bir kaynak olduğunu ayrıca konvansiyonel yöntemle alternatif olarak ultrases destekli ekstraksiyon yönteminin kullanılması ile daha kısa sürede ve daha yüksek verimde bu bileşenlerin elde edilip kullanılabileceğini göstermektedir.

## Etik Standartlar ile Uyumluluk

**Çıkar çatışması:** Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

**Etik izin:** Araştırma niteliği bakımından etik izne tabii değildir.

**Veri erişilebilirliği:** Veriler istek üzerine sağlanacaktır.

**Finansal destek:** -

**Teşekkür:** -

**Açıklama:** -

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## Besinlerde melanoidinlerin oluşumu ve sağlık üzerine etkileri

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### ÖZ

Melanoidinler, gıdanın ısıl işlemi sırasında meydana gelen maillard reaksiyonunun son aşamalarında oluşan indirgen şekerler ve proteinler veya amino asitlerden üretilen kahverengi, yüksek moleküler ağırlıklı bileşiklerdir. Beslenmemizde melanoidinlerin başlıca kaynağı ekmek ve unlu mamullerdir. Melanoidinlerin kimyasal yapıları tam olarak bilinmemekle birlikte diyetle günlük yaklaşık 10 g alındığı tahmin edilmektedir. Melanoidinler oluştukları gıdanın bileşimine bağlı olarak melanosakkaritler (kahve, bira ve kakao) ve melanoproteinler (ekmek ve unlu mamuller) şeklinde bulunur. Besinlerde melanoidinlerin oluşumu ürünün bileşimi, ısıl işlem süresi ve sıcaklığı, pH değeri, su içeriği ve reaktanların aktivitesi gibi koşullara bağlıdır. Melanoidinlerin insan sağlığı üzerinde; prebiyotik, antioksidan, antimikrobiyal, antikarsinojenik, antihipertansif, ksenobiyotik enzim aktivitesi, iştah ve enerji alımını düzenleme gibi birçok faydalı etkileri bulunmaktadır. Melanoidinler gastrointestinal sistemde, bifidobakterilerin büyümesini sağlayarak diyet posası olarak da davranırlar. Ayrıca, melanoidinler gıdalardan doku ve lezzetini katkı sağlayarak gıdanın kalitesini koruyabilir. Bu derleme yazının amacı; melanoidinlerin oluşumunu, etki mekanizması, besinlerdeki miktarları ve insan sağlığı üzerine olası etkilerini incelemektir.

**Anahtar Kelimeler:** Melanoidinler, Sağlık, Antioksidanlar, Prebiyotikler

### ABSTRACT

#### Formation of melanoidins in foods and effects on health

Melanoidins are brown, high molecular weight compounds generated in the late stages of the Maillard reaction from reducing sugars and proteins or amino acids during thermal food processing. The primary source of melanoidin is bread and bakery products in the diet. Although the chemical structures of melanoidins are not precisely known, the daily dietary intake is estimated at approximately 10 g. Melanoidins exist as monosaccharides (coffee, beer, and cocoa) and melanoproteins (bread and bakery products), depending on the food composition they form. The formation of melanoidins in foods depends on conditions such as composition of products, heat treatment time and temperature, pH, water and activity of reactants. Melanoidins have many beneficial effects on health, such as prebiotic, antioxidant, antimicrobial, anticarcinogenic, antihypertensive, xenobiotic enzyme activity, appetite regulation, and energy intake. In the gastrointestinal tract, melanoidins behave as dietary fibre by promoting the growth of bifidobacteria. In addition, melanoidins can preserve the quality of food by contributing to the texture and flavour of food. This review examines the formation, amount of food, mechanism of action and effects on human health of melanoidins.

**Keywords:** Melanoidins, Health, Antioxidants, Prebiotics

## Giriş

Gıda ürünleri işleme, pişirme veya depolama sırasında ısıl işleme tabii tutulur ve tüketiciler tarafından kabul edilebilirliği artıran aroma, renk ve tattan sorumlu bir dizi dönüşüm gerçekleşir. Bu dönüşümlerden biri, ilk kez 1912'de tanımlanan Fransız kimyager Louis Maillard'ın adını taşıyan Maillard reaksiyonudur. Maillard reaksiyonu, indirgeyici şekerler ve amino asitler gibi karbonil ve amino bileşikleri içeren karmaşık bir reaksiyonlar ağıdır (Maillard, 1912). Maillard reaksiyonunun ilk aşamasında amadori ürünleri, ikinci aşamasında akrilamid, hidroksimetilfurfural, strecker aldehitleri veya pirazinler gibi ara ürünler oluşurken son aşamada melanoidinler oluşmaktadır (Hodge, 1953). Melanoidinler, termal olarak işlenmiş gıdalarda yaygın olarak bulunan, nitrojen içeren, anyonik, polimerik, yüksek moleküler ağırlıklı kahverengi bileşiklerdir (Morales, 2002). Molekül ağırlıkları ısıtma süresi ve derecesine bağlı olarak birkaç kDa'dan 100 kDa'ya kadar değişkenlik gösterir. Melanoidinler oldukça hidrofildir ve genellikle negatif yüklüdür (Wang ve ark., 2011). Melanoidinlerin çözünürlüğü, reaktanların doğasına ve polimerin boyutuna bağlı olup çok yüksek moleküler ağırlığa sahip olan melanoidinler genellikle çözünmezler (Morales ve ark., 2012). Melanoidinlerin kimyasal yapıları, reaksiyonda üretilen ürünlerin karmaşıklığı nedeniyle büyük ölçüde bilinmemektedir (Kim ve Lee, 2008). Fakat model sistemlerde ve gıdalarda birkaç ön çalışma yapılarak yüksek moleküler ağırlıklı melanoidin yapıları incelenmiştir. Termal bozunmadan sonra elde edilen melanoidinler, esas olarak karbonil bileşikler, piroller, pirazinler ve piridinlerin eşlik ettiği furanlardan oluşmaktadır (Wang ve ark., 2011). Melanoidinler, yapısındaki bileşenlere göre melanosakkaritler ve melanoproteinler olmak üzere iki sınıfa ayrılır. Melanosakkaritler; amino asitler ve polisakkaritlerin reaksiyonundan oluşur, genellikle negatif yüklüdür, suda oldukça çözünürdür, düşük moleküler ağırlıklı moleküllerden oluşur ve en çok kahve, bira ve kakaoda bulunur. Melanoproteinler; proteinlerin ve şekerlerin çapraz bağlanmasından elde edilir ve büyük ölçüde çözünmeyen, son derece yüksek moleküler ağırlıklı moleküllerden oluşur ve en çok ekmek, unlu mamuller ve kahvaltılık gevreklerde bulunur (Sharma ve ark., 2021). Melanoidinlerin, sık tüketilen gıdalardaki yüksek içeriği nedeniyle diyetle günlük alımı İspanya'da 12.2 g/kişi/gün (Pastoriza ve Rufián-Henares, 2014), Brezilya'da 10.7 g/kişi/gün (Alves ve ark., 2020) olarak hesaplanmıştır ve melanoidin alımına en büyük katkı kahve, ekmek ve biradan gelmektedir. Bu nedenle melanoidinlerin insan sağlığına olası yararlarını veya risklerini bilmek oldukça önemlidir. Melanoidinlere prebiyotik, antioksidatif, antimikrobiyal, antihipertansif aktivite, ksenobiyotik enzim aktivitesi, anjiyotensin I dönüştürücü enzime karşı inhibitör aktivite gibi birçok farklı biyolojik etki atfedilmiştir

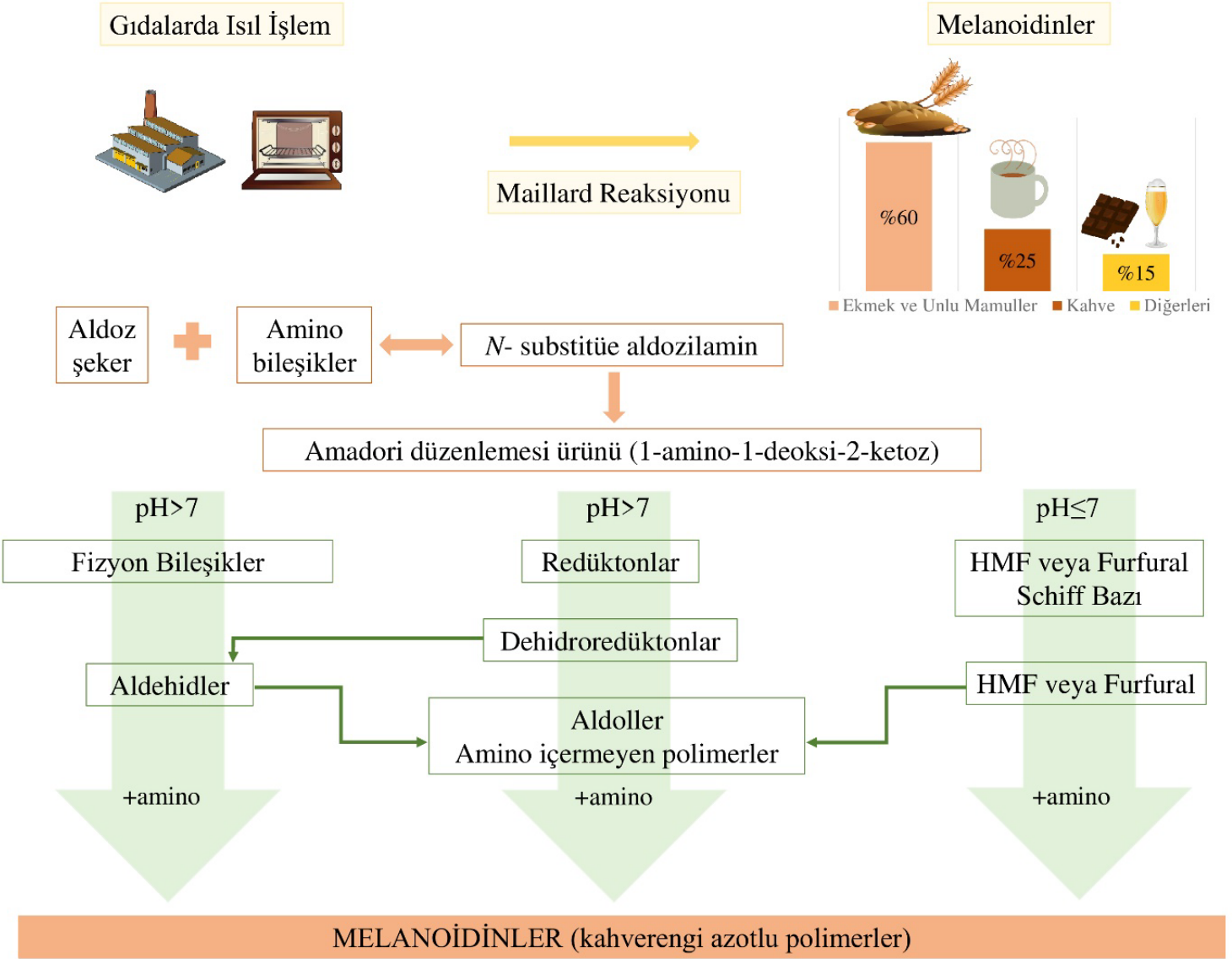
(Morales ve ark., 2012; Wang ve ark., 2011). Bu derleme yazının amacı; melanoidinlerin oluşumu, etki mekanizması ve insan sağlığına etkilerini incelemektir.

## Besinlerde Melanoidinlerin Oluşumu ve Tayini

Besinlerde melanoidinlerin oluşumu ürünlerin bileşimi (amino asit veya şeker tipi), ısıtma süresi, sıcaklığı, pH değeri, su içeriği ve reaktanların aktivitesi gibi koşullara bağlıdır (Wang ve ark., 2011). Melanoidinlerin oluşumu ile ilgili birçok teori ortaya atılmıştır ve oluşum üç aşamada şematize edilmiştir (Şekil 1). İlk aşamada çoğunlukla Amadori ürünleri olmak üzere başlangıç aşaması bileşikler oluşur. Maillard reaksiyonunda aldozların karbonil grubu ile amino asitin serbest amino grubu bir yoğunlaştırma reaksiyonuna girer ve azot içerikli aldozil amin oluşur. Aldozil amin, 1-amino-1-deoksi 2-ketoza dönüşür. İkinci aşamada; aldozil amin, asidik koşullarda Schiff bazı oluşturur, amadori bileşiğinde pentozlardan furfural ve heksozlardan ise 5-hidroksimetil furfural oluşur. Alkali koşullarda asetol, piruvataldehit ve diasetil gibi fizyon bileşikler oluşur. Son aşamada, strecker degradasyonu meydana gelerek karbonil grupları ile serbest amino grupları aldehitleri oluşturur. Aldehitler kahverengi bileşikler oluşturmak için reaksiyona girer ve amino asitler ile doymamış karbonil bileşikler melanoidinleri oluşturur (Shaheen ve ark., 2022).

Besinlerde melanoidinlerin oluşumu ile ilgili sağlıklı melanoidinler üretmek ve tüketmek için farklı yaklaşımlar öne sürülmüştür (Shaheen ve ark., 2022). Bunlar;

1. Kanserojen madde oluşumuna ve serbest fenolikler azaltarak süpürme kapasitesinde bir düşüşe yol açtığından, pişirme sıcaklıkları 250°C'nin üzerine çıkarılmamalıdır.
2. Maillard reaksiyonunu hızlandırdığından ve mutajenlerin oluşumuna yol açtığından yüksek konsantrasyonlarda içilebilir alkol ile yemek pişirilmemelidir.
3. Alkali sulu ortamda (pH 7.0-9.0) glikoz ile çiğ et ve balıktaki yüksek kreatin seviyelerinden elde edilen amino asitler önemli miktarlarda mutajenik madde içerdiğinden mutajenik madde oluşumu engellenmelidir.
4. Diğer besin maddelerini korumak için melanoidin oluşumunu etkileyen faktörler (sıcaklık, şekerler/amino asit tipi ve seviyesi, pH) optimize edilmelidir.
5. Heterosiklik aminlerin alımını ve kötü tatların oluşumunu azaltmak için pişirme sırasında maillard reaksiyonu oluşumu azaltılmalıdır.
6. Melanoidinlerin aşırı tüketimi, enzimlerin özelliklerini ve peptidik kökenli hormonları kaybedebilir ve immünolojik davranışlarını değiştirebilir. Bu nedenle melanoidin kaynaklarının aşırı tüketiminden kaçınılmalıdır.



**Şekil 1.** Besinlerde melanoidinlerin oluşumu (HMF; hidroksimetil furfural)

**Figure 1.** Formation of melanoidins in foods (HMF; hydroxymethyl furfural)

Kahve ve unlu mamuller, diyet melanoidinlerinin ana kaynaklarını temsil eder. Diğer melanoidin kaynakları arasında ise kakao, bira, ızgara et, balzamik sirke, şarap ve işlenmiş domates yer almaktadır (Morales ve ark., 2012). Tablo 1’de bazı besinler ve içerdikleri melanoidin miktarları verilmiştir. Kahve melanoidin kaynakları arasında en yaygın şekilde araştırılan gıda ürünüdür. Kahvenin kuru maddesi demlenmiş kahvenin yaklaşık %25’ini oluşturur ve bu içeceğin viskozitesinin yanı sıra kahvenin belirli organoleptik özelliklerinin bir kısmından melanoidinler sorumludurlar. Melanoidinlerin

bileşimi, kahvede bulunan polisakkaritlere (galaktomannanlar ve arabinogalaktanlar), amino asitlere, proteinlere ve fenolik bileşiklere (klorojenik, kafeik veya ferulik asitler) bağlıdır. Düşük moleküler ağırlıklı melanoidinler, tüm kahve melanoidinlerinin %41’ini ve yüksek moleküler ağırlıklı melanoidinler %59’unu oluşturur (Nunes ve Coimbra, 2010). Kahve çekirdeklerinin kavrulması kahve melanoidinlerinin oluşumunda önemli bir rol oynar. Kahve ne kadar çok kavrulursa yüksek moleküler ağırlıklı melanoidinlerin miktarı o kadar artar (Borrelli ve ark., 2002). Kahve ile ilgili olarak, en

yüksek melanoidin miktarı çözünebilir kahvede bulunmaktadır (Tablo 1). Orta (960 mL/gün) ve yüksek düzeyde (1440 mL/gün) kahve tüketen bireylerde günlük melanoidin alımı sırasıyla 0.5 ile 2.0 g arasında değişmektedir (Fogliano ve Morales, 2011). Unlu mamullerdeki melanoidinler, renkli maillard reaksiyon ürünleri ile çapraz bağlanan gluten proteinleri tarafından oluşturulur. Melanoidinler unlu mamullerin tad, renk ve doku özelliklerini etkiler ve antioksidan aktiviteleri ile raf ömrünü uzatır. Unlu mamullerde melanoidinler ağırlıklı olarak ekmekek kabuklarında bulunurken, bisküvilerde ürünün tamamında bulunur (Manzocco ve ark., 2000). Ekmekek kabuğundaki melanoidin miktarı, ekmeğin türüne bağlı olarak 100 g kabukta 14 ile 30 g arasında değişirken, bütün ekmekek 100 g'da 4.4 g'a kadar düşmektedir (Fogliano ve Morales, 2011; Pastoriza ve Rufián-Henares, 2014). Unlu mamullerde en yüksek melanoidin miktarı ekşi mayalı ekmekek türünde ve kahvaltılık gevreklerde bulunmaktadır (Tablo 1). Ekmekek melanoidinleri suda çözünmezler, bu nedenle yalnızca kapsamlı enzimatik sindirimden sonra verimli bir şekilde ekstrakte edilebilirler (Borrelli ve ark., 2003).

Gıda melanoidinlerinin izolasyonu ve saflaştırılması için farklı prosedürler uygulanmaktadır. Melanoidinlerin moleküler ağırlığının tahmini, ultrafiltrasyon veya diyaliz, kütle spektrometrisi ve farklı kesme boyutlarına sahip membranlar aracılığıyla santrifüjleme yoluyla gerçekleştirilir. Günümüzde en yaygın olarak kullanılan yöntem; 3, 5 veya 10 kDa'da bir moleküler ağırlık kesme boyutlarına sahip membranlar ile ultrafiltrasyon tekniklerinin kullanılmasını içerir. Filtrasyondan sonra melanoidin fraksiyonları liyofilize edilir ve içerikleri, ilk gıdanın kuru madde ağırlığına orantılanarak ifade edilir. Bu yaklaşım, yüksek moleküler ağırlıklı bileşiğin diğer yüksek moleküler ağırlıklı bileşikleri (reaksiyona girmemiş polisakkaritler, lif veya proteinler gibi) içermesi anlamında sınırlıdır. Bu durum, gıdadaki melanoidin içeriğinin tahmini hakkında kesin bir sonuca engel olmakla birlikte bugüne kadar gıda melanoidinlerinin tahmininde kullanılan en iyi yöntem budur (Borrelli ve ark., 2003; Tagliazucchi ve Bellesia, 2015).

## Melanoidinlerin Sağlık Etkileri

Melanoidinlerden zengin yiyecek ve içeceklerin tüketiminden sonra, bu tür bileşikler mide ve bağırsak lümeninde yüksek konsantrasyonlarda bulunabilir (Tagliazucchi ve Bellesia, 2015). Sıçanlarda oral olarak verilen yüksek moleküler ağırlıklı melanoidinlerin (>10 kDa) %70-90'ı dışkı ve idrarla atılıp sadece %1-5'i emilmiştir (Finot ve Magnenat, 1981). Düşük moleküler ağırlıklı melanoidinler ise %30 oranında emilmektedir (Delgado-Andrade, 2014). Bu nedenle melanoidinlerin insan sağlığına olası yararlarını veya risklerini bilmek oldukça önemlidir. Melanoidinlere prebiyotik,

antioksidatif, antimikrobiyal, antihipertansif aktivite, antikarsinogenik, ksenobiyotik enzim aktivitesi, anjiyotensin I dönüştürücü enzime karşı inhibitör aktivite gibi birçok farklı biyolojik etki atfedilmiştir (Langner ve ark., 2011, Morales ve ark., 2012, Shaheen ve ark., 2022, Wang ve ark., 2011).

**Tablo 1.** Bazı besinlerin melanoidin içerikleri

**Table 1.** Melanoidin content of some foods

Besinler	Melanoidin Miktarı (g/100g)	Kaynak
Beyaz ekmekek	10-12.5	(Alves ve ark., 2020)
Tam buğday ekmeği	10-12.2	(Alves ve ark., 2020)
Ekşi mayalı ekmekek	28-32	(Fogliano ve Morales, 2011)
Mısır ekmeği	4.8-7.2	(Alves ve ark., 2020)
Açma/poğaç	1-4.5	(Alves ve ark., 2020)
Tuzlu kraker	7.2-10.8	(Alves ve ark., 2020)
Tatlı bisküvi	6-12	(Alves ve ark., 2020)
Kek	2-8.8	(Alves ve ark., 2020)
Kurabiye	4.1-5.8	(Alves ve ark., 2020)
Kahvaltılık gevrek	10.2-13/25.5	(Alves ve ark., 2020; Pastoriza ve Rufián-Henares, 2014)
Kahvaltılık gevrek (tam buğday)	7.9-10	(Alves et al., 2020)
Kakao	12.5-15/22	(Alves et al., 2020; Bellesia ve Tagliazucchi, 2014)
Çikolata	15	(Pastoriza ve Rufián-Henares, 2014)
Gofret	3.2-6	(Alves ve ark., 2020)
Süt reçeli	1.6-1.8	(Alves ve ark., 2020)
Filtre kahve	7.2	(Fogliano ve Morales, 2011)
Espresso	7.2	(Fogliano ve Morales, 2011)
Çözünür kahve	21-22.5	(Alves ve ark., 2020)
Kafeinsiz kahve	6.7-7.8	(Alves ve ark., 2020)
Mate çayı	1.3-2.6	(Alves ve ark., 2020)
Siyah bira	5-7.6	(Alves ve ark., 2020)
Sarı bira	2.5-5.8	(Alves ve ark., 2020)
Şarap	11-17	(Pastoriza ve Rufián-Henares, 2014)
Balzamik sirke	74-93	(Tagliazucchi ve Verzelloni, 2014)
Izgara köfte	1.3	(Obretenov ve ark., 1993)
Şinitzel	1.2	(Obretenov ve ark., 1993)
Domates püresi/salça	73.6 mg	(Adams ve ark., 2005)

## Prebiyotik Etki

Yüksek moleküler ağırlıklı melanoidinlerin düşük emilimlerinden dolayı kan dolaşımında veya organlarda biyolojik aktif bileşikler olarak hareket etmesi olası değildir. Bu nedenle tüketilen melanoproteinlerin çoğu mide-bağırsak yolunda kalır ve biyolojik etkilerini bu bölgede gösterirler (Rajakaruna ve ark., 2022). Melanoidinler gıdaların ısı işlemleri sırasında oluştuğu ve amino asit içerdikleri için tam olarak diyet posası olarak kabul edilemezler. Bununla birlikte, melanoidinler ve posa, bazı fiziksel, kimyasal ve fizyolojik işlevleri bakımından benzerdir. Silván ve ark. (2010), melanoidin kavramını “maillardize posa” olarak yeniden tanımlamıştır. Melanosakaritler ve melanoproteinler; bağırsak florasına katkıda bulunur, bağırsak bakterileri tarafından fermente edilerek asetat, propiyonat ve bütirat oluşumunu sağlar (Rajakaruna ve ark., 2022). Borrelli ve Fogliano (2005), ekmekten izole edilen farklı melanoidinlerin potansiyel prebiyotik aktivitesini araştırmış ve anaerobik bakterilerin, özellikle bifidobakteri suşlarının, karbon kaynağı olarak ekme kabuğu melanoidinlerini kullanabildiğini göstermiştir. Kahveden izole edilen melanoidinlerin bağırsak bakteri popülasyonu üzerindeki etkisini araştırmayı amaçlayan iki in vivo çalışmada da benzer etki gözlemlenmiştir. Üç hafta boyunca günde üç fincan kahve tüketiminin insanlarda bifidobakteri suşlarını olumlu yönde etkilediği (Jaquet ve ark., 2009), üç gün boyunca kahve ile beslenen farelerde proksimal kolonda *Escherichia coli* ve *Clostridium spp.* sayılarının önemli ölçüde azaldığı ve aynı bölgede bifidobakteri suşlarının arttığı gözlenmiştir (Nakayama ve Oishi, 2013).

## Antioksidan Etki

Melanoidinler, gıdanın ısı işlemleri sırasında oluşan ikincil antioksidanlar olarak rol oynar. Natella ve ark. (2002), bireylerin 200 mL normal kahve alımından 2 saat sonra kan örneklerinin plazma antioksidan kapasitesinde istatistiksel olarak anlamlı %7' lik bir artış bildirmiş ve bu etkinin melanoidinlerden geldiğini öne sürmüştür. Melanoidinlerin gözlenen antioksidan davranışından sorumlu spesifik bileşenler henüz bilinmemekle birlikte, antioksidatif etkileri, radikal süpürücü aktivitelerinden ve metal katyonları ile stabil kompleksler oluşturabilen metal şelatlama kapasitesinden oluşmaktadır (Tagliazucchi ve Bellesia, 2015). Suda çözünen gıda melanoidinlerinin gastrik koşullar altında (pH 2; 37°C) ABTS (2,2-azinobis 3-etilbenzothiazollin-6-sulfonik asit) radikalini süpürücü etkisi, yapılarında fenolik grubun varlığına atfedilmiştir (Tagliazucchi ve Verzelli, 2014). Bunun yanı sıra demlenmiş kahve ve ekme ekstraktının fenolik olmayan fraksiyonunun ABTS radikalini süpürücü aktivitesinin, uzun süreli kavurma veya fırınlama ile arttığı ve sirke melanoidin-

lerinin hidroksil radikal süpürme aktivitesinin, sirkenin kaynatılması, saklanması ve bekletilmesi sırasında arttığı gözlemlenmiştir (Sacchetti ve ark., 2009). Diyetle alınan melanoidinler  $Ca^{2+}$ ,  $Pb^{2+}$ ,  $Zn^{2+}$ ,  $Cu^{2+}$  ve  $Fe^{2+}$ 'yi bağlayabilir. Gıda melanoidinlerinin şelat oluşturma yeteneği, güçlü bir şekilde asitlik derecesine bağlı olan anyonik doğalarından kaynaklanmaktadır (Tagliazucchi ve Verzelli, 2014). Melanoidinler, metal iyonları dışında heterosiklik aminler gibi gıda işleme sırasında oluşan zararlı bileşikler de bağlayabilmektedir. Bichler ve ark. (2007) tarafından yapılan çalışmada gönüllü bireylere beş gün boyunca günde 600 mL normal kahve verilmiş ve tüketimden önce ve sonra kan örnekleri analiz edilerek DNA hasarı değerlendirilmiştir. DNA hasarı, müdahaleden önceki indüklenen hasara kıyasla kahve tüketiminden sonra önemli ölçüde azalmış, süperoksit dismutaz aktivitesinde %38 artış gözlemlenmiştir. Melanoidinlerin heterosiklik aromatik aminler tarafından indüklenen DNA hasarına karşı koruduğu bildirilmiştir. Ayrıca melanoidinlerin in vitro ACE (angiotensin converting enzyme-anjiyotensin dönüştürücü enzim) inhibitör aktivitesi üzerindeki etkileri ile antihipertansif olduğu söylenebilir. Etki mekanizmaları tam olarak bilinmemekle birlikte, ACE inhibitörleri Zn'ye bağımlı bir enzim olduğundan, gıda ve model melanoidinlerin inhibitör aktivitesinin bir kısmının metal şelatlama özelliklerinden gelebileceği düşünülmektedir (Rufián-Henares ve Morales, 2007). Melanoidinler gıdalarda, doymamış yağlar, vitaminler, polifenoller ve flavonoidler gibi fonksiyonel gıda bileşenlerinin oksidasyonunu önleyebilir (Wang ve ark., 2011).

## Antimikrobiyal Etki

Melanoidinlerin antimikrobiyal aktivitesi için iki farklı mekanizma öne sürülmüştür. Melanoidinler, düşük konsantrasyonlarda, kültür ortamında demirin şelatlanmasının aracılık ettiği bakteriyostatik bir etki, yüksek konsantrasyonlarda, dış zardan  $Mg^{2+}$  iyonlarını şelatlayarak hem dış hem de iç zarları bozarak bakterisidal etki gösterirler (Rufián-Henares ve de la Cueva, 2009). Antimikrobiyal aktivite melanoidin konsantrasyonuna, ortamın pH ve sıcaklık seviyelerine, maillard reaksiyonu ürünlerinin moleküler ağırlığına ve yapısına bağlıdır. Gıda melanoidinleri, bu özelliklere bağlı olarak hem Gram-pozitif (*Streptococcus mutans* gibi) hem de Gram-negatif (*Escherichia coli* gibi) bakterilere karşı etki gösterebilmektedir (Morales ve ark., 2012).

Bazı içecek ve yiyecekler, glukoziltransferaz aktivitelerini inhibe ederek dış yüzeylerini *Streptococcus mutans*'a karşı korur. *Streptococcus mutans*'ın dış yüzeyine yapışması, dış çürüğünün başlamasına ve gelişmesine neden olmaktadır. Yapılan bir çalışmada, 6 mg/mL konsantrasyonda kavrulmuş kahve melanoidinlerin yüksek moleküler ağırlıklı fraksiyonu-

nun (>3.5 kDa), yapışmayı önleyici güçlü aktivite (%91), antimikrobiyal aktivite ve *S. mutans* biyo-film oluşumuna karşı inhibe edici (%100) aktivite gösterdiği bildirilmiştir (Stauder ve ark., 2010).

Hem düşük hem de yüksek moleküler ağırlıklı gıda melanoidinleri, bağırsak mikrobiyotasında istenmeyen ve şiddetli ishale neden olabilen Gram negatif bir bakteri olan *Escherichia coli*'ye karşı antimikrobiyal aktivite göstermektedir (Morales ve ark., 2012).

## Antikarsinojenik Etki

Gastrointestinal tümörler, özellikle kolorektal kanserler, insanları etkileyen neoplastik hastalıkların en yaygın türlerinden biridir. Mide, bağırsak kanserlerinin ana nedenleri arasında beslenme alışkanlıkları ve yaşam tarzı faktörleri yer almaktadır. Aşırı protein, yağ ve alkol alımı mide-bağırsak kanseri riskini artırır. Beslenme, mide-bağırsak kanserlerinin başlaması için sadece bir risk faktörü değil, aynı zamanda önleyici de olabilir. Sebzeler, içecekler ve meyveler gibi bazı gıdaların mide ve bağırsak sisteminde koruyucu etkiye sahip olduğu gösterilmiştir (Thanikachalam ve Khan, 2019). Yüksek moleküler ağırlıklı gıda melanoidinlerinin antikarsinojenik etkileri kanıtlanmıştır. Melanoidinler, mide karsinoma hücrelerinin proliferasyonuna müdahale ederek hücre döngüsünün durmasını sağlar, apoptoza neden olarak in vitro insan karsinoma hücrelerinin büyümesini inhibe eder ve ksenobiyotik enzim aktivitesinde Faz I ve Faz II detoksifikasyon enzimlerini uyarır (Tagliazucchi ve Bellesia, 2015). Ksenobiyotik gibi davranan gıda veya ilaçlarda bulunan toksik bileşikler, kanser de dahil olmak üzere birçok hastalıkla önemli ölçüde bağlantılıdır. Ksenobiyotiklerin dokularda birikmesini önlemek için, bu bileşikler idrar yoluyla kolayca atılabilen polar, suda çözünür yapılara dönüştürmek için Faz I ve Faz II detoksifikasyon enzimlerinden oluşan karmaşık bir sistem mevcuttur. Kahve ve ekme melanoidinleri ile yapılan hayvan deneylerinde melanoidinlerin Faz I ve Faz II enzimlerini modüle ettiği gösterilmiştir. Enterositlerde ve böbreklerde Faz II glutatyon S transferaz (GST) aktivitesini artırmıştır (Morales ve ark., 2012; Tagliazucchi ve Bellesia, 2015). Soya sosu ve pişmiş patates melanoidinlerinin kolon ve mide kanseri hücrelerinin in vitro ortamda büyümesini engellemiştir. Ayrıca pişmiş patates melanoidinlerinin kanser hücresi motilitesini azalttığı, apoptotik hücre ölümünü indüklediği ve insülin benzeri büyüme faktörünün uyarıcı etkisini tersine çevirdiği ortaya konulmuştur (Langner ve ark., 2011).

## Melanoidinlerin Diğer Olası Etkileri

Melanoidinler iştahı ve enerji alımını etkileyebilmektedir. Bu etkilerin araştırıldığı bir çalışmada bireylere kahvaltıda 3 g

kahve melanoidini veya 3 g ekme melanoidini ile zenginleştirilmiş ekme veya geleneksel beyaz ekme verilmiştir. Kahve melanoidini ile zenginleştirilmiş ekme tüketen bireylerin enerji alımı %26 oranında azalmıştır. Kahve melanoidinleri, kan şekeri tepe noktasını, insülini, melanosit uyarıcı hormonu, oreksin-A'yı,  $\beta$ -endorfini düşürmüştür. Kahvaltıda tüketilen kahve melanoidinlerinin günlük enerji alımını azalttığı, yemek sonrası glisemi ve diğer biyobelirteçleri modüle ettiği sonucuna varılmıştır (Walker ve ark., 2020). Yüksek moleküler ağırlıklı melanoidin fraksiyonlarının *Helicobacter pylori* kolonizasyonunu baskılayarak enfeksiyonunun önlenmesinde antibiyotik aktivite göstermektedir (Hiramoto ve ark., 2004). Klorojenik asit ve izomerlerine ek olarak, kahve melanoidinlerinin pH 2'de tükürük nitrit ve tiyosiyanat ile reaksiyona girerek mide lümeninde tükürük ve mide sıvılarının karışımını taklit ettiği bildirilmiştir. Melanoidinler mukozal akışı, mukoza oluşumunu ve mide hareketliliğini düzenleyebilen nitrik oksit (NO) üretir (Takahama ve Hirota, 2008).

Gıda melanoidinlerinin sağlığa faydalı özellikleri, prebiyotik, antioksidan, antimikrobiyal, antiinflamatuvar ve antihipertansif aktivite gibi çeşitli aktivitelerle ilişkilendirilmiştir. Bununla birlikte, melanoidinlerin sağlık üzerindeki olumsuz etkilerine ilişkin çok az çalışma yapılmıştır. Melanoidinlerin iyonları bağlama özellikleri, mide-bağırsak sisteminde olumsuz sağlık etkilerine neden olabilir ve iyonların emilimini ve biyoyararlanımını azaltabilir. Maillard reaksiyonu ürünleri açısından zengin bir diyet ile beslenenlerde demir biyoyararlanımının 2.7 kat daha az olduğu belirlenmiştir (García ve ark., 2009). Bu durumun melanoidinler dışında diğer maillard reaksiyonu ürünlerinden kaynaklandığı öne sürülmüştür. Yüksek karboksimetil lizin, diyabet ve kardiyovasküler hastalıkları teşvik ederken, akrilamid tümörleri tetikler. Melanoidinler inatçı moleküllerdir ve mikroorganizmalar tarafından biyolojik olarak parçalanması zordur. Bira fabrikalarının ve içki fabrikalarının endüstriyel atıkları melanoidinler açısından zengindir. Melanoidinler, çevreyi kirletici olarak hareket eder ve nihayetinde dolaylı yoldan insan sağlığını olumsuz etkileyebilir (Sharma ve ark., 2021).

## Sonuç

Melanoidinler maillard reaksiyonunun son aşamasında oluşur. Düşük moleküler ağırlıklı olan melanosakkaritlerin emilimi daha yüksektir ve antioksidan aktivite, enerji alımının düzenlenmesi, faz I ve II enzimlerini modüle edici aktivite gibi yararlı etkilere sahiptir. Yüksek moleküler ağırlıklı melanoproteinler daha çok bağırsakta prebiyotik, antimikrobiyal ve antioksidan aktivite gösterirler. Ayrıca melanoidinlerin saklama sırasında gıdanın kalitesini koruyucu etkisi vardır. Maillard reaksiyonu sırasında oluşan bu bileşiklerin saflaştırılması ve tanımlanmasındaki zorluklar nedeniyle, belirli bir

melanoidinin hangi spesifik etkiye sahip olabileceği konusu net olarak bilinmemektedir. Gıdalardaki melanoidinlerin faydalarını daha iyi anlayabilmek için, bu bileşiklerin kimyasal olarak karakterize edilmesi ve yapıya özgü sağlık etkilerine göre kategorize edilmesi gerekir. Ayrıca, in vivo sağlık etkileri hakkında daha fazla bilgi edinmek için bileşiklerin biyolojik etkileri daha kapsamlı bir şekilde incelenmelidir. Böylelikle, yararlı melanoidinlerin üretimini artırmak için gıda işleme teknolojilerinin gelişimi sağlanabilir. Bu derleme yazıda özetlenen araştırmaların sonuçlarına göre melanoidinlerin biyolojik aktiviteleri nedeniyle gelecekte sağlıklı beslenme ile ilgili yapılacak çalışmalarda daha çok gündeme geleceğine inanılmaktadır.

### Etik Standartlar ile Uyumluluk

**Çıkar çatışması:** Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

**Etik izin:** Araştırma niteliği bakımından etik izne tabii değildir.

**Veri erişilebilirliği:** Makalede açıklanan araştırma için hiçbir veri kullanılmamıştır.

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**Teşekkür:** -

**Açıklama:** -

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