Food Chemistry: X 12 (2021) 100151

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x

Tombul hazelnut (*Corylus avellana* L.) peptides with DPP-IV inhibitory activity: *In vitro* and *in silico* studies

Ahmet Furkan Çağlar [a], Ayşe Gülden Göksu [a], Bilal Çakır [b,c,*], İbrahim Gülseren [a,c]

[a] Department of Food Engineering, İstanbul S. Zaim University (İZÜ), Halkalı – Küçükçekmece, İstanbul, Turkey
[b] İstanbul S. Zaim University (İZÜ), Halal Food R&D Center, Halkalı, Küçükçekmece, İstanbul, Turkey
[c] İZÜ Food and Agricultural Research Center (GTÜAM), Halkalı Campus, 34303 Küçükçekmece, İstanbul, Turkey

ARTICLE INFO

Keywords:
Hazelnut proteins
Valorization
Bioactive peptides
Antidiabetic activity
Molecular docking

ABSTRACT

Cold press technology generates high quality value-added oil products along with highly stable oilseed cakes. Hazelnut cakes are characterized by high protein concentrations that can be industrially valorized. Here, using an aqueous extraction scheme along with enzymatic proteolysis and FPLC (fast protein liquid chromatography)-based fractionation, a variety of hazelnut peptide fractions with varying bioactive properties were manufactured and their sequences were determined based on mass spectrometry. DPP-IV inhibitory attributes were determined based on an *in vitro* DPP-IV assay and *in silico* techniques were administered for for the analysis of overall bioactive potential and DPP-IV inhibitory characteristics of peptides. Based on these investigations, 256 peptides were identified in 81 different fractions. The majority of fractions were characterized with low to moderate DPP-IV inhibitory activity possibly due to their dilute nature. Some hazelnut peptides were characterized by comparable IC_{50} values as the positive control (Diprotin-A). The most influential 7 peptides were shown to generate higher docking scores than the control. The main interaction mechanism between hazelnut peptides and DPP-IV possibly depended on hydrophobic interactions. While further concentration could enhance the DPP-IV inhibitory potential of hazelnut peptides, hazelnut cakes represent a sustainable resource of potentially antidiabetic peptides.

Introduction

Diabetes is the most common non-communicable disease caused by the inability of the pancreas to produce insulin or the ineffectiveness of the secreted insulin. While the global number of diabetic patients was 108 million in 1980, that increased to 422 million in 2014 and is projected to increase to 642 million by 2040 (Agarwal and Gupta, 2016; Arrutia et al., 2016; Sebokova et al., 2007). Incretin-based therapies that provide glucose and weight control have been developed in the treatment of diabetes. These treatments are based on Glucagon-like peptide-1 (GLP-1) receptor agonists and dipeptidyl peptidase-IV (DPP-IV) inhibitors (Holst and Gromada, 2004).

Dipeptidyl peptidase IV (DPP-IV; EC.3.4.14.5) is a multifunctional transmembrane glycoprotein and serine protease involved in various metabolic processes. The action of DPP-IV inhibitors lead to extended stability of GLP-1 and improved metabolic systems without the risk of hypoglycemia (Singh et al., 2021). Bioactive molecules with such inhibitory activities can potentially be utilized in preventive applications (Singh et al., 2021). Previously, hazelnut consumption was linked to reduction in high-density lipoprotein-cholesterol concentrations in patients with type 2 diabetes (Damavandi et al., 2013). Hazelnut protein hydrolysates were shown to potentially exert anti-diabetic effects *in silico* (Gülseren, 2018; Li et al., 2021) and *in vitro* (Simsek, 2021).

Hazelnut (*Corylus avellana* L.) is a perennial cultivar that grows in bush form and belongs to the genus Corylus of the Betulaceae family (Başaran, 2015). Hazelnuts grow in the temperate zones of the Northern hemisphere from Japan to China, the Caucasus, Turkey, Europe and North America. Currently Turkey generates approximately 70 % of the world hazelnut production based on a 10 year average (approx. 550,000 tons) (TİM, 2018). Hazelnuts are consumed in their natural state, either roasted or blanched, processed into hazelnut paste, flour, and oil, or used as cracked hazelnuts. The industrial utilization for hazelnut

Abbreviations: DPP-IV, Dipeptidyl peptidase-IV; FPLC, Fast protein liquid chromatography; IC_{50}, Half maximal inhibitory concentration; LC-Q-TOF/MS, Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry.

* Corresponding author at: İstanbul S. Zaim University (İZÜ), Halal Food R&D Center, Halkalı, Küçükçekmece, İstanbul, Turkey.
E-mail address: bilal.cakir@izu.edu.tr (B. Çakır).

https://doi.org/10.1016/j.fochx.2021.100151
Received 15 August 2021; Received in revised form 21 September 2021; Accepted 28 October 2021
Available online 29 October 2021

Table 1
Number of peptides identified in each proteolytic hydrolysate of hazelnut protein isolates.

Protease used	Number of identified peptides
Thermolysin	104
Trypsin	67
Bromelain	49
Papain	20
Pepsin	14
Chymotrypsin	8
Peptides generated by multiple proteases	6

ingredients widely lies in dairy, pastry, bakery, chocolate and confectionery sectors (Başaran, 2015).

Hazelnut cakes are characterized with high concentrations of protein after oil removal. Hazelnut proteins contain all essential amino acids, and their digestibility could account for up to 80–90 % (Özdemir, 1997). Protein concentrates extracted from hazelnut meal demonstrated significant antioxidative activity, iron chelation capacity, angiotensin converting enzyme inhibitory and antiproliferative activities (Aydemir et al., 2014). Liu et al. (2018a, 2018b) identified ACE-inhibitory peptides in Asian hazelnuts (*Corylus heterophylla* Fisch.). In our previous studies, a series of ACE-, DPP-IV inhibitory and antioxidative peptides were predicted to exist in proteolytic digests of hazelnut proteins *in silico* (Gülseren, 2018). Experimental studies conducted by our team have also demonstrated ACE-inhibitory activity of hazelnut peptides (Çağlar et al., 2021). In this study, the potential DPP-IV inhibitory activities of hydrolysate fractions and peptide sequences present therein were investigated. While there is previous literature on *in vitro* DPP-IV inhibitory activities of hazelnut peptides, extensive mass spectrometry data were utilized here to support the *in vitro* findings and using *in silico* methods, both the activity potential of the peptides and their corresponding interactions with DPP-IV were analyzed.

Materials and methods

Materials

Cold press deoiled hazelnut (*Corylus avellana* L., Giresun, Turkey, "tombul" hazelnuts) cakes were provided by a local company (Neva Foods Ltd., İstanbul, Turkey). All chemicals and enzymes were analytical grade and purchased from Sigma Chemical Corp. The product numbers for the enzymes used here include bromelain (B4882), chymomotrypsin (C4129), papain (P4762), pepsin (P6887), thermolysin (P1512), trypsin (T4799), and DPP-IV (D4943).

Manufacture of hazelnut protein isolates

Protein extraction from cold press deoiled hazelnut cakes (approx. 50 % protein as determined by a Dumas analysis technique) was based on the alkaline extraction-isoelectric precipitation (AE-IP) technique described by Coşkun et al. (2019).

Proteolytic hydrolysis

Six different proteolytic enzymes (3 gastrointestinal and 3 non-gastrointestinal) were separately used for enzymatic hydrolysis of hazelnut protein isolates which contained approx. 94.8 ± 2.9 % protein as determined by a Kjeldahl analysis technique (NMKL, 2003). Aqueous dispersions of proteins (20 $mg.mL^{-1}$) were prepared in appropriate buffer solutions. Proteases were added to protein solutions at an enzyme:substrate ratio of 1:100 for trypsin (100 $mmolL^{-1}$ Tris-HCl, pH 8), papain (20 $mmolL^{-1}$ sodium phosphate, pH 7.0), bromelain (30 $mmolL^{-1}$ sodium acetate buffer, pH 4.5), pepsin (distilled water, where pH was set to 2.0 using 1 $molL^{-1}$ HCl) and chymotrypsin (100 $mmolL^{-1}$ Tris-HCl, pH 8), and 1:50 for thermolysin (100 $mmolL^{-1}$ Tris-HCl, pH 8). In order to standardize the treatments, the enzymatic processes were carried out at a mixing rate of 1,000 rpm using a thermomixer. Immediately afterwards, trypsin, chymotrypsin, papain, bromelain and pepsin hydrolysates were heated at 95 °C for 5 min. For the inhibition of thermolysin, 0.5 % formic acid was added to the hydrolysates. The sample tubes containing the hydrolysates were centrifuged at 5,000xg for 30 min to remove the insoluble matter (25 °C). Finally, the supernatant was filtered through PVDF (Polyvinylidene fluoride) syringe filters (0.45 μm) (Millex-HV, Merck-Millipore, Germany) (Gülseren and Corredig, 2013) and the filtered hydrolysates were stored at −80 °C until further use.

Fractionation of hydrolysates

Hydrolyzed hazelnut proteins were fractionated using AKTA-Pure 25-L1 fast protein liquid chromatography (FPLC) system (GE Healthcare Life Sciences, Sweden). HiTrap Capto Q or Capto DEAE anion exchange columns were utilized for fractionation (GE Healthcare). Hydrolysate samples were injected into the column at a rate of 1 CV. min^{-1}. Salt containing 20 or 100 $mmolL^{-1}$ Tris-HCl buffers (0.6 $mmolL^{-1}$

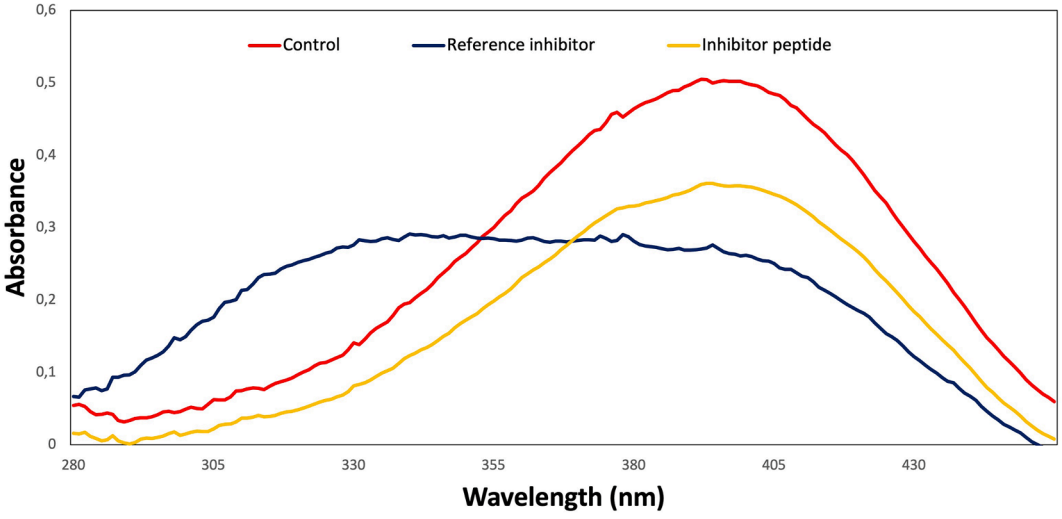


Fig. 1. Absorbance spectrum for DPP-IV inhibition in hazelnut protein hydrolysates. The calculations were based on absorbance values at 405 nm and Diprotin-A was used as the reference inhibitor.

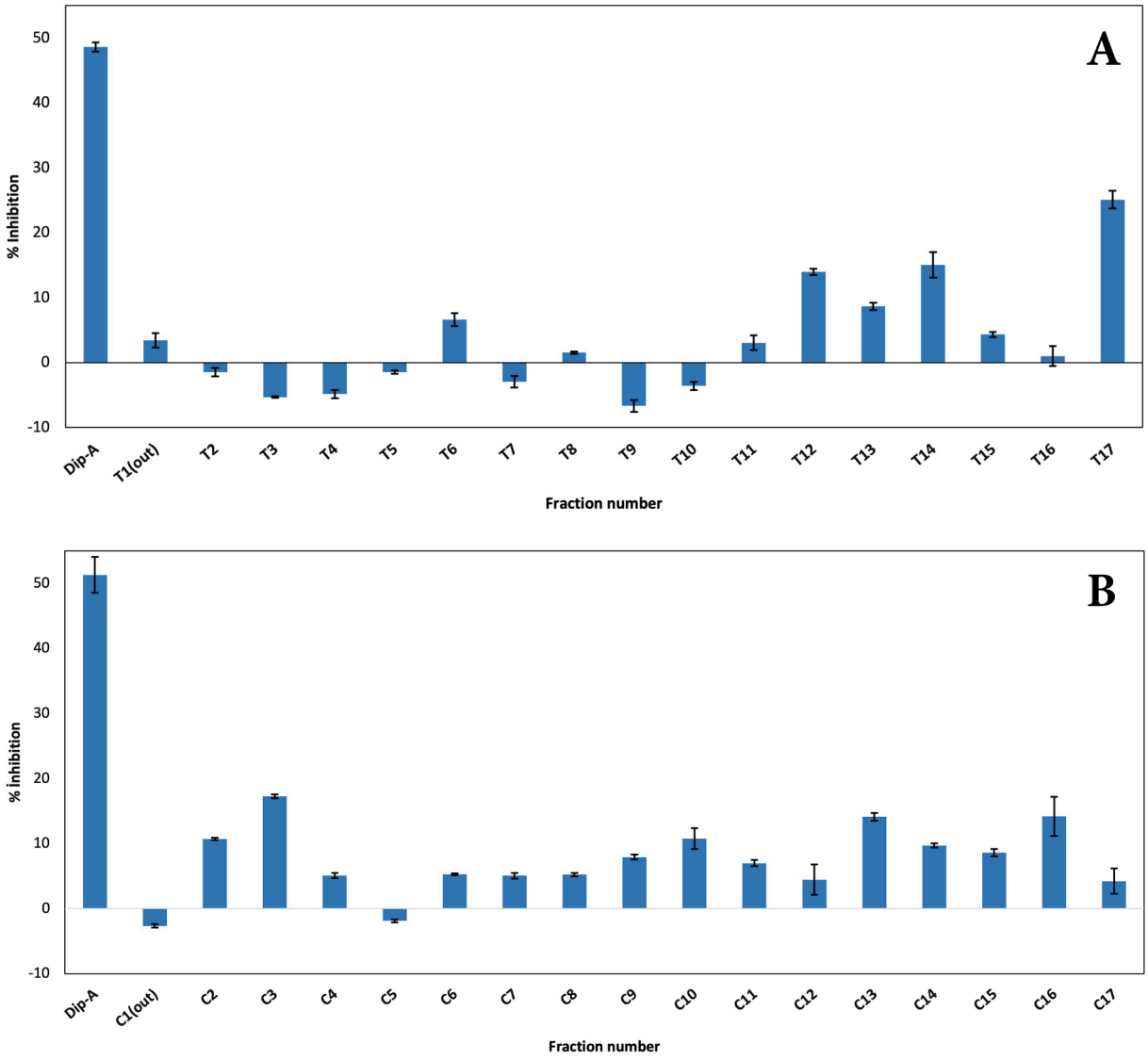


Fig. 2. % DPP-IV inhibitory activities of hazelnut protein hydrolysate fractions (X: fraction number, Y: % inhibition) (A) Trypsin hydrolysate fractions, (B) Chymotrypsin hydrolysate fractions, (C) Thermolysin hydrolysate fractions, (D) Papain hydrolysate fractions, (E) Bromelain hydrolysate fractions, (F) Pepsin hydrolysate fractions. (Dip-A: reference inhibitor).

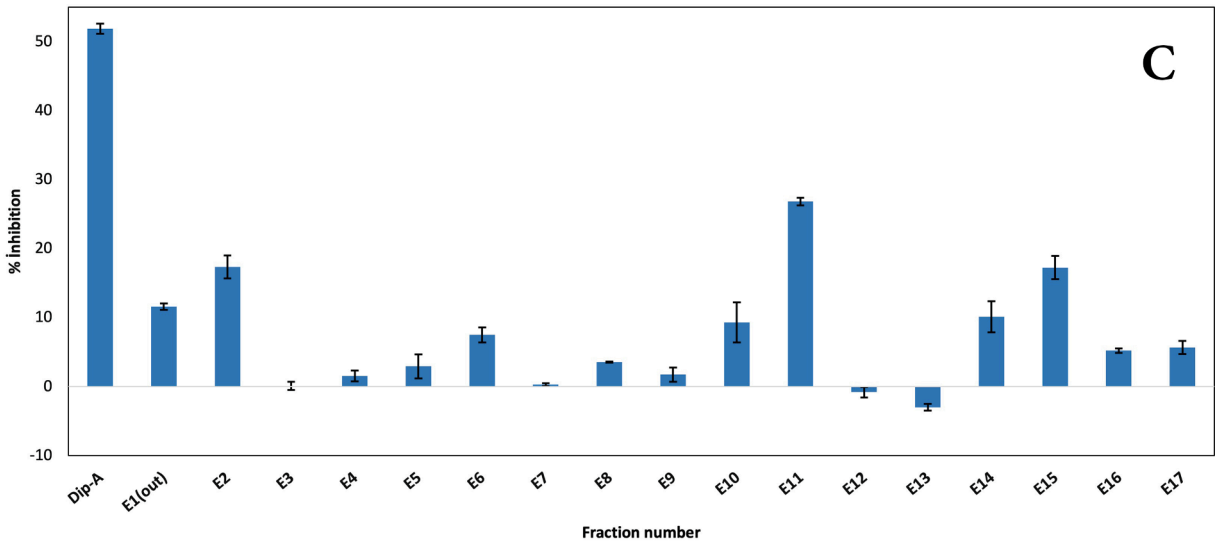
or 0.8 mmolL^{-1} NaCl, set to pH 8) were used for the elution of the column-bound compounds. The elution was carried out utilizing a linear salt gradient over 25 or 32 CV for papain, bromelain and pepsin hydrolysates vs. trypsin, chymotrypsin and thermolysin hydrolysates, respectively, which were fractionated and numbered sequentially. Prior to the experiments, a variety of other ion exchange (HiTrap Capto Q, DEAE FF, Capto DEAE, Capto-S) and hydrophobic interaction (HiTrap Phenyl FF, Butyl-S FF, Octyl FF) columns were tested in pre-trials. Absorbance (280 nm) was measured by means of a UV detector. In addition, medium pH, conductivity, column and system pressure values were also monitored. The collected fractions were stored at −20 °C until further use.

DPP-IV inhibitory activity test

To determine the DPP-IV inhibitory activities of the peptide fractions, the method of Nongonierma et al. (2018) was used with minor revisions. Fifty µl of aliquots from each fraction and 50 µl of 0.8 mmolL^{-1} substrate (Gly-Pro-pNA, G0513) were mixed in mini-centrifuge tubes. This mixture was pre-incubated at 37 °C in a thermomixer (1,000 rpm) for 10 min. Immediately afterwards, 100 µl of DPP-IV (0.01 U.mL^{-1}) was added to the mixture to initiate the reaction. After 1 h, the reaction was stopped by adding 200 µl of 1 molL^{-1} sodium acetate (pH 4.0) buffer. The amount of pNA released from the substrate was measured using a spectrophotometric method (405 nm) (SP-3000 nano, Optima, Japan). Diprotin A (Ile-Pro-Ile, CAS 90614–48-5) was used as a reference inhibitor at a level of 8 µM. Unless otherwise stated, all reagents were prepared in 100 mmolL^{-1} Tris-HCl pH 8 buffer solutions. Percent inhibition values for the fractions were calculated in comparison to negative controls.

Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-TOF/MS) analysis of DPP-IV-inhibitory fractions

All MS analysis was carried out using the default settings of Labmed, Acıbadem University, İstanbul, Turkey, using a Xevo G2-XS QTof (Waters) device. Samples treated with different proteases were incubated with 10 mmolL^{-1} DTT at 55 °C for 10 min to ensure the reduction of the peptides. The reduced peptide mixtures were then alkylated with 20 mmolL^{-1} iodoacetamide (IAA) in the dark at ambient temperature



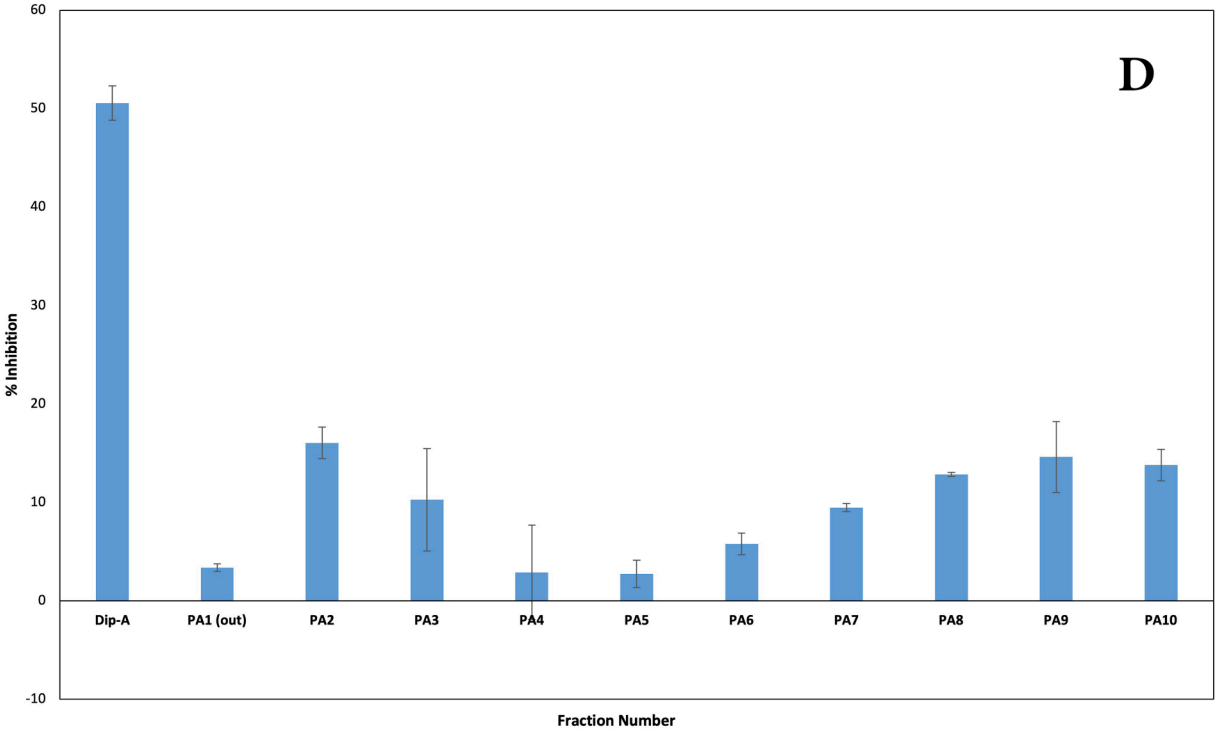


Fig. 2. (*continued*).

conditions. Samples were filtered through 30 kDa filters. Peptide concentration of the samples was determined and the samples were taken into vials for LC-Q-TOF/MS analysis at 1 mg per injection.

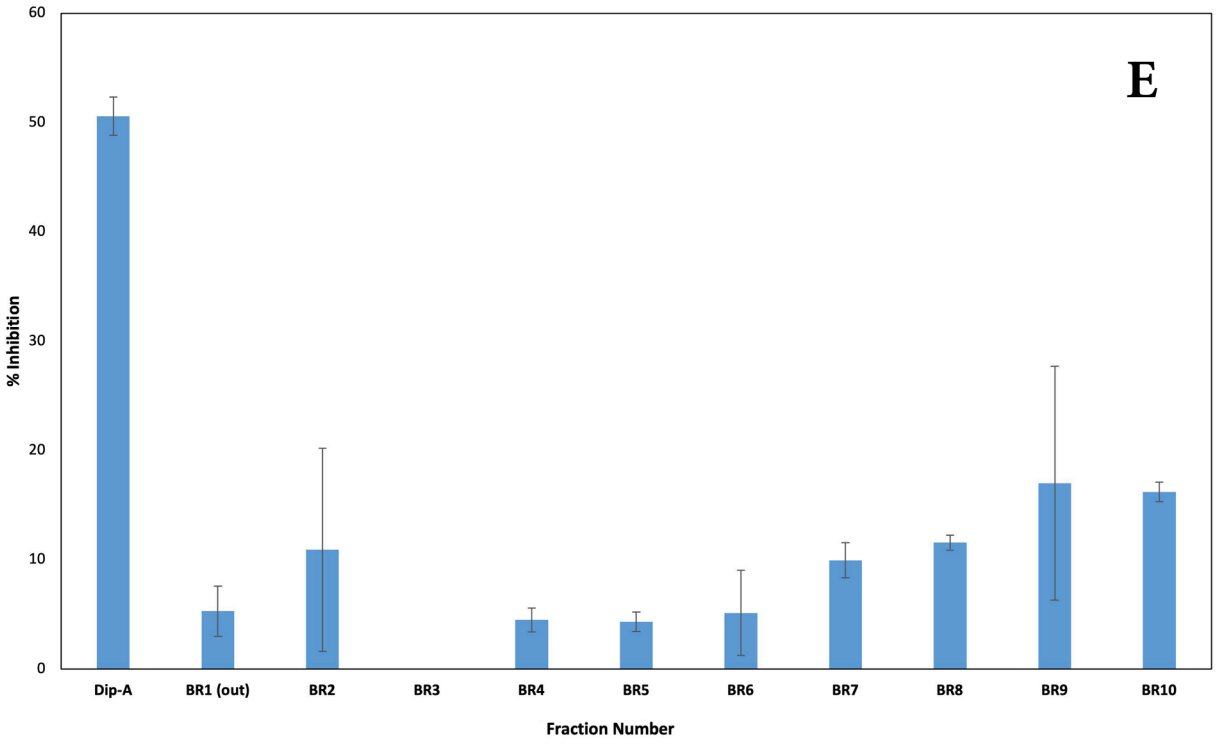
LC separation was based on an HSS T3 Column (1.8 μm, 2.1 × 150 mm), detection wavelength of 214 nm and a column temperature of 60 °C. A flow rate of 0.2 mL.min^{-1} was administered. MS conditions were based on a sample cone voltage of 80 V, source temperature of 120 °C, desolvation temperature of 300 °C and desolvation gas flow rate of 800 L.h^{-1}.

Prior to the analyses, the detector and calibration settings were made via the MassLynx software specific to the Xevo G2-XS QTof device where the analyses were performed. The peptide fractions were further fractionated with an acetonitrile gradient (5–35 %) in an HSS T3 column based on their hydrophobicity and the separated peptides were analyzed by mass spectrometry upon electrospray ionization. MS analysis was performed for 0.7 s and information was collected about the entire peptide. Afterwards, MS/MS analysis was performed for 0.7 *sec* and the peptide fragmentation and sequence information were obtained. For protein identification, appropriate protein databanks were used for each sample.

Peptides and proteins were identified using ProteinLynx Global Server (PLGS 3.0) software. Analysis was performed using the appropriate databank for each sample type. "False positive rate" was set to 1 %. Further details are not presented here to ensure brevity.

In silico analyses

The determined peptide sequences were analyzed *in silico* for their probability of being bioactive (Mooney et al., 2012), and potential DPP-IV inhibitory activities (Minkiewicz et al., 2019). Parameter A, which is



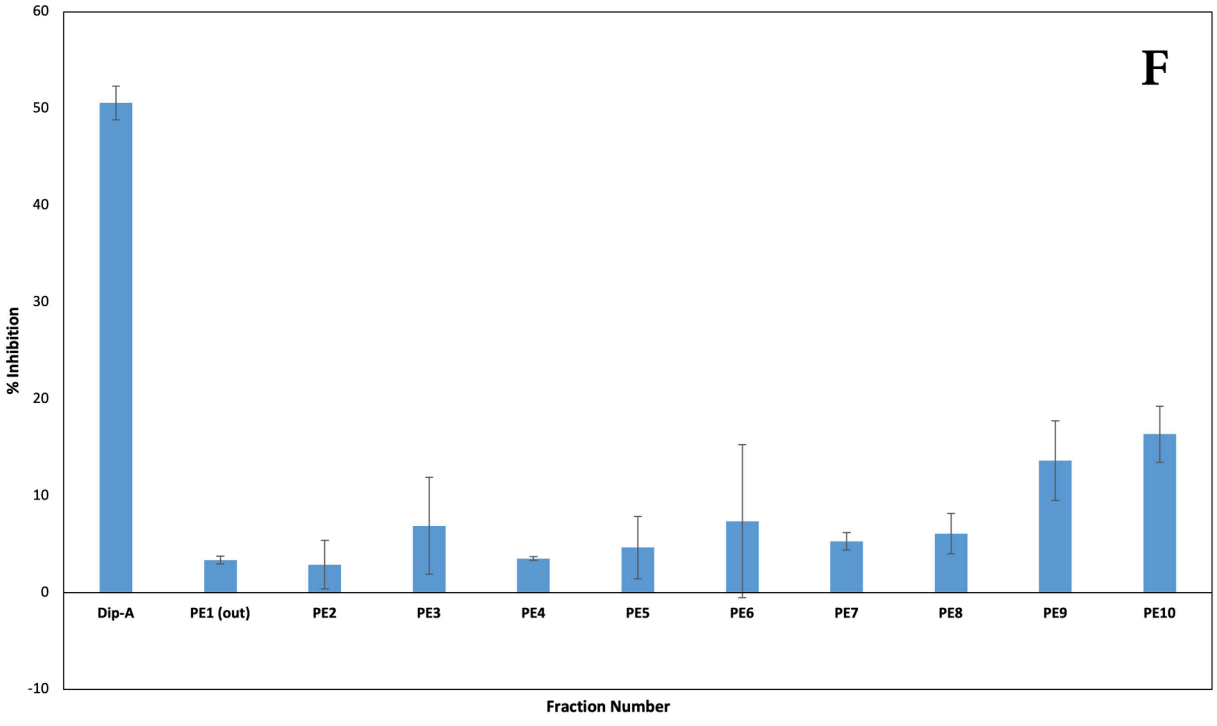


Fig. 2. (*continued*).

defined as the relative frequency of active residues (0–1) in a given sequence, was calculated (Minkiewicz et al., 2019). Using a molecular docking approach, peptide-protein interactions were studied, which generated docking scores and 3D protein-peptide interaction images (Zhou et al., 2018). The site of interactions between the relatively short hazelnut peptides (i.e., ≤10 residues in length) and DPP-IV were predicted using PepSite2 (Petsalaki et al., 2009).

Statistical analysis

The data collected in the current investigations were reported as sample means ± standard deviations based on at least triplicate experiments. Whether differences existed between various treatments were studied based on statistical significance ($p < 0.05$).

Table 2
Docking scores of the predicted protein-peptide interactions between DPP-IV from human and listed hazelnut peptides with a PeptideRanker score > 0.9.

Peptide No	Sequence	Docking Parameter (Best match)
Positive control	IPI (Diprotin A)	−114.062
1	PGHF	−188.526
2	FMRWRDRFL	−290.843
3	APGHF	−182.136
4	NSMVGNMIFWFFFCILGQPMCVLLYYHDLMNR	−371.575
5	FFFPGPNK	−233.726
6	LSVPNLYVWLCMFY	−270.187
7	LILVSFSLCLLVLFNGCLG	−271.013

Results and discussion

Isolation and identification of hazelnut peptides

The extent of proteolysis in gastrointestinal digestion is determined by enzymatic specificity, and governing physiological properties. Primary structure of food-based proteins have a bearing on the sequence and bioactivity of released peptides (Amigo and Hernández-Ledesma, 2020). Consequently, the spectrum of peptides that can be generated by gastrointestinal enzymes is limited and the usage of alternative (i.e., non-gastrointestinal) enzymes could enhance the potential for generating bioactive sequences (Gülseren, 2018).

First of all, 6 different hydrolysates were prepared using 6 different proteases, namely, bromelain, chymotrypsin, papain, pepsin, thermolysin, and trypsin (Çağlar et al., 2021; Göksu et al., 2021). In these studies, 81 different FPLC fractions were generated and using appropriate LC-Q-TOF/MS techniques, 256 different hazelnut peptides were identified. The identified sequences and their corresponding fractions were listed in the Supplementary Data section (Table S1).

The number of hazelnut peptides identified in each fraction was listed on Table 1. Thermolysin generated approx. 40.6% of all identified peptides (*i.e.*, 104 out of 256), whereas in chymotryptic hydrolysates only 8 sequences could be identified. The majority of the peptides were specific to a certain proteolytic treatment, while only 6 peptide sequences were identified in multiple hydrolysates (Table 1 and S1). For example, ADIYTEQVGR was found in both T1 (out) and PA1 (out) fractions.

In our previous studies, hazelnut peptides have been demonstrated to perform significant ACE-inhibitory activities (Çağlar et al., 2021; Göksu et al., 2021). In addition, *in silico* findings demonstrated that hazelnut peptides could potentially act as DPP-IV inhibitors (Gülseren, 2018). Based on these findings, an attempt was made to analyze the *in vitro* and *in silico* DPP-IV inhibitory potential of hazelnut peptides.

In vitro DPP-IV inhibitory activity assay

DPP-IV inhibitory activities of hydrolysate fractions were determined via the monitoring of pNA released from the substrate (Gly-Pro-pNA). A sample absorbance spectrum was shown on Fig. 1, which was representative of the current measurements. DPP-IV inhibitory activities of the fractions from each hydrolysate were presented in Fig. 2. In average, the control samples (Diprotin-A) accounted for a DPP-inhibitory activity of approx. 50.6 % under the current test conditions. The highest DPP-IV inhibitory activities in trypsin hydrolysate fractions were generally observed in later numbered strong anionic fractions ranging between T10-T17 (Fig. 2A). Among these fractions, T17 fraction showed the highest DPP-IV inhibitory activity of approx. 25 %. For chymotrypsin hydrolysate fractions, some extent of activity was observed in all fractions except for C1 (out) and C5 fractions (Fig. 2B), while the highest activity was attained by the C3 fraction (approx. 17 %). In a comparable manner, thermolysin treated E11 fraction demonstrated 26 % inhibition (Fig. 2C).

In papain treated fractions, the highest extent of activities (approx. 14–16 %) were observed in PA2, PA9 and PA10 fractions demonstrating that the anionic character of the fractions was weakly related to their potential DPP-IV inhibitory activity (Fig. 2D). However, in bromelain treated samples, later (i.e., highly anionic) fractions (BR 8–10) were characterized with the higher activities ranging between 11.5 and 17 % (Fig. 2E). Once again, similar results were attained among pepsin fractions, since PE10 (16.4 %) and PE9 (13.7 %) were the most effective treatments (Fig. 2F).

In order to determine the relative effectiveness of the DPP-IV inhibitory activity of hydrolysates and their fractions, the IC_{50} value of the positive control (i.e., Diprotin A) was investigated at a concentration range of 1–2,000 $\mu molL^{-1}$ and calculated as 9.76 $\mu g.mL^{-1}$ (*i.e.*, 28.59 $\mu molL^{-1}$). In the previous literature, similar (24.7 $\mu molL^{-1}$ in Huang et al., 2012) or somewhat comparable results were found (8.49 $\mu molL^{-1}$ in Nongonierma and FitzGerald, 2013; 3.5 $\mu molL^{-1}$ in Jia et al., 2020).

While the current fractions demonstrated low activity compared to the positive controls, that is in part due to the relatively dilute nature of the fractions. Once the fractions were lyophilized in bulk quantities and analyzed for their IC_{50} values, some of them were shown to have comparable activity as the control. For example, PA2 was characterized by an IC_{50} value of 5.58 $\mu g.mL^{-1}$, whereas IC_{50} for BR9 was as low as 0.07 $\mu g.mL^{-1}$. Consequently, we regard the current hydrolysates as a significant source of DPP-IV inhibitory peptides. Previously, the IC_{50} values of the LTFPGSAED (Lammi et al., 2018), KTYGL (Mojica, Luna-Vital and González de Mejía, 2017) and QPF (Taga et al., 2017) peptides obtained from food samples were reported as approx. 194.2, 34.4 and 28 $\mu g.mL^{-1}$, respectively.

DPP-IV inhibitory activity is potentially related to the composition and sequence of amino acids in a certain peptide (Huang et al., 2012), whereas the influence of anionic character was relatively weak. The current fractions demonstrated varying extents of DPP-IV inhibitory activity, which in turn enhances their potential for being utilized in commercial applications without further purification. To enhance our understanding on the activities of these complex hydrolysates, *in silico* analyses including molecular docking were carried out.

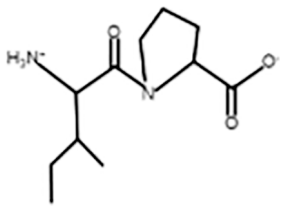
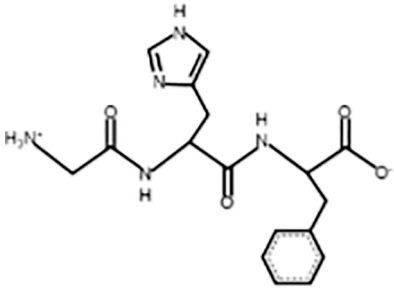
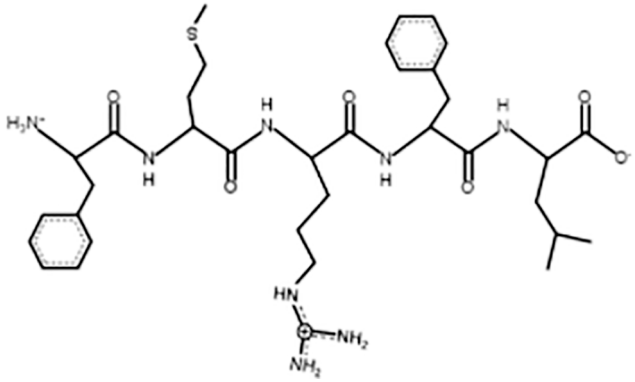
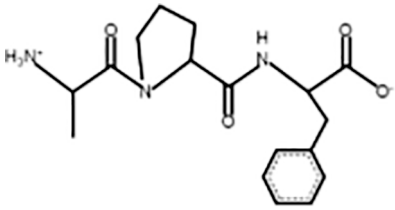
In silico prediction of DPP-IV inhibitory activity

As detailed on Table S1, DPP-IV inhibitory potential of the current peptides were investigated using PeptideRanker and BIOPEP tools. Along with a few exceptions, the majority of all hazelnut peptides were classified as DPP-IV inhibitors, while the potential activity largely varied. In addition, Parameter A calculated by BIOPEP (Minkiewicz et al., 2019), was also listed on Table S1. In the majority of cases (57.81 %), Parameter A was > 0.5. Furthermore, 12.5 % of the peptides had a Parameter A > 0.75.

In addition, PeptideRanker scores of the current peptides were determined, which is a commonly utilized parameter for potential bioactivity of peptide sequences (Mooney et al., 2012). Based on PeptideRanker predictions, 45 peptides demonstrated a score > 0.5 indicating potential bioactivity. Among these 45 peptides, 7 of them were characterized by a PeptideRanker score of > 0.9 indicating highly potential bioactivity. These 7 sequences were also characterized by relatively high Parameter A (>0.5) as evaluated by BIOPEP and they were further investigated using molecular docking tools (HPEPDOCK, Zhou et al., 2018).

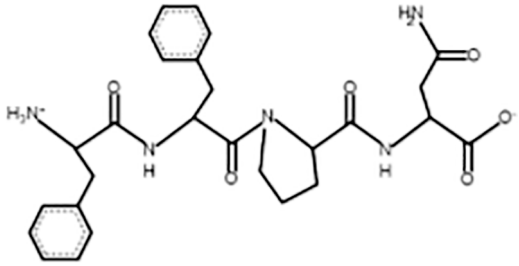
Based on the most probable model, all 7 potentially DPP-IV-inhibitory peptides were predicted to bind to human DPP-IV and yield relatively high docking scores and hence, potentially induce inhibition. For comparative purposes, DPP-IV inhibitory Diprotin-A (*i.e.*, IPI) was investigated as a reference peptide and its docking score was compared to the current peptides (Table 2). In all cases, the current hazelnut peptides and IPI were predicted to generate significant binding to

Table 3
The interacting sites on the hazelnut peptides and DPP-IV, and the significance level (p-value) of this interaction. The interactions were predicted using PepSite2 software, where peptides that have a length of ≤ 10 residues can be analyzed. Active residues of the peptides were drawn as a single peptide. The interacting residues on DPP-IV that were shared between IPI (Diprotin-A) and the hazelnut peptides were shown in bold font.

Peptide Sequence	p-value	Active Residues on the Peptide, and Their Corresponding Hydrophobicity Value and Illustration as a Single Peptide	Interacting Residues on DPP-IV
IPI	0.03277	Ile-1, Pro-2 GRAVY =1.45	**Tyr-48,** Trp-627, **Trp-629**, **His-748**, **Tyr-752**
			
PGHF	0.02934	Gly-2, His-3, Phe-4 GRAVY =-0.267	Glu-206, Tyr-547, Ser 630, Tyr-631, Tyr-662, Tyr-666
			
FMRWRDRFL	0.01208	Phe-1, Met-2, Arg-7, Phe-8, Leu-9 GRAVY =1.36	**Tyr-48,** Phe-357, Tyr-547, **Trp-629**, Ser-630, Tyr-631, Val-656, Trp-659, Tyr-662, Tyr-666, His-740, Gly-741, His-748, **Tyr-752**
			
APGHF	0.01272	Ala-1, Pro-2, Phe-5 GRAVY =1	**Tyr-48**, Tyr-547, **Trp-629**, **His-748**, **Tyr-752**
			
FFFPGPNK	0.01754	Phe-1, Phe-3, Pro-6, Asn-7 GRAVY = 0.125	Phe-357, Tyr-547, Trp-627, **Trp-629**, Tyr-666, **Tyr-752**

(*continued on next page*)

Table 3 (*continued*)

Peptide Sequence	p-value	Active Residues on the Peptide, and Their Corresponding Hydrophobicity Value and Illustration as a Single Peptide	Interacting Residues on DPP-IV
			

human DPP-IV, while hazelnut peptides generated higher docking scores than IPI. These *in silico* findings were also coherent with the *in vitro* experiments and calculated IC_{50} parameters. The potential interactions between the 7 hazelnut peptides and DPP-IV were also depicted visually on Table S2.

Using PepSite2 software (Petsalaki et al., 2009), the interactions between the active residues on Table 2 peptides and DPP-IV were investigated (Table 3). Only the most significant model (p-value $\ll$0.05) was presented and the active residues of the peptides were drawn as a single peptide. The interacting residues on DPP-IV that were shared between IPI (Diprotin-A) and the hazelnut peptides were shown in bold font. Finally, the GRAVY values, which are indicators of hydropathy, were listed. In average, the interacting residues on hazelnut peptides were predicted to constitute uncharged and low polarity sequences. The majority (75%) of the interacting amino acids on DPP-IV was predicted to be aromatic amino acids (Phe, Tyr, Trp) (i.e., 27/36 of the residues in all cases). Among the interacting residues, there was only a single charged residue (R) when 5 different peptides (4 hazelnut peptides and the positive control) were considered (Table 3).

Based on these *in silico* predictions and *in vitro* findings listed in Section 3.2, we hypothesize that electrostatics were not a critical factor in hazelnut peptide-DPP-IV interactions. Due to the low polarity nature of interacting hazelnut peptides and the aromatic nature of interacting DPP-IV residues, hydrophobic interactions are potentially the key mechanism in DPP-IV inhibiton. This argument seems to be accurate for Diprotin-A as well.

Conclusion

The literature on the bioactivity of plant protein hydrolysates is rapidly expanding. Here, a detailed analysis on the sequences and potential activity mechanisms of hazelnut peptides in their interactions with DPP-IV was carried out *in vitro* and *in silico*. Although the peptide fractions were relatively dilute, significant DPP-IV-inhibitory was observed in many cases. While purification and concentration of bioactive peptides generate high activity products, utilization of fractions with DPP-IV-inhibitory characteristics could be more suitable for industrial exploitation due to lower costs. Current work from our group is underway on the industrial utilization of bioactive (i.e., ACE-, DPP-IV-inhibitory and antioxidative) hazelnut peptide fractions in hazelnut paste and hazelnut cocoa cream manufacture, while alternative opportunities might develop in food supplement or cosmetic industries.

Declaration of Competing Interest

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

Acknowledgements

Hazelnut cakes were donated by Neva Foods. The authors would like to thank Prof. Dr. Ahmet Tarık Baykal and Ms. Emel Akgün for their technical support at Labmed. This study was funded by a grant from TÜBİTAK 1001 Program, Turkey (Grant No. 217O063).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2021.100151.

References

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

Amigo, L., & Hernández-Ledesma, B. (2020). Current evidence on the bioavailability of food bioactive peptides. *Molecules, 25*(19), 4479.

Arrutia, F., Puente, Á., Riera, F. A., Menéndez, C., & González, U. A. (2016). Influence of heat pre-treatment on BSA tryptic hydrolysis and peptide release. *Food Chemistry, 202*, 40–48.

Aydemir, L. Y., Gökbulut, A. A., Baran, Y., & Yemenicioğlu, A. (2014). Bioactive, functional and edible film-forming properties of isolated hazelnut (*Corylus avellana* L.) meal proteins. *Food Hydrocolloids, 36*, 130–142.

Başaran, E., (2015). Fındıkta Uygulanan Ultrases Ön İşleminin Fındık Yağı Verimine ve Kalitesine Etkisi. İstanbul Technical University, MS Thesis, İstanbul, Turkey (in Turkish).

Coşkun, Ö., Çakır, B., Vahapoğlu, B., & Gülseren, İ. (2019). Influence of extraction conditions on structural and functional characteristics of black cumin protein concentrates and ACE-inhibition in their hydrolysates. *Journal of Food Measurement and Characterization, 13*(3), 2328–2338.

Çağlar, A. F., Çakır, B., & Gülseren, İ. (2021). LC-Q-TOF/MS based identification and in silico verification of ACE-inhibitory peptides in Giresun (Turkey) hazelnut cakes. *European Food Research and Technology, 247*(5), 1189–1198.

Damavandi, R. D., Eghtesadi, S., Shidfar, F., Heydari, I., & Foroushani, A. R. (2013). Effects of hazelnuts consumption on fasting blood sugar and lipoproteins in patients with type 2 diabetes. *Journal of Research in Medical Sciences: the official journal of Isfahan University of Medical Sciences, 18*(4), 314–321.

Göksu, A.G., Çakır, B., Gülseren, İ. (2021). Industrial utilization of bioactive hazelnut peptide fractions in the manufacture of functional hazelnut paste: ACE-inhibition and allergy suppression. *Submitted to* Waste and Biomass Valorization.

Gülseren, İ., & 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.

Gülseren, İ. (2018). *In silico* methods to identify ACE and DPP-IV inhibitory activities of ribosomal hazelnut proteins. *Journal of Food Measurement and Characterization, 12*(4), 2607–2614.

Holst, J. J., & Gromada, J. (2004). Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. *American Journal of Physiology-Endocrinology and Metabolism, 287*(2), E199–E206.

Huang, S.-L., Jao, C.-L., Ho, K.-P., & Hsu, K.-C. (2012). Dipeptidyl-peptidase IV inhibitory activity of peptides derived from tuna cooking juice hydrolysates. *Peptides, 35*(1), 114–121.

Jia, C.-l., Hussain, N., Joy Ujiroghene, O., Pang, X.-Y., Zhang, S.-W., Lu, J., … Lv, J.-P. (2020). Generation and characterization of dipeptidyl peptidase-IV inhibitory peptides from trypsin-hydrolyzed α-lactalbumin-rich whey proteins. *Food Chemistry, 318*, 126333. https://doi.org/10.1016/j.foodchem.2020.126333

Lammi, C., Bollati, C., Ferruzza, S., Ranaldi, G., Sambuy, Y., & Arnoldi, A. (2018). Soybean-and lupin-derived peptides inhibit DPP-IV activity on *in situ* human intestinal Caco-2 cells and *ex vivo* human serum. *Nutrients, 10*(8), 1082.

Li, C., Qi, R., Yuan, J., Han, L., Wang, S., Li, W., & Han, W. (2021). *In silico* study to predict potential precursors of human dipeptidyl peptidase-IV inhibitors from hazelnut. *Journal of Biomolecular Structure and Dynamics*.

Liu, C., Fang, L.i., Min, W., Liu, J., & Li, H. (2018a). Exploration of the molecular interactions between angiotensin-I-converting enzyme (ACE) and the inhibitory peptides derived from hazelnut (*Corylus heterophylla* Fisch.). *Food Chemistry, 245*, 471–480.

Liu, C., Ren, D., Li, J., Fang, L.i., Wang, J.i., Liu, J., & Min, W. (2018b). Cytoprotective effect and purification of novel antioxidant peptides from hazelnut (*C. heterophylla* Fisch) protein hydrolysates. *Journal of Functional Foods, 42*, 203–215.

Minkiewicz, P., Iwaniak, A., & Darewicz, M. (2019). BIOPEP-UWM Database of Bioactive Peptides: current opportunities. *International Journal of Molecular Sciences, 20*, 5978.

Mojica, L., Luna-Vital, D. A., & González de Mejía, E. (2017). Characterization of peptides from common bean protein isolates and their potential to inhibit markers of type-2 diabetes, hypertension and oxidative stress. *Journal of the Science of Food and Agriculture, 97*(8), 2401–2410.

Mooney, C., Haslam, N. J., Pollastri, G., Shields, D. C., & Kurgan, L. (2012). Towards the improved discovery and design of functional peptides: Common features of diverse classes permit generalized prediction of bioactivity. *PloS One, 7*(10), e45012.

NMKL Nitrogen determination in foods and feeds according to Kjeldahl, NMKL method Nordic Commitee on Food Analysis No. 6 4th ed., 2003 Norway.

Nongonierma, A. B., & FitzGerald, R. J. (2013). Dipeptidyl peptidase IV inhibitory and antioxidative properties of milk protein-derived dipeptides and hydrolysates. *Peptides, 39*, 157–163.

Özdemir, M. (1997). Critical evaluation of properties of Turkish hazelnuts. *Gıda Teknolojisi, 10*, 1–9.

Petsalaki, E., Stark, A., García-Urdiales, E., Russell, R. B., & Rost, B. (2009). Accurate prediction of peptide binding sites on protein surfaces. *PLoS Computational Biology, 5*(3), e1000335.

Sebokova, E., Christ, A. D., 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.

Simsek, S. (2021). Angiotensin I-converting enzyme, dipeptidyl peptidase-IV, and α-glucosidase inhibitory potential of hazelnut meal protein hydrolysates. *Journal of Food Measurement and Characterization, 15*(5), 4490–4496.

Singh, A. K., Yadav, D., Sharma, N., & Jin, J. O. (2021). Dipeptidyl Peptidase (DPP)-IV inhibitors with antioxidant potential isolated from natural sources: A novel approach for the management of Diabetes. *Pharmaceuticals, 14*(6), 586.

Taga, Y., Hayashida, O., Kusubata, M., Ogawa-Goto, K., & Hattori, S. (2017). Production of a novel wheat gluten hydrolysate containing dipeptidyl peptidase-IV inhibitory tripeptides using ginger protease. *Bioscience, Biotechnology, and Biochemistry, 81*(9), 1823–1828.

TİM, (2018). Türkiye İhracatçılar Merkezi., URL:https://www.tim.org.tr/tr/ihracat-rakamlari.html. Access date:08.10.2018.

Zhou, P., Jin, B., Li, H., & Huang, S. Y. (2018). HPEPDOCK: A web server for blind peptide–protein docking based on a hierarchical algorithm. *Nucleic Acids Research, 46*(W1), W443–W450.