Turkish Journal of Agriculture and Forestry

Volume 47 | Number 2 Article 7

1-1-2023

Antioxidant, antidiabetic, and antihypertensive effects of peptides from some Quercus species

MUHAMMED YUSUF ÇAĞLAR

MUHAMMET ARICI

Follow this and additional works at: https://journals.tubitak.gov.tr/agriculture

 Part of the Agriculture Commons, and the Forest Sciences Commons

Recommended Citation

ÇAĞLAR, MUHAMMED YUSUF and ARICI, MUHAMMET (2023) "Antioxidant, antidiabetic, and antihypertensive effects of peptides from some Quercus species," *Turkish Journal of Agriculture and Forestry*: Vol. 47: No. 2, Article 7. https://doi.org/10.55730/1300-011X.3078
Available at: https://journals.tubitak.gov.tr/agriculture/vol47/iss2/7

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Agriculture and Forestry by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2023) 47: 196-205
© TÜBİTAK
doi:10.55730/1300-011X.3078

Antioxidant, antidiabetic, and antihypertensive effects of peptides from some *Quercus* species

Muhammed Yusuf ÇAĞLAR[1,2,*], **Muhammet ARICI**[2]

[1]Department of Food Engineering, Faculty of Engineering and Natural Sciences, İstanbul Sabahattin Zaim University, İstanbul, Turkey
[2]Department of Food Engineering, Faculty of Chemical and Metallurgical Engineering, Yıldız Technical University, İstanbul, Turkey

Received: 10.10.2022 • **Accepted/Published Online:** 04.01.2023 • **Final Version:** 06.04.2023

Abstract: In the present study, the antihypertensive, antidiabetic, and antioxidant properties of oak peptides were determined in vitro. For this purpose, samples from most common oak species (*Quercus coccifera*, *Quercus ilex*, and *Quercus cerris*) were collected, the proteins were extracted and the bioactive properties of 48 different peptide fractions were monitored using a fast protein liquid chromatography. The results showed that acorn peptides had no remarkable antioxidant or antihypertensive effects. Comparing the bioactive peptides of all oak species, the peptides of *Q. coccifera* generally had higher DPP-IV inhibition activity than those of *Q. cerris* and *Q. ilex*. The highest DPP-IV inhibition activity was determined in *Q. coccifera* second peptide fraction (50.10%). To sum up, acorn peptides could positively contribute to the human health, and they could be evaluated as functional food ingredients for the prevention of type 2 diabetes.

Key words: Bioactive peptides, antihypertensive, antidiabetic, antioxidative, *Quercus*, oak

1. Introduction

Quercus spp. (*Fagaceae* family) is an important group of evergreen or deciduous trees that mostly grow in the regions with high temperate or tropical climate. The genus *Quercus* consists of approximately 450 species worldwide, often differing in flowering and fruiting dynamics (Tejerina et al., 2011). These species produce a fruit commonly described as an acorn (Vinha et al., 2016). Türkiye is one of the richest regions in the world for oak taxa and also has the world's largest span of them (Yaltırık, 1984). Oak represents approximately 25.9% of the broad-leaved trees in the forests of Türkiye. Therefore, it is a dominant tree in central and eastern Anatolia (Kayacık, 1977).

In traditional medicine, acorns were used as astringents (antidiarrhea) and antidotes (Popović et al., 2013). Acorns contain important macronutrients and proteins, which are one of the most important compounds for human metabolism (Gea-Izquierdo et al., 2008). Bioactive peptides can be present as free or are simply described as the organic substances structured by amino acids along with peptide or amide bonds. Bioactive peptides (BP) are organic substances formed by amino acids joined by covalent bonds known as amide or peptide bonds. Although some BPs exist free in their natural source, the vast majority of known BPs are encrypted in the structure of the parent proteins and are released mainly by enzymatic processes. Bioactive peptides are responsible for health-promoting characteristics of proteins (Gobbetti et al., 2007). In vivo and in vitro studies reported that bioactive peptides are beneficial for the prevention of hypertension, diabetes, hypocholesterolemia, and cancer (Coda et al., 2012).

Diabetes is one of the most common noncommunicable diseases caused by an insufficiency of the pancreas in insulin production or secretion. Because the number of diabetes patients has been increasing in recent years, many researchers have investigated potential treatments for this disease (Agarwal and Gupta, 2016). Today, incretion-based therapies have been developed for the treatment of diabetes via glucose and weight control (Sebokova et al., 2007). These treatments are based on dipeptidyl peptidase-IV (DPP-IV) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists (Sebokova et al., 2007).

ACE (angiotensin I-converting enzyme) is a fundamental compound of the renin- angiotensin system which restrains the blood pressure and provides a water-salt balance in the arteries (Volpe et al., 2002). ACE plays a crucial role as a catalyst in the conversion of the angiotensin I hormone (inactive) that degrades bradykinin, a vasodilator to angiotensin II hormone (active). Angiotensin I-converting enzyme (ACE)

* Correspondence: muhammedyusufcaglar@gmail.com

This work is licensed under a Creative Commons Attribution 4.0 International License.

inhibitory peptides are bioactive peptides that have the potential to provide lower blood pressure (Gülseren et al., 2019).

Antioxidative peptides are known as compounds that preventing the oxidation and formation of free radicals (Gu et al., 2015). Due to the adverse effects of synthetic antioxidants, both academic and industrial researchers have investigated natural antioxidants, which are preferred by consumers because of their bioavailability, low molecular weight, and high activity (Xie et al., 2008).

In the present study, three different defatted acorn meal protein hydrolysates were obtained by trypsin hydrolysis. The antioxidant activity was determined by the superoxide anion retention activity, the antihypertensive effects were examined by the ACE inhibition activity, and the antidiabetic effects were demonstrated by the DPP-IV inhibition activity.

2. Material and methods

2.1. Materials

Quercus cerris and *Quercus coccifera* were collected by hand from the forests in Isparta region, and *Quercus ilex* was collected by hand from the forests in Antalya region in 2019, in Türkiye. All the other chemicals and reagents were obtained from Merck (Merck, Darmstadt, Germany) and Sigma Chemicals (Sigma-Aldrich, Steinheim, Germany).

2.2. Isolation of proteins from vegetable pulp

For the isolation of proteins, the method of Karaca et al. (2011) was used. Ten grams of acorn seeds were extracted for deoiling using hexane for 6 h in a Soxhlet system according to the AOCS method (AOCS, 1993). In this method, deoiled acorn seed was dispersed by mixing with water (1:15 by mass; sample: water). The pH of the medium was adjusted to pH 9.5 using a 1N NaOH solution, and the dispersion was allowed to stand for 1 h at 500 rpm with a magnetic stirrer at room temperature. The mixture was then centrifuged at 5000 × *g* for 30 min. The solute was collected, and the pH of the medium was fixed to 4.5 with 1 N HCl. Thus, isoelectric precipitation was promoted. The precipitated fraction was collected and stored at –20 °C until lyophilization (Stone et al., 2015).

2.3. Proteolytic hydrolysis of isolated proteins

Hydrolysis of the acorn protein was performed by trypsin enzyme. Acorn proteins were prepared in a 100 mM Tris-HCl pH: 8 buffer containing 1% in solution. The enzyme was added to the protein solutions using Eppendorf tubes (1:100 unit for trypsin, enzyme:substrate). The enzymatic process was carried out using a suitable thermomixer at 37 °C 1000 rpm for one night (18 h). In addition, solutions for enzyme inhibition were heated at 95 °C for 5 min. Afterwards, the solutions were put in an ice bath and cooled rapidly. Finally, the samples were centrifuged (30 min, 5000 × *g*) and passed through a 0.45-µm filter (Gülseren and Corredig, 2013).

2.4. Fractionation of peptides

The peptide fraction of the hydrolyzed acorn proteins was performed by the ÄKTA-Pure 25-L1 FPLC chromatography system (USA). The process was carried out on ion exchange columns (HiTrap DEAE FF, Capto-Q, Capto DEAE, Capto-S) and hydrophobic interaction columns (HiTrap Phenyl FF, HiTrap Butyl-S FF, HiTrap Octyl FF). For acorn samples, anion exchange columns were preferred for fractionation because the binding rate of the anion exchange columns was high, the elution was easy, and the resulting fractions did not require pretreatment in activity tests. In addition, according to the experiments, it was decided to inject 30% of the column binding capacity (Caglar et al., 2021).

2.5. DPP-IV inhibitory activity (antidiabetic)

DPP-IV inhibitory activity assays were described in Nongonierma et al. (2018). In this study, samples of a protein hydrolysates fractionated with 100 mM Tris-HCl (pH 8) buffer were used. DPP-IV inhibitor Diprotin A was used as the reference molecule. The sample and substrate (0.2 mM Gly-Pro-pNA) were mixed into an Eppendorf tube of 25 µL. The mixture was allowed to preincubate in a thermomixer at 37 °C for 10 min. At the end of this period, 50 µL of DPP-IV enzyme (0.01 unit/mL) was added, and the reaction was started. The amount of pNA released from the substrate after a 1-h reaction period was measured at 405 nm by spectrophotometer (Shimadzu® BioSpec, Japan). All reagents were prepared in a 100 mM Tris-HCl buffer (pH 8). The negative control was similarly prepared with only substrate and buffer solution. The % inhibition values of the samples were calculated by comparing with the negative control sample.

2.6. Angiotensin-converting enzyme (ACE) inhibitory activity

In vitro ACE-inhibitory activity was performed using the method of Sinha et al. (2007). In this study, using the ODS-3 C18 column, the analysis was performed using HPLC (Shimadzu Corporation International Marketing Division, Tokyo, Japan). In the HPLC system, the liquid chromatography LC-20AD consists of a solvent dispenser module, a SIL-20A HT autosampler, a DGU-20A5R degasser, an SPD-20A UV-VIS detector, and a CTO-10AS VP column furnace. The salt concentrations of peptide fractions previously purified in the 20 mM Tris-HCl buffer were adjusted to contain 0.6M NaCl. The 1.68 mU of 250 ACL ACE and 250 µL of peptide fraction prepared in sodium borate (0.1 M, pH 8.3) buffer were mixed and preincubated in a thermomixer for 5 min at 37 °C 500 rpm. After this, 3.94 mM HHL (15 µL) was added to the mixture, and the reaction was continued for 1 h. At the end

of the time, 1M HCl (500 µL) was added to the mixture to stop the reaction.

The ACE inhibition was monitored at 228 nm by injecting 10 µL of this mixture directly into the column. 0.1% TFA prepared in 50% methanol was used as the mobile phase. The isocratic flow rate was used as 0.6 mL min and 20 mM Tris-HCl + 0.6 M NaCl buffer was used as control. At the control value, HHL is assumed to be 100% HA in the presence of ACE. Fraction samples in which the decrease in HA was investigated were compared with the control sample, and the percentage of inhibition values were calculated. In addition, a captopril inhibitor was used as positive control.

2.7. Superoxide anion retention activity test

In this study, the method performed by Marklund and Marklund (1974) was modified and used. Two milliliters of peptide solutions fractionated in Tris-HCl buffer solution (20 mM, pH 8.3) were mixed with 40 mL of 45 mM pyrogallol (prepared in 10 mM HCl). The absorbance at 420 nm was measured in the spectrophotometer (Shimadzu UV-1280 model, UK) in the first 5 min of the mixing time. Trolox (0.02 mg/mL) and BHT (0.03 mg/mL) were used as positive controls.

2.8. LC/MS-MS analysis

Mass scanning of peptides were performed on the Agilent Infinity 1290 HPLC system (Agilent Technologies, Santa Clara, CA, USA) consisting of a binary pump (G4220A), column compartment (G1316C) and an autosampler (G7167B) coupled with 6470 triple quadrupole mass spectrometry (6470A, Agilent Technologies, Santa Clara, CA, USA) in the range of 100–1500 m/z (amu) for positive polarity modes. The solutions of peptides were placed in the autosampler and directly infused to triple quadrupole mass spectrometer without utilizing an analytical column. During infusion, the composition of eluent was 30% mobile phase A (20 mM ammonium format in water) and 70% mobile phase B (methanol) in a constant flow at 0.2 mL/min. The mass spectrometer settings of the scanning method were as follows: drying gas temperature 350 °C, drying gas flow 10 L/min, nebulizer pressure 40 psi, sheath gas temperature 400 °C, sheath gas flow 10 L/min, and capillary voltage for positive and negative, 3000 V and 2500 V, respectively (Wang et al., 2010).

2.9. Statistical analysis

Statistical analysis was performed using the JMP 16.0 (USA) statistical package program. In the study, single factor (ANOVA) analysis of variance was used to determine the differences between the parameters ($p < 0.01$).

3. Results and discussion

3.1. Inhibitory activity of acorn peptides against DPP-IV

The inhibitory activity of three acorn peptides is given in Table 1. As can be seen in the table, six fractions of CER, 15 fractions of CO, and 10 fractions of M showed antidiabetic effects. Among the peptide fractions, while the CO2 fraction (2nd fraction of *Q. coccifera*) had the highest antidiabetic activity (50.10% ± 0.18), the CE13 fraction (13th fraction of *Q. cerris*) had the lowest antidiabetic activity (0.47% ± 0.07). When the DPP-IV inhibitions of the *Quercus* species peptides were compared, *Q. coccifera* was generally found to have a higher antidiabetic activity than those of *Q. cerris* and *Q. ilex*.

In this study, the peptide sequence of the CO2 fraction was identified as SSLLIIK and NYGR (Figure 1). The probability of these sequences' bioactivity is 41% and 48%, respectively (identified by peptide ranker) (Mooney et al., 2012). The SSLLIIK peptide sequence contained Ser-Leu, Ile-Ile, Leu-Ile dipeptides, and the DPP-IV inhibitory activity of these peptides is mentioned in some studies (Nongonierma and Fitzgerald, 2013; Nongonierma et al., 2013; Lan et al., 2015). The NYGR sequence also contained Asn-Tyr and Tyr-Gly dipeptides. Lan et al. (2015) studied 337 dipeptides to evaluate their DPP-IV inhibitory effect, and they reported that Asn-Tyr and Tyr-Gly dipeptides had DPP-IV inhibitory activity. Considering these results, the antidiabetic effect of the CO2 peptide fraction may depend on these dipeptides.The peptide sequence of CO14 fraction was identified as AMHAVIDR and GGLDFTH. The probabilities of bioactivity of these sequences were 28% and 50%, respectively (identified by peptide ranker) (Mooney et al., 2012). The AMHAVIDR peptide sequence consisted of Ala-Val, Asp- Arg, Met-His, and Val-Ile dipeptides. Some studies reported DPP-IV inhibitory activities with these peptides (Nongonierma and Fitzgerald, 2013; Nongonierma et al., 2013; Lan et al., 2015). The other sequence was found to have Gly-Leu, Gly-Gly and Thr-His dipeptides and similarly had DPP-IV inhibitory activity (Nongonierma et al., 2013; Lan et al., 2015) (Figure 2).

Xu et al. (2018) indicated that the polyphenols of *Quercus liaotungensis* leaves had inhibitory effect on α-glucosidase, protein tyrosine phosphatase 1B and protective effects on normal pancreatic beta cells. Ahmed et al. (2017) examined the α-amylase inhibition effect of 14 extracts of *Q. dilatata*, and they concluded that chloroform extracts were more effective on α-amylase inhibition than ethanol + ethyl acetate, chloroform + methanol, ethanol + ethyl acetate extracts. Lyophilized extracts of *Quercus brantii* was examined for α-glycosidase activity in rat small intestine and polyphenols of *Quercus brantii* showed antidiabetic activity (Dogan et al., 2015). Although many studies regarding the antidiabetic effect of acorn polyphenols have been reported, no study on the antidiabetic properties of bioactive peptides derived from acorn proteins has yet been reported.

Vealvarde-Salcedo et al. (2013) examined the DPP-IV inhibitory effect of amaranth, soybean, black bean,

Table 1. Antidiabetic activity of *Quercus* species peptide fraction.

Fraction of species	Antidiabetic activity (%)	Fraction of species	Antidiabetic activity (%)	Fraction of species	Antidiabetic activity (%)
Diprotein A	46.11 ± 0.98^{A}	Diprotein A	46.11 ± 0.98^{B}	Diprotein A	46.11 ± 0.98^{A}
CER1	10.95 ± 0.22 ^{D}	CO1	25.05 ± 0.22 ^{F}	M1	8.51 ± 0.43 ^{G}
CER2	17.49 ± 0.22 ^{C}	CO2	50.10 ± 0.18 ^{A}	M2	11.35 ± 0.29 ^{F}
CER3	n.d.	CO3	25.31 ± 0.31 ^{F}	M3	n.d.
CER4	n.d.	CO4	32.34 ± 0.24 ^{D}	M4	n.d.
CER5	n.d.	CO5	29.69 ± 0.97 ^{E}	M5	n.d.
CER6	n.d.	CO6	12.08 ± 0.26 ^{J}	M6	n.d.
CER7	n.d.	CO7	6.61 ± 0.42 ^{KL}	M7	7.33 ± 0.30 ^{H}
CER8	n.d.	CO8	19.11 ± 0.38 ^{H}	M8	n.d.
CER9	n.d.	CO9	7.86 ± 0.25 ^{K}	M9	7.09 ± 0.12 ^{H}
CER10	n.d.	CO10	14.32 ± 0.41 ^{I}	M10	14.42 ± 0.31 ^{E}
CER11	1.18 ± 0.19 ^{E}	CO11	21.56 ± 0.27 ^{G}	M11	22.93± 0.57 ^{D}
CER12	10.72 ± 0.32 ^{D}	CO12	22.14 ± 0.22 ^{G}	M12	13.71 ± 0.36 ^{EF}
CER13	0.47 ± 0.07 ^{E}	CO13	33.49 ± 0.42 ^{D}	M13	24.82 ± 0.73 ^{C}
CER14	n.d.	CO14	36.82 ± 0.19 ^{C}	M14	29.08 ± 0.22 ^{A}
CER15	n.d.	CO15	15.21 ± 0.31 ^{I}	M15	25.53 ± 0.10 ^{C}
CER16	20.88 ± 0.38 ^{B}	CO16	n.d.	M16	n.d.

$^{A-D}$: Levels not connected by the same letter are significantly different ($p < 0.01$).
CER1 - CER16: Peptide fraction of *Quercus cerris*, CO1 - CO16: Peptide fraction of *Quercus coccifera*, M1 - M16: Peptide fraction of *Quercus ilex*, ± standard deviation (n = 3).

and wheat and reported that the bioactive peptides of amaranth showed the highest antidiabetic effect which was attributed to globulin peptides and which hinder active dimer formation. In a related study, the DPP-IV inhibitory effects of bioactive peptides of rice bran were tested, and Ile-Pro dipeptide was found to have higher antidiabetic activity than the other peptides (Hatanaka et al., 2012). Thus, vegetable bioactive peptides have potential for the prevention of diabetes.

3.2. Inhibitory activity of acorn peptides against angiotensin I-Converting enzyme

The ACE inhibitory effects of acorn peptides are given in Table 2. The results showed that the majority of acorn peptide fractions did not inhibit ACE, and only four peptide fractions including the *Q. ilex* M4, *Q. cerris* CE7, *Q. cerris* CE8, and *Q. coccifera* CO7 showed ACE inhibition. Out of all peptide fractions, the CE7 fraction had the highest ACE inhibition activity (5.88% ± 0.69) (Figure 3), and the CO7 fraction showed the lowest ACE inhibition (0.01% ± 0.63) (Figure 4) (Table 2) ($p < 0.01$). In the literature, Gulseren et al. (2019) compared the ACE inhibition activity of the untreated hazelnut protein concentrate and trypsin-treated hazelnut protein concentrate, and their activities were 7.6% and 40%, respectively. The ACE inhibition activities of acorn peptides studied in our research were not as high as that of the hazelnuts. This difference might be attributed to the sample matrix and the hydrolyzation time of proteins which was 4 h with trypsin. In another study, Wu and Muir (2008) reported that the ACE inhibitory effects of isoflavones-enriched soybean peptides were not significant, as expected. The ɣ-aminobutyric acid of fermented soy milk was found to be more effective on the reduction of hypertension than its peptides (Tsai et al., 2006).

In another study, Dumandan et al. (2014) studied the ACE inhibition activity of pistachio nuts and determined the trypsin enzyme to be 70.83% at 24 h. Compared to the present study, this difference might originate from enzyme treatment time and difference in food. The ACE inhibition activity of peptides was based on short peptide sequences (Hernández-Ledesma et al., 2011). Many bioactive peptides were isolated and identified for the ACE inhibition activity of protein in plants, microorganisms, and animals, and the positive effect on

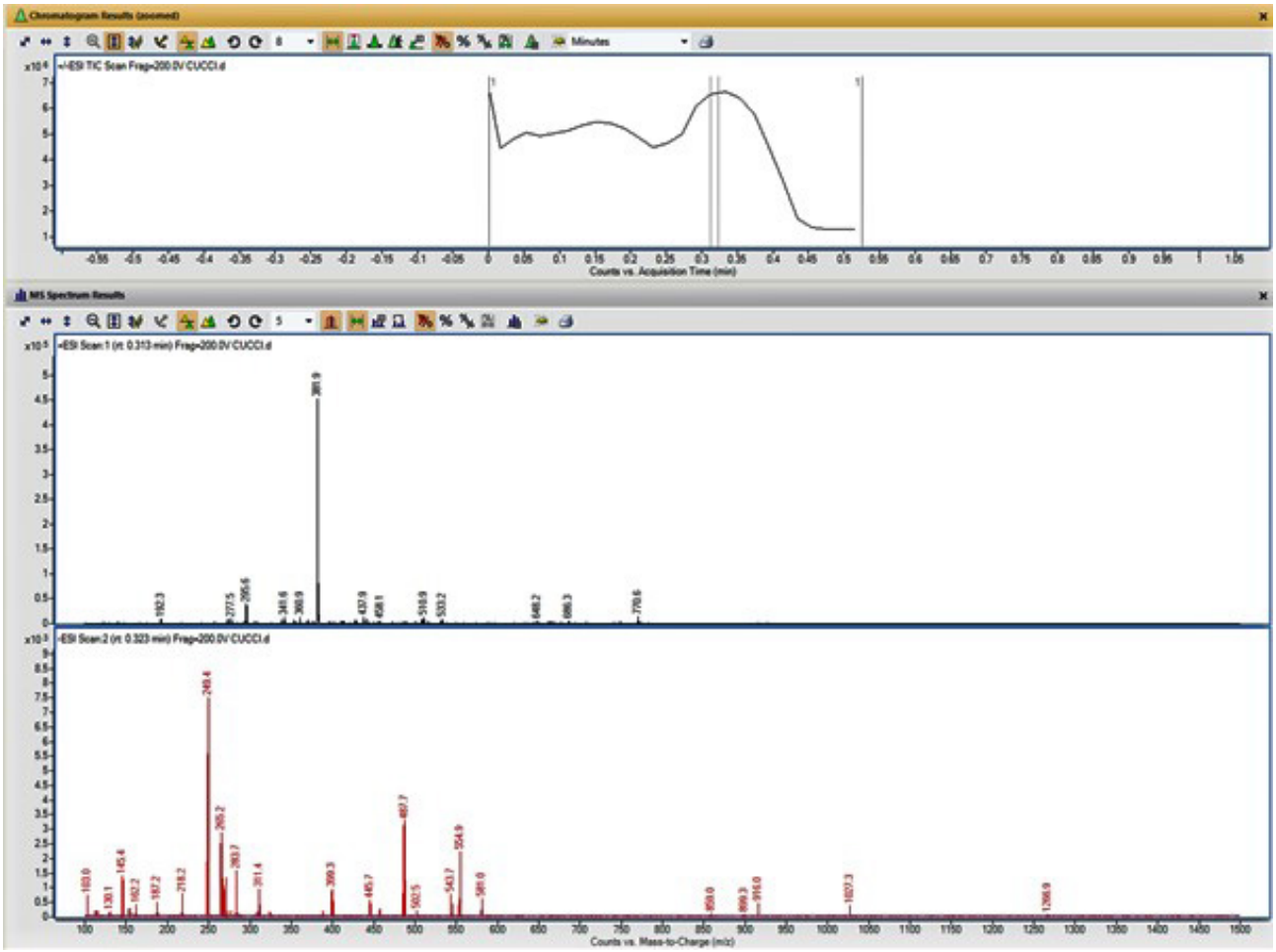


Figure 1. LC/MSMS chromatogram of CO2 peptide fraction.

hypertension was indicated in both animal and human studies (Bhat et al., 2017; Bougle and Bouhallab, 2017). Overall, all the studies performed on the ACE inhibition effects of different plant peptides were found to be higher than that of our study.

3.3. Antioxidant activity of acorn peptides

The antioxidant activity of acorn peptides is shown in Table 3 where it may be seen that four out of 48 acorn peptide fractions showed antioxidant activity; these were M1, M2, M3, and M4 fractions of the *Q. ilex* species. Among the all-peptide fractions, while the M2 fraction showed the highest antioxidant activity (13.33% ± 0.31), the M4 fraction showed the lowest antioxidant activity (1.75% ± 0.07) (Table 3) ($p < 0.01$). Although the peptide fraction of the *Q. cerris* and *Q. coccifera* species had antidiabetic and antihypertensive activity, they did not show antioxidant activity. It was estimated that this might be related to the amino acid sequence and peptide structure. Also, the antioxidative effect of peptides was mostly associated with the structure, hydrophobicity, and electron transfer capability (Lemes et al., 2016). Besides the number and types of amino acids in the peptide structure, the locations of these amino acids in the peptide sequence are also important for the antioxidative effect (Arcan and Yemenicioğlu, 2007). In the in silico study conducted by Gülseren (2018) on hazelnut (*Corylus avellana* L.) peptides, antioxidant activity can be observed to some extent and less frequently than other bioactivities (e.g., ACE-inhibitory and DPP-4 inhibitory activities). Of the 138 proteolytic scenarios, antioxidant activity was predicted in only 44 cases (31.9%). Our 48 proteolytic scenarios (16 peptide fractions × 3 *Quercus* species), only four peptide fractions had antioxidant activity (8.33%). Therefore, this difference might be attributed to the enzyme type and food matrix used in the study.

The antioxidant activities of peptides are based on the amino acids they contain (Srivastava, 2013). For example, the antioxidant activities of aromatic amino acids such as tyrosine, phenylalanine and tryptophan, and cysteine

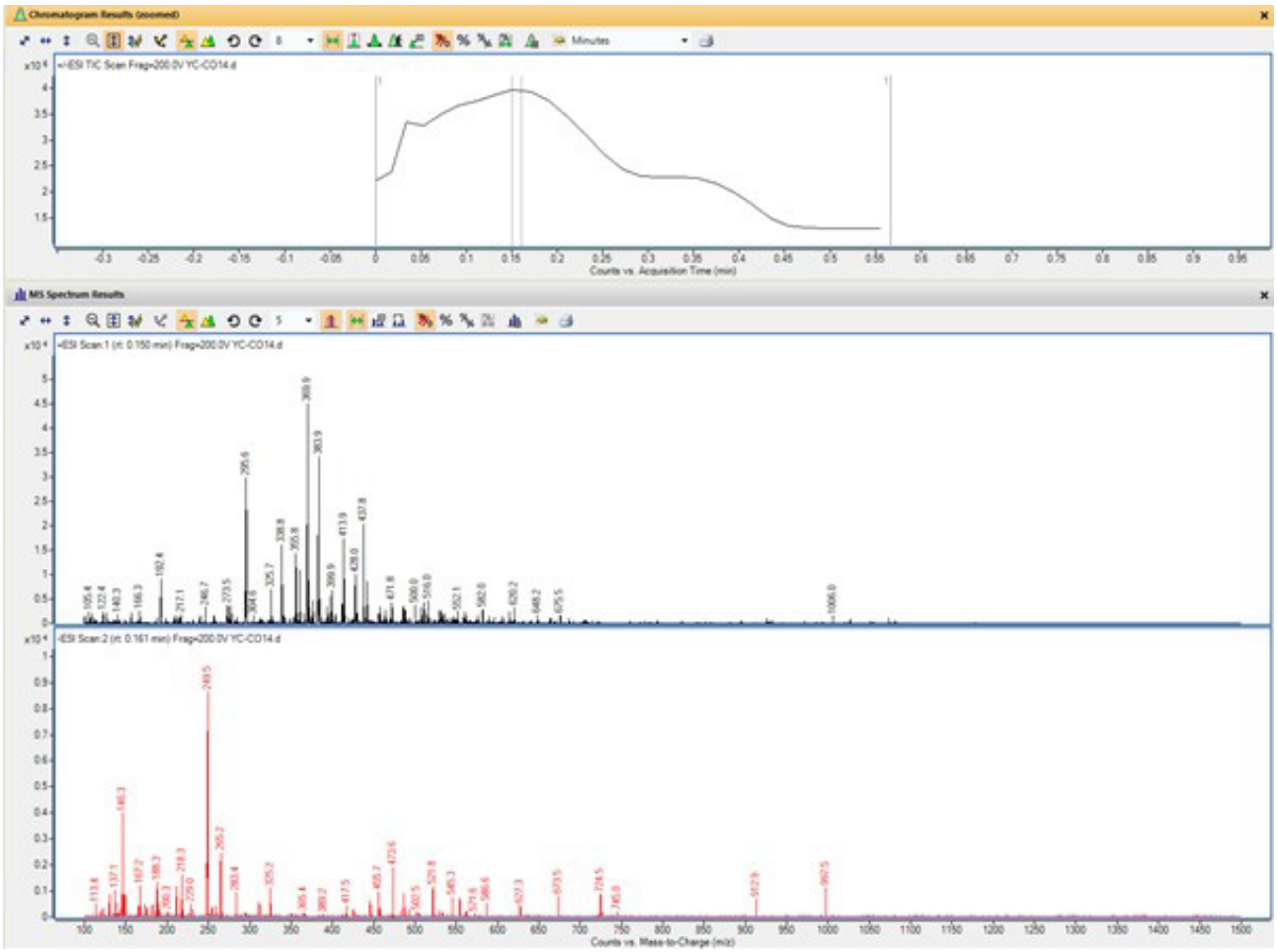


Figure 2. LC/MSMS chromatogram of CO14 peptide fraction.

Table 2. ACE inhibitory effects of *Quercus* species peptide fraction.

Fraction of species	**ACE inhibition (%)**
Captopril	53.23 ± 0.91 [A]
CE7	5.88 ± 0.69 [B]
CE8	4.77 ± 0.49 [C]
CO7	0.01 ± 0.63 [E]
M4	0.06 ± 0.57 [D]

[A-D]: Levels not connected by the same letter are significantly different ($p < 0.01$).
CER: Peptide fraction of *Quercus cerris*, CO: Peptide fraction of *Quercus coccifera*, M: Peptide fraction of *Quercus ilex*, ± standard deviation (n = 3).

are connected to their ability to deliver protons to free radicals (Srivastava, 2013). Basic amino acids such as lysine and arginine and acidic amino acids such as aspartic acid and glutamic acid exhibit antioxidant activity based on the ability to form chelates with metal ions (Chen et al., 1996). The bioactivities and benefits of oak species were indicated by some researchers (Sagdic et al., 2021; Morales, 2022).

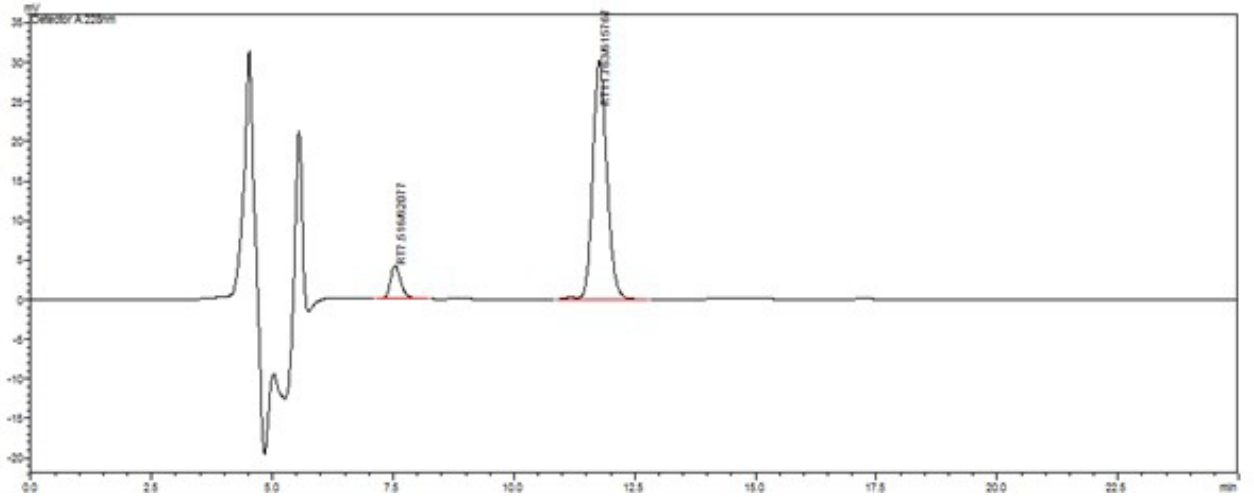


Figure 3. HPLC choromatogram of ACE inhibition activity of CE7 peptide fraction.

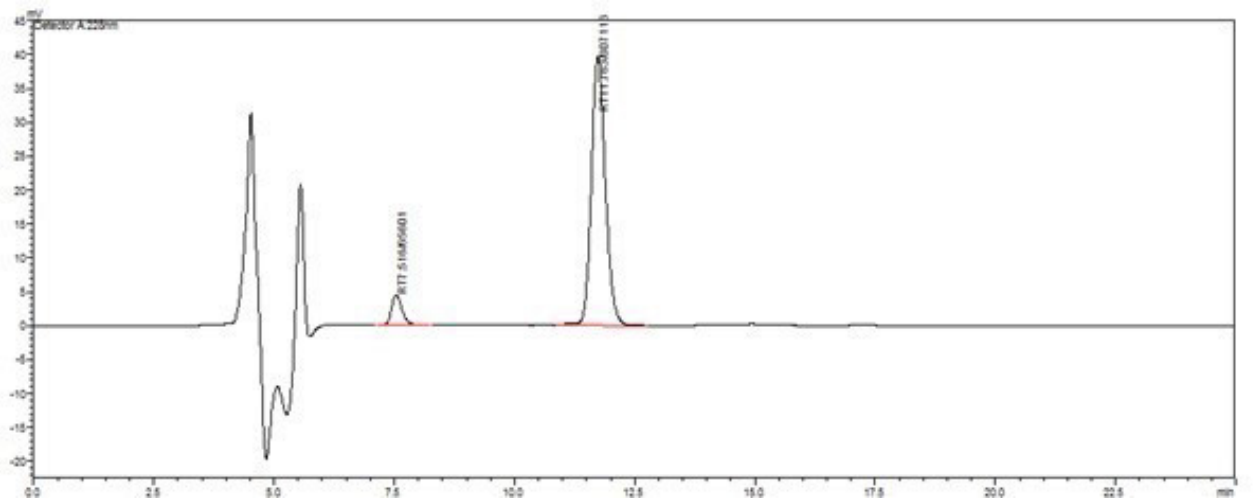


Figure 4. HPLC choromatogram of ACE inhibition activity of CO7 peptide fraction.

Table 3. Antioxidant capacity of *Quercus* species peptide fractions.

Fraction of species	**Superoxide anion retention activity (%)**
BHT	48.20 ± 0.56 ^{A}
M1	10.42 ± 0.03 ^{C}
M2	13.33 ± 0.31 ^{B}
M3	7.79 ± 0.16 ^{D}
M4	1.75 ± 0.07 ^{E}

$^{A-D}$: Levels not connected by the same letter are significantly different ($p < 0.01$).
M: Peptide fraction of *Quercus ilex*, ± standard deviation (n = 3).

4. Conclusion

In this study, the potential bioactivity of peptides obtained by the hydrolysis of *Quercus coccifera*, *Quercus ilex*, and *Quercus cerris* proteins was studied. In the literature, the presence of antioxidant, antidiabetic and antihypertensive properties in *Quercus* polyphenols has been investigated, but this study is the first to investigate the peptides of *Quercus*. In addition to the studies that improve these activities in acorns, new studies can be conducted on different bioactive properties such as antimicrobial and anticancer activities. In the future, these active peptides could be subject to pharmacological studies, or their usefulness as a functional compound in foods that can be consumed uncontrollably could be investigated. In this way, it would be possible to make important contributions to the fight against hypertension and diabetes.

Author contributions

Muhammed Yusuf Çağlar and Muhammet Arici contributed to the planning and conception. Analyses were carried out by Muhammed Yusuf Çağlar. Muhammed Yusuf Çağlar and Muhammet Arici participated in the writing of the manuscript.

Conflict of interest

The authors declare no potential conflicts of interest.

References

Agarwal P, Gupta R (2016). Alpha-amylase inhibition can treat diabetes mellitus. Research and Reviews Journal of Medical and Health Sciences 5 (4): 1-8.

Ahmed M, Fatima H, Qasim M, Gul B (2017). Polarity directed optimization of phytochemical and in vitro biological potential of an indigenous folklore: *Quercus dilatata* Lindl. ex Royle. BMC Complementary Medicine and Therapies 17 (1): 386. https://doi.org/10.1186/s12906-017-1894-x

AOCS (1993) Official methods and recommended practices. American Oil Chemists' Society Champaign.

Arcan I, Yemenicioğlu A (2007). Antioxidant activity of protein extracts from heat-treated or thermally processed chickpeas and white beans. Food Chemistry 103 (2): 301-312. https://doi.org/10.1016/j.foodchem.2006.07.050

Bhat ZF, Kumar S, Bhat HF (2017). Antihypertensive peptides of animal origin: A review. Critical Reviews in Food Science and Nutrition 57 (3): 566-578. https://doi.org/10.1080/10408398.2014.898241

Bougle D, Bouhallab S (2017). Dietary bioactive peptides: Human studies. Critical Reviews in Food Science and Nutrition 57 (2): 335-343. https://doi.org/10.1080/10408398.2013.873766

Bukya A, Vijayakumar, TP (2013). Food Proteomics. In Y. Srivastava (Ed.), Advances in Food Science and Nutrition (pp. 77-96). Oba Ile: Science and Education Development Institute.

Caglar AF, Goksu AG, Cakir B, Gulseren I (2021). Tombul hazelnut (*Corylus avellana* L.) peptides with DPP-IV inhibitory activity: In vitro and in silico studies. Food Chemistry X 12:100151. https://doi.org/10.1016/j.fochx.2021.100151

Chen HM, Muramoto K, Yamauchi F, Nokihara K (1996). Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein. Journal of Agricultural and Food Chemistry 44 (9): 2619-2623. https://doi.org/10.1021/jf950833m

Coda R, Rizzello CG, Pinto D, Gobbetti M (2012). Selected lactic acid bacteria synthesize antioxidant peptides during sourdough fermentation of cereal flours. Applied and Environmental Microbiology 78 (4): 1087-1096. https://doi.org/10.1128/AEM.06837-11

Dogan A, Celik I, Kaya MS (2015). Antidiabetic properties of lyophilized extract of acorn (*Quercus brantii* Lindl.) on experimentally STZ-induced diabetic rats. Journal of Ethnopharmacology 176: 243-251. https://doi.org/10.1016/j.jep.2015.10.034

Dumandan NG, Angelia MRN, Belina-Aldemita MD, Torio MAO (2014). Extraction and characterization of bioactive peptides derived from the hydrolysates of total soluble proteins of pistachio nuts (*Pistacia vera* L.). Kimika 25 (1): 2-10. https://doi.org/10.26534/kimika.v25i1.1-10

Elias RJ, Kellerby SS, Decker EA (2008). Antioxidant activity of proteins and peptides. Critical Reviews in Food Science and Nutrition 48 (5): 430-441. https://doi.org/10.1080/10408390701425615

Gea-Izquierdo G, Cañellas I, Montero G (2006). Acorn production in Spanish holm oak woodlands. Forest Systems 15 (3): 339-354. https://doi.org/10.5424/srf/2006153-00976

Gobbetti M, Minervini F, Rizzello CG (2007). Bioactive peptides in dairy products. In Y. H. Hui (Ed.), Handbook of Food Products Manufacturing (pp. 489-517). https://doi.org/10.1002/9780470113554.ch70

Gu M, Chen HP, Zhao MM, Wang X, Yang B et al. (2015). Identification of antioxidant peptides released from defatted walnut (Juglans Sigillata Dode) meal proteins with pancreatin. LWT-Food Science and Technology 60 (1): 213-220. https://doi.org/10.1016/j.lwt.2014.07.052

Gülseren İ, Çakır B, Çağlar AF (2019). Preliminary investigations in vitro ace-inhibitory activities of tryptic peptides produced from cold press deoiled hazelnut meals. Gıda 44 (2): 309-317. https://doi.org/10.15237/gida.GD18125

Gülseren I, Corredig M (2013). Storage stability and physical characteristics of tea-polyphenol-bearing nanoliposomes prepared with milk fat globule membrane phospholipids. Journal of Agricultural and Food Chemistry 61 (13): 3242-3251. https://doi.org/10.1021/jf3045439

Gupta S, Kapoor P, Chaudhary K, Gautam A, Kumar R et al. (2013). In silico approach for predicting toxicity of peptides and proteins. PloS one, 8 (9). https://doi.org/10.1371/journal.pone.0073957

Hatanaka T, Inoue Y, Arima J, Kumagai Y, Usuki H et al. (2012). Production of dipeptidyl peptidase IV inhibitory peptides from defatted rice bran. Food Chemistry 134 (2): 797-802. https://doi.org/10.1016/j.foodchem.2012.02.183

Hernández-Ledesma B, del Mar Contreras M, Recio I (2011). Antihypertensive peptides: production, bioavailability and incorporation into foods. Advances in Colloid and Interface Science 165 (1): 23-35. https://doi.org/10.1016/j.cis.2010.11.001

Karaca AC, Low N, Nickerson M (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. Food Research International 44 (9): 2742-2750. https://doi.org/10.1016/j.foodres.2011.06.012

Kayacık H (1977). An overview of Turkey oak forest and their thoughts about the future. Forestist, 27 (2): 32–40.

Lan VTT, Ito K, Ohno M, Motoyama T, Ito S et al. (2015). Analyzing a dipeptide library to identify human dipeptidyl peptidase IV inhibitor. Food Chemistry 175: 66-73. https://doi.org/10.1016/j.foodchem.2014.11.131

Lemes AC, Sala L, Ores JDC, Braga ARC, Egea MB et al. (2016). A review of the latest advances in encrypted bioactive peptides from protein-rich waste. International Journal of Molecular Sciences 17 (6): 950. https://doi.org/10.3390/ijms17060950

Marklund S, Marklund G (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry 47 (3): 469-474.

Minkiewicz P, Dziuba J, Iwaniak A, Dziuba M, Darewicz M (2008). BIOPEP database and other programs for processing bioactive peptide sequences. Journal of AOAC International 91 (4): 965-980. https://doi.org/10.1093/jaoac/91.4.965

Mooney C, Haslam NJ, Pollastri G, Shields DC (2012). Towards the improved discovery and design of functional peptides: common features of diverse classes permit generalized prediction of bioactivity. PloS one, 7 (10): 1-12. https://doi.org/10.1371/journal.pone.0045012

Morales D (2021). Oak trees (*Quercus* spp.) as a source of extracts with biological activities: A narrative review. Trends in Food Science & Technology 109 116-125. https://doi.org/10.1016/j.tifs.2021.01.029

Murray BA, FitzGerald RJ (2007). Angiotensin converting enzyme inhibitory peptides derived from food proteins: biochemistry, bioactivity and production. Current Pharmaceutical Design 13 (8): 773-791. https://doi.org/10.2174/138161207780363068

Nongonierma AB, FitzGerald RJ (2013). Dipeptidyl peptidase IV inhibitory and antioxidative properties of milk protein-derived dipeptides and hydrolysates. Peptides 39: 157-163. https://doi.org/10.1016/j.peptides.2012.11.016

Nongonierma AB, Mooney C, Shields DC, FitzGerald RJ (2013). Inhibition of dipeptidyl peptidase IV and xanthine oxidase by amino acids and dipeptides. Food Chemistry 141(1): 644-653. https://doi.org/10.1016/j.foodchem.2013.02.115

Nongonierma AB, Paolella S, Mudgil P, Maqsood S, FitzGerald RJ (2018). Identification of novel dipeptidyl peptidase IV (DPP-IV) inhibitory peptides in camel milk protein hydrolysates. Food Chemistry 244: 340-348. https://doi.org/10.1016/j.foodchem.2017.10.033

Park EY, Murakami H, Mori T, Matsumura Y (2005). Effects of protein and peptide addition on lipid oxidation in powder model system. Journal of Agricultural and Food Chemistry 53 (1): 137-144. https://doi.org/10.1021/jf040221e

Popović BM, Štajner D, Ždero R, Orlović S, Galić Z (2013). Antioxidant characterization of oak extracts combining spectrophotometric assays and chemometrics. The Scientific World Journal 2013:134656. https://doi.org/10.1155/2013/134656

Sagdic O, Polat B, Yetim H (2022). Bioactivities of some wild fruits grown in Turkey. Erwerbs-Obstbau 64 (2) 299-305. https://doi.org/10.1007/s10341-021-00631-0

Sebokova E, Christ AD, Boehringer M, Mizrahi J (2007). Dipeptidyl peptidase IV inhibitors: the next generation of new promising therapies for the management of type 2 diabetes. Current Topics in Medicinal Chemistry 7 (6): 547-555. https://doi.org/10.2174/156802607780091019

Shahidi F, Zhong Y (2008). Bioactive peptides. Journal of AOAC International 91 (4): 914-931.

Sheih IC, Fang TJ, Wu TK (2009). Isolation and characterization of a novel angiotensin I-converting enzyme (ACE) inhibitory peptide from the algae protein waste. Food Chemistry 115 (1): 279-284. https://doi.org/10.1016/j.foodchem.2008.12.019

Sinha R, Radha C, Prakash J, Kaul P (2007). Whey protein hydrolysate: Functional properties, nutritional quality and utilization in beverage formulation. Food Chemistry 101 (4): 1484-1491. https://doi.org/10.1016/j.foodchem.2006.04.021

Stone AK, Karalash A, Tyler RT, Warkentin TD, Nickerson MT (2015). Functional attributes of pea protein isolates prepared using different extraction methods and cultivars. Food Research International 76 (1): 31-38. https://doi.org/10.1016/j.foodres.2014.11.017

Tejerina D, García-Torres S, de Vaca MC, Vázquez F M, Cava R (2011). Acorns (*Quercus rotundifolia* Lam.) and grass as natural sources of antioxidants and fatty acids in the "montanera" feeding of Iberian pig: Intra-and inter-annual variations. Food Chemistry 124 (3): 997-1004. https://doi.org/10.1016/j.foodchem.2010.07.058

Trabuco LG, Lise S, Petsalaki E, Russell RB (2012). PepSite: prediction of peptide-binding sites from protein surfaces. Nucleic Acids Research 40 (1): 423-427. https://doi.org/10.1093/nar/gks398

Tsai JS, Lin YS, Pan BS, Chen TJ (2006). Antihypertensive peptides and γ-aminobutyric acid from prozyme 6 facilitated lactic acid bacteria fermentation of soymilk. Process Biochemistry 41 (6): 1282-1288. https://doi.org/10.1016/j.procbio.2005.12.026

Velarde-Salcedo AJ, Barrera-Pacheco A, Lara-González S, Montero-Morán GM, Díaz-Gois A et al. (2013). In vitro inhibition of dipeptidyl peptidase IV by peptides derived from the hydrolysis of amaranth (*Amaranthus hypochondriacus* L.) proteins. Food Chemistry 136 (2): 758-764. https://doi.org/10.1016/j.foodchem.2012.08.032

Vinha AF, Barreira JC, Costa AS, Oliveira MBP (2016). A new age for *Quercus* spp. fruits: review on nutritional and phytochemical composition and related biological activities of acorns. Comprehensive Reviews in Food Science and Food Safety 15 (6): 947-981. https://doi.org/10.1111/1541-4337.12220

Volpe M, Savoia C, De Paolis P, Ostrowska B, Tarasi D et al. (2002). The renin-angiotensin system as a risk factor and therapeutic target for cardiovascular and renal disease. Journal of the American Society of Nephrology 13 (3): 173-178. https://doi.org/10.1097/01.ASN.0000032549.36050.78

Wang H, Chang-Wong T, Tang HY, Speicher DW (2010). Comparison of extensive protein fractionation and repetitive LC-MS/MS analyses on depth of analysis for complex proteomes. Journal of Proteome Research 9 (2): 1032-1040. https://doi.org/10.1021/pr900927y

Wu J, Muir AD (2008). Isoflavone content and its potential contribution to the antihypertensive activity in soybean angiotensin I converting enzyme inhibitory peptides. Journal of Agricultural and Food Chemistry 56 (21): 9899-9904. https://doi.org/10.1021/jf801181a

Xie Z, Huang J, Xu X, Jin Z (2008). Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. Food Chemistry 111 (2): 370-376. https://doi.org/10.1016/j.foodchem.2008.03.078

Xu J, Cao J, Yue J, Zhang X, Zhao Y (2018). New triterpenoids from acorns of *Quercus liaotungensis* and their inhibitory activity against α-glucosidase, α-amylase and protein-tyrosine phosphatase 1B. Journal of Functional Foods 41: 232-239. https://doi.org/10.1016/j.jff.2017.12.054

Yaltırık F (1984). Turkey oak diagnostic manual. - Innovation printing house, Istanbul.