EDIBLE BIRD NEST HYDROLYSATES AS NATURAL ANTIOXIDATIVE PEPTIDES

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Introduction

Edible bird nest (EBN) mainly comprises a secretion of the salivary gland of Aerodramus genus is commercially available in form as whole cleaned nests or EBN soup. Nowadays, *Aerodramus fuciphagus*nest has been used for centuries whether as a tonic or a health food, forming the basis of a multi-billion dollar industry worldwide. These beneficial health effects may be attributed to numerous known peptides sequences exhibiting antioxidative, antimicrobial and antihypertensive activities. This study focused on producing glycopeptides via enzymatic hydrolysis of irradiated EBN powder, followed by enzymatic hydrolysis toexplore their potential bioactivities (antioxidative and antihypertensive).

Materials and Methods

Materials

Edible bird nests from Pahang area were supplied by Nest Excel Resources Sdn. Bhd. and grounded into microparticulate powder.

Gamma Irradiation

The EBN powder was irradiated at doses of 0.0, 1.0, 2.0, 5.0, 7.5, 10.0, 20.0 and 30.0 kGy using cobalt-60 irradiator (220 Gammacell ® Excel) at a rate of 2.17 $kGy.h^{-1}$.

Enzymatic Hydrolysis

EBN peptides were prepared using alcalase enzyme (30079 Sigma) according to the method reported by (Choa et al., 2004) with some modifications.

Amino Acid Profile

Determination of amino acid profiles was carried out according to the method proposed by Fontaine (2003).

Microbiological Analysis

Total plate count, *Staphylococcus aureus* count, *Salmonella* spp. detection and yeast and fungi counts were conducted using the method proposed by Roberts and Greenwood (2003) with minor modifications. *Coliforms* and *Escherichia coli* (*E. coli*) count were performed according to the method of Thermo Fisher Scientific Inc. (2001-2003) with minor modifications.

Degree of Hydrolysis, Protein Solubility and Peptide Content

The degree of hydrolysis (DH), protein solubility and peptide content were estimated using methods described by Hoyle & Merrit (1994), Lowry (1951) and Church et al. (1983) respectively with minor modification.

Antioxidative Assay

The antioxidant activity was determined by three different methods; 2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) proposed by Yang et al. (2009), 2,2'-Azinobis-(3-Etilbenzthiazolin-6-Asid Sulfonik)diammonium salt (ABTS) proposed by Wiriyaphan et al.(2012) and ferric reducing antioxidant power (FRAP) reported by Hogan et al. (2009) with slight modifications.

Results And Discussion

Table 1 showed the microbiological count of irradiated EBN powder at different doses.The TPC was significantly decreased with the increasing dose of gamma irradiation. The coliform count was significantly reduced ($P<0.05$) by 2.08 log $CFU.g^{1}$ after irradiated at 1.0 and 2.0kGy respectively. By increasing the irradiation dose to 5.0kGy, the coliform count of EBN microparticulated powder was greatly reduced to <100 $CFU.g^{-1}$ sample. The *E. coli* counts was reduced to <100 $CFU.g^{-1}$ at doses as low as 1.0kGy. At 2.0kGy, the number of *S. aureus* in the irradiated EBN powder was reduced by 1.96 log cycle respectively and the microbe was not detected or reduced to <100 $CFU.g^{-1}$ samples at 5.0kGy and higher. *Salmonella typhimurium* strain (ATCC 14028) was used as the positive control for the detection of *Salmonella* spp. Results showed that there was no *Salmonella* spp. detected in the irradiated EBN powder. The yeast and mould count decreased significantly ($P<0.05$) with the increasing of irradiation doses. Gamma irradiations at the dose of 5.0kGy and above was effective to reduce yeast and fungi counts (<1000$CFU.g^{-1}$).

Table 1. Microbiological quality of irradiated edible bird nest (EBN) powder at different irradiation doses.

Irradiation doses (kGy)	TPC (log $CFU.g^{-1}$)	*Coliform* count (log CFU.g-1)	*E.coli* count (log $CFU.g^{-1}$)	*S. aureus* count (log $CFU.g^{-1}$)	Yeast and mould count (log $CFU.g^{-1}$)
0.0	7.64 ± 0.00^{aA}	5.95 ± 0.01^{aA}	2.47 ± 0.10^{A}	4.55 ± 0.04^{aA}	5.10 ± 0.02^{aA}
1.0	7.09 ± 0.03^{bA}	3.80 ± 0.00^{bB}	< 2.0	3.72 ± 0.01^{bA}	4.74 ± 0.01^{bA}
2.0	6.29 ± 0.02^{cA}	3.16 ± 0.02^{bB}	< 2.0	2.59 ± 0.16^{bA}	3.39 ± 0.13^{cA}
5.0	4.90 ± 0.02^{dB}	< 2.0	< 2.0	< 2.0	< 3.0
7.5	4.24 ± 0.02^{dA}	< 2.0	< 2.0	< 2.0	< 3.0
10.0	3.84 ± 0.05^{dA}	< 2.0	< 2.0	< 2.0	< 3.0
20.0	< 2.0	< 2.0	< 2.0	< 2.0	< 3.0
30.0	< 2.0	< 2.0	< 2.0	< 2.0	< 3.0

Each data represents average value and ±standard deviation of triplicate replication, n=3.
a-d Means between column are significantly different ($p < 0.05$). A – B Means between line are significantly different ($p < 0.05$).
< 2.0 log cfu/g = No microorganism detected at dilution -2.

Amino acid profiling of the irradiated EBN powder was carried out to determine the effect of irradiation on nutritional quality of EBN. Result showed that gamma irradiation at doses as high as 10.0 and 20.0kGy did not significantly affect ($P>0.05$) the amino acid profile of the samples.The nutritional effect of irradiated EBNmight be related to the dose of irradiation as low level of doses gave no effect on nutritional quality. Matloubi et al. (2004) reported that, irradiation does not create a serious problem from a nutritional standpoint because the amino acids itself are protected from damage by irradiation in a complete protein structure.

Table 2 showed the degree of hydrolysis (DH), protein solubility and peptide content of EBN hydrolysate at different times of hydrolysis. By increasing the time of hydrolysis (h) to 1.5h, it increased the DH, protein solubility and peptide content to 82.7%, 104.1mg/g and 86.68mg/g respectively. The degree of hydrolysis indicated the percentage of peptides breakdown and it is usually used as a parameter to control the level of proteolysis (Adler-Nissen, 1979). Accordingly, after an initial rapid phase of hydrolysis up to 1.5h, the rate of hydrolysis tend to decrease and entered a stationary phase. At this point, increasing the time of hydrolysis did not result in higher degree of hydrolysis because the concentration of peptide bonds available for hydrolysis may be the limiting factor. Montecalvo et al. (1984) reported that increasing of DH also caused increase in cleavage of peptide bonds which then increases the peptides solubility. The high value at 1.5 h of hydrolysis indicates that more peptide bonds were broken down by the alcalase enzyme at that duration.

Table 2. Degree of hydrolysis (DH), protein solubility, peptide content and antioxidant activity of EBN in different time of hydrolysis.

Sample (h)	Degree of hydrolysis (%)	Protein solubility (mg/g)	Peptide content (mg/g)
0.0	7.6 ± 5.0^{c}	25.5 ± 2.8^{e}	27.7 ± 0.2^{e}
0.5	71.2 ± 1.8^{b}	62.0 ± 0.7^{d}	81.2 ± 0.9^{d}
1.0	72.0 ± 3.7^{b}	74.3 ± 1.4^{b}	85.7 ± 0.3^{c}
1.5	82.7 ± 1.9^{a}	86.7 ± 0.4^{a}	104.1 ± 0.1^{a}
2.0	73.5 ± 1.9^{b}	69.3 ± 0.1^{c}	87.8 ± 0.9^{b}
3.0	73.3 ± 3.2^{b}	76.0 ± 2.0	86.2 ± 0.8^{c}
4.0	72.6 ± 3.2^{b}	70.1 ± 2.0^{c}	81.9 ± 1.8^{d}

Each data represents average value ± standard deviation of triplicate replication, n=3. ($P<0.05$). $^{a-e}$ Means between time of hydrolysis are significantly different.

Hydrolysis is necessary in order to release ROS inhibitory peptides from an inactive form within the sequence of edible bird nest protein. During hydrolysis, a wide variety of smaller peptides and free amino acids is generated, depending on enzyme specificity. Antioxidant activities, as determined by DPPH, ABTS and FRAP assays of hydrolysates were shown in Table 3. EBN hydrolyzed at 1.5h (82.7% DH) showed the highest DPPH and ABTS radical-scavenging activities with value of 59.45%and 69.45%respectively. This indicated that hydrolysate produced at 1.5h have the high ability as hydrogen donor to produce non radical species. The varying scavenging radicals capabilities might be caused by the difference in chain length and amino acid composition of the peptides in the different hydrolysates. The highest FRAP reducing power activities recorded by EBN hydrolyzed at 2.0h (73.5%DH) with value of 0.57. Hidrolysis time of 2.0h produce hydrolysate with high potential of electron donor to reduce reactive species of Fe^{3+} to stable species of Fe^{2+}.

Table 3. Antioxidant activity of EBN hydrolysates at different time of hydrolysis at concentration of 5mg mL^{-1}

Sample (h)	Antioxidant activity		
	DPPH (%)	ABTS (%)	FRAP
0.0	27.17 ± 0.20^{f}	29.30 ± 0.6^{d}	0.27 ± 0.03^{e}
0.5	41.72 ± 1.2^{e}	51.65 ± 0.1^{c}	0.31 ± 0.04^{d}
1.0	42.45 ± 0.3^{d}	52.01 ± 0.6^{c}	0.45 ± 0.02^{b}
1.5	59.45 ± 0.8^{a}	69.45 ± 0.2^{a}	0.46 ± 0.01^{b}
2.0	56.29 ± 0.8^{b}	69.28 ± 0.5^{a}	0.57 ± 0.02^{a}
3.0	55.90 ± 0.2^{c}	60.60 ± 0.8^{b}	0.44 ± 0.04^{c}
4.0	55.74 ± 1.2^{c}	60.24 ± 0.3^{b}	0.45 ± 0.01^{b}

Each data represents average value ± standard deviation of triplicate replication, n=3. ($P<0.05$). $^{a-e}$ Means between time of hydrolysis are significantly different.

Conclusion

Bioactive EBN hydrolysates were produced with alcalase enzymatic hydrolysis of low dose irradiated micrparticulate EBN powder. The hydrolysates demonstrated significant antioxidative activities.

Acknowledgements

Centre of Excellence (CoE) Faculty of Veterinary Medicine, Universiti Putra Malaysia (6371400-10301), Universiti Kebangsaan Malaysia (STGL-004-2013) and Innovation Centre for Confectionary Technology Universiti Kebangsaan Malaysia (MANIS).

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