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ORIGINAL PAPER

# In silico methods to identify ACE and DPP-IV inhibitory activities of ribosomal hazelnut proteins

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## Abstract

In this study, an in silico attempt was made to evaluate the biological functionality of hazelnut (*Corylus avellana* L.) peptides, using three gastrointestinal (GI) and three non-GI enzymes. As of March 2017, 469 hazelnut proteins were listed on UniProt database. Here, a small subset (i.e., 23 ribosomal proteins) was investigated. Using in silico proteolysis, the efficacy of gastrointestinal proteases (i.e., trypsin, pepsin, chymotrypsin) were compared to various other non-GI proteases such as thermolysin, papain and bromelain for the generation of bioactive peptides. In most cases, gastrointestinal proteases (i.e., trypsin, pepsin, chymotrypsin) were shown to be less efficient compared to various other non-GI proteases such as thermolysin, papain and bromelain in the generation of bioactive peptides. After in silico proteolysis, the extent of angiotensin-converting enzyme (ACE)-inhibitory peptide content (A′) accounted for approximately 5.1, 7.9 and 9.1% of all amino acids present for bromelain, thermolysin and papain treatments, respectively, while comparable results were obtained for dipeptidyl peptidase-IV (DPP-IV) inhibitory activity (4.8, 8, and 10.9%). In all cases, ACE- and DPP-IV inhibitory activities were dominant to all other activities. Based on the current findings, ribosomal hazelnut proteins could be considered as a valuable source of bioactive peptides.

**Keywords** Plant protein peptides · Common hazelnut (*Corylus avellana* L.) · Ribosomal proteins · In silico proteolysis

## Introduction

Plant proteins are a sustainable source of proteins fit for human consumption. The conversion ratio of plant proteins to animal proteins is approximately 15% [1] which underlines the importance of plant protein utilization in food systems and other consumer products, especially since the global demand for protein is constantly rising. Furthermore, increasing demand in the usage of animal-based foods were shown to limit the universal availability of foods to all humans [2]. One of the research priorities in our group is the valorization of cold press deoiled plant meals of oil fruits and oil seeds in order to generate a variety of value-added plant protein products and their hydrolysates. Since protein content in these plants are relatively high and proteins are further concentrated upon deoiling, they represent a viable resource for protein manufacture.

Hazelnuts (*Corylus avellana* L.) are among the major agricultural products of Turkey, where approximately 80% of the global production is being harvested. The total production was roughly 550,000 and 646,000 tonnes per year in 2013 and 2015, respectively [3, 4]. Other Mediterranean countries and western USA are among the other major producers. While hazelnuts are high in calories due to their oil content (~ 60%), a variety of bioactive effects including antioxidant, anti-inflammatory, anti-proliferative and hypocholesterolemic activities were previously demonstrated for hazelnut oil and other hazelnut bioactives [5]. For example, the cardioprotective effects of hazelnuts were attributed in part to their mono- and poly-unsaturated fatty acid contents, especially that of oleic and linoleic acids [6], Oleic acid and α-tocopherol are the primary fatty acid and vitamin in hazelnuts, whereas hazelnut samples account for a pronounced gallic acid equivalency (GAE) value as well [7]. Polyphenols, and squalene are also among the major bioactive compounds of hazelnuts [8–10], while phytosterols

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and phytostanols contribute to their cholesterol lowering and antioxidant properties [11].

As industrial production of cold press hazelnut oil is increasing, the deoiled meal represents a great potential of valorization (up to 45% protein in the meal, based on the composition presented in Alphan et al. [12]) and various fractions can be extracted from the gently processed meals. Compared to its other bioactive constituents, there is considerably less work on the analysis of hazelnut proteins and the peptides that could be generated from them. Most of the literature on hazelnut proteins were focused on the reduction of food allergies [13–15], while the relative digestive stability of hazelnut allergens were found to be lower compared to peanut allergens [14]. Regression modelling of allergy severity [16] and gastrointestinal fate of allergens in hazelnuts [17] have also been recently studied. Aydemir et al. [18] carried out a detailed study on the bioactive and mechanical properties of isolated hazelnut meal proteins, where the isolates were shown to demonstrate antioxidative, anti-carcinogenic and anti-hypertensive properties. These investigators, however, did not analyze the bioactive characteristics of the corresponding peptides.

In foods, a wide variety of bioactivities were demonstrated based on protein hydrolysates from dairy, plant, fungal, marine etc. resources, including ACE- and DPP-IV inhibitory activities. Angiotensin I-converting enzyme (ACE) is a constituent of the renin-angiotensin system [19]. While renin first transforms angiotensinogen into angiotensin-I, ACE converts angiotensin I to angiotensin II, which is a vasoconstrictor. In addition, ACE hydrolyzes bradykinin, which is a vasodilator. Consequently, ACE action elevates blood pressure in humans [20, 21]. ACE-inhibitory peptides have the capabilities of reducing blood pressure and the likelihood of hypertension [21]. Although bioactive peptides are usually not as efficient as synthetic drug molecules, due to the absence of pronounced side effects, they can conveniently be utilized in functional foods or food supplements [22].

Similarly, dipeptidyl protease IV (DPP-IV) is a critical enzyme that is found in a variety of biochemical reactions. Based on its proteolytic activities, for example, DPP-IV causes the activation or inactivation of peptides. Due to DPP-IV action, the inactivation of incretin hormones that are utilized in the treatment of Type-2 diabetes occurs. In order to prevent this reaction, a variety of synthetic DPP-IV inhibitors are commercially available. Once again, a preferred treatment could be the utilization of bioactive peptides instead of synthetic inhibitors [23].

In this study, an attempt was made to predict the potential bioactivities of peptides generated from ribosomal hazelnut proteins in silico using both gastrointestinal and non-gastrointestinal enzymes. Due to the large amount of hazelnut proteins that were listed in the current protein databases, a small subset (i.e., ribosomal proteins) of proteins were investigated in this first report. Since ribosomal proteins constituted ~5% of all known hazelnut proteins, they were analyzed as a specific sub-category. Although the results presented here are not exhaustive by any means, this early attempt made it possible to generate funding and currently, a comprehensive in vitro study on the influence of proteolysis on bioactivities and allergenic characteristics of hazelnut proteins is currently being executed in our laboratories.

## Methods

The analyses carried out in this study are summarized on Fig. 1.

### Sequences of ribosomal hazelnut proteins

The primary sequences of the ribosomal hazelnut proteins were obtained from UniProtKB database (http://www.uniprot.org) on March 10th, 2017. Ribosomal proteins represented 23 out of 469 proteins (~5% of all proteins) for the common hazelnut (*Corylus avellana* L.) on this database. The basic data including the names, accession numbers and number of amino acids in each protein were listed on Table 1. To initiate a preliminary round of investigations, this selection was carried out due to the time and labor-intensive nature of the analysis and would fully represent the case where only a ribosomal protein isolation method would be applicable.

### In silico proteolysis and the analysis of bioactive sequences

In silico proteolysis of the ribosomal hazelnut proteins were carried out using the "enzyme action" tool of the BIOPEP webserver (http://www.uwm.edu.pl/biochemia/index.php/pl/biopep) [24]. Based on the general proteolytic characteristics of every enzyme, BIOPEP predicts the fragments that will be generated from every input sequence. Consequently, for every input sequence, an output sequence with varying number of peptides was computed [24].

Proteolytic simulations were conducted separately using six different proteases for each protein listed on Table 1 based on the "enzyme action" tool. These enzymes were three gastrointestinal proteases (trypsin, chymotrypsin and pepsin) and three proteases from other resources (thermolysin, bromelain and papain). This selection was based on the previous work of Udenigwe et al. [25] where the activities of Rubisco were studied in detail and also was based on the immediate commercial availability of these enzymes. For every enzyme, the fragments were analyzed for bioactive properties based on the "profiles of biological activity tool" [24]. BIOPEP database provides cataloging of previously

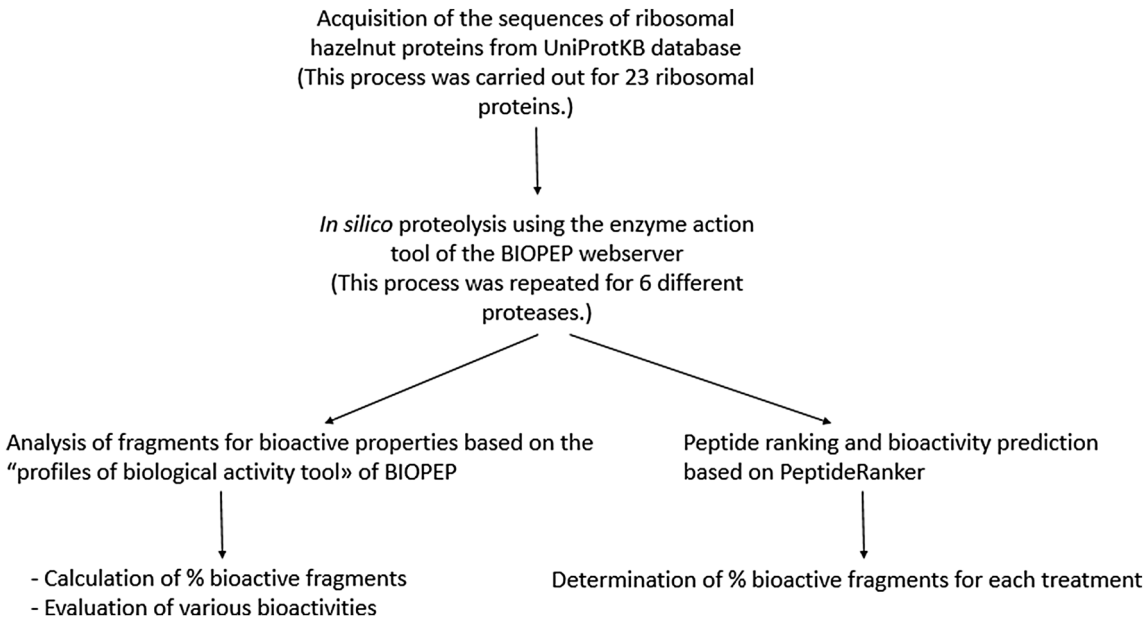


**Fig. 1** Analytical setup of the current study

**Table 1** Randomly assigned order numbers, accession numbers for protein databases and lengths of ribosomal proteins from the common hazelnut (*Corylus avellana* L.)

| Order number | UniProtKB Swiss-Prot or TrEMBL accession number | Protein names | Number of amino acids |
|---|---|---|---|
| 1 | Q9TGB0 | Ribosomal protein small 3 (Fragment) | 25 |
| 2 | A0A1I9RG92 | Ribosomal protein S19 | 92 |
| 3 | A0A1I9RG90 | Ribosomal protein L23 | 93 |
| 4 | A0A1I9RG93 | Ribosomal protein S12 | 124 |
| 5 | A0A1I9RG73 | Ribosomal protein S7 | 155 |
| 6 | A0A1I9RG76 | Ribosomal protein L32 | 56 |
| 7 | A0A1I9RG53 | Ribosomal protein S18 | 101 |
| 8 | A0A1I9RG91 | Ribosomal protein L2 | 286 |
| 9 | A0A1I9RG19 | Ribosomal protein S16 | 65 |
| 10 | A0A1I9RG65 | Ribosomal protein L14 | 122 |
| 11 | A0A1I9RG35 | Ribosomal protein S4 | 201 |
| 12 | A0A1I9RG66 | Ribosomal protein L16 | 136 |
| 13 | A0A1I9RG31 | Ribosomal protein S14 | 100 |
| 14 | A0A1I9RG67 | Ribosomal protein S3 | 219 |
| 15 | A0A1I9RG62 | Ribosomal protein S11 | 138 |
| 16 | A0A1I9RG70 | Ribosomal protein L2 | 287 |
| 17 | A0A1I9RG52 | Ribosomal protein L33 | 73 |
| 18 | A0A1I9RG25 | Ribosomal protein S2 | 241 |
| 19 | A0A1I9RG63 | Ribosomal protein L36 | 42 |
| 20 | A0A1I9RG68 | Ribosomal protein L22 | 169 |
| 21 | A0A1I9RG85 | Ribosomal protein S15 | 90 |
| 22 | A0A1I9RG64 | Ribosomal protein S8 | 134 |
| 23 | A0A1I9RG54 | Ribosomal protein L20 | 117 |

established bioactive peptide sequences and compares the input fragments to the listed active sequences in order to compare their bioactive potential primarily based on sequence-based similarities.

Almost in all cases, the ACE and DPP-IV inhibitory properties were dominant based on their corresponding % bioactive sequence values and were reported here in detail. The number of bioactive sequences, total number of amino acids in these bioactive sequences and their corresponding % frequencies compared to the total number of amino acids in each protein were determined.

### Peptide ranking and bioactivity prediction

For all sequences generated in the previous section ("In silico proteolysis and the analysis of bioactive sequences"), a PeptideRanker score was computed (PeptideRanker in the Bioware webserver at http://bioware.ucd.ie/~compass/biowareweb hosted by the University College, Ireland) [26]. Based on the previous literature, PeptideRanker determines the likelihood of bioactivity to occur in a given sequence based on established structure–function patterns. This output, however, is not bioactivity specific and expresses the potential of being bioactive for every input sequence. A PeptideRanker score between 0 and 1 was assigned to every input sequence, where higher scores represented increasing possibility of being bioactive. Since a score of 0.5 represented 50% likelihood of being bioactive, the number of sequences with a score of $\geq 0.5$ were determined [26]. Furthermore, the total number of amino acids in these sequences and their corresponding % frequencies in their parent proteins were also calculated.

## Results and discussion

The basic information about the ribosomal proteins of the hazelnut including their names, number of residues they contain and accession numbers were summarized on Table 1. The number of residues in the proteins ranged between 25 and 286 amino acids and the average number of amino acids in 23 ribosomal proteins was 133.3 residues per protein. The amino acid sequences of these proteins were exported from the UniProt database and utilized in further analysis.

Using BIOPEP [24], 23 different proteins were treated individually with different proteases in silico. Three of these enzymes were gastrointestinal (GI) enzymes (pepsin, trypsin, and chymotrypsin), whereas the other three enzymes were non-gastrointestinal enzymes (bromelain, thermolysin, and papain). Since non-GI enzymes can be utilized in the manufacture of bioactive peptides [27], these enzymes were also included in the current study. In all cases, enzymatic action generated multiple peptides from each protein. Peptide sequences that contained two or more amino acids were recorded for further analysis, whereas single amino acid sequences were not taken into account.

Using the "profiles of biological activity tool" on BIOPEP [24], the potential bioactivities of all peptide fragments were analyzed (Tables 2, 3, 4; Fig. 2). In approximately all cases (> 90%), the dominant bioactivities by % bioactive sequences were ACE- and/or DPP-IV inhibition unless the peptide fragments exhibited no bioactivity at all. Among all 178 cases (23 proteins × 6 proteases = 178 proteolytic scenarios), in only 44 cases (31.9%) antioxidant activity was predicted. Antioxidant activity was generally due to multifunctional dipeptides such as IR, which also could demonstrate ACE- and DPP-IV inhibitory activities, whereas the antioxidative dipeptide LK (i.e., single activity peptide) was also commonly observed in the current analyses.

Potential ACE- and/or DPP-IV inhibitory activities of the ribosomal proteins were reported on Tables 2 and 3, respectively. The theoretical frequency of ACE-inhibitory peptides (A) ranged between 16.67 and 50.23 (Table 2). The relation between A and B values were relatively weak as also reported by Minkiewicz et al. [24] in both cases (Tables 2, 3). However, theoretical A values were higher for DPP-IV inhibitory activity (50.68–76.34) and the corresponding B values were considerably lower compared to ACE-inhibitory counterparts. In all cases, as expected, after the proteolysis, the frequency of released bioactive peptides (A′) were much lower compared to the theoretical values (A). Using the gastrointestinal (GI) proteases, the frequency of released ACE peptides ranged between 1.33–2.5%, whereas considerably higher frequencies were observed with the non-GI proteases (5.07–9.09) (Table 2). Similar results were obtained for DPP-IV inhibitory activity, although the frequency values were generally higher (Table 3). Consequently, DPP-IV inhibitory activity was the dominant activity, followed by ACE-inhibitory activity, while the antioxidative activity was determined to be a distant third category. All the other bioactivities were predicted in much lower frequencies (data not shown).

As an example, on Fig. 2, the distribution of DPP-IV inhibitory sequences in the papain hydrolysate of ribosomal protein S7 was reported. In this particular case, all the active sequences were composed of dipeptides, which was not necessarily the case for all digests.

Finally using PeptideRanker [26], all peptide sequences generated by enzymatic actions (Tables 2, 3) were analyzed for their potential bioactivities regardless of the type of bioactive action predicted by BIOPEP. On Table 4, the average number of bioactive peptides on each protein, their corresponding average number of residues and % of bioactive residues in comparison to the size of each protein were listed. In this computation, a bioactive peptide was defined as "a sequence that has a $\geq 50\%$ probability of being bioactive" based on its PeptideRanker score [26]. Once again, the number of potentially bioactive sequences for non-GI enzymes was higher than GI proteases, which

**Table 2** The extent (%) of released ACE-inhibitory peptides from ribosomal proteins of the common hazelnut (*Corylus avellana* L.) by the utilization of various proteases

| Number | A (%) | B ($\mu M^{-1}$) | Trypsin | Pepsin | Chymotrypsin | Bromelain | Thermolysin | Papain |
|---|---|---|---|---|---|---|---|---|
| 1 | 40.00 | 0.0121 | nd | 8.00 | nd | 20.00 | 8.00 | 28.00 |
| 2 | 39.13 | 0.0114 | nd | 2.17 | 2.17 | 6.52 | 6.52 | 4.35 |
| 3 | 41.94 | 0.0133 | 2.15 | nd | nd | 2.15 | 12.90 | 11.83 |
| 4 | 41.94 | 0.0201 | 3.23 | nd | nd | 8.87 | 8.87 | 15.32 |
| 5 | 43.23 | 0.0163 | 1.29 | 1.29 | 7.10 | 9.03 | 7.74 | 12.90 |
| 6 | 48.21 | 0.0094 | nd | nd | 3.57 | 3.57 | 17.86 | 7.14 |
| 7 | 34.65 | 0.0059 | nd | nd | nd | 1.98 | 7.92 | 5.94 |
| 8 | 49.65 | 0.0184 | 1.40 | 2.10 | 4.20 | 7.69 | 6.64 | 7.69 |
| 9 | 40.00 | 0.0135 | nd | nd | nd | 6.15 | 18.46 | 6.15 |
| 10 | 49.18 | 0.0250 | nd | 1.64 | 6.56 | 3.28 | 8.20 | 5.74 |
| 11 | 39.80 | 0.0115 | 1.00 | 1.99 | 5.47 | 2.49 | 8.96 | 9.45 |
| 12 | 47.06 | 0.0271 | 2.94 | nd | nd | 4.41 | 11.76 | 7.35 |
| 13 | 46.00 | 0.0226 | 6.00 | 2.00 | 4.00 | 2.00 | 7.00 | 8.00 |
| 14 | 50.23 | 0.0117 | 0.91 | 0.91 | 1.83 | 4.57 | 14.16 | 13.24 |
| 15 | 44.93 | 0.0080 | 3.62 | 1.45 | 1.45 | 2.90 | 1.45 | 13.77 |
| 16 | 49.83 | 0.0184 | 1.39 | 2.09 | 4.18 | 7.67 | 6.62 | 7.67 |
| 17 | 27.40 | 0.0024 | 2.74 | 2.74 | 2.74 | 2.74 | 5.48 | 5.48 |
| 18 | 42.74 | 0.0206 | 0.83 | 0.83 | 0.83 | 0.83 | 1.66 | 1.66 |
| 19 | 16.67 | 0.0028 | nd | nd | nd | nd | nd | 4.76 |
| 20 | 40.83 | 0.0384 | 7.69 | 1.18 | 3.55 | 5.33 | 2.37 | 10.06 |
| 21 | 36.67 | 0.0037 | 4.44 | 2.22 | 4.44 | 2.22 | 6.67 | 6.67 |
| 22 | 38.81 | 0.0111 | nd | nd | 3.73 | 4.48 | 8.96 | 8.96 |
| 23 | 43.59 | 0.0110 | 5.13 | nd | 1.71 | 7.69 | 3.42 | 6.84 |
| Average (%) | | | 1.95 | 1.33 | 2.50 | 5.07 | 7.90 | 9.09 |

In silico proteolysis of 23 ribosomal hazelnut proteins was carried out using 6 different proteases. Comparison to the theoretical frequency of bioactive residues (A, %) and potential bioactivity of protein fragments (B, $\mu M^{-1}$) were also carried out.

*nd* not detected

implied that non-GI hydrolysates could also be more bioactive for all sorts of bioactive functions including ACE- and DPP-IV inhibitory activities. However, when the number of amino acids and their corresponding % frequencies in these digests were compared (Table 4), there were no clear trends which implied that some of the larger peptides generated by the GI proteases could be expected to demonstrate bioactivity as well. For example, some of the antimicrobial peptides tend to be larger but still demonstrate activity, since their antimicrobial function does not necessitate digestion and absorption [28]. That could imply that GI peptides could demonstrate some additional activities as well. Meanwhile the majority of peptides generated by non-GI proteases were small peptides as exemplified on Fig. 2. The PeptideRanker data were also illustrated in further detail on Fig. 3. In the majority of cases, papain was the most effective protease for the generation of bioactive peptides both in terms of the number of bioactive peptides (Fig. 3a) and the % frequency of bioactive sequences (Fig. 3b) compared to their corresponding parent sequences (Table 1).

## Conclusion

In this first report, the potential bioactivities of ribosomal hazelnut proteins were investigated in silico. In the majority of cases, non-GI-proteases (especially papain) were predicted to be more effective than GI-proteases in the generation of bioactive peptides. Again, in most cases, DPP-IV inhibitory activity, followed by ACE-inhibitory activity, were the most probable bioactive properties. To some extent, antioxidative activity could also be observed, whereas all the other bioactivities were predicted to be less frequent.

Compared to the recent work of Udenigwe et al. [25], ribosomal hazelnut proteins had a higher frequency of DPP-IV inhibitory peptides than most food proteins (egg, soy and milk proteins), whereas ACE-inhibitory peptide contents were relatively lower (i.e., ~60% ACE inhibitors for beta-casein vs. 16.67–50.23% for ribosomal hazelnut proteins). Compared to RuBisCO proteins as well [25], frequency of released bioactive peptides were generally higher, although the global abundance of hazelnut proteins are considerably lower. Based on the current findings, ribosomal proteins

**Table 3** The extent (%) of released DPP IV-inhibitory peptides from ribosomal proteins of the common hazelnut (*Corylus avellana* L.) by the utilization of various proteases

| Number | A (%) | B ($\mu M^{-1}$) | Trypsin | Pepsin | Chymotrypsin | Bromelain | Thermolysin | Papain |
|---|---|---|---|---|---|---|---|---|
| 1 | 56 | – | nd | nd | nd | nd | 8.00 | 8.00 |
| 2 | 70.65 | 0.002 | nd | 2.17 | 2.17 | 6.52 | 6.52 | 6.52 |
| 3 | 76.34 | 0.0004 | nd | nd | nd | 2.15 | 6.45 | 6.45 |
| 4 | 66.94 | 0.0003 | 6.45 | nd | nd | 6.45 | 4.84 | 16.13 |
| 5 | 67.1 | 0.0006 | 1.29 | nd | 3.87 | 10.32 | 9.03 | 24.52 |
| 6 | 55.36 | 0.0002 | 3.57 | nd | 3.57 | 7.14 | 21.43 | 17.86 |
| 7 | 60.40 | 4.96 E−5 | 3.96 | 7.92 | 5.94 | 5.94 | 3.96 | 9.90 |
| 8 | 66.43 | 0.0002 | 0.70 | 1.40 | 2.80 | 11.19 | 8.39 | 11.89 |
| 9 | 61.54 | 0.0002 | nd | 6.15 | 12.31 | 3.08 | 15.38 | 3.08 |
| 10 | 63.93 | 0.0005 | nd | 1.64 | 6.56 | 1.64 | 9.84 | 4.92 |
| 11 | 66.17 | 0.0003 | 3.98 | 2.99 | 8.46 | 1.99 | 8.46 | 8.96 |
| 12 | 65.44 | 0.0004 | 4.41 | nd | 1.47 | 5.88 | 7.35 | 17.65 |
| 13 | 61 | 0.0007 | 2.00 | 2.00 | 4.00 | 6.00 | 4.00 | 12.00 |
| 14 | 63.01 | 0.0004 | 1.83 | 2.74 | 1.83 | 5.48 | 11.87 | 10.96 |
| 15 | 65.94 | 8.45 E−5 | nd | 1.45 | 1.45 | 4.35 | 7.25 | 11.59 |
| 16 | 66.2 | 0.0002 | 0.70 | 1.39 | 2.79 | 11.15 | 8.36 | 11.85 |
| 17 | 50.68 | 2.24 E−5 | 2.74 | 2.74 | 2.74 | 2.74 | 5.48 | 10.96 |
| 18 | 62.66 | 0.0005 | 0.83 | 0.83 | 0.83 | 0.83 | 1.66 | 3.32 |
| 19 | 59.52 | 2.54 E−5 | nd | nd | nd | 4.76 | 4.76 | 4.76 |
| 20 | 63.91 | 0.0002 | 1.18 | nd | 2.37 | 2.37 | 5.92 | 9.47 |
| 21 | 60 | 0.0002 | 11.11 | 2.22 | 4.44 | nd | 6.67 | 15.56 |
| 22 | 67.91 | 0.0003 | 2.99 | 4.48 | 5.97 | 5.97 | 11.94 | 11.94 |
| 23 | 70.94 | 7.99 E−5 | 6.84 | nd | 1.71 | 5.13 | 6.84 | 11.97 |
| Average (%) | | | 2.37 | 1.74 | 3.27 | 4.83 | 8.02 | 10.88 |

In silico proteolysis of 23 ribosomal hazelnut proteins was carried out using 6 different proteases. Comparison to the theoretical frequency of bioactive residues (A, %) and potential bioactivity of protein fragments (B, $\mu M^{-1}$) were also carried out. The abbreviation "nd" stands for "not detected"

**Table 4** Average number of bioactive peptides, amino acids in those peptides and their corresponding % frequencies compared to the total number of amino acids in the parent protein

| | Trypsin | Pepsin | Chymotrypsin | Bromelain | Thermolysin | Papain |
|---|---|---|---|---|---|---|
| Average number of bioactive peptides | 4.09 | 3.13 | 3.96 | 4.83 | 6.09 | 7.78 |
| Average number of amino acids in bioactive peptides | 22.96 | 17.48 | 17.17 | 18.78 | 18.43 | 24.09 |
| Percentage of amino acids in bioactive peptides (%) | 17.22 | 13.11 | 12.88 | 14.09 | 13.83 | 18.07 |

The average number of amino acids in 23 ribosomal proteins was 133.3 residues per protein. In silico proteolysis of 23 ribosomal hazelnut proteins was carried out using 6 different proteases. A bioactive peptide was defined as "a sequence that has a ≥ 50% probability of being bioactive" based on its PeptideRanker score

1 MSRRG**TA**EEK **TA**KSDPIYR**N** **R**LVNMLVNRI LKHGKKSLAY QIIYRA**MK**KI
51 QQK**TE**TNPLS VLR**QAIR**GVT PDIA**VK**ARR**V** **G**GSTHQVPIE IGSA**QG**KA**LA**
101 **IR**WLLGASRK R**PG**RNMAFKL SSELVDAAKG SGDA**IR**KKEE **TH**R**MA**EA**NR**A
151 **FA**H**FR**

**Fig. 2** The distribution of DPP-IV inhibitory sequences (shown in bold) that are released by papain from ribosomal protein S7. Note that in this specific case, all the active sequences including 64–65 and 66–67 in the hydrolysate were dipeptides

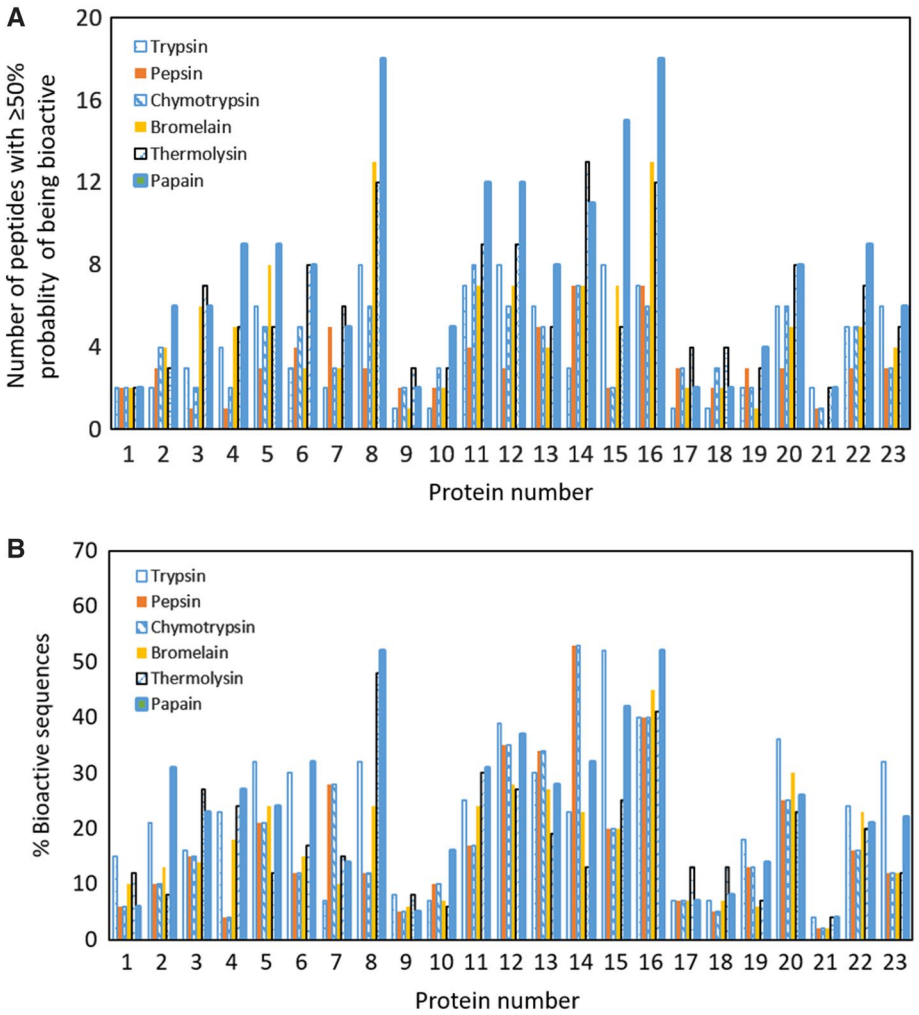


**Fig. 3** **a** The number of potentially bioactive sequences, and **b** their corresponding % frequencies in the parent proteins. In silico proteolysis of 23 ribosomal hazelnut proteins was carried out using 6 different proteases. The likelihood of being bioactive was determined by the PeptideRanker. A bioactive peptide was defined as "a sequence that has a ≥ 50% probability of being bioactive" based on its PeptideRanker score

from the common hazelnut could be considered as a valuable source of bioactive peptides. A comprehensive in vitro study on the influence of proteolysis on bioactivities and allergenic characteristics of all hazelnut proteins is currently being executed in our laboratories.

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## Compliance with ethical standards

**Conflict of interest** The author declares that he has no conflict of interest.

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