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COMPARISON OF AMINO ACIDS PROFILE AND ANTIOXIDANT ACTIVITIES BETWEEN EDIBLE BIRD NEST AND CHICKEN EGG

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ABSTRACT

Edible bird's nest (EBN) and eggs are sources of high quality protein. The objectives of this study were to compare the amino acid profile between EBN and eggs to determine the differences in nutrition composition and antioxidant properties. Amino acid profile and antioxidant properties (DPPH, ABTS and FRAP) of four samples, raw EBN (EBN_{raw}), EBN hydrolysates (EBN_h), commercial chicken egg (Egg_{comm}) and free range chicken egg (Egg_{FR}) were determined and correlated with one another. It was found that EBN_{raw} and EBN_h have significantly higher ($p<0.05$) DPPH and ABTS activities compared to Egg_{comm} and Egg_{FR}, whereas Egg_{comm} and Egg_{FR} have significantly higher ($p<0.05$) FRAP activities compared to EBN_{raw} and EBN_h. Amino acid profile analysis showed that Egg_{comm} and Egg_{FR} have significantly higher ($p<0.05$) methionine, cysteine, lysine and isoleucine content compared to EBN_{raw} and EBN_h. Histidine, proline, phenylalanine and tryptophan in EBN were found to have significant ($p<0.01$) positive correlation with DPPH and ABTS antioxidant activities assays, while methionine and cysteine in chicken eggs have significant ($p<0.01$) positive correlation with FRAP activity. In conclusion, both EBN and chicken eggs are good sources of proteins and essential amino acids, but EBN showed higher antioxidant activities.

Key words: Antioxidant activity, amino acid profile, edible bird's nest, chicken eggs

INTRODUCTION

Amino acids are essential in many physiological functions such as biosynthesis and neurotransmitter transporter. Protein, which comprises of amino acid monomers, are components of body tissues, such as hormones and enzymes, as well as essential nutrients and energy source. Proteins' nutritional value is fundamentally determined by its amino acid compositions. Amino acids consist of both the amino group and the acid group. Besides, amino acids also consist of an asymmetric carbon and exhibit optical activity except for glycine (Wu, 2009). In other words, amino acids contain carboxylic acid (-COOH), amines ($-NH_2$), a hydrogen atom and an R group that is unique for every amino acid. Amino acids are made up of carbon, hydrogen, oxygen and nitrogen. Amino acids exist freely and formed peptides via dehydration condensation with covalent peptides bonds linking between amino acids. Numerous amino acids present as proteins (polypeptides) that exist in the body of organisms. Peptides are compounds which consists of multiple amino acids linked by peptide bonds. Oligopeptides are referred to peptides with 2-20 amino acids, whilst polypeptides are referred to those consisting of more than 20 amino acids (Takahashi *et al.*, 2011). Takahashi *et al.* (2011) also reported that most dietary amino acids are found in protein.

Human body naturally has enzymatic and non-enzymatic antioxidant defenses system that counteracts the damage from oxidants and disease-causing free radicals (Alam *et al.*, 2012). Anti-oxidants are normally obtained via chemical synthesis and extracted from plant and animal tissues (Govindarajan *et al.*, 2003). Typically, plants are potential natural antioxidant sources and they also produce different antioxidative phytochemicals that counteract reactive oxygen species (ROS) (Akbarirad *et al.*, 2016). However, there are natural antioxidants from animal sources, such as fish, meat, keratin and collagen. It gives physiological effect in the human body such as antihypertensive,

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immunomodulating, anti-thrombic, antioxidative, anticancer and antimicrobial (Vercruysse *et al.*, 2005).

The main component in edible bird's nest (EBN) is glycoprotein which exhibits properties related to health, vigour and beauty (Babji, 2014). EBN contains high levels of nutrients such as mineral salts, proteins and amino acids. EBN is rich in proteins and comprises of all the essential amino acids including valine, phenylalanine, threonine and proline (Yu-Qin *et al.*, 2000). Furthermore, EBN stimulates epidermal growth, strengthens immunity, as well as improves digestive and respiratory systems (Quek *et al.*, 2015). EBN consists of abundant amount of 18 different amino acids. However, some of them cannot be synthesised by the human body and must be obtained through dietary sources.

Although there are various species of swiftlets, i.e. more than 24 species, however, only a few species of swiftlets produce nests using its saliva, which are edible by human. Most EBN traded worldwide come from two heavily exploited species, *Aerodramus fuciphagus* and *Aerodramus maximus,* which are the white-nest swiftlet (Babji *et al.*, 2016). These swiftlets are endemic in the Southeast Asian region. The nests are made of saliva from sublingual salivary glands. Nests are typically including feather but they only amount to approximately 10% of dry weight (Kathan & Weeks, 1969).

Chicken eggs are considered as an economical source of animal protein for humans. The egg proteins are superior in nutrition and have an ideal proportion of essential amino acids (Nimalaratne *et al.*, 2016). Chicken eggs contain antioxidant compounds in which the egg yolk is richer in antioxidants as compared to egg white (Nimalartne & Wu, 2015). In comparison with other animal protein sources, eggs are higher in lutein and zeaxanthin which enhance the bioavailability of nutrients as well as saturated fat that normally found in the yolk. Eggs are also the main source of dietary cholesterol. Therefore, eggs are deemed good for modulating various body functions and reducing risks of cancer as well as coronary heart disease (Surai & Sparks, 2001).

This study compared the nutritional values of EBN (salivary mucus glycoprotein) and chicken eggs to determine their amino acids profiles. This study also analysed the antioxidant activities of EBN and eggs using 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethybenzothiazoline-6-sulphonic acid) (ABTS) and ferric reducing anti-oxidant power (FRAP) assays. Subsequently, correlations study were performed on the amino acids profiles and antioxidative activities.

MATERIALS AND METHODS

Materials

Commercial chicken egg (Egg_{comm}) and free range chicken egg (Egg_{FR}) was manufactured by Ng Loon Em & Sons Poultry Farm (M) Sdn. Bhd while raw EBN was purchased from Mobile Harvester's Malaysia Sdn. Bhd.

Chicken egg preparation

Eggs (yolk and white without shell) were homogenised using Ultra-Turrax Homogeniser for 5 mins, and frozen at -18°C for 24 hours and then freeze-dried (Christ Alpha 1-4 LD Plus, Germany) for 72 hours. The dried eggs were subsequently ground at high speed for 2 min using a commercial blender (WARNING 240V Torrington, CT, USA) and then filtered through a stainless steel sieve (40-mesh grid). The ground samples were kept in air-tight bottles and stored at -20°C until used.

Edible bird's nest preparation

Raw EBN (EBN_{raw}) was cleaned by soaking in water until softened and tweezers were used to remove small feathers and fine plumage. Cleaned EBN_{raw} was ground into microparticulate powder (size: 300 μm) using Buchi Mixer Homogenizer (B-400, Switzerland).

Enzymatic hydrolysis of edible bird's nest

The EBN suspension was incubated with the alcalase enzyme (Novozymes, Denmark) (1:100 w/v, pH 8, 60°C) for 2 hours. The enzyme to EBN protein fractions ratio was set at 1:100 (w/w). The temperature was then increased to 90°C for 5 minutes to cease the enzymatic hydrolysis activity. After cooling, the EBN hydrolysate (EBN_h) produced was then filtered (Whatman 4 filter paper) and the filtrate was freeze-dried (Christ Alpha 1-4 LD Plus, Germany) for 72 hours (Nurfatin *et al.*, 2016).

Determination of amino acids composition

Amino acids compositions were determined based on Murad *et al.* (2013). In order to determine all the amino acids present, three different hydrolysis methods were performed. The acid hydrolysis method (6 N HCl at 110°C for 24 hours) was performed to determine aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine. On the other hand, cysteine and methionine were analysed using performic oxidation (formic acid:hydrogen peroxide, 9:1 v/v; 4°C 16 hours; added with HBr, 4°C 30 minutes; and subsequently 6 N HCl at 110°C for 24 hours), while tryptophan was analysed using alkaline hydrolysis (4.3 N LiOH, 120°C 16

hours). Both the acid and performic acid hydrolysis methods were derivatised using AccQ Flour reagent, with α-aminobutyric acid (AABA) as an internal standard. Samples and standards (10 µL) were injected into HPLC (Waters) and analyses of amino acids were performed with AccQ Tag column, with Eluent A (200 mL AccQ Tag to 2 L of Milli-Q water) and the Eluent B (60% acetonitrile) as mobile phase with the linear gradient condition. Fluorescence detector (λ excitation and λ emission at 250 nm) was used to detect the amino acid eluted. As for the alkaline hydrolysis samples, the hydrolysates were injected into HPLC equipped with Nova Pak C18 column, eluted using 0.0085 M sodium acetate (pH 4.0) and methanol at a ratio of 86.7:13.3. Elusion was detected using a fluorescence detector (λ excitation at 285 nm and λ emission at 345 nm).

DPPH assay

DPPH assay was performed according to Khalafu *et al.* (2017) and Lim *et al.* (2017), with modifications. Samples (20 µL; 10 mg/mL) were mixed with 200 µL of 0.01 mM DPPH methanolic solution and added with 80 µL methanol. The mixtures were incubated the in dark for 1 hour at room temperature. The blank was prepared by substituting the samples with methanol. Samples were prepared in triplicates and the absorbance at 517 nm was measured using a microplate reader spectrophotometer. Samples were measured in triplicates and DPPH radical scavenging activity (%) was calculated using the following formula:

$$DPPH\ radical\ scavenging\ activity\ (\%) = 1 - \frac{Abs_{517}\ sample}{Abs_{517}\ DPPH\ solution} \times 100$$

ABTS assay

ABTS assay was performed based on Murad *et al.* (2013). Stock ABTS reagent was prepared by adding 7 mM ABTS solution to 2.45 mM potassium persulphate solution at a ratio of 1:1, and then incubated in the dark for 16 hours at room temperature. The obtained solution was then measured using spectrophotometer at 734 nm and diluted with methanol accordingly until it achieves absorbance of approximately 0.7. Then, 0.1 mL of samples (10 mg/mL) were added with 1 mL ABTS solution and the mixtures were incubated in the dark for 10 minutes at room temperature. The reduction of absorbance was read at 734 nm using microplate spectrophotometer. Trolox (5–300 µM) was used as a standard curve, and samples were measured in triplicates, and the ABTS radical scavenging activity (%) of all samples were determined using the following formula:

$$ABTS\ radical\ scavenging\ activity\ (\%) = 1 - \frac{Abs_{734}\ sample}{Abs_{734}\ ABTS\ solution} \times 100$$

Ferric reducing antioxidant power

All samples were prepared at 10 mg/mL using distilled water, and 0.5 mL of them were added to 2.5 mL of FRAP reagent (0.156g TPTZ, 0.27g $FeCl_3$, 50 mL acetate buffer). The samples were then incubated in the dark at room temperature for 10 minutes. The mixtures were then measured at 593 nm using a microplate spectrophotometer. FRAP activity was determined using the following formula:

$$Reducing\ power = Abs_{593}\ sample - Abs_{593}\ FRAP\ reagent$$

Statistical analysis

Data obtained were analysed statistically using one-way ANOVA and Duncan Test by using SPSS Version 22 (IBM) to compare differences between means. Correlations between the anti-oxidant activities and amino acids of the samples were established using Pearson's correlation coefficient (r).

RESULTS AND DISCUSSION

Amino acids profile of edible bird's nest and chicken eggs

The amino acids profile of EBN_{raw}, EBN_h, Egg_{comm} and Egg_{FR} are shown in Table 1. The highest essential amino acids content in both EBN_{raw} and EBN_h was valine, with values of 3.07 g/100 g and 2.61 g/100 g respectively. Threonine was the second highest essential amino acids content in both EBN_{raw} and EBN_h, recorded values of 2.49 g/100 g and 2.04 g/100 g respectively. However, the highest essential amino acids compositions in Egg_{comm} and Egg_{FR} was leucine, with values of 3.42 g/100 g and 3.28 g/100 g respectively. Lysine recorded the second highest content of essential amino acids in both Egg_{comm} and Egg_{FR}, with values of 2.89 g/100 g and 2.87 g/100 g respectively.

The most concentrated hydrophobic amino acids in EBN_{raw} was proline (3.63 g/100 g) while valine was the most concentrated hydrophobic amino acids in EBN_h (2.61 g/100 g). A similar result was obtained by Kathan and Weeks (1969) who reported that proline and valine are the major amino acids in the edible bird nest. Marcone (2005) also reported that EBN contains a high concentration of valine, isoleucine and tyrosine, which are essential amino acids. This showed that EBN is deemed as a good source of essential amino acids. For both Egg_{comm} and Egg_{FR}, leucine was the most con-centrated hydrophobic amino acids, with values of 3.42 g/100 g and 3.28 g/100 g respectively. The most concentrated sulphur amino acids in both EBN_{raw} and EBN_h was cysteine, with values of 0.67

Table 1. Amino Acids compositions (g/100g) of raw edible bird's nest (EBN_{raw}), hydrolysate edible bird's nest (EBN_h), commercial chicken egg (Egg_{comm}) and free range chicken egg (Egg_{FR}) (n=2)

Amino acids	EBN_{raw}	EBN_h	Egg_{comm}	Egg_{FR}
Aspartic acid	3.59 ± 0.99^{ab}	3.06 ± 0.13^{b}	4.71 ± 0.26^{a}	4.76 ± 0.09^{a}
Serine	3.74 ± 1.12^{a}	3.15 ± 0.13^{a}	3.36 ± 0.22^{a}	3.17 ± 0.06^{a}
Glutamine	3.01 ± 0.84^{b}	2.52 ± 0.09^{b}	5.85 ± 0.40^{a}	5.73 ± 0.24^{a}
Glycine	1.52 ± 0.44^{a}	1.26 ± 0.03 ^{a}	1.67 ± 0.10^{a}	1.66 ± 0.09^{a}
Histidine	1.53 ± 0.46^{a}	1.23 ± 0.06^{a}	1.29 ± 0.09^{a}	1.19 ± 0.06^{a}
Arginine	2.56 ± 0.87^{a}	2.01 ± 0.03^{a}	2.73 ± 0.15^{a}	2.59 ± 0.11^{a}
Threonine	2.49 ± 0.71^{a}	2.04 ± 0.09^{a}	2.18 ± 0.17^{a}	2.08 ± 0.13^{a}
Alanine	1.32 ± 0.46^{b}	1.07 ± 0.02^{b}	2.78 ± 0.24^{a}	2.72 ± 0.11^{a}
Proline	3.63 ± 0.75^{a}	2.27 ± 0.06^{ab}	1.57 ± 0.12^{ab}	1.53 ± 0.09^{b}
Tyrosine	1.93 ± 0.47^{a}	1.80 ± 0.11^{a}	1.66 ± 0.09^{a}	1.70 ± 0.04^{a}
Valine	3.07 ± 0.83^{a}	2.61 ± 0.09^{a}	2.78 ± 0.19^{a}	2.75 ± 0.09^{a}
Lysine	1.26 ± 0.29^{b}	1.11 ± 0.02^{b}	2.89 ± 0.23^{a}	2.87 ± 0.11^{a}
Isoleucine	1.16 ± 0.33^{b}	0.97 ± 0.04^{b}	2.21 ± 0.15^{a}	2.15 ± 0.08^{a}
Leucine	1.97 ± 1.1 ^{a}	2.00 ± 0.07^{a}	3.42 ± 0.24^{a}	3.28 ± 0.08^{a}
Phenylalanine	2.12 ± 0.56^{a}	1.82 ± 0.08^{a}	2.12 ± 0.15^{a}	2.10 ± 0.06^{a}
Tryptophan	0.61 ± 0.10^{a}	0.54 ± 0.01^{a}	0.60 ± 0.39^{a}	0.45 ± 0.13^{a}
Cysteine	0.67 ± 0.03^{b}	0.55 ± 0.02^{c}	0.71 ± 0.01^{b}	0.78 ± 0.02^{a}
Methionine	0.03 ± 0.10^{b}	0.03 ± 0.00^{b}	1.62 ± 0.09^{a}	1.60 ± 0.07^{a}
Total AAs	35.64 ± 10.95^{a}	30.58 ± 1.81^{a}	44.17 ± 3.32^{a}	43.13 ± 1.68^{a}
EAA	14.24 ± 4.39^{a}	12.37 ± 0.48^{a}	19.12 ± 1.72^{a}	18.48 ± 0.82^{a}
Non-EAA	20.98 ± 5.98^{a}	18.21 ± 1.33^{a}	25.05 ± 1.61^{a}	24.65 ± 0.85^{a}

Note: Total AAs: total amino acids; EAA: essential amino acid; EBN: Edible bird's nest; non-EAA: non-essential amino acids.
[a-c] Different letters in the same row denotes significant differences at $p<0.05$.

Table 2. Antioxidant activities of raw edible bird's nest (EBN_{raw}), hydrolysate edible bird's nest (EBN_h), commercial chicken egg (Egg_{comm}) and free range chicken egg (Egg_{FR})

Antioxidant activities	EBN_{raw} (%)	EBN_h (%)	Egg_{comm} (%)	Egg_{FR} (%)
DPPH	35.00 ± 1.00^{b}	45.00 ± 2.00^{a}	9.0 ± 1.00^{d}	26.0 ± 2.00^{c}
ABTS	81.50 ± 1.50^{b}	87.0 ± 2.00^{a}	63.0 ± 1.00^{c}	65.0 ± 1.00^{c}
FRAP	0.06 ± 0.01^{b}	0.05 ± 0.02^{b}	2.18 ± 0.15^{a}	2.00 ± 0.50^{a}

Note: DPPH: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activities, ABTS: 2,2'-Azino-Bis 3 ethylbenzothiazoline 6 Sulfonic Acid (ABTS) Radical Scavenging Activity, FRAP: Ferric Reducing Antioxidant Power
[a-d] Different letters in the same row denotes significant differences at $p<0.05$.

g/100 g and 0.55 g/100 g respectively. However, methionine was the most concentrated sulphur amino acids in both Egg_{comm} (1.62 g/100 g) and Egg_{FR} (1.60 g/100 g). Lewis *et al.* (1950) reported that the amino acids methionine and cysteine are the sulphur amino acids in egg protein. Both EBN_{raw} and EBN_h had a higher content of proline with values of 3.63 g/100 g and 2.27 g/100 g respectively as compared to Egg_{comm} and Egg_{FR}.

Antioxidant activities of edible bird's nest and chicken eggs

DPPH radical scavenging activities

EBN_h showed the significantly ($p<0.05$) highest DPPH radical scavenging activity, followed by EBN_{raw}, Egg_{FR} and Egg_{comm} as shown in Table 2. The different amino acid composition may be the reason for the variation observed among the four samples studied in this study. The DPPH radical scavenging activities increased from 35.0 ± 1.0% in raw EBN to 45.0 ± 2.0% in EBN_h. This is most likely due to the different quantity of amino acids present in EBN. Among the amino acids that exhibit antioxidant activities are cysteine, methionine, tyrosine, tryptophan, phenylalanine and histidine. EBN_h showed the highest DPPH and ABTS radical scavenging activities as compared to EBN_{raw} as it has a high ability as a hydrogen donor to produce non-radical species (Etty Syarmila *et al.*, 2014). Lower DPPH radical scavenging activities were observed in Egg_{comm} (9.0 ± 1.0%) and Egg_{FR} (26.0 ± 2.0%). This suggested that aromatic amino acids such as phenylalanine, tyrosine and tryptophan in egg yolk contributed minimally to the DPPH radical scavenging activity (Nimalaratne *et al.*, 2011).

Higher antioxidant activities in Egg_{FR} have been due to the higher content of cysteine content with a value of 0.78 ± 0.02 g/100 g compared to Egg_{comm} with a value of 0.71 ± 0.0 g/100 g. There were significant differences ($p<0.05$) between all samples in DPPH, ABTS and FRAP values. EBN_{raw} and EBN_h have higher values of DPPH and ABTS indicated that these antioxidant activities in EBN may be due to the presence of high composition of certain amino acids such as proline, which was found to be lower in Egg_{comm} and Egg_{FR}. Zambrowicz (2012) reported that several types of chicken eggs had shown lower values of DPPH and ABTS. This might be partially due to egg white which losing the primary structure of the antioxidative compounds.

ABTS radical scavenging activity

Table 2 shows that the EBN_h has the significantly highest ($p<0.05$) ABTS radical scavenging activity among all samples with a value of 87.0 ± 20.0%, followed by EBN_{raw}, Egg_{FR} and Egg_{comm} with values of 81.5 ± 1.5%, 65.0 ± 1.0% and 63.0 ± 1.0% respectively. In general, EBN showed higher ABTS radical scavenging activity as compared to chicken eggs. Etty Syarmila *et al.* (2014) reported that the high scavenging antioxidant activities in EBN might have been due to the high content of amino acids such as tryptophan and proline. Nurul Nadia *et al.* (2017) also reported that antioxidant activity showed in EBN was highly related to the presence of amino acid residues including hydrophobic amino acid (AAH) such as proline and aromatic amino acid (AAR) such as tryptophan and tyrosine which contributed to the antioxidant properties.

Ferric reducing antioxidant power (FRAP)

Table 2 shows that Egg_{comm} and Egg_{FR} had significantly ($p<0.05$) higher FRAP activities with values of 2.18 ± 0.15% and 2.00 ± 0.50% respectively, as compared to EBN_{raw} (0.06 ± 0.01%) and EBN_h (0.05 ± 0.02%). This showed that chicken egg had the ability to produce high potential electron donor which reduced reactive species of Fe^{3+} to stable species of ferrous ion (Fe^{2+}) (Benzie & Strain, 1996), as compared to EBN.

Correlation of amino acids and antioxidant activities

Both EBN_{raw} and EBN_h showed high DPPH and ABTS radical scavenging activities and it showed a strong positive correlation ($p<0.01$) between amino acids with these two radical scavenging activities. The amino acids which showed strong positive correlations with antioxidant activities are histidine, proline, phenylalanine and tryptophan. The correlation of DPPH radical scavenging activity with histidine, proline, phenylalanine and tryptophan in EBN_{raw} have r-values of 0.822, 0.932, 0.940 and 0.962 respectively while for ABTS radical scavenging activity in EBN_{raw} have r-values of 0.845, 0.867, 0.852 and 0.901 respectively. Moreover, the correlation of DPPH radical scavenging activity with histidine, proline, phenylalanine and tryptophan in EBN_h with r-values of 0.942, 0.889, 0.807 and 0.967 respectively while the ABTS radical scavenging activity in EBN_h with r-values of 0.872, 0.912, 0.810 and 0.964 respectively. However, there was no significant correlation between amino acids and DPPH/ABTS radical scavenging activities found for both eggs samples. Both EBN_{raw} and EBN_h showed a strong negative correlation ($p<0.01$) of IRAP with cysteine and methionine, with r-values of -0.868 and -0.873 respectively in EBN_{raw} whereas -0.742 and -0.846 respectively in EBN_h. On the other hand, egg samples have shown a strong positive correlation ($p<0.01$) of IRAP with cysteine and methionine, with r-values of 0.930 and 0.897 in Egg_{comm} whereas 0.872 and 0.996 in Egg_{FR}. There is no significant correlation between DPPH and ABTS radical scavenging activities with the amino acids in both Egg_{comm} and Egg_{FR}. The greater FRAP activity could be due to the presence of cysteine and methionine, which contributed to the antioxidant activities and this is similar to the result obtained by Nimalaratne *et al.* (2011). Ghassem *et al.* (2017) reported that two pentapeptides sequence found in EBN, i.e. Pro-Phe-His-Pro-Tyr and Leu-Leu-Gly-Asp-Pro, were responsible for the antioxidation activities of EBN. This corresponds with our findings where EBN which showed significant antioxidant activities, also showed significant amounts of these amino acids, especially histidine, proline, phenylalanine and tryptophan.

CONCLUSIONS

The highest amount of essential amino acids in both raw EBN and EBN hydrolysates was valine, while for both commercial chicken egg and free range chicken egg was leucine. However, chicken egg contained significantly higher amount of aspartic acid, glutamine, alanine, lysine, isoleucine, methionine and cysteine. EBN samples have significantly ($p<0.05$) higher ABTS and DPPH scavenging activities, while chicken eggs have significantly ($p<0.05$) higher FRAP activities. The amino acids in EBN (histidine, proline, phenylalanine and tryptophan) had shown significant positive correlations ($p<0.01$) with radical scavenging activity of ABTS and DPPH while amino acid in chicken egg (cysteine and methionine) had shown a positive correlation ($p<0.01$) with FRAP activities.

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REFERENCES

Akbarirad, H., Gohari, A.A., Kazemeini, S.M. & Mousavi, K.A. 2016. An overview on some of important sources of natural antioxidants. *International Food Research Journal,* **23(3)**: 928-933.

Alam, M., Bristi, N. & Rafiquzzaman, M. 2012. Review on in vivo and vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal,* **21**: 143-152.

Babji, A.S. 2014. Development of downstream products from edible bird's nest. *Edible Bird Nest Industry Conference 2014*. 25-26 November 2014, Putrajaya.

Babji, A.S., Etty Syarmila, I.K., Nurfatin, H., Najafian, L. & Nur Aliah, D. 2016. Bioactive glycoprotein components from white hydrolysed edible bird nest. *Presentaion at the 20th World Congress on Clinic Nutrition (WCCN) Rama Gardens Hotel, Bangkok*, 14-16 December 2016.

Benzie, I.F.F. & Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry,* **239**: 70-76.

Etty Syarmila, I.K., Nurfatin, M.H., Masitah, M., Farahniza, Z., Mohd. Khan, A., Zalifah, M.K. & B. Abdul Salam. 2014. *Edible bird nest Industry Conference 2014*, 25-26 November 2014, Putrajaya.

Ghassem, M., Arihara, K., Mohammadi, S., Sania, N.A. & Babjia, A.S. 2017. Identification of two novel antioxidant peptides from edible bird's nest (*Aerodramus fuciphagus*) protein hydrolysates. *Food and Function,* **8**: 2016.

Govindarajan, R., Rastogi, S., Vijayakumar, M., Shirwaikar, A., Rawat, A.K.S., Mehrotra, S. & Pushpangadan, P. 2003. Studies on the antioxidant activities of *Desmodium angeticum*. *Biological & Pharmaceutical Bulletin,* **26(10)**: 1424-1427.

Kathan, R.I. & Weeks, D.I. 1969. Structures studies of Collocalia mucoid carbohydrate and amino acid composition. *Archives of Biochemistry and Biophysics,* **134**: 572-6.

Khalafu, S.H.S., Wan Aida, W.M., Lim, S.J. & Maskat, M.Y. 2017. Effects of deodorisation methods on volatile compounds, chemical properties and antioxidant activities of fucoidan isolated from brown seaweed (*Sargassum* sp.). *Algal Research*, **25**: 507-515.

Lewis, J.C., Snell, N.S., Hirschmann, D.J. & Fraenkel-Conrat, H. 1950. Amino-acid composition of egg proteins. *Journal of Biological Chemistry,* **186**: 23-35.

Lim, S.L., Khalafu, S.H.S., Wan Aida, W.M. & Lim, S.J. 2017. Effects of Different Precipitation Method on Physicochemical Properties and Antioxidant Activities of Alginates from *Sargassum* sp. *Sains Malaysiana*, **46(10)**: 1807-1816

Marcone, M.F. 2005. Characterization of the edible bird's nest the "Caviar of the East". *Food Research International,* **38(10)**: 1125-1134.

Murad, M., Abdullah, A. & Wan Aida, W.M. 2013. Antioxidant capacity and amino acid profiles of egg tofu. *American Journal of Applied Sciences*, **10**: 1315-1324.

Nimalaratne, C., Lutz, D.L., Schieber, A. & Wu, J. 2011. Free aromatic amino acids in egg yolk show antioxidant properities. *Food Chemistry,* **129**: 155-161.

Nimalaratne, C., Schieber, A. & Wu, J. 2016. Effect of storage and cooking on antioxidant capacity of laying hen egg. *Food Chemistry,* **194**: 111-116.

Nimalaratne, C. & Wu, J. 2015. Hen egg as an antioxidant food commodity: a review. *Nutrients,* **7**: 8274-8293.

Nurfatin, M.H., Etty Syarmila, I.K., Nur 'Aliah, D., Zalifah, M.K., Babji, A.S. & Ayob, M.K. 2016. Effect of enzymatic hydrolysis on Angiotensin converting enzyme (ACE) inhibitory activity in swiftlet saliva. *International Food Research Journal,* **23(1)**: 141-146.

Nurul Nadia, M., Babji, A.S., Ayub, M.K. & Nur Aliah, D. 2017. Effect of enzymatic hydrolysis on antioxidant capacity of cave edible bird's nests hydrolysates. *International Journal of ChemTech Research,* **10(2)**: 1100-1017.

Quek, M.B., Chin, N.L., Yusof, Y.A., Tan, S.W. & Law, C.L. 2015. Preliminary nitrite, nitrate and colour analysis of Malaysian edible birds nest. *Processing in Agriculture,* **2**: 15.

Surai, P. & Sparks, N. 2001. Designer egg: form improvement of egg composition functional food. *Food Science & Technology,* **12**: 7-16.

Takahashi, T., Toda, E., Singh, R.B., Meester, F.D., Wilczynska, A., Wilson, D. & Juneja, L.R. 2011. Essential and non-essential amino acids in relation to glutamate. *The Open Nutraceuticals Journal,* **4**: 205-212.

Vercruysse, L., Van Camp, J. & Smagghie, G. 2005. ACE inhibitory peptides derived from enzymatic hydrolysates of animal muscle protein: A review. *Journal of Agriculture and Food Chemistry,* **53**: 8106-8115.

Wu, G. 2009. Amino acids: metabolism, functions, and nutrition. *Amino acids,* **37(1)**: 1-17.

Yu-Qin, Y., Liang, X., Hua, W., Hui-Xing, Z., Xin-Fang, Z. & Bu-Sen, L. 2000. Determination of edible bird's nest & its products by gas chromatography. *Journal of Chromatographic Science,* **38(1)**: 27-32.

Zambrowicz, A., Pokora, M., Eckert, E., Szoltysik, M., Dabrowska, A., Chrzanowska, J. & Trziszka, T. 2012. Antioxidant and antimicrobial activtiy of several types chicken egg. *Functional Foods in Health and Disease,* **2(12)**: 487-500.