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Assessment on bioactive components of hydrolysed edible bird nest

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Abstract

Edible bird's nest (EBN) is one of the most highly valued food products of South East Asia. It is widely consumed as a health food due to its beneficial effects to human health and has been considered to be one of the most precious food items by the Chinese for thousands of years. As more products-containing EBN are being developed, that include the food, beauty and skincare industries, the future of EBN seems promising. EBN contains rich sources of structurally diverse nutritional components. Major components of edible bird nest were explored upon their functional characters to promote consumer's health. This paper presents an overview study which has been done on bioactive components generated from EBN through enzymatic hydrolysis process; which related to antioxidant, inhibitory of angiotensin converting enzyme (ACE), and microbiological quality. In addition, extracted edible bird nest components with antimicrobial properties are insignificant, yet promote the growth of bacteria, which showed potential prebiotic ingredients. As the bioactive components may be used in food formulations to improve human's health, they might as well improve beneficial microbes in human's gut.

Introduction

Most edible bird nests (EBNs) traded worldwide come from two heavily exploited species, the White-nest swiftlet *(Aerodramus fuciphagus)* and the Black-nest swiftlet *(A. maximus)*. The swiftlet species commonly found in Malaysia is *A. fuciphagus* which construct their nest with glutinous strands of starch-like saliva produced by a pair of salivary glands under their tongue; thereafter functioned to protect their young in the nest (Goh *et al*., 2001). The order of composition (from lowest to highest) in EBN is: lipid (0.14–1.28%), ash (2.1%), carbohydrate (25.62–31.40%) and protein (60–66%), thus making EBN a good source of protein (Marcone, 2005; Saengkrajang *et al*., 2013; Zainab *et al.*, 2013). Carbohydrates in the EBN consisted of sialic acid (9.0%), galactosamine (7.2%), glucosamine (5.3%), galactose (16.9%) and fucose (0.7%) (Kathan and Weeks, 1969; Ma and Liu, 2012). EBN are a valuable source of high quality functional proteins (Babji *et al*., 2015). Micro-sized EBN had been explored to study the therapeutic and nutraceutical value in response of delivery system to the target organs and its effectiveness. This study reviews functional bioactive quality of EBN that have been generated through enzymatic hydrolysis and possible benefits of glycoprotein within EBN as potential prebiotic activities.

Materials and Methods

Preparation of EBN

Edible bird nests from Pahang area were supplied by Nest Excel Resources Sdn. Bhd. Raw EBN was soaked in a water with ratio 1:100 (w/v) before incubated at 4°C for 16 hours. The sample was then boiled at 100°C for 30 minutes and cooled to room temperature before adjusted to the suitable pH for enzymatic hydrolysis.

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 30.0kGy using cobalt-60 irradiator (220 Gammacell® Excel) at a rate of 2.17kGy.h^{-1}.

Amino acid profile

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

Enzymatic hydrolysis

Enzyme alcalase was added to substrate with ratios 1% of enzyme to substrate and the hydrolysis

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was carried out for 4 hours. The hydrolysates were heated in boiling water for 5 mins to inactivate the enzyme, and then centrifuged at 4°C and 4,000rpm for 10 mins. The supernatant was filtered using Whatman No.1 and the filtrate was freeze-dried before stored for further analysis.

Ultrafiltration process

The high and low molecular weight fractions of EBN protein hydrolysates were separated by ultrafiltration at 4°C after filtered with a 0.2 µm membrane. The hydrolysates were passed through ultrafilter membranes (Vivaflow 200, Sartotius, Germany) with 10 kDa and 3 kDa molecular weight cut-off (MWCO), accordingly. The hydrolysates were designated as <10 kDa and <3 kDa fractions. All fractions were lyophilised and stored at −20°C until further use.

Microbiological analysis

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

Antioxidant and angiotensin converting enzyme (ACE) inhibitory assays

The antioxidant activity was determined by three different methods; 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay proposed by Yang *et al*. (2009), 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS) radical scavenging assay proposed by Wiriyaphan *et al.* (2012) and ferric reducing antioxidant power (FRAP) assay based on Hogan *et al.* (2009). ACE inhibitory assay was performed using the method of Cushman and Cheung (1971).

Statistical analysis

Data obtained were analysed statistically with analysis of variance and Duncan test using SPSS Version 20 (SPSS 2011) to identify the significance difference among the samples ($^{*}p<0.05$). Data reported from triplicate determination.

Results and Discussion

Determination of bioactive components extracted from edible bird nest

Bioactive components have been defined as specific components such as protein fragments that have a positive impact on body functions or conditions and may ultimately influence health (Kitts and Weiler, 2003). According to Leh (2001), EBN has a long shelf-life period, thus it will not turn mouldy even when left in a moist environment for a couple of days. Based on the stated observation, antimicrobial study had been conducted on the EBN glycoprotein and its hydrolysates. Yet, results as shown in Table 1 demonstrated low antimicrobial activities of crude aqueous EBN extract and its hydrolysate fractions, which indicate insignificant activity. This result indicates that other components (such as sialic acid that was partially lost during EBN washing process) or factors (such as low water activity) might be involved in the self-mechanism of EBN protection from being used by fungi and moulds.

Table 1. Antimicrobial activity of crude aqueous EBN extract and hydrolysate fractions.

Bacterial strain	Inhibition diameters (mm)				
	Crude extract	>30 kDa	10-3 kDa	<3 kDa	Gentamycin (15 µg/disc)
Bacillus cereus	6	6	8	10	17
Enterococcus faecalis	6	6	8	9	10
Staphylococcus aureus	6	6	12	15	18
Escherichia coli	6	6	6	6	22
Klebsiella pneumoniae	6	6	6	6	17
Salmonella typhimurium	6	6	10	12	17

Diameter disc (6 mm) included
Gentamycin as a reference

Table 2. Microbiological quality of irradiated EBN powder at different irradiation doses.

Irradiation doses (kGy)	Total plate count (log CFU.g-1)	Coliform count (log CFU.g-1)	*E. coli* count (log CFU.g-1)	*S. aureus* count (log CFU.g-1)
0	7.64 ± 0.00	5.95 ± 0.01	2.47 ± 0.10	4.55 ± 0.04
1	7.09 ± 0.03	3.80 ± 0.00	< 2.0	3.72 ± 0.01
2	6.29 ± 0.02	3.16 ± 0.02	< 2.0	2.59 ± 0.16
5	4.90 ± 0.02	< 2.0	< 2.0	< 2.0
7.5	4.24 ±0.02	< 2.0	< 2.0	< 2.0
10	3.84 ± 0.05	< 2.0	< 2.0	< 2.0
20	< 2.0	< 2.0	< 2.0	< 2.0
30	< 2.0	< 2.0	< 2.0	< 2.0

< 2.0 log CFU/g = No microorganism detected at dilution -2.

In a way to make sure the EBN is safe to be kept and further to be consumed, Abdullah Sani *et al.* (2015) has conducted a study to lower the high bacterial population in EBN by irradiation method, with view to ensure safety, resulting in safe level of total plate count (TPC) as well as for pathogenic bacterial count significantly. This study also showed that gamma irradiation improved the microbiological quality of EBN, as in Table 2, without affecting its amino acid profile.

In another study, edible bird nest hydrolysates showed strong antioxidant and ACE-inhibitory activities via a few different in vitro assays. The assays on EBN hydrolysates prepared using alcalase

Table 3. Antioxidant and ACE-inhibitory activities of EBN hydrolysates at different time of hydrolysis.

Sample (h)	Antioxidant activity			ACE-I activity	
	DPPH (%)	ABTS (%)	FRAP (abs)	ACE-I (%)	IC_{50} value (mg/ml)
0	27.17 ± 0.20^{d}	29.30 ± 0.6^{d}	0.27 ± 0.03^{d}	6.88 ± 4.85^{d}	-
0.5	41.72 ± 1.2^{c}	51.65 ± 0.1^{c}	0.31 ± 0.04^{c}	83.07 ± 2.4^{b}	0.07^{b}
1	42.45 ± 0.3^{c}	52.01 ± 0.6^{c}	0.45 ± 0.02^{b}	86.24 ± 3.2^{a}	0.02^{a}
1.5	59.45 ± 0.8^{a}	69.45 ± 0.2^{a}	0.46 ± 0.01^{b}	71.43 ± 4.2^{c}	0.15^{c}
2	56.29 ± 0.8^{b}	69.28 ± 0.5^{a}	0.57 ± 0.02^{a}	81.48 ± 4.0^{b}	0.09^{b}
3	55.90 ± 0.2^{b}	60.60 ± 0.8^{b}	0.44 ± 0.04^{b}	73.54 ± 5.7^{c}	0.18^{c}
4	55.74 ± 1.2^{b}	60.24 ± 0.3^{b}	0.45 ± 0.01^{b}	71.43 ± 4.0^{c}	0.19^{c}

$^{a-d}$ Means between time of hydrolysis are significantly different ($p < 0.05$).

Table 4. Antioxidant and ACE-inhibitory activities of different fractions of EBN hydrolysates.

Fractions	Molecular weight (kDa)	ABTS (%)	DPPH (%)	FRAP (abs)	Antioxidant EC_{50} (mg/g)	ACE-I IC_{50} (mg/g)
Unhydrolyzed EBN	>30	29.30 ± 0.6^{d}	27.17 ± 0.20^{d}	0.27 ± 0.03^{c}	-	3.5^{d}
Hydrolyzed EBN	<30	69.45 ± 0.2^{b}	59.45 ± 0.8^{b}	0.46 ± 0.01^{b}	1.186^{b}	2.2^{c}
Ultrafiltrate	≤10	75.25 ± 1.3^{a}	62.25 ± 1.0^{a}	0.52 ± 0.8^{a}	0.898^{a}	1.6^{b}
	≤3	39.47 ± 1.1^{c}	30.15 ± 0.9^{c}	0.28 ± 1.0^{c}	1.723^{c}	0.06^{a}

$^{a-d}$ Means between samples are significantly different ($p < 0.05$).

enzyme, was significantly higher compared to the crude EBN, as shown in Table 3. Further study with ultra-filtrated fractions (results as in Table 4), EBN hydrolysates with low molecular weight less than 10 kDa showed higher antioxidant activities, followed with hydrolysate without ultra-filtrate and hydrolysates with molecular weight less than 3 kDa (Etty Syarmila *et al*., 2014). On the other hand, the EBN hydrolysates fraction with molecular weight less than 3 kDa demonstrated the highest ACE-inhibitory activity (Nurfatin *et al*., 2016).

Studies on amino acid profile from soluble EBN hydrolysates demonstrated that there were significant increase ($p<0.05$) of aromatic amino acid composition (histidine, phenylalanine, and tyrosine) and essential free amino acid such as valine and threonine in hydrolysate compared to the raw EBN, as in Table 5. The low molecular weight of EBN hydrolysate fractions contained higher content of hydrophobic free amino acids which showed a correlation with the observed biological activities in all evaluated in vitro assays (Etty Syarmila *et al.*, 2014). Furthermore, the peptides with molecular weight less than 3 kDa was then purified using gel filtration chromatography (GPC) and reverse-phase high performance liquid chromatography (RP-HPLC) to purify the peptides that give the highest value of activities.

The most active fraction was subjected to LC-ESI-TOF mass spectrometry for peptide identification. In this study, peptides with sequence of PFHPY and LLGDP, corresponding to f134-138 and f164-168 of cytochrome b of A. fuciphagus, indicating the highest ORAC values, with significant ($p<0.05$) increase of aromatic amino acid (His, Phe, and Tyr) composition, valine (Val), serine (Ser), proline (Pro), threonine (Thr) and phenylalanine (Phe), were identified. These results demonstrated the effects of specific enzymatic hydrolysis used may release the functional bioactive components of EBN compared to crude EBN (Ghassem *et al.*, 2017). Furthermore, EBN fractions showed potentials as a source of functional and bioactive components that can be used in the development of natural food preservatives, functional food ingredients and nutraceuticals with significant health benefits and enhanced shelf-life quality.

Glycoprotein from edible bird nest as potential prebiotic ingredients

Glycoproteins from the EBN might have a potential to be a prebiotic on growth of probiotic microorganisms. Prebiotics may function as food ingredients to selectively stimulate growth of both beneficial bacteria which are already established in the colon as well as externally administered probiotic bacteria. Possible benefits that can get from the consumption of the prebiotics such as gut health maintenance, colitis prevention, cancer inhibition, immuno-stimulation, cholesterol removal, reduction of cardiovascular disease, prevention of obesity and constipation, based on symbiotic benefits of microbial fermentation in the gut environment (Patel and Goyal, 2012). Prebiotics are usually mixtures of indigestible oligosaccharides; except for inulin, a mixture of fructooligo- and polysaccharides (Manning and Gibson, 2004). Possible benefits for the gut microbiota that could be obtained from EBN

Table 5. Amino acid profile of raw EBN and hydrolysed EBN

Amino acid	Raw EBN (%)	Hydrolysed EBN (%)
Aspartic acid	10.39 ± 0.110[a]	10.07 ± 0.001[a]
Serine	3.84 ± 0.008[b]	9.27 ± 0.030[a]
Glutamine	15.70 ± 0.946[a]	7.09 ± 0.026[b]
Glycine	4.13 ± 0.774[b]	4.34 ± 0.494[a]
Histidine	2.25 ± 0.073[b]	3.43 ± 0.494[a]
Arginine	6.15 ± 0.347[a]	6.29 ± 0.305[a]
Threonine	4.70 ± 0.254[b]	6.93 ± 0.017[a]
Alanine	6.34 ± 0.071[a]	2.92 ± 0.546[b]
Proline	4.75 ± 0.378[b]	7.43 ± 0.163[a]
Tyrosine	4.56 ± 0.347[b]	6.66 ± 0.167[a]
Valine	5.29 ± 0.002[b]	7.81 ± 0.108[a]
Lysine	9.11 ± 0.546[a]	5.21 ± 0.677[b]
Isoleucine	5.52 ± 0.288[a]	3.17 ± 0.056[b]
Leucine	8.47 ± 0.086[a]	7.00 ± 0.021[b]
Phenylalanine	4.34 ± 0.314[b]	6.54 ± 0.010[a]
Tryptophan	1.23 ± 0.009[a]	1.43 ± 0.067[a]
Cysteine	2.60 ± 0.148[a]	3.23 ± 0.157[a]
Methionine	0.63 ± 0.081[a]	1.18 ± 0.256[a]
Total Amino Acids (%)	100	100
Total EAA (%)	36.02	39.53
Total Non-EAA (%)	63.98	60.47

[a-b] Means between samples are significantly different ($p < 0.05$).

glycoprotein and its bioactive components such as glycoprotein, glycopeptides, peptides and glycan in relation to functional food could be studied.

The consumption of different plant- and animal-derived dietary glycans (in the form of poly- or oligosaccharide) by human being, most of which cannot be degraded by enzymes encoded in the human genome. Microbial fermentation may transform these indigestible glycans into short-chain fatty acids (SCFAs), which may serve as nutrients for human body (Koropatkin *et al.*, 2012). Potential prebiotic oligosaccharides can be classified according to their chemical constituents and degree of polymerization such as manno-oligosaccharides (Zentek *et al.*, 2002), pectic-oligosaccharides (Olano-Martin *et al.*, 2003), soybean-oligosaccharides (Saito *et al*., 1992), isomalto-oligosaccharides (Morgan *et al.,* 1992), xylo-oligosaccharides (Campbell *et al*., 1997) and lactulose (Tuohy *et al.*, 2002).

It is well-known that specific prebiotics encourage specific colon microbial populations *in vitro* and *in vivo* (Gibson and Roberfroid, 1995). For example, lactulose has been reported to support the growth of bifidobacteria and decrease the number of bacteroides in a culture model as well as in the human colon (Owen, 1997; Bouhnik *et al*., 2004). Faecal levels of *Bifidobacterium lactis* increases by oral administration of galactooligosaccharides (Malinen, 2002). On the contrary, β-glucans support the growth of *Lactobacilli* spp. more than the *Bifidobacteria* spp. (Jaskari *et al*., 1998). The EBN mucus is mainly constituted of sialic acid, rich in O-glycosidically linked oligosaccharides (Hanisch and Uhlenbruck, 1984), which have a potential to serve as prebiotic oligosaccharides.

Conclusion

Most of EBN's nutritional value was stored in the glycoprotein and its nature had been identified. This is based on variety of glycoprotein isolation and purification methods that have been developed specifically for EBN. However, there is still much work need to be done. Studies in Universiti Kebangsaan Malaysia over the last 3 years explored the potential of glycopeptides in EBN as functional bioactives such as antimicrobial, antioxidant and ACE-inhibitory components present in the hydrolysates. A study on the possible benefits of EBN and its hydrolysates as prebiotics are currently being investigated. Preliminary results on the probiotic activities indicated EBN hydrolysate has a potential to be prebiotic with higher probiotic bacterial growth count compared to uncleaned as well as cleaned crude EBN. A series of studies had been conducted and with the exception on antimicrobial properties, other functional activities showed beneficial results after enzymatic hydrolysis of the EBN.

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