Research Article

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Evaluation of antioxidant, antimicrobial, and bioactive properties and peptide sequence composition of Malatya apricot kernels

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Abstract

BACKGROUND: This study used four different apricot (*Prunus armeniaca*) kernels cultivated in Malatya during two consecutive years. The varieties were Hacihaliloglu, Hasanbey, Kabaasi, and Zerdali. The physicochemical properties of the kernels were determined, and the bioactive content of the kernels was evaluated using kernel hydrolysates prepared using trypsin.

RESULTS: With regard to the physicochemical properties of the kernels, the dry matter ratio and protein content were the highest in the Hacihaliloglu variety; the ash ratio was the highest in the Kabaasi variety, and the free oil ratio was the highest in the Hasanbey variety. The bioactive compound content changed according to kernel variety. Angiotensin-converting enzyme inhibitors activity was found to be the highest in the Hacihaliloglu and Hasanbey varieties, which had the lowest amygdalin content, and Zerdali had the highest amygdalin content. The antioxidant and antimicrobial effects of the kernels varied, with Hasanbey and Kabaasi generally having the highest content in both analyses. Moreover, a concentration of 20 mg mL^{-1} of the hydrolysate was determined to have a destructive effect for the microorganisms used in this study. The storage protein of the kernels, except Hacihaliloglu, was found to be Prunin 1, with the longest matching protein chain in the kernels being R.QQQGGQLMANGLEETFCSLRLK.E.

CONCLUSION: The results suggest that the peptide sequences identified in the kernels could have antihypertensive, antioxidative, and Dipeptidyl peptidase IV (DPP-IV) inhibitory effects. Consequently, apricot kernels show potential for use in the production of functional food products. Of the kernels evaluated in this study, Hacihaliloglu and Hasanbey were deemed the most suitable varieties due to their higher bioactive content and lower amygdalin content.
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Keywords: apricot; *Prunus armeniaca* L.; extraction; optimization; hydrolyzation

INTRODUCTION

Most of the world's apricot production occurs in Türkiye, especially in Malatya, a province of Türkiye. Malatya is strongly associated with apricot production due to its geography and soil characteristics.[1,2]

Apricot exhibits different characteristics in various geographical locations, and these distinct traits are manifested in different apricot varieties.[3] These varieties have similarities and differences. Their kernels also have some differences.[4]

Bioactive peptides are naturally available in many foods. As secondary metabolites, they become active during digestion of food and exhibit various effects and benefits. The differences among them are related to the sequences of amino acids in their structure. Short amino acid chains show greater bioactivity and offer more health benefits.[5] These peptides must be activated in order to exert their effects and there are three ways to achieve this. These methods include hydrolysis, which occurs during digestion of foods, fermentation, and the food's own enzymes. Most studies using hydrolysis to activate bioactive peptides have utilized alcalase, pepsin and trypsin as enzyme types.[2,5]

Antioxidants react by donating electrons to free radicals and by forming bonds with them. Components in foods can exhibit antioxidant activity at different rates. It was reported that the antioxidant activity of the apricot kernel cultivated in the Himalaya region was in the range of 50–200 mg L^{-1} ferrous sulfate,[3]

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whereas others stated that the antioxidant activity of the apricot kernel grown in Egypt was as low as 1 mg iron sulfate.[6] On the other hand, it was determined that apricot kernels cultivated in the same region could have significantly different antioxidant content.[7] The hydrolysis process also has a significant effect on antioxidant effects of foodstuffs,[2] primarily because the type of enzyme used in the hydrolysis process affects the activity. This is due to the presence of peptides with antioxidant effects in the plant's kernels. Most of the antioxidant peptides detected in foods have molecular weights ranging from 500 to 1800 Da.[6]

Several studies have reported bioactive content, including antioxidative, antimicrobial, phenolic content, and Angiotensin-converting enzyme inhibitors (ACE-i) activity in protein hydrolysates from kernels of various fruits.[5,6,8–11] It has also been noted that the bioactive content can vary based on factors such as the type of enzyme used in hydrolyzation, the degree of hydrolysis, the molecular weight of peptides and the amino acid sequence. However, a comprehensive study related to the bioactive content of apricot kernel has not been conducted. In the current study, the kernels of some selected apricot (*Prunus armeniaca* L.) varieties including Hasanbey, Hacihaliloglu, Kabaasi, and Zerdali, were evaluated with regard to their physicochemical properties (moisture, ash, protein, and oil content), total phenolic content, antioxidant activity, amygdalin amount, antimicrobial activity, peptide chain structures, and protein structures, to investigate significant differences that occurred during the 2 year period 2018 and 2019.

MATERIALS AND METHODS

Material

This study used the Hacihaliloglu, Kabaasi, Hasanbey, and Zerdali apricot varieties, harvested from different apricot trees in the garden of Apricot Research Institute Directorate in Malatya, Türkiye, between June and August in 2018 and 2019. Prior to commencing the analysis, the samples were kept in the laboratory at 20 °C. A total of 250 g of the kernel from different trees was weighed, pulverized, and mixed. The resulting mixed apricot kernel powder was used in the subsequent analyses.

Method

Physicochemical analysis of apricot kernels

The total protein, ash, moisture, and oil content of apricot kernels from the different varieties was determined as described in a previous work.[12]

Extraction conditions of proteins

Alkaline extraction and acid precipitation methods were used to extract the proteins in the kernel powders, following a procedure described in a previous work,[5] with some modification. To enhance the extraction yield, parameters including extraction time, extraction temperature, and the solvent were evaluated.

The defatted kernel powder was mixed with water at a ratio of 1:10 (w:v), and then the pH of the solution was adjusted to 9.0. After homogenization, the extraction was performed under the optimized conditions. The optimized conditions used water as a solvent, and maintained a temperature of 40 °C and an extraction time of 3 h. After extraction, the solution was centrifuged (Hettich universal 320 R, Tuttlingen, Germany) at 6500 × *g* at 4 °C for 30 min, and the upper liquid phase was collected in tubes. The pH of the removed solution was adjusted to 4.5. The second centrifugation to separate the precipitate was performed at 8000 × *g* for 30 min at 4 °C. The separated precipitate was mixed with water (1:2; w:v) and its pH was adjusted to 7. The extract was lyophilized at −50 °C for 18 h for further use.

Preparation of crude proteins

The hydrolyzation process was performed according to a method described earlier.[5] It was performed in two stages. In the first stage, optimized parameters were used in the hydrolysis process following the single-factor experiment principle. For this purpose, different enzymes (pepsin, trypsin, chymotrypsin, and alcalase), different enzyme:substrate ratios (1:5, 2:5, 3:5, 1:10, 1:50, and 1:100), and different hydrolysis times (3 and 6 h) were evaluated. The cadmium-ninhydrin method given below was used to determine the best parameters.

In the second stage, the hydrolysis process of the extracts belonging to the different apricot kernel varieties was performed under the optimized conditions. Briefly, the extracts of apricot kernel varieties were mixed with a suitable buffer solution at a 1:1 (w:v) ratio (pH 1.5 for pepsin; pH 8.4 for trypsin, alcalase, and chymotrypsin) while the enzymes were preincubated in a shaking water bath at a suitable temperature (50 °C for alcalase; 37 °C for pepsin, trypsin and chymotrypsin). The hydrolyzation was performed under the optimized conditions, which included a duration of 3 h at 37 °C with trypsin at 3:5 ratio. The hydrolyzation process was stopped at 85 °C for 10 min to inactivate the enzyme. Thereafter, the supernatants were collected and lyophilized at −50 °C for 18 h for further use.

Determination of hydrolysis level by cadmium-ninhydrin method

The analysis was performed to determine the hydrolysis levels at the first and end stages of hydrolyzation as described in a previous work.[13] The data were collected using a spectrophotometer (model uv-1800, Shimadzu, Kyoto, Japan) and the sample with the highest absorbance value at 507 nm was considered to have the highest level of hydrolyzation.

Fractionation of hydrolysates

The method was adapted from Mirzapour *et al.*[5] The lyophilized samples were dissolved at a ratio of 1:1 in phosphate buffer. Fractions in the mixture were separated according to their molecular size by centrifuging with 3 kDa filters (Amicon, Ultra-15 centrifugal filter unit, Darmstadt, Germany) at 3300 × *g* for 30 min at 4 °C.

Angiotensin-converting enzyme inhibitory and antioxidant activity

The ACE-i activity was measured according to a method described in an earlier work.[5] Results were expressed using the following formula:

$$\text{ACE\%inhibition rate} = \frac{(B-A)}{B} \times 100$$

where *A* was the absorbance for the solution with ACE and HHL, and without the sample (Control) and *B* was the absorbance for the solution with ACE, HHL, and the sample.

Three different methods, namely 2,2-azino bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and Ferric ion reducing antioxidant power (FRAP), were used to determine the antioxidant activity of the hydrolysates. The ABTS and DPPH antioxidant activity was assayed by methods described in an earlier work[14] and the results were expressed as mg Trolox equivalent (TE) g^{-1} dm (dry matter).

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The FRAP was performed as described in an earlier study[15] and the results were expressed as μmol FE II g^{-1} dm.

Determination of amygdalin content

The analysis was performed according to a method described in a previous work[16] using reverse-phase high-performance liquid chromatography (RP-HPLC) (Shimadzu) at an isocratic flow rate of 0.8 mL min^{-1} using the mobile phase with the mixture of ultrapure water and acetonitrile (80:20 v/v). The separation was carried out using a Zorbax C18 column (4.6 × 250 mm × 5 μm; Agilent, Santa Clara, CA, USA) at 218 nm in a photo diode array (PDA) detector (Shimadzu). The standard curve of amygdalin was constructed with various concentrations (5–400 mg L^{-1}), resulting in the calibration curve of $y = 14\,824x + 14\,359$, showing good linearity ($R^2 > 0.998$).

Determination of total phenolic content

Total phenolic content (TPC) in the hydrolysates was determined according to a method described in a previous work,[3] using a spectrophotometer (model uv-1800, Shimadzu). The results were expressed as mg gallic acid equivalent (GAE) 100 g^{-1} dm. The standard curve for gallic acid was constructed with a concentrations range of 1–40 mg L^{-1}, resulting in the calibration curve of $y = 0.0183x–0.0018$ ($R^2 = 0.9965$).

Determination of peptide profile by RP-HPLC

The method used by Sulejmani and Hayaloglu[17] was applied using RP-HPLC (Shimadzu). A Phenomenex Jupiter C18 column (250 × 4.6 mm × 5 μm, 300 Å pore size; Phenomenex Co., Torrance, CA, USA) was used. The solvents were prepared as 0.1% (v/v) trifluoroacetic acid (TFA), sequencing grade (Sigma-Aldrich Laborchemikalien GmbH, Taufkirchen, Germany) in deionized HPLC-grade water (Milli-Q system; Waters Corp., France) for solvent A and 0.1% (v/v) TFA in 100% acetonitrile (HPLC grade; Merck, Darmstadt, Germany) for solvent B at a flow rate of 0.75 mL min^{-1}. A 10 mg aliquot of freeze-dried water-soluble fraction of the sample was dissolved in solvent A (10 mg mL^{-1}) and filtered through a 0.45 μm cellulose acetate filter; an aliquot (80 μL) of filtrate was then injected into the column. The samples were eluted with 100% A for 10 min, then from 0% to 50% B and 50% to 60% B for 80 and 5 min, respectively. The elute was monitored at 214 nm.

Determination of protein structures and peptide chain structures

Protein structures in apricot kernel hydrolysates were identified according to the method described by Ghorab *et al.*[18] and peptide sequences in the hydrolysates were determined using a method described in an earlier study.[19] The peptide mass fingerprints were generated using a Matrix-assisted lazer desorption ionization time of flight mass spectrometry (MALDI-ToF-MS) system (Bruker, Billerica, MA, USA). Peptide sequences of the most abundant ions ($P < 0.05$) were determined from the spots obtained in MS/MS with respect to the MASCOT evaluation. In brief, the lyophilized sample was dissolved at the ratio of 1:10 (sample: matrix) in 1 mL of water containing 0.1% TFA. The matrix was saturated alpha-cyano-4-hydroxy cinnamic acid (HCCA). The mixture was dried after it was dropped (1 μL) onto the plates; thereafter, the plate was placed into the MALDI-ToF-MS. Bruker peptide calibration mix 700–3500 Da was utilized for calibration before each analysis using positive ion reflectron mode. The Matrix-assisted laser desorption/ ionization (MALDI) spectra were collected by the accumulation of 10 000 smart beam pulsed laser shots and interpreted using Bruker FlexAnalysis software. The results were evaluated using UniProt Knowledgebase (www.uniprot.org).

Antimicrobial activity

The antimicrobial effects of the hydrolysates from various apricot kernel varieties were determined using disk diffusion and minimum inhibition concentration (MIC) methods. In antimicrobial activity analysis, six different microorganisms including two gram positive (g+), namely *Bacillus cereus* and *Staphylococcus aureus*, two gram negative (g−), *Escherichia coli* and *Salmonella enteritidis*, and two yeast species, namely *Saccharomyces cerevisiae* and *Candida albicans* were utilized.

Disk diffusion method

Bacterial cultures were cultivated at 37 °C for 24 h in test tubes containing Brain Heart Infusion broth (BHI) (Lab M, Lancashire, UK), and yeast cultures were cultivated at 30 °C for 48 h in test tubes with Saboraud Dextrose Broth (SDA) (Lab M) as described in a previous study.[20] The bacterial and yeast cultures were diluted to 10^6 cfu mL^{-1} and 10^5 cfu mL^{-1} respectively.

The BHI and SDA were poured into 90 mm sterile petri dishes to a height of 3 ± 0.5 mm. Then, 250 μL of the culture inoculum was uniformly spread with a glass swab stick on BHI for the bacteria and SDA for the yeasts. Additionally, 20 μL of the sample was absorbed on sterile discs, which were subsequently placed on agar at regular intervals. The petri dishes were incubated at suitable temperature (48 h at 30 °C for yeasts; 24 h at 37 °C for bacteria). As for the positive control, namely antibiotic (oxytetracycline dihydrate, Sigma, USA), it was prepared in sterile distilled water at a concentration of 1 mg mL^{-1}.

Minimum inhibition concentration

The minimum inhibition concentration (MIC) of the extract for each apricot kernel hydrolysate at different concentrations (1000, 500, 250, 125, 62.5, and 31.25) was evaluated according to the method described by Qin *et al.*[14] under the same conditions. Sample solutions were prepared at a concentration of 6 g L^{-1}. Tetracycline, ampicillin and gentamycin were used as positive controls. The MIC was defined as the lowest concentration of the solution that would inhibit the growth of the culture.

Statistical analysis

Statistical analyses were performed using IBM SPSS software, version 29. Results are presented as descriptive statistics (mean, percentage, standard deviation). At a significance level of least $P < 0.05$ was accepted for all analyses using Duncan's multiple range test or Dunnett's C test.

The data were analyzed with Shapiro–Wilk and Kolmogorov–Smirnov tests. Data showing homogenous distribution were evaluated using parametric tests, and non-parametric tests were used for data showing non-homogeneous distribution. Pearson's or Spearman's correlation tests were employed to assess potential correlations between two quantitative variables.[21]

RESULTS

Physicochemical analysis of apricot kernels

The physicochemical properties of apricot kernel varieties are shown in Table 1. These properties significantly changed depending on the variety, with no significant effect observed by year, except for the moisture content of Kabaasi, the ash content of Zerdali, and the protein content of Hacihaliloglu and Zerdali.

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Extraction conditions of proteins

Parameters including solvent type, temperature, and time were optimized using the single factor experiment principle. Initially, the solvent type was determined. Solvents containing less than 75% of water were unable to extract proteins from the kernels. However, the solvents containing 75% of water or more successfully extracted the proteins, with the 100% water solvent extracting significantly higher protein content than other solvents. The 100% water solvent was therefore chosen for extraction. Temperature and time did not affect protein extraction significantly in general but a significant change was observed when using 100% water solvent. The highest protein content was achieved at a temperature of 40 °C and a time of 3 h. Consequently, the parameters of 100% water solvent, 40 °C temperature and 3 h duration were selected for the extraction process.

Preparation of crude proteins

Parameters including enzyme type, time, and enzyme-to-substrate ratio were optimized using the single factor experiment principle based on the result of the cadmium-ninhydrin method by the determination of free amino acid. The highest absorbance was measured using the optimized parameters and trypsin was used as the enzyme. The enzyme-to-substrate ratio was 3:5 and the hydrolyzation time was 3 h.

Determination of ACE-i activity

The ACE-i activity of the fractions (<3 kDa and 3 kDa and >3 kDa) belonging to each apricot kernel variety is shown in Table 2. The ACE-i activity of the fractions did not change significantly by year, except for the fractions from Hacihaliloglu and the fraction below 3 kDa from Zerdali. The variety had a significant effect on the ACE-i activity of the hydrolysates. The highest ACE-i activity was observed in the Hacihaliloglu variety among the fractions less than 3 kDa and for the Hasanbey variety among the 3 kDa and the fractions greater than 3 kDa.

The ACE-i activity was affected by type of fraction, *P*-values for both 2018 and 2019 being less than 0.001 ($P < 0.05$), with the fractions less than 3 kDa fractions having significantly higher ACE-i activity than the fractions that were 3 kDA or greater.

Determination of peptide profile by RP-HPLC

A comparison of peptide profiles from apricot kernel hydrolysates was performed based on the chromatograms obtained through RP-HPLC. These chromatograms are shown in Figs 1 and 2. Visual identification was made with these chromatograms. The chromatograms are quite similar to each other. The short-retention peptides were generally similar across the varieties, a distinctive peak between 7 and 8 min, detected in Zerdali and Hasanbey, was not observed in other varieties. It proves that this particular peptide is only available in these varieties. Most of the peptides were eluted with a retention time of 45–75 min, and the peaks corresponding to the peptides retained for 65 and 75 min were more prominent than the other peaks.

Differences were noted in the peak levels of the varieties. The peaks of the peptides retained at 72 min in Hacihaliloglu and at 69 min in Zerdali were higher than the peaks of other varieties at the same times.

Table 1. Physicochemical properties of apricot kernel varieties

Harvest Year	Apricot kernel type	Moisture, %	Ash, %	Protein, %	Oil, %
Harvested in 2018	Hacihaliloglu	3.39 ± 0,17b	2.46 ± 0,13a	28.80 ± 0,10d	40.78 ± 0,34a
	Hasanbey	2.94 ± 0.05a	2.87 ± 0.07b	26.50 ± 0.10c	50.20 ± 1.75c
	Kabaasi	3.02 ± 0.03a	3.98 ± 0.06c	23.20 ± 0.20a	42.70 ± 1.27a
	Zerdali	2.98 ± 0.20a	3.02 ± 0.12b	24.20 ± 0.10b	47.40 ± 1.43b
Harvested in 2019	Hacihaliloglu	3.47 ± 0,20b	2.53 ± 0,03a	28.40 ± 0.40c	40.99 ± 0.63a
	Hasanbey	3.04 ± 0.12a	2.96 ± 0.11b	26.50 ± 0.10b	50.35 ± 2.67b
	Kabaasi	3.11 ± 0.02a	4.16 ± 0.43c	23.80 ± 0.20a	41.33 ± 2.44a
	Zerdali	3.08 ± 0.16a	3.23 ± 0.05b	24.30 ± 0.10a	47.01 ± 3.27b
t-test by year		0.159	0.297	0.862	0.419

Note: Different letters in the same column for the year variance indicate a significant difference ($P < 0.05$).

Table 2. ACE-I activity of the fractions below 3 and above 3 kDa in the hydrolysates of apricot kernel varieties

	<3 kDa		>3 kDa	
Type of apricot kernel	2018	2019	2018	2019
Hacıhaliloğlu	%85.92 ± 0.1c	%89.50 ± 0.1b	%24.50 ± 0.3a	%29.13 ± 0.20a
Hasanbey	%77.30 ± 6.3b	%83.87 ± 7.5b	%58.50 ± 9.5c	%62.30 ± 10.7b
Kabaaşı	%62.44 ± 3.5a	%66.17 ± 4.4a	%40.40 ± 6.9b	%42.87 ± 7.40a
Zerdali	%80.30 ± 0.8b,c	%73.83 ± 1.9a	%40.20 ± 2.3b	%40.67 ± 6.70a
ANOVA	<0.001	<0.001	<0.001	0.004
t-test by year	0.325		0.309	

Note: Different letters in the same column for the year indicate significant difference among the varieties ($P < 0.05$).

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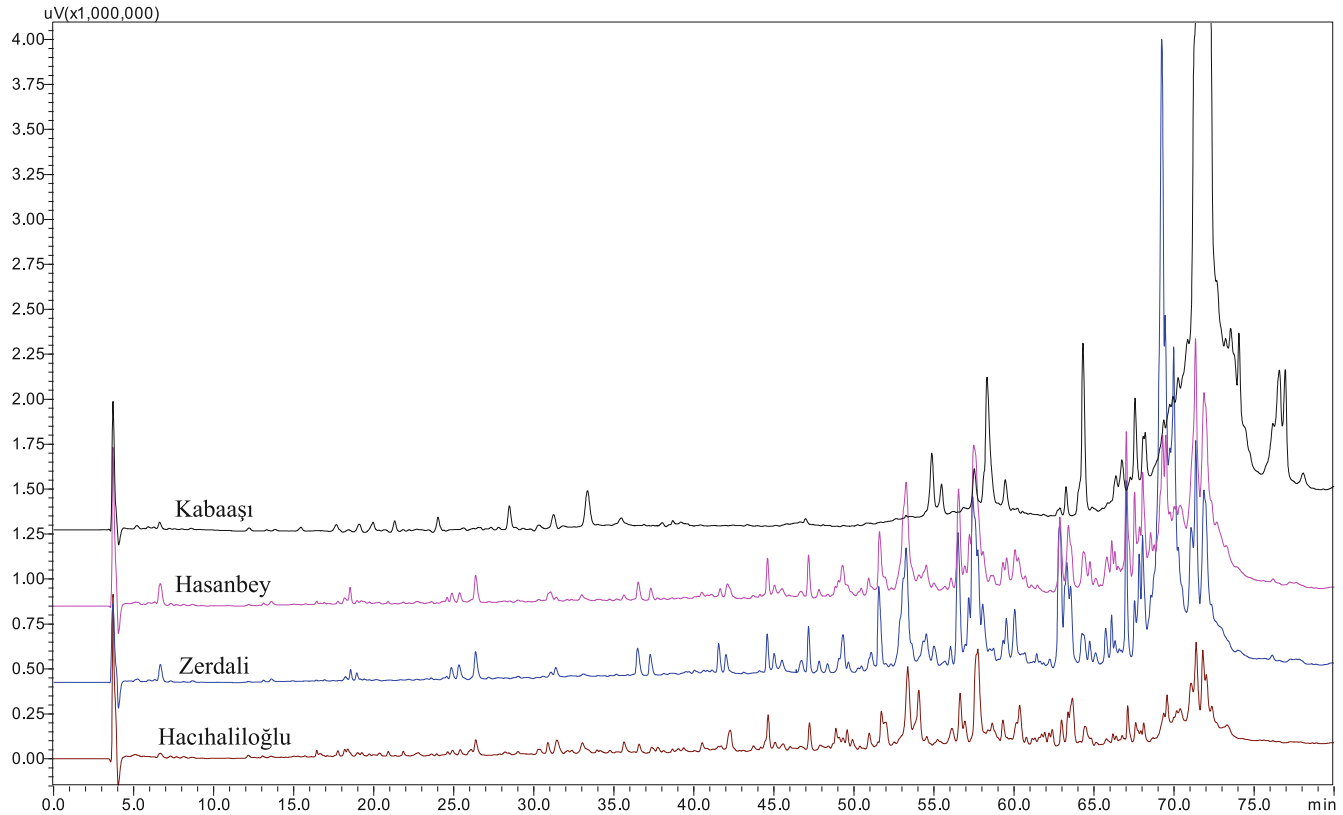


Figure 1. Comparison of peptide profiles of hydrolysates from apricot kernel harvested in 2018.

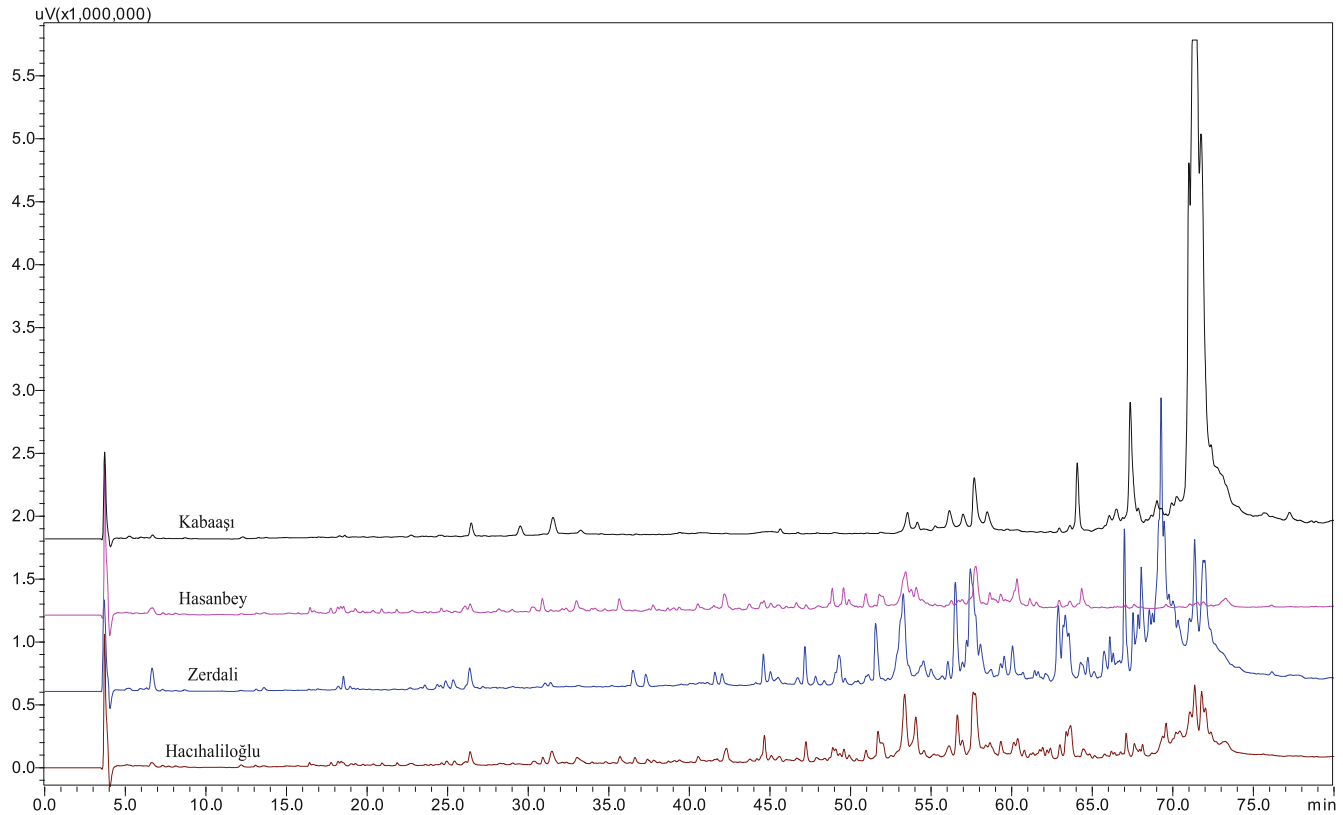


Figure 2. Comparison of peptide profiles of hydrolysates from apricot kernel harvested in 2019.

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Determination of amygdalin amount

The amygdalin contents of the hydrolysates are shown in Table 3. They did not exhibit significantly changes by variety and the year, with Zerdali having the highest amygdalin content in both years (56.33 mg g^{-1} for year 2018; 50.67 mg g^{-1} for year 2019).

Determination of protein structure and peptide chain structure of apricot kernel

The storage protein of the hydrolysates, except for Hacihaliloglu, is Prunin 1. The protein structures detected by MALDI-ToF-MS are presented in Tables 4 and 5. Apricot kernel hydrolysates contain different peptide structures, belonging to the different protein groups. The protein chain structures defined by MALDI-TOF spectrometer are shown in Fig. 3.

Peptides exhibit different physiological roles depending on their peptide chains. In this study, the possible roles of peptide chains detected in apricot kernel hydrolysates are evaluated according to the findings of previous studies. Peptides containing fewer than three amino acid residues do not clearly belong to a single protein.[18] Only peptide chains containing at least three amino acid residues were considered in the evaluation. The longest peptide chain in the samples was R.QQQGGQLMANGLEETFCSLRLK.E. Table 6 shows the peptide chains found in the samples along with their potential functional bioactive aspects. The only short-chain peptide found in apricot kernel hydrolysates was GFFYR.

Antioxidant activity

Optimization for ABTS and DPPH antioxidant activity was performed before the analysis. The times of 15, 30, 45, and 60 min were examined for both analyses. There was not any significant difference in the antioxidant activity by time for either method, ABTS or DPPH ($P > 0.05$).

The antioxidant activity of the hydrolysates changed significantly changed depending on the variety (Table 7), ($P < 0.05$). The year variable did not exhibit a significant effect on the antioxidant activity in the tests using the DPPH and FRAP methods; however, the ABTS antioxidant activity was significantly influenced by the year variable.

Possible correlations between antioxidant activity of the hydrolysates were tested. No correlation was observed between ABTS DPPH ($r = 0.186$) or between ABTS-FRAP ($r = 0.049$) variables. Moreover, a positive and moderately significant correlation between DPPH and FRAP antioxidant activity ($r = 0.671$) was observed.

Total phenolic content

The TPC of the hydrolysates is shown in Table 8. The year did not affect the TPC of the samples significantly, except for the Hasanbey variety, which has a significant influence on the TPC. Notably, TPC of Hasanbey hydrolysate exhibited a significant change by the year ($P < 0.05$).

Possible correlations between antioxidant activity and TPC were investigated. Although there is no correlation between TPC and ABTS ($r = -0.114$), a positive and strong correlation between TPC and FRAP ($r = 0.726$) and TPC and DPPH ($r = 0.595$) was determined.

Antimicrobial activity

Two methods were used for the evaluation of the hydrolysates' antimicrobial activity. These are the disk diffusion method and the minimum inhibition concentration (MIC).

Disk diffusion method

Antimicrobial activity of the hydrolysates is shown in Table 9 and the inhibition diameter (mm) of the antibiotics against microorganisms is given in Table 10. Zerdali and Kabaasi were the most effective varieties and Hacihaliloglu was the least effective variety against the microorganisms used in this study. The antifungal activity of the hydrolysates regardless of the variety was less effective than their antimicrobial activity. The inhibition zone of the sample derived from Kabaasi hydrolysates against *E. coli* was also larger than those of the antibiotics.

Minimum inhibition concentration

The effect of the antibiotics against microorganisms used in this study are shown in Table 11. The MIC values of the hydrolysates were found to be 6 mg mL^{-1} for the bacteria and 3 mg mL^{-1} for the yeast. Neither the variety nor the year variables were found to affect the MIC values of the hydrolysates. The zones of inhibition were consistently generated at concentrations of 20 mg mL^{-1} and above.

DISCUSSION

Physicochemical analysis of apricot kernels

Previous studies examining the physicochemical properties of apricot kernels reported similar results to this study.[29,30] However, Juhaimi *et al.*[29] found that Cataloglu, Hasanbey, Kabaasi, and Sogancioglu varieties had moisture contents of 14.68, 15.38, 16.56, and 14.41%, respectively. Other researchers[30] reported that the Hacihaliloglu variety had a lower oil level than the findings in the present study. The differences among the studies can be attributed to various factors including the growing conditions of

Table 3. Amygdalin content of apricot kernel varieties in 2018 and 2019

	Amygdalin content (mg g^{-1})		
Apricot kernel	2018	2019	Mann–Whitney *U*-test
Hacihaliloglu	1.77 ± 0.03	1.77 ± 0.03	0.683
Hasanbey	1.77 ± 0.04	1.69 ± 0.07	0.121
Kabaasi	1.55 ± 0.12	1.40 ± 0.06	0.121
Zerdali	56.33 ± 7.81	50.67 ± 0.08	0.121
Kruskal–Wallis group	0.106	0.083	
U-test of the years	0.370		

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Table 4. Proteome of apricot kernel hydrolysates cultivated in 2018

Apricot Kernel type	Matching protein	Metabolic function	References
Hacihaliloglu	YCF2 SOLLC	Protein	Buxbaum[22]
	YCF2 SOLBU	Protein	Buxbaum[22]
	YCF2 SOLTU	Protein	Buxbaum[22]
	YCF2B ATRBE	Protein	Buxbaum[22]
	YCF2A ATRBE	Protein	Buxbaum[22]
	YCF2 NICSY	Protein	Buxbaum[22]
	RCA PHAVU	It involves the ATP-dependent carboxylation.	Buxbaum[22]
	RR19 EPIVI	It binds to rRNA	Wang *et al.*[23]
	RIR2 TOBAC	It is necessary for DNA synthesis.	Buxbaum[22]
	UGPA PYRPY	It is responsible for the migration of the substrates (glycerone-3-phosphate) throughout the membrane.	Ghorab *et al.*[18]
Kabaasi	KAD1 SOLTU	It plays an important role in cellular energy homeostasis.	Buxbaum[22]
	MATK MAGFI	It encodes DNA for plant varieties and shows signal transduction properties for various metabolic functions. It also performs important biological functions in mRNA processes, RNA splicing and tRNA processes.	Barthet and Hilu[24]
	MATK MICAL	It is encoded in the trnK tRNA gene intron.	Buxbaum[22]
	PRU01 PRUDU	It is an allergen; it is the main allergen in peach kernel and takes part in plant defense against microbial attacks.	Ghorab *et al.*[18]
	MATK MAGCH	It is encoded in the trnK tRNA gene intron.	Buxbaum[22]
	LGB1 LUPLU	It is formed by plants in response to nitrogen-fixing bacteria called rhizobia when the bacteria colonize in root of the plant.	Luck *et al.*[25]
Zerdali	LONP2 SPIOL	It breaks down proteins in the peroxisome matrix damaged by oxidative stress, thus providing protein recovery.	Luck *et al.*[25]
	YCF2 SOLTU	Protein	Buxbaum[22]
	MATK MAGFI	It encodes DNA for plant varieties and to show signal transduction properties for various metabolic functions. It also performs important biological functions in mRNA processes, RNA splicing, and tRNA processes.	Barthet and Hilu[24]
	YCF2 SOYBN	Protein	Buxbaum[22]
	KAD1 SOLTU	It plays an important role in cellular energy homeostasis.	Buxbaum[22]
	RK14 SORBI	It binds to rRNA	Wang *et al.*[23]
	RIPS PHYAM	It is an antiviral, which inhibits replication of the virus in a host cell and prevents the spread of the virus to the other cells.	Chen *et al.*[7]
	PRU01 PRUDU	It is an allergen; it was the main allergen in peach kernel and takes part in plant defense against microbial attacks.	Chen *et al.*[7]
Hasanbey	PRU01 PRUDU	It is an allergen; it was the main allergen in peach kernel and takes part in plant defense against microbial attacks.	Chen *et al.*[7]
	YCF2 SOLLC	Protein	Buxbaum[22]
	LGB4 MEDSA	It is formed by plants in response to nitrogen-fixing bacteria called rhizobia when the bacteria colonize in root of the plant.	Luck *et al.*[25]
	RBS ZANAE	It has been reported to have an effect against Covid19-Cov.	Luo *et al.*[26]
	KAD1 SOLTU	It plays an important role in cellular energy homeostasis.	Buxbaum[22]

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Table 5. Proteome of apricot kernel hydrolysates cultivated in 2019

Apricot Kernel type	Matching protein	Metabolic function	References
Hacihaliloglu	YCF2 SOYBN	Protein	Buxbaum[22]
	ACT1 MAIZE	It belongs to the actin group, which plays an important role in cytoplasmic flow and has a role in cell shape, cell division, and the movement of organelle.	Ghorab *et al.*[18]
	ADF2 MAIZE	It is one of the essential actin-binding proteins to control actin dynamics in eukaryotes.	Ghorab *et al.*[18]
	PCKA MAIZE	It participates in catalyst activity.	Buxbaum[22]
	LGB1 LUPLU	It is formed by plants in response to nitrogen-fixing bacteria called rhizobia when the bacteria colonize in root of the plant.	Luck *et al.*[25]
	NU5C CALFG	It transmits electrons in NAD(P)H:plastoquinone to quinones in the photosynthetic chain via iron–sulfur (Fe-S) centers.	Ghorab *et al.*[18]
	RK22 CUCSA	It binds to rRNA	Wang *et al.*[23]
	YCF2 DAUCA	Protein	Buxbaum[22]
	YCF2 SOLBU	Protein	Buxbaum[22]
	YCF2 SOLTU	Protein	Buxbaum[22]
Zerdali	YCF2 SOLLC	Protein	Buxbaum[22]
	KAD1 SOLTU	It plays an important role in cellular energy homeostasis.	Buxbaum[22]
	YCF2 SOYBN	Protein	Buxbaum[22]
	RSSA OSTLU	It is needed for processing of the 20S rRNA.	Buxbaum[22]
	GRP HORVU	It binds single-stranded DNA and homoribopolymers of guanine, uracil and adenine. It is also involved in RNA transcription	Wang *et al.*[23]
	MPIM1 PINBN	It has an active role in the plant defense mechanisms.	Buxbaum[22]
Hasanbey	PRU01 PRUDU	It is an allergen, which was the main allergen in peach kernel and takes part in plant defense against microbial attacks.	Chen *et al.*[7]
	EF1G PRUAV	It plays an important role in enzymatic delivery of aminoacyl tRNAs to the ribosome.	Ghorab *et al.*[18]
	LONP2 SPIOL	It breaks down proteins in the peroxisome matrix damaged by oxidative stress, thus providing protein recovery.	Luck *et al.*[25]
	YCF2 SOLLC	Protein	Buxbaum[22]
	COX5C IPOBA	It plays a crucial role in mitochondrial electron transport.	Laxa[27]
	EXB11 MAIZE	It loosens the cell wall, this facilitates penetration of the pollen tube.	Ghorab *et al.*[18]
	PRU01 PRUDU	It is an allergen; it was the main allergen in peach kernel and takes part in plant defense against microbial attacks.	Chen *et al.*[7]

the kernels, climatic effects, the genetic characteristics of the kernel, and soil structure in which the apricot was cultivated.[27]

Preparation of crude proteins

The initial step in the preparation of crude proteins from the kernels was to disrupt the biological tissue to release the protein into the extract. Subsequently, the extract was isolated using a specific enzyme that can cleave target substrates efficiently.[5,9] The reaction conditions were specific to each enzyme for its optimal activity.[9] Extraction and hydrolyzation parameters showed different extraction yields in this study ($P < 0.05$). In agreement with the result of this study, previous studies investigating the optimization of the hydrolysis process for the kernels of different fruits have reported that variations in the optimized parameters showed differences in the extraction and hydrolyzation mechanisms specific to each type of fruit kernel.[8,9] The main reason for

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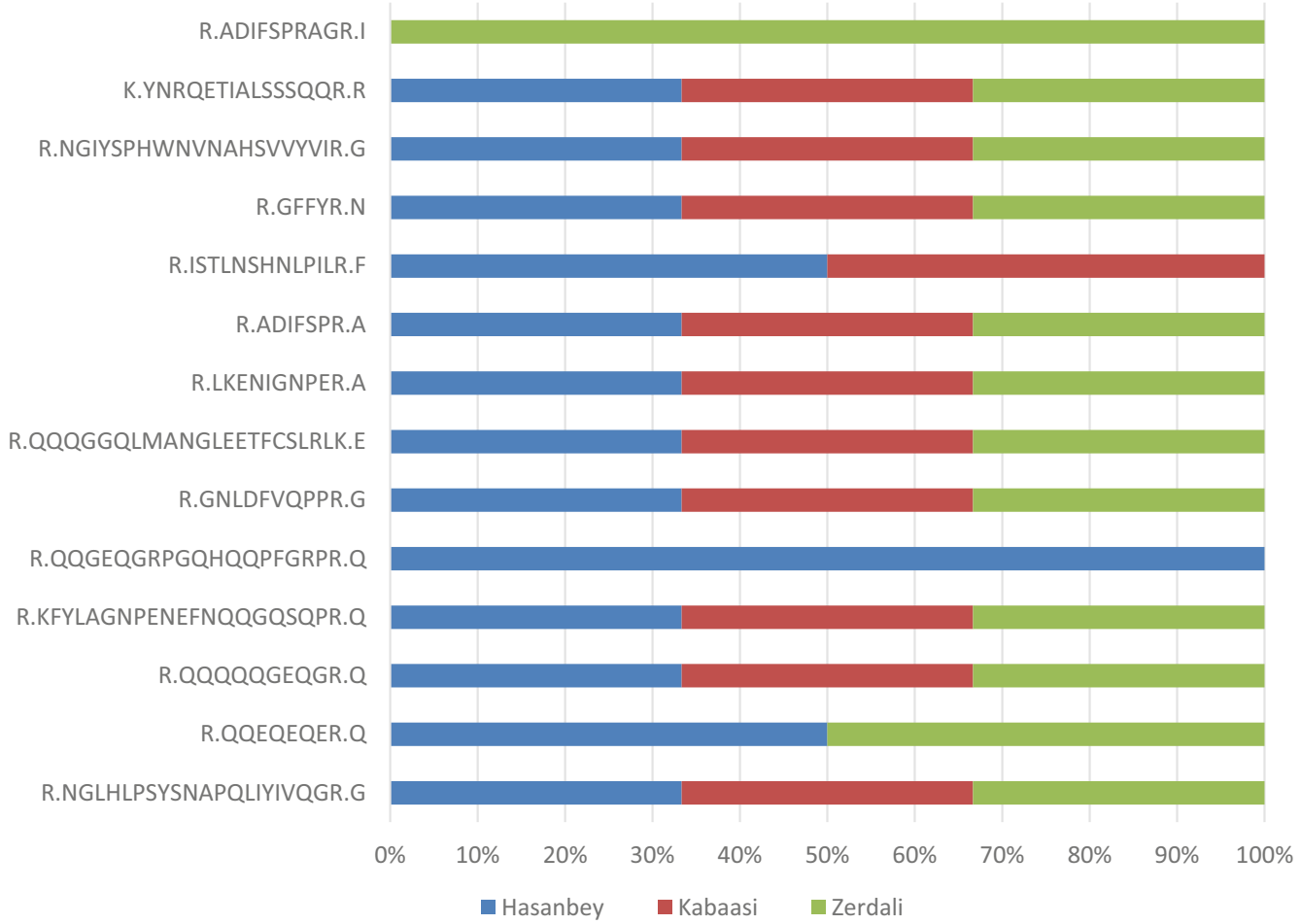


Figure 3. Peptide chain structures defined by MALDI-TOF spectrometer and mascot database ($P < 0.05$).

the change in extraction and hydrolyzation mechanisms is related to mass transfer and solubility of biocompounds.[31] Moreover, the properties of the solvent – the solvent-to-solid ratio, temperature, and duration – can also affect extraction and hydrolyzation yield.[5,32] For solvent used in extraction, based on the law of intermiscibility, solvents with a polarity close to the polarity of the solute better perform. High temperature and time can increase the solubility; however, excessively high temperatures lead to the extraction of undesirable impurities and the extraction yield increases until the equilibrium of the solute is reached inside and outside the solid material.[5,6,12] In addition, extended extraction time increases the probability of oxidation, epimerization and degradation of bioactive compounds. There is no standard method due to the diverse physicochemical properties of bioactive compounds in foodstuffs, and the choice of the best extraction parameters to extract selected bioactive compound with high efficiency is an the most important part of extraction.[9]

Determination of ACE-i activity

The result is in accordance with previous studies that have evaluated the ACE-i activity of various fruit's kernel hydrolysates in literature.[5,8,9,33] The reason for higher ACE-i activity of the fractions with lower molecular weight is that structures with low molecular weight experience less damage to their beneficial biological properties during hydrolysis.[34] The type of enzyme also plays a crucial role in the ACE-i activity of fruit kernel hydrolysates.[35] Similar findings have been observed in other studies using different varieties of fruit kernels.[35–37] Different enzymes cleave the peptide chain from different locations as a result of their activity, leading to the formation of various peptide components with different ACE-i activity.[37]

Reverse phase high-performance liquid chromatography peptide profile

Late eluting peaks in the chromatogram (Figs 1 and 2), occurring between 50 and 70 min, are known to be hydrophobic peptides.[38] The delayed elution of hydrophobic peptides is attributed to the interaction between these peptides and the chromatographic column. Hydrophobic peptides tend to interact more with the column than hydrophilic peptides.[18] In addition, the peak levels of the peptides with retention times of 45, 53, and 63 min in the 2018 apricot kernel hydrolysates were higher than those at the same points in the 2019 apricot kernel hydrolysates. The differences could be attributed to climatic conditions, which can affect composition of bioactive compounds, for example, some peptides.[39] The peak level of the peptides with retention time of 72 min in Hacihaliloglu and 69 min in Zerdali was higher than the peaks of other varieties at the same retention times. This difference may be attributed to the presence of specific amino acids or low molecular weight peptides formed through the action of the trypsin enzyme.[40]

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Table 6. Bioactive peptide chain structures as the result of hydrolysis with trypsin

Peptide sequence	Apricot kernel variety	ACE-I activity	Antioxidant activity	DPP-IV inhibition activity	References
R.NGLHLPSYSNAPQLIYIVQGR.G	Hasanbey, Kabaasi Zerdali	GLHLPS, LHLP, LPSY, LIY, NAPQLIY and APQLIY	LHLPS		Mirzapour *et al.*[5]; Vasquez *et al.*[9]
R.QQQGGQLMANGLEETFCSLRLK.E	Hasanbey, Kabaasi Zerdali	SLR			Huang *et al.*[2]
R.ISTLNSHNLPILR.F	Hasanbey Kabaasi	LPILR	SHNLPILR, NSHNLPILR and HNLPIL	NLPIL	Huang *et al.*[2]; Gonzalez *et al.*[8]; Vasquez *et al.*[9]; Ghorab *et al.*[18]
R.QQGEQGRPGQHQQPFGRPR.Q	Hasanbey	GRP		GRPR and QQGEQGRPGQH	Ghorab *et al.*[18]
R.NGIYSPHWNVNAHSVVYVIR.G	Hasanbey, Kabaasi Zerdali	VVYV and GIYSPH	PHW and IYSPH	NVNAH and SPHW	Ghorab *et al.*[18]; Gonzalez *et al.*[8]; Vasquez *et al.*[9]
R.KFYLAGNPENEFNQQGQSQPR.Q	Hasanbey, Kabaasi Zerdali	YLA	LAGNPENE		Vasquez-Villanueva *et al.*[9]
R.ADIFSPR.A	Hasanbey Zerdali	IFSPR			Gonzalez *et al.*[8]
R.ADIFSPRAGR.I	Kabaasi Zerdali	IFSPR			Gonzalez *et al.*[8]

Levels of amygdalin

Although no studies evaluating the amygdalin content of apricot kernel hydrolysates were found for comparison, previous studies using extracts of apricot kernel varieties have reported that the variety significantly affected the amygdalin content.[16,41,42] It is therefore assumed that hydrolyzation process prevents the variety-originated differences on amygdalin content.

Protein structure and peptide chain structure of apricot kernels

The storage protein of the hydrolysates, except for Hacihaliloglu, was Prunin 1, which has also been identified as storage protein in hydrolysates from the kernels of different fruits.[8,18,43] The protein structures identified in this study were mostly hydrophobic proteins.[18] The protein structures detected by MALDI-ToF-MS are presented in Tables 4 and 5. Apricot kernel hydrolysates contain different protein structures, belonging to the different protein groups.

Peptides exhibit different physiological roles depending on their peptide chains. In this study, the possible roles of peptide chains detected in apricot kernel hydrolysates are evaluated according to the finding of previous studies. Peptides containing less than 3 amino acid residues do not clearly belong to a single protein.[18] Only peptide chains containing at least three amino acid residues were therefore considered in the evaluation. The longest peptide chain in the samples was R.QQQGGQLMANGLEETFCSLRLK.E. Table 6 shows the peptide chains found in the samples along with their potential functional bioactive aspects. The only short-chain peptide found in apricot kernel hydrolysates was GFFYR.

Prunin belongs to cupin family and is related to 11S globulins.[18] The majority of the protein structures in apricot kernel hydrolysates belong to the genus *Prunus*, showing homology with plum, peach, and almond kernels. Furthermore, the protein structures belonging to some plants such as *Oryza sativa*, *Setaria italica*, *Triticum aestivum*, *Vitis vinifera*, and *Arabidopsis thaliana* were also observed in the hydrolysates. Gonzalez-Garcia *et al.*[8] used homologies (*Glycine max*, *Vitis vinifera*, *Zea mays*, and *Populus thichocarpa*) to identify the protein structures of *Prunus* kernel.

Apricot kernel hydrolysates contain different protein structures, belonging to the different protein groups. In the Zerdali and Kabaasi varieties, the proteins (GRP HORVU and MATK MAGFI) are the parts of the GRAS protein and the TFIIB/ zinc finger protein gene families. In Hacihaliloglu and Hasanbey, the proteins (NU5C CALFG, EF1G PRUAV, UGPA PYRPY, and COX5C IPOBA) belong to the ABC transporter gene family. The ABC transporter gene family, which is the largest protein family found in all living organisms, is responsible for transporting a wide variety of substrates between the mitochondrial outer membrane and the cytosol.[22] The ABC transporter family shows hydrolytic activity and performs various functions, including DNA repair and RNA translocation.[18] Moreover, the proteins (ACT1 MAIZE, ADF2 MAIZE, EXB11 MAIZE) in Hacihaliloglu and Hasanbey varieties related the cell wall were identified.

Some protein structures that are involved in the cell defense mechanisms were identified. Antimicrobial proteins (RIPS PHYAM, PRU01 PRUDU, RBS ZANAE, MPIM1 PINBN) were detected in Zerdali, Kabaasi, and Hasanbey. The RIP gene family, to which these proteins belong, enzymatically and irreversibly damage the ribosome.[7] Musarra-Pizzo *et al.*[11] found that the proteins belonging to the RIP gene family in peanuts displayed their activity at a concentration of 0.4–0.8 mg mL^{-1}. Stress proteins (LGB1 LUPLU,

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Table 7. Antioxidant activity of hydrolysates of apricot Kernel varieties

	Antioxidant activity					
	ABTS (mg TE/g)		DPPH (mg TE/g)		FRAP (μmol FE/g)	
Kernel variety	2018	2019	2018	2019	2018	2019
Hacihaliloglu	485.5 ± 10a	805.5 ± 15a	204.6 ± 4a	205.3 ± 3a	6.49 ± 0.31c	6.83 ± 0.54c
Hasanbey	565.5 ± 10c	985.5 ± 20c	219.1 ± 1c	219.4 ± 3b	10.94 ± 0.65d	12.92 ± 0.31d
Kabaasi	525.5 ± 20b	915.5 ± 15b	213.2 ± 3b,c	220.1 ± 5b	1.78 ± 0.15a	1.01 ± 0.65a
Zerdali	650.5 ± 25d	1155.5 ± 10d	211.2 ± 3b	209.2 ± 4a	2.29 ± 0.46b	2.63 ± 0.39b
ANOVA	<0.001	<0.001	0.005	0.003	0.018	0.016
t-test by year	<0.001		0.300		0.795	

Note: Different letters in the same column for the year indicate significant difference among the varieties ($P < 0.05$).

Table 8. Total phenolic content (TPC) of hydrolysates of apricot kernel varieties

	Total phenolic content (mg GAE/g)	
Kernel variety	2018	2019
Hacihaliloglu	5.78 ± 0.14a	5.83 ± 0.16a
Hasanbey	8.08 ± 0.38b	7.37 ± 0.29b
Kabaasi	7.48 ± 0.54b	6.93 ± 0.17b
Zerdali	6.27 ± 0.58a	6.33 ± 0.20a
ANOVA	<0.001	<0.001
t-test by year	0.212	

Note: Different letters in the same column for the year indicate significant differences among the varieties ($P < 0.05$).

LGB4 MEDSA, LONP2 SPIOL, MPIM1 PINBN) were also found in all the hydrolysates and these proteins are formed as a part of the cells' defense mechanism.[25] Even though they were described as responding to heat, they are produced in response to various physical and chemical stimuli during plant development and kernel growth.[44] Stress proteins in tomatoes, for example, are known to influence fruit development and ripening.[44]

The PTGS gene family is effective in the prevention of many diseases. The protein structure (COX5C IPOBA) belonging to the PTGS gene family has been identified in Hasanbey. The root symbol 'Cox' describes its function in the respiratory chain complex; hence, it is deemed as target protein structure of inflammatory and painkiller drugs.[28] The PTGS gene family consists of many subunits, and COX5C is the nuclear encoded subunit of the COX gene family and was first found in sweet potato.[45]

Short-chain peptides, which have less than six amino acid residues, make a greater contribution to human health because they reach blood vessels with less activity of protease.[5] Peptides containing the amino acid R (arginine) at the C-terminal are known to show possible ACE-i activity.[18] Thus, it is predicted that the only short-chain peptides in the samples have ACE-i activity.

Peptides that display antioxidant activity generally contain Tyr in their structure and have been reported to possess radical scavenging activity.[8] Some peptides also show their antioxidant activity by chelating metal ions, especially in the presence of both acidic (glutamic acid and aspartic acid) and basic (arginine and lysine) amino acids.[9] Among the peptides determined in the hydrolysates of apricot kernel varieties, there is only one peptide chain, KFYLAGNPENEFNQQGQSQPR, which might have the ability to chelate metal ions. Moreover, some peptides were determined to display antioxidant activity due to histidine (H), which shows antioxidant activity by scavenging free radicals within the chain.[33] The peptide chain ISTLNSHNLPILR is predicted to have antioxidant activity according to the findings,[2,8] even though it lacks an aromatic amino acid residue in the chain.

The chain length is an important parameter for the DPP-IV inhibitory effect of the peptides. Short-chain peptides show greater DPP-IV inhibitory activity.[46] In this study, all peptides that may show DPP-IV inhibitory activity, contained more than six amino acid residues in their structures. Moreover, the peptides displaying DPP-IV activity have Pro residue in the chain.[46] Peptides with potential DPP-IV activity have a Pro residue in their chains, as reported in some studies.[2,5,9,18]

Antioxidant activity

Table 7 shows the antioxidant activity of different apricot kernels used in this study. The genotype of the kernel has a significant effect on the extent of antioxidant activity.[3,6] Previous studies examining the antioxidant activity of apricot kernel hydrolysates reported similar results to those of this study, indicating that antioxidant activity of apricot kernel varieties changed depending on the variety.[3,4,47] Different results were obtained between studies using the same seed types.[1,4] The reason is generally related to growing conditions, climatic conditions, soil structure and solvent type used for the extraction of antioxidative content.[27,48]

The year variable significantly affected the antioxidant activity of the samples in one of the antioxidant activity assays (ABTS antioxidant activity). The variation is attributed to the impact of different soluble flavonoids in the reaction media.[49] In the case of ABTS, all hydrolysates significantly exhibited different antioxidant activity depending on the year but their antioxidant activity remained unchanged with the DPPH method. Regarding the FRAP method, Hasanbey is the only variety that showed a significant change dependent on the year. Specific genetic attributes of Hasanbey are believed to play a crucial role in these differences.[3]

No correlation between ABTS-DPPH and ABTS-FRAP indicates that ABTS antioxidant activity does not change depending on DPPH and FRAP antioxidant activity in the hydrolysates. The ABTS antioxidant activity is measured at neutral pH whereas FRAP and DPPH antioxidant activity are evaluated at an acidic pH.[6] The correlation between DPPH and FRAP antioxidant activity means that the compounds that react with DPPH radical are the same as the compounds that react with the Fe^{+3}-TPTZ complex.[50] Previous

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Table 9. Inhibition diameter (mm) of the hydrolysates of apricot kernel varieties against the microorganisms

Microorganisms	Apricot kernel variety	2018 Inhibition diameter (mm)	2019 Inhibition diameter (mm)
Escherichia coli	Hacıhaliloğlu	6 ± 0.5	6 ± 0.3
	Hasanbey	5 ± 0.7	6 ± 0.5
	Kabaaşı	9 ± 1.4	13 ± 0.6
	Zerdali	8 ± 0.7	8 ± 0.7
Salmonella *enteritidis*	Hacıhaliloğlu	-	5 ± 0.7
	Hasanbey	-	10 ± 0.3
	Kabaaşı	10 ± 0.4	9 ± 0.5
	Zerdali	11 ± 0.6	12 ± 0.5
Bacillus cereus	Hacıhaliloğlu	-	-
	Hasanbey	-	-
	Kabaaşı	9 ± 0.5	8 ± 0.6
	Zerdali	7 ± 1.3	6 ± 0.7
Staphylococcus *aureus*	Hacıhaliloğlu	-	-
	Hasanbey	11 ± 1	9 ± 0.5
	Kabaaşı	10 ± 0.3	9 ± 0.5
	Zerdali	18	13 ± 2.5
Candida albicans	Hacıhaliloğlu	10 ± 1.5	-
	Hasanbey	-	-
	Kabaaşı	7 ± 0.7	-
	Zerdali	-	-
S. cerevisiae	Hacıhaliloğlu	8 ± 0.5	9 ± 0.6
	Hasanbey	-	-
	Kabaaşı	-	10 ± 0.4
	Zerdali	5 ± 0.3	-

Table 10. Inhibition diameter (mm) of the antibiotic against microorganisms

			Apricot kernel variety			
Microorganisms		Negative control	Hacıhaliloglu	Hasanbey	Kabaasi	Zerdali
Gram (+)	*S. aureus*	+	28	24	30	28
	B. cereus	+	27	30	30	20
Gram (–)	*E. coli*	+	10	15	12	12
	Salmonella	+	20	20	20	20
Yeast	*C. albicans*	+				
	S. cereviciae	+				

Table 11. The effect of the antibiotics against the microorganisms used in this study

			Positive control		
Microorganisms		Negative control	Tetracycline	Ampicillin	Gentamycin
Gram (+)	*S. aureus*	+	1.56	3.12	1.56
	B. cereus	+	1.56	6.25	1.56
Gram (–)	*E. coli*	+	1.56	12.50	6.25
	Salmonella	+	1.56	6.25	3.12
Maya	*C. albicans*	+			
	S. cereviciae	+			

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studies examining the relationship between DPPH-FRAP antioxidant activity of fruit kernels also reported a positive and strong correlation.[3,47]

Total phenolic content

A similar result of this study was also reported by others,[10] which stated that the year variable significantly changed TPC of the hydrolysates from Chilean *Araucaria araucana* kernel. The year variance did not change the TPC of the Hasanbey hydrolysate, meaning genotype plays an important role in the bioactive content of fruit kernels.[3] Moreover, previous studies, which are consistent with the result of this study, mentioned that the TPC of apricot kernel hydrolysates[6] and extracts[3] significantly changed according to the variety. These differences can also be attributed to growing conditions, geographical location, and the soil composition in which the fruit kernels are cultivated.[27] The type of solvent and extraction conditions were also observed to affect TPC of fruit kernels hydrolysates significantly.[1,48] With regard to apricot kernel hydrolysates, the TPC of other fruit kernel hydrolysates differed significantly depending on the variety.[10,49] Moreover, the correlations between TPC-DPPH and TPC-FRAP mean that phenolics are the main components responsible for the DPPH and FRAP antioxidant activity observed in the hydrolysates. The result of the present study agrees with a previous study,[3] which observed the same correlations for the extract of apricot kernel varieties.

Antimicrobial activity

Disk diffusion method

The finding of this study was consistent with the results from previous studies examining the antimicrobial activity of the extract[48] and hydrolysates[6] of apricot kernel varieties. Yeasts were reported to be more resistant to the extracts or hydrolysates belonging to fruit or parts of fruit than the bacteria.[51] Antimicrobial activity against gram negative bacteria was also higher than that of gram-positive bacteria, which aligns with a similar result reported in previous studies.[1,48] These differences in susceptibility are related to the presence of an outer cell membrane composed of lipopolysaccharides in gram-negative bacteria, making them more resistant to the antibiotics and the substances having antimicrobial effect.[20]

Various factors influence antimicrobial and antifungal activity of the hydrolysates produced from fruits or fruit parts.[1] One of these factors is the solvent type, as polar solvents are more effective in extraction of antimicrobial and antifungal substances from plant sources.[48] In this study, the inhibition zones of the hydrolysates were greater than those reported in previous studies for both extracts[48] and the hydrolysates.[1,6] Polyphenols are also identified as influencing antimicrobial activity.[11] The samples from Kabaasi and Hasanbey varieties, which have higher TPC than other varieties, exhibited larger inhibition zones. Consistent with this result, previous studies have reported a correlation between TPC and antimicrobial activity of apricot kernel extracts.[48] The hydrolyzation process enhances the antimicrobial activity of plant sources.[52] There is a specific concentration at which the effect on all microorganisms is irreversible. In the case of methanol, a concentration of 30 mg mL^{-1} was found to be effective in destroying *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*.[48] However, in this study, 20 mg mL^{-1} of the hydrolysates was observed to have the same destructive effect on these microorganisms.

An inhibition zone is a circular area around the antibiotic spot in which microorganisms do not grow. Typically, plant sources tend to have smaller inhibition zones than antibiotics.[36] However, the inhibition zone of the sample derived from Kabaasi hydrolysates against *E. coli* was larger than those of the antibiotics. There is thus a potential for Kabaasi hydrolysate to be considered as an alternative to antibiotics.

Minimum inhibition concentration

The MIC concentration (<20 mg mL^{-1}) observed in this study inhibited the growth of the microorganisms but did not completely destroy them. Previous studies have indicated different MIC values for extracts and hydrolysates of apricot kernel varieties.[6,48] Several factors play an essential role in these differences. The choice of the proper solvent type is important for extracting with maximum efficiency.[48] For instance, the MIC value for apricot kernel extracts have been reported to range between 2.5 and 5 mg mL^{-1} against *Candida albicans*.[48] Genotype has also been mentioned as a factor influencing the MIC value of plant sources.[1] However, in this study, no difference in MIC value of the hydrolysates was observed. Likewise, the study[48] also reported no difference in the MIC value of apricot kernel extracts.

CONCLUSION

In this study, the kernels of Hasanbey, Zerdali, Hacihaliloglu, and Kabaasi apricot varieties cultivated in Malatya were utilized. The physicochemical properties of the kernels, as well as TPC, antioxidant and antimicrobial activity, peptide sequences, amygdalin content, ACE-i activity and protein structures of the hydrolysates of the kernels were compared with each other with year variance. The peptide sequences identified in the kernels were predicted to have antihypertensive, antioxidative, and DPP-IV inhibitory effect. Sweet apricot kernel varieties are therefore estimated to be more suitable for the production of functional foods. There is a need for scientific studies conducted in the laboratory to determine the impact of incorporating bioactive substances from apricot kernel into functional foods on human health. This is a public health concern.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

CONFLICT OF INTEREST

There is no conflict of interest to report.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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