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IN SILICO ANALYSIS OF EDIBLE BIRD'S NEST PROTEINS AS POTENTIAL PRECURSORS FOR BIOACTIVE PEPTIDES

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ABSTRACT

The present study aimed to perform an *in silico* evaluation of edible bird's nest protein as potential precursors of bioactive peptides, as well as to determine whether such peptides can be released by selected proteolytic enzymes. Six edible bird's nest (EBN) protein sequences from a previous study were chosen as potential precursors to produce bioactive peptides via *in silico* method using the BIOPEP database. AMCase protein sequences gave the highest number of bioactivities (16 to 18) and nucleobindin-2 protein gave the lowest number of bioactivities (9) among the other protein sequences. It was found that the most potential bioactive peptides from EBN proteins are angiotensin-converting enzyme (ACE) inhibitors and dipeptidyl peptidase-IV (DPP-IV) inhibitors. Furthermore, *in silico* proteolysis using six selected enzymes was employed to release both dominant bioactivities in EBN proteins, which were ACE and DPP-IV inhibitors. This study shows that a combination of enzymes, chymotrypsin, and papain, produced the highest number of activities for both ACE and DPP-IV inhibitor peptides with the frequency of occurrence of bioactive peptides of 0.0968 and 0.1104, respectively. The toxic prediction tool, ToxinPred, found that all EBN peptides derived by *in silico* analysis were non-toxic. The current study proposed that EBN can serve as a potential source of bioactive peptides.

Key words: ACE inhibitor, bioactive peptides, DPP-IV inhibitor**,** edible bird's nest, *in silico*

INTRODUCTION

Bioactive peptides are peptides with unique sequences of between 2 to 30 amino acids, which contribute to positive health effects for humans when ingested (Liu *et al*., 2016). There is a growing interest in developing natural and potent bioactive peptides without undesirable side effects. Bioactive peptides derived from food protein are considered to be natural, milder, and safer for human consumption than synthetic drugs. Enzymatic protein hydrolysis using commercial proteases is commonly used to release bioactive peptides from food proteins (Korhonen & Pihlanto, 2006). Bioactive peptides from dietary proteins via enzymatic hydrolysis have demonstrated diverse bioactivities, including antioxidant, antidiabetic, antihypertensive, and antithrombotic properties (Hall *et al*., 2018).

Advances in proteomics have resulted in the development of *in silico* proteolysis tools such as the BIOPEP and ExPASy Peptide Cutter. Both online BIOPEP and ExPASy Peptide Cutter can predict theoretical bioactive peptides from various proteins with known sequences and can select suitable proteases to release them. BIOPEP is a tool that contains databases of protein sequences, and bioactive and sensory peptides. It can also be used to predict the proteolytic hydrolysate and allergenic peptides by choosing certain enzymes and proteins (Iwaniak *et al*., 2005). Meanwhile, ExPASy Peptide Cutter is a software that predicts potential cleavage sites in a protein sequence that could be cleaved by proteases or chemicals (Gasteiger *et al*., 2005). The advantage of using *in silico* analysis compared to *in vitro* hydrolysis are it can minimize the time and cost of bioactive peptides screening in diverse food protein sources (Udenigwe, 2014; Aluko, 2017). BIOPEP has been successfully used for the prediction of various proteins exhibiting bioactivities such as an angiotensin-converting enzyme (ACE) inhibitory activity, dipeptidyl peptidase-IV inhibitors (DPP-IV) (Iwaniak *et al*., 2005; Garg et. al., 2018), opioid, immunostimulating, antimicrobial (Nur 'Aliah *et*

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al., 2016), antithrombotic, hypercholesterolemic and antioxidative (Marciniak *et al*., 2018). Prediction and design of bioactive peptides from various food proteins via *in silico* analyses have been reported such as from bovine collagen (Fu *et al*., 2016), crude barley (Gangopadhyay *et al*., 2016), yak milk (Lin *et al*., 2018) and rice bran (Udenigwe, 2016).

Edible bird's nest (EBN) is a therapeutic medicine that has been used for several hundred years in China. EBN mainly comprises saliva excretion of several swiftlet species of the *Aerodramus* genus (formerly *Collocallia*), such as *A. fuciphagus* and *A. maximus*. These swiftlets are found predominantly in Southeast Asia, including Thailand, Vietnam, Indonesia, Malaysia, and the Philippines (Marcone, 2005). EBN was reported rich in protein by the various author that there was about 62% protein in the form of glycoprotein (Marcone, 2005), 61.0 to 66.9% protein of essential amino acid (Saengkrajang *et al*., 2013), and 67.53% of total amino acids (Quek *et al*., 2018). Due to the high protein content of EBN, it can be used as a good source of bioactive peptides. Amiza *et al*. (2014) reported on the optimum enzymatic hydrolysis condition of EBN using Alcalase® to obtain maximum ACE inhibitory activity. The identification of two antioxidative peptides that were Pro-Phe-His-Pro-Tyr (PFHPY) and Leu-Leu-Gly-Asp-Pro (LLGDP) from EBN using pepsin-trypsin hydrolysis was reported by Ghassem *et al*. (2017). Wong *et al*. (2018) have isolated 31 proteins from EBN, and have identified the amino acid sequence of six main proteins (with nine accession numbers) using a monoclonal antibody approach. To date, no work has been reported on the *in silico* analysis of EBN proteins as potential precursors for bioactive peptides. Thus, this work aimed to predict potential bioactive peptides from EBN proteins as reported by Wong *et al*. (2018) and to determine the appropriate proteases to perform the proteolysis to prepare dominant bioactivity, using the BIOPEP database application.

MATERIALS AND METHODS

Protein sequences of edible bird's nest (EBN)

Six EBN proteins as identified by Wong *et al*. (2018), with 9 different protein sequences were selected from the NCBI database. The accession numbers were obtained for acidic mammalian chitinase-like (XP_010006620.1, XP_010005363.1, XP_009995766.1), mucin-5AC-like (XP_009994736.1, XP_010002210.1), 3 ovoinhibitor-like (XP_010000294.1), 4 nucleobindin-2 (XP_009993696.1), 45 kDa calcium-binding protein (XP_010003465.1) and lysyl oxidase homolog 3 (XP_010006484.1).

Evaluation of edible bird's nest as a precursor for bioactive peptides

The potential for the selected EBN protein sequences to release bioactive peptides was determined using the BIOPEP database. The frequency of occurrence of the bioactive fragments in EBN protein sequence (A), the total frequency of fragments exhibiting all biological activities in protein sequences available in the BIOPEP database (∑A), and the potential biological activity of the protein (B) were described in the BIOPEP website. The number of potential bioactive for each subclasses bioactivity was counted manually from BIOPEP analysis for five bioactivities (bioactivities where B values are available in BIOPEP). BIOPEP only gives B values for ACE inhibitor, DPP-IV inhibitor, a hypotensive, alpha-glucosidase inhibitor, and opioid.

In silico proteolysis to release ACE and DPP-IV inhibitory peptides

The most effective proteases to prepare bioactive peptides with the two most dominant bioactivities from EBN (the highest A value or bioactive fragment frequency occurrence), namely ACE and DPP-IV inhibitory peptides, were evaluated using *in silico* proteolysis application in the BIOPEP database. Six proteases (chymotrypsin, trypsin, papain, thermolysin, pepsin, & proteinase K) were chosen. The single enzyme, a combination of 2 enzymes, and combinations of 3 enzymes were applied in *in silico* proteolysis to obtain the maximum amount of ACE inhibitory peptide fragments from EBN. The $\Sigma A_{ACE\ inhibition}$ values of the different proteolysis combinations were obtained.

Prediction of toxicity for ACE and DPP-IV inhibitory peptides from EBN

The toxicity of peptides derived from EBN was evaluated using ToxinPred http://www.imtech.res.in/raghava/toxinpred/. Several motifs were present in the toxic peptides. ToxinPred used this motif information to predict the toxicity of peptides. For toxicity prediction, the support vector machine (SVM) based prediction method was chosen where 0.0 was the threshold value and the cut-off value for the motif-based method was 10 (Gupta *et al*., 2013).

RESULTS AND DISCUSSION

Analysis of sequence and amino acid of edible bird's nest

The six different proteins with nine accession numbers used in this study are shown in Table 1. The protein sequences were obtained from the library of *Chaetura pelagica*, which is a bird species in the same family as *A. fuciphagus.* This was due to the lack of a full sequence in proper protein identification of the *Aerodamus* genome (Wong *et al*., 2018).

Table 1. Protein name, accession number used *in silico* analysis, and the sum of potential bioactivity of each protein in EBN

Identified Protein	Entry name (accession number)	Amino acid residues	Molecular mass (kDa)	∑A
Acidic mammalian chitinase-like (AMCase)	XP_010006620.1	359	39.92	1.4140
	XP_010005363.1	426	46.97	1.3658
	XP_009995766.1	384	42.94	1.3707
Mucin-5AC-like	XP_009994736.1	1545	169.64	1.0561
	XP_010002210.1	501	55.96	1.1778
Ovoinhibitor-like	XP_010000294.1	471	50.99	1.1848
Nucleobindin-2	XP_009993696.1	455	53.62	1.0002
45 kda calcium-binding protein	XP_010003465.1	356	41.87	1.2050
Lysyl oxidase homolog 3	XP_010006484.1	776	85.68	1.2367

EBN as a potential precursor for bioactive peptides

BIOPEP gave values of A, ∑A, and B and also lists of potential bioactive peptides from each protein sequence. Table 2 shows A values for 19 subclasses of bioactivity from EBN proteins. A value gave the frequency of encrypted bioactive peptides occurring in a particular protein (Minkiewicz *et al*., 2008). The frequency of bioactive peptides is important due to the correlation of the frequency of each peptide fragment with the biological activity (Cherkasov *et al*., 2014). The higher the A value, the higher the probability for particular bioactivity to occur in the protein. BIOPEP contains 48 major classes of peptide bioactivity. However, BIOPEP analysis of EBN protein sequences only gave 19 subclasses of potential bioactivity. AMCase protein sequences gave the highest number of biological activities compared to other protein sequences (16 to 18 bioactivities), while nucleobindin-2 protein gave the lowest number of potential bioactivities (9 bioactivities). Among the 19 subclasses of bioactivities, 9 of these bioactivities were present in all 9 EBN protein sequences which were dipeptidyl peptidase (DPP) IV inhibitor, ACE inhibitor, activating ubiquitin-mediated proteolysis, antioxidative, bacterial permease ligand, hypotensive, neuropeptide, regulating and stimulating. The other ten bioactivities present in at least any one of the EBN protein sequences were alpha-glucosidase inhibitor, anorectic, antiamnestic, antibacterial, antithrombotic, chemotactic, immunomodulating, immunostimulating, inhibitor, and opioid.

Based on the A values, the predominant bioactivity for all EBN proteins is DPP-IV inhibitor (0.5541-0.6771), ACE inhibitor (0.3163-0.4977), and antioxidative (0.0498-0.0858). This finding is consistent with previous studies on *in vitro* hydrolysis of EBN releasing ACE inhibitory and antioxidative activity (Amiza *et al*., 2014; Ghassem *et al*., 2017). However, no study has been reported for DPP-IV inhibitory activity from EBN.

ACE inhibitor

The ACE inhibitor is the second main peptide bioactivity in EBN proteins. ACE plays important role in regulating blood pressure in the renin-angiotensin system (RAS) and kallikrein-kinin system. In RAS, ACE converts angiotensin I to an active vasoconstrictor angiotensin II, resulting in a blood pressure increase. Inhibition of ACE activity is mainly used to prevent hypertension (Shahidi & Zhong, 2008). ACE inhibitors such as captopril are widely used as pharmaceutical drugs for the treatment of cardiovascular diseases. However, they often cause side effects such as coughing, skin rashes, and taste disturbances (Lee & Hur, 2017). As a result of inhibiting ACE in the bradykinin system, the ACE inhibitor drugs allow increased levels of bradykinin which would normally be degraded. This mechanism can explain the two most common side effects seen with ACE inhibitors such as angioedema and cough. Natural ACE inhibitory peptides are a natural alternative to synthetic drugs. ACE-inhibitory peptides usually contain hydrophobic (proline) and aliphatic amino acids (isoleucine & leucine) at the N-terminal (Lee & Hur, 2017).

DPP IV inhibitor

DPP-IV inhibitor is the main peptide bioactivity in EBN proteins. DPP-IV (EC 3.4.1.4.5), a serine protease cleaves dipeptides of X-Pro or X-Ala from the N terminal (Hildebrandt *et al*., 2000). Inhibition of DPP-IV activity has a positive effect on type 2 diabetes (Agirbasli & Cavas, 2017). Diabetes is a chronic metabolic disorder that resulted in high blood sugar levels over a prolonged period. In recent years, diabetes has become one of the leading causes of death worldwide. According to the International Diabetes Federation (IDF), in 2017, about 425 million people were living with diabetes globally. However, synthetic DPP-IV drugs are reported to have some adverse effects such as gastrointestinal adverse effects, allergic reactions, skin-related side effects,

and musculoskeletal disorders (Liu *et al*., 2019). Many DPP-IV inhibitory peptides have been discovered in various food protein hydrolysates, including milk proteins (Uchida *et al*., 2011), rice bran (Hatanaka *et al*., 2012), oat (Bleakly *et al*., 2017), and fish proteins (Huang *et al*., 2012; Sila *et al*., 2016).

Table 1 shows that the highest value of ∑A value was given by acidic mammalian chitinase such as (AMCase) (1.3658 -1.414), while that of nucleobindin-2 gave the lowest ∑A value with 1.0002. According to Wong *et al*. (2018), AMCase can be found in many species such as humans, mice, and birds. From the BIOPEP database, it was also found that *C. pelagica* proteins have various bioactive peptides mainly ACE inhibitor, antioxidative, antithrombotic, and dipeptidyl peptidase IV inhibitor.

The potential biological activity of a particular bioactivity

BIOPEP also provides the potential biological activity (B value) for particular bioactivity. However, B values were only available for five subclasses of bioactivities, as stated in Table 3. Table 2 shows the numbers of potential bioactive peptides of identified proteins and their potential biological activity.

Table 3 shows that considering both A and B values, the most potent bioactivity in EBN proteins was shown in ACE inhibitor, followed by DPP-IV inhibitor. Although the DPP-IV inhibitor had the highest A value, its B value was much lower than that of the ACE inhibitor. On the other hand, although the ACE inhibitor had a lower A value than the DPP-IV inhibitor, however, its B value was much higher.

The third highest value of A was given by antioxidative activity, however, its B value was not available. Furthermore, the BIOPEP database also showed that the 13 antioxidant peptides sequences from EBN reported by Ghassem *et al*. (2017) did not show any similarity.

***In silico* proteolysis of EBN protein to release ACE and DPP-IV inhibitory peptides**

Enzymatic hydrolysis is the most common approach to releasing biologically active peptides (Lin *et al*., 2018). In the BIOPEP database, there are 33 types of enzymes, but in this study, only six proteases were chosen for the *in silico* proteolysis of ACE inhibitory peptides. Figures 1 and 2 show the proteolysis results for ACE inhibitory and DPP-IV inhibitory activity, respectively.

Figure 1 shows that for single enzyme proteolysis, papain and pepsin gave the highest $\sum A_{\text{ACE inhibition}}$ value with 0.0608 followed by proteinase K and thermolysin with 0.0507 and 0.0405, respectively. Meanwhile, for the combination of two enzymes, the results indicated that chymotrypsin and papain gave the highest value (0.0968), while the lowest value was the combination of trypsin and pepsin (0.027). The combination of three enzymes, which were chymotrypsin, papain, and thermolysin gave the highest $\sum A_{\text{ACE inhibition}}$ value (0.0711) compared to the other three combinations of enzymes. The combination of two enzymes of chymotrypsin and papain gave the highest $\sum A_{\text{Ace inhibition}}$ compared to a single enzyme and other combinations. The *in silico* analysis where papain produced the highest score supported the finding by Agirbasli and Cavas (2017). According to Agirbasli and Cavas (2017), papain was effective in releasing ACE inhibitors and antioxidative peptides from wheat gluten, bovine muscle proteins, patatin, and quinoa. On the other hand, proteolysis action of chymotrypsin, trypsin, and pepsin was lower compared to thermolysin and papain in sorghum (Udenigwe *et al*., 2013).

Figure 2 shows that for a single enzyme, papain gave the highest $\sum A_{\text{DPP-IV inhibition}}$ (0.0798) followed by proteinase K (0.0551), and thermolysin (0.0495), chymotrypsin (0.0473), pepsin (0.009) and finally trypsin (0). Meanwhile, the combination of two enzymes indicated that chymotrypsin and papain gave the highest $\sum A_{\text{DPP-IV inhibition}}$ (0.1104), followed by papain and thermolysin (0.0878), papain and trypsin (0.0811), pepsin and chymotrypsin (0.0788), pepsin and papain (0.0766), pepsin and thermolysin (0.0653), and finally, trypsin and pepsin (0.0293). When three enzymes were combined, the highest $\sum A_{\text{DPP-IV inhibition}}$ was given by chymotrypsin, papain, and thermolysin (0.0819), followed by proteinase K, chymotrypsin, and thermolysin (0.0631). Both combinations of pepsin, thermolysin, and papain and proteinase K, thermolysin, and papain gave similar $\sum A_{\text{DPP-IV inhibition}}$. These results showed that a combination of two enzymes of chymotrypsin and papain gave the highest $\sum A_{\text{DPP-IV inhibition}}$ compared to a single enzyme and other combinations. In contrast, Lacroix and Li-Chan (2012) reported a higher $\sum A_{\text{DPP-IV inhibition}}$ value in ovotransferrin (0.111), while ovalbumin (0.104) and ovomucoid (0.075) had values quite similar to EBN proteins. The *in silico* analysis in this study showed that the sequences of all DPP-IV inhibitors consist of only two amino acids. Dipeptidyl peptidase IV (DPP-IV) inhibitors are generally found to have short sequences of amino acids and are less than five amino acids in length (Lafarga *et al*., 2014; Hall *et al*., 2018).

The combination of enzymes gave an effective effect and the combination of enzymes was purposely performed to release ACE inhibitory peptides from the EBN protein to obtain the highest $\sum A_{\text{Ace inhibition.}}$ Enzyme combination may produce more ACE inhibitory peptides than a single enzyme (Lin *et al*., 2018). Tu *et al*. (2018) reported that a single enzyme exhibited limited proteolysis where the certain protein has a unique structure that could resist proteolysis with only one single enzyme, which is closely related to the functionality and bioactivity of the hydrolysate. It was also noted that a combination of three enzymes gave a lower number of ACE inhibitory peptides than two

Table 2. The frequency of occurrence of the bioactive fragment (A) for each type of bioactivity in six EBN proteins

Protein name	Acidic mammalian chitinase-like			Mucin-5AC-like		Ovo inhibitor-like	Nucleo bindin-2	45 kda calcium-binding protein	Lysyl oxidase homolog 3
Accession no.	XP_009995766.1	XP_010005363.1	XP_010006620.1	XP_009994736.1	XP_010002210.1	XP_010000294.1	XP_009993696.1	XP_010003465.1	XP_010006484.1
Activity									
ACE inhibitor	0.4740	0.4977	0.4652	0.3420	0.3852	0.3385	0.3163	0.4512	0.3624
Dipeptidyl peptidase IV inhibitor	0.6771	0.6714	0.6713	0.5643	0.5749	0.5912	0.5541	0.6105	0.6039
Hypotensive	0.0469	0.0305	0.0474	0.0200	0.0160	0.0330	0.0085	0.0167	0.0393
Alpha-glucosidase inhibitor	0.0026	0.0023	0.0028			0.0022		0.0013	
Opioid	0.0026	0.0023		0.0019					
Activating ubiquitin-mediated proteolysis	0.0078	0.007	0.0084	0.0052		0.0022	0.0064	0.0129	0.0056
Anorectic	0.0026	0.0023	0.0028						
Antiamnestic	0.0182	0.0211	0.0167	0.0084	0.0180	0.0132		0.0077	
Antibacterial	0.0026			0.0013	0.0020				
Antioxidative	0.0755	0.0587	0.0696	0.0498	0.0858	0.0835	0.0637	0.0733	0.0787
Antithrombotic	0.0182	0.0211	0.0167	0.0090	0.0200	0.0154		0.0077	0.0028
Bacterial permease ligand	0.0026	0.0023	0.0056	0.0019	0.0040	0.0154	0.0085	0.0026	0.0084
Chemotactic	0.0026	0.0023	0.0028						
Immunomodulating	0.0026	0.0023	0.0028	0.0006				0.0013	
Inhibitor	0.0078	0.0047	0.0084	0.0071	0.0080	0.0154		0.0026	0.0253
Neuropeptide	0.0026	0.0023	0.0028	0.0052	0.0040	0.0066	0.0042	0.0039	0.0028
Regulating	0.0208	0.0305	0.0195	0.0090	0.0180	0.0176	0.0021	0.0090	0.0028
Stimulating	0.0469	0.0235	0.0279	0.0304	0.0399	0.0484	0.0382	0.0347	0.0073
Immunostimulating		0.0023				0.0022		0.0013	

enzymes (chymotrypsin and papain). A combination of three enzymes may damage the original ACE inhibitory peptides which were produced from the previous proteolysis and may produce fewer new ACE inhibitory peptides. Moreover, Klompong *et al*. (2007) found that extensive proteolysis may release peptides with no bioactive properties. The finding was similar to the report by Lin *et al*. (2018) on yak milk casein and Ambigaipalan *et al*. (2015) on date seed protein. Therefore, the BIOPEP database simulation is a guide for further wet laboratory research into producing more bioactive peptides.

The result of the online tool using ToxinPred in Table 4 shows that all peptides derived from *in silico* analysis were non-toxic. The results of the toxicity analysis revealed that none of the peptides chosen were predicted to be toxic, implying that these peptides could potentially be used as functional food ingredients for human consumption. This study also showed that EBN is a potential substrate of bioactive peptides to be incorporated in food, nutraceutical, and cosmeceutical products.

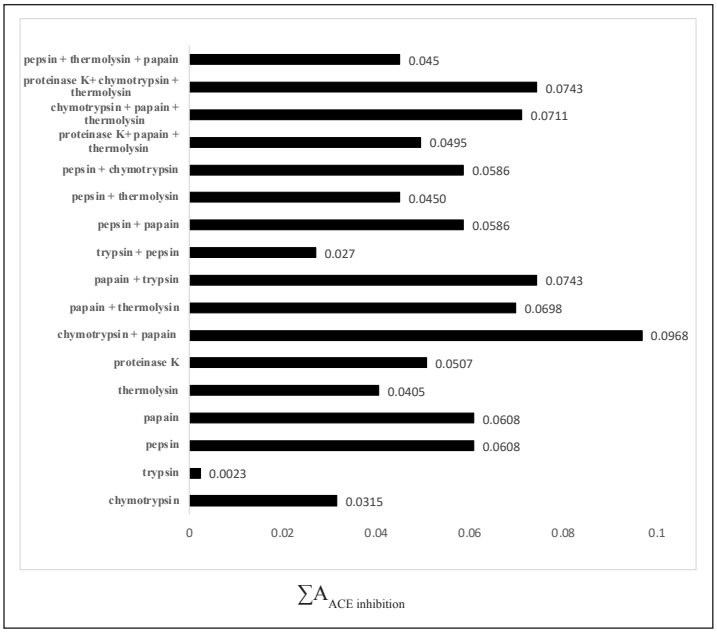

Fig.1. The sum of ACE inhibitory frequency in EBN protein sequence using a single enzyme and a combination of enzymes.

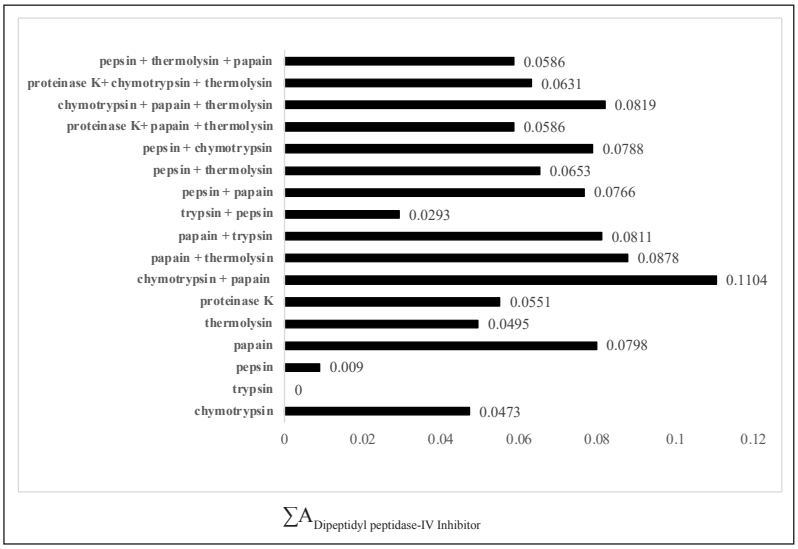

Fig. 2. The sum of DPP-IV inhibitory frequency in EBN protein sequence using a single enzyme and a combination of enzymes.

Table 3. Number of potential bioactive peptides of identified proteins and their potential biological activity (in parentheses)

Protein name	Acidic mammalian chitinase-like			Mucin-5AC-like		Ovo inhibitor-like	Nucleo bindin-2	45 kda calcium-binding protein	Lysyl oxidase homolog 3
Accession no.	XP_009995766.1	XP_010005363.1	XP_010006620.1	XP_009994736.1	XP_010002210.1	XP_010000294.1	XP_009993696.1	XP_010003465.1	XP_010006484.1
ACE inhibitor	177 (0.0189)	210 (0.0182)	165 (0.0195)	412 (0.0146)	166 (0.0146)	147 (0.0090)	132 (0.0142)	127 (0.0139)	347 (0.0104)
Dipeptidyl peptidase IV inhibitor	256 (0.0005)	283 (0.0004)	239 (0.0005)	859 (0.0004)	253 (0.0006)	257 (0.0003)	240 (0.0003)	212 (0.0004)	470 (0.0004)
Hypotensive	19 (0.0007)	13 (0.0003)	17 (0.0006)	31 (0.0005)	6 (0.0002)	4 (0.0007)		13 (0.0003)	13 (0.0014)
Alpha-glucosidase inhibitor	1 (0.0001)	1 (0.0001)	1 (0.0001)						
Opioid	1 (0.0001)	1 (0.0001)							

*The value of the potential biological activity of the protein (B) for specific activities was rounded off to the 4th decimal place.

Table 4. Toxicity Predicted from edible bird's nest protein

Identified Protein	Entry name (accession number)	Molecular weight (g/mol)	Toxicity
Acidic mammalian chitinase-like	XP_010006620.1	39918.98	Non-Toxin
	XP_010005363.1	46963.28	Non-Toxin
	XP_009995766.1	42931.60	Non-Toxin
Mucin-5AC-like	XP_009994736.1	169840.89	Non-Toxin
	XP_010002210.1	55951.41	Non-Toxin
Ovoinhibitor-like	XP_010000294.1	50986.56	Non-Toxin
Nucleobindin-2	XP_009993696.1	53603.21	Non-Toxin
45 kda calcium-binding protein	XP_010003465.1	41863.99	Non-Toxin
Lysyl oxidase homolog 3	XP_010006484.1	85888.63	Non-Toxin

CONCLUSION

In silico study showed that the selected EBN proteins in this study are potential precursors for ACE inhibitory peptides and DPP-IV inhibitors. AMCase protein sequences gave the highest number of bioactivities. The most potential bioactive peptides from EBN proteins are angiotensin-converting enzyme (ACE) inhibitors and dipeptidyl peptidase-IV (DPP-IV) inhibitors. It was found that the most suitable enzymes to prepare both peptides are a combination of chymotrypsin and papain. In addition, the peptides produced by *in silico* analysis for both bioactivities are predicted to be toxic-free.

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