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Differentiation of Malaysian Farmed and Commercialised Edible Bird's Nests through Nutritional Composition Analysis

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ABSTRACT

The growing demand of edible bird's nest (EBN) worldwide as well as competition from neighbouring countries has made the EBN industry one of the rising industries in Malaysia with stringent exportation requirement by China. However, as majority of the EBN products in the market is in commercialised form, studies on the nutritional composition of these commercialised EBN in comparison with farmed, raw EBN are limited. The farmed EBN samples were taken from 4 different regions of Malaysia: Perak (central), Kelantan (eastern), Johor (southern) and Sarawak (west Borneo) while the commercialised sample was obtained from a local drug store. Proximate, amino acid and elemental composition were performed on these EBNs. Farmed EBNs mainly comprised protein followed by carbohydrate while the commercialised EBN had similar protein and carbohydrate composition. The total collection of essential amino acid in commercialised EBN was higher (237.9 mg/g protein) compared to the farmed EBN which was between 156.81 – 236.6 mg amino acid/g protein. Among the essential amino acids, valine was found

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to be highest in both commercialised and farmed EBN. The differences between the nutritional compositions of EBNs could be due to the process of commercialisation of the EBN as well as seasonal, breeding sites and diet of the swiftlets. Farmed EBN therefore can be considered to be more nutritional due to higher protein levels.

Keywords: Amino acid analysis, commercialised EBN, edible bird's nest (EBN), elemental analysis, farmed EBN, food analysis, food composition, proximate analysis

INTRODUCTION

Edible bird's nest (EBN) is a well-known and precious delicacy among Chinese communities around the world produced by swiftlets. Swiftlets are birds (Apodidae) similar to swallows and sparrows but are not closely related. They consume a wide range of aerial insects like the other species however, they are able to fly at a higher velocity and manoeuvrability (Lim, 2007). Swiftlets weigh between 6 to 40 g which makes them relatively small in size (Ibrahim et al., 2009). They are mainly found in South East Asia countries from the coastal regions of Malaysia, Thailand, Vietnam, Islands in the Philippines and South-Eastern part of China (Aowphol et al., 2008; Phach & Oisin, 2007). Among the many species that produce EBN, *Aerodramus fuciphagus* and *Aerodramus maximus* are of commercial interest as the nest produced by these swiftlets are most pure hardened cement with minor trails of feathers and contaminants (Zukefli et al., 2017).

EBN has been eaten in traditional Chinese cuisine from as far back as the Tang Dynasty (907 AD). Traditionally EBN is prepared by double boiling with rock sugar and is known to maintain general health and youthful looking skin. It has been proven in many of the research studies on the health benefits of the EBN such as the proliferative effects of the corneal keratocytes and human adipose-derived stem cells (hADSCs). EBN was also found to inhibit the hemagglutination of the human erythrocytes caused by the influenza A virus. EBN extract also appeared to promote bone strength and improvement of skin complexion (Haghani et al., 2016).

The history of the swiftlet industry in Malaysia is unique and has grown since the 18th century where most of the nests were collected from the caves. With rapid urbanisation, the availability of the swiftlet nesting site has reduced and therefore the use of buildings that imitate cave-like environment as a swiftlet nesting site has been promoted this is called swiftlet farming. This industry has grown sizeable over the years and has a market value of USD 1000 to USD 10 000 per kilogram(Chua & Zukefli, 2016; Looi & Omar, 2016). The high price of the EBN has led to the widespread of counterfeiting and adulteration of the EBN. The commonly used materials include *Tremella* fungus, gum karaya, red seaweed, gelatine, agar and starch (Ma & Liu, 2012).

In contrast to the rapid growth of the swiftlet industry, comparative scientific investigation on the nutritional and medicinal properties between farmed, unprocessed

EBN and commercialised, ready-to-eat EBN is not as rapid. It had been reported that the major macro nutrients components were found out to be carbohydrates and proteins with 10-27% and 40-60% of the total mass respectively (Ma & Liu, 2012). Additionally, EBN is a mucin glycoprotein; with a polypeptide backbone and polysaccharides chains with a molecular weight between 40-130 kDa (Shim et al., 2016). All essential amino acids have been reported to be present in EBN with two of amino acid markers were suggested to differentiate between house and cave EBN; namely tyrosine and glutamic acid (Seow et al., 2016). Mineral composition of different types of EBN has also been reported (Marcone, 2005) and amount of minerals in EBN is highly correlated with the higher ash content in the sample (Chua & Zulkefli, 2016). However, to ease EBN consumption, many EBN products have been prepared in a more commercialised manner. Adulteration of these EBN products with gum karaya, red seaweed, *Tremella* fungus may alter its nutritional composition and therefore, this paper plans to investigate the nutritional composition of both commercialised and farmed EBN collected from various parts of Malaysia.

MATERIALS AND METHOD

Edible Bird's Nest Preparation

Farmed EBN in this paper is defined as house, uncleaned, raw EBN while commercialised EBN is defined as commercially cleaned, processed nests which are being sold to the public for consumption. Around 30 pieces of farmed nest per region were collected from several regions throughout Malaysia to represent different regions of Malaysia namely Perak (central), Kelantan (eastern), Johor (southern) and Sarawak (west of Malaysia) before pooled to depict each of the regions. These nests were cleaned by soaking in distilled water and manually to remove dirt, feathers and other foreign materials with a use of forceps. After cleaning process, the nests were air dried before being ground into powder using a mortar and pestle. The finely grounded EBN was then filtered through different size filters to remove left over feathers and other foreign materials. The commercialised EBN collected from swiftlet houses of a reputable brand claimed to be originated from Sarawak was obtained from a local pharmacy store. Conventional cleaning process for this type of EBN consisted of separating the feathers and dirt with immersion of the nests in high grade reverse osmosis water. Separation of large feathers and other impurities were achieved by using forceps and for smaller feathers, vegetable oil was used to float the feathers. Before drying in the oven, the separated strands of EBN and broken filaments were molded into leaf shape depending on the producer's preference. Upon reception, these samples were finely ground as described before and labelled accordingly before being kept in an air-tight bottle until further analysis. About 15 pieces of commercialised EBN were utilised in this study.

Proximate Composition Determination

The determination of moisture, protein, fibre, ash and fat contents of the ground EBN samples were performed following the official methods of the Association of Official Analytical Chemistry (AOAC, 2005). The moisture content of the samples was determined according to AOAC by oven drying (method 952.45). The dried samples from the previous analysis were used to determine the ash content by inserted into a muffle furnace at 550 ˚C for 5 h. The micro Kjeldahl method was used to determine the crude protein content in the samples, using 6.25 as a conversion factor (method 945.16). Fibre was determined after digesting a known weight of a fat free sample in refluxing with 1.25% sulfuric acid and 1.25% sodium hydroxide (method 926.09). The crude fat content was measured for the extracted lipid fraction of the EBN samples (Igwe et al., 2007). The crude carbohydrate content was calculated by deducting the sum of protein, fat, fibre and ash from 100%).

Elemental Analysis

Two grams of EBN sample was burned at 550°C for 5 h until no black particle was present. The sample was then added with concentrated hydrochloric acid (HCl) and was boiled until near dryness. The residue was then resuspended in 2 N HCl and filtered through a filter paper and was made up to 100 mL with deionised water. The adsorption of the solution was measured using an Atomic Absorption Spectrometer (AAS) according to the AOAC method 965.09 (AOAC, 2005).

The standard solution was prepared by dissolving 1.249 g of $CaCO_3$ in a minimum amount of 3 N HCL and diluting with deionized water to 1 L. The final stock solution was prepared by diluting 50 mL of the previous solution to 1 L with deionized water. One gram of pure zinc metal was dissolved in 10 mL of 6 N HCl and diluted with deionized water to 1 L. The standard was then diluted with dilution factors of 5x, 20x and 50x. The absorption of the solution was measured using an atomic absorption spectrophotometer (AAS) according to AOAC method 965.09 (AOAC, 2005).

Amino Acid Analysis

The amino acids content of the samples was determined according to Waters™ PICO.TAG™ method (P/N 88131). An internal standard (AABA) was prepared by dissolving 0.2578 g using 0.1 M HCl and adjusting the volume to 1 L. Mobile phase A contained 0.1 M ammonium acetate at pH 6.5, and mobile phase B had 0.1 M ammonium acetate with acetonitrile and methanol (44:46:10) at pH 6.5. The pH was adjusted using acetic acid. The preparation of redrying solution was performed by mixing methanol, water and triethylamine with a ratio of 2:2:1, and the derivation reagent contained phenylisothiocyanate (PITC), deionized water, trimethylamine and methanol with a ratio of 1:1:1:7.

For analysis, a 4/x g of sample was weighed, where x represented the percentage of protein in the respective samples, which were determined earlier. The samples were placed in test tubes with covers, and 15

mL of 6 N HCL was added to the sample and vortexed. The test tubes were put in an oven at 110°C for 24 h. After cooling, 10 mL AABA (internal standard) was added to the test tubes. The hydrolysed samples were poured into 50-mL volumetric flasks and brought to volume with deionized water, then filtered through filter paper (0.2 µm cellulose membrane filter). A 10 µL aliquot of the hydrolysed sample was put into a Durham tube. The same amount of mixed amino acids standard was also inserted into another Durham tube. The tubes were dried under vacuum for 30 min, and 20 µL of redrying solution was added to each tube before mixing the mixtures. The tubes were vacuum dried for another 30 min and were then mixed with 20 µL of derivatization reagent and vortexed. The tubes were left at room temperature for 20 min and were vacuum dried for 30 min until dryness. High pressure liquid chromatography (HPLC) with an RP 18 column (3.9 mm x 15 cm) was used to identify and quantify the amino acids. Before injection into the HPLC, the samples and standards were mixed with 100 µL of mobile phase A and vortexed for 15 min. The injection volumes of the standard, blank and samples were 8, 8 and 20 µL, respectively.

Statistical Analysis

All variables were tested for normality by applying the Kolmogorov-Sminov test and results were expressed as mean ± standard deviation (n=2). The data was statistically treated by using the Fisher least significant different method and $p < 0.05$ considered to be statistically significant. The statistical analysis was performed using GraphPad Prism (GraphPad Software, Inc. USA) and Minitab 17 Statistics software (USA).

RESULTS AND DISCUSSION

Proximate Analysis

The proximate composition of the EBN collected different regions of Malaysia is shown in Table 1. From Table 1, there was significant difference in the moisture content of EBN from Johor, Kelantan, Perak, Sarawak with the commercialised EBN. The EBN from Johor showed the highest moisture with 17.7% whereas the commercialised EBN had the lowest

Table 1
Proximate analysis of farmed EBN samples collected from various regions of Malaysia in comparison to commercialised EBN

Origin of EBN	Proximate composition (% dry matter, n=2)					
	Moisture	Ash	Fibre	Crude fat	Crude protein	Carbohydrate
Commercialised	0.9±0.01^{d}	3.9±0.04^{c}	0.5±0.03^{b}	0.4±0.03^{b}	46.6±2.35^{b}	47.7±2.39^{a}
Johor	17.7±0.04^{a}	5.1±0.1^{b}	0.1±0.01^{c}	0.3±0.01^{c}	55.3±0.45^{a}	21.5±0.41^{b}
Kelantan	15.3±0.23^{c}	5.0±0.07^{b}	0.0±0.00^{c}	0.3±0.01^{c}	56.3±0.34^{a}	23.1±0.19^{b}
Perak	17.6±0.01^{ab}	5.4±0.02^{a}	0.1±0.01^{c}	0.3±0.01^{c}	54.2±0.18^{a}	22.4±0.19^{b}
Sarawak	16.7±0.01^{b}	5.4±0.09^{a}	0.7±0.11^{a}	1.9±0.04^{a}	55.2±0.83^{a}	20.1±0.98^{b}

* Means in category column that do not share a letter are significantly different

content of 0.9%. The amount of moisture contents found in commercialised EBN was significantly lower than in all farmed EBN while EBN from Kelantan appeared to be having the least moisture content in comparison to EBN collected from Johor, Perak and Sarawak. Although Ma and Liu (2012) reported to have lower range of moisture content (7.5 - 12.9%), Saengkrajang et al. (2013) had similar range of moisture content as reported in this study (17.8 - 24.3%).

The protein content was found to be the highest macro component found in farmed EBN originated from Johor, Kelantan, Perak and Sarawak with 55.3%, 56.3%, 54.2% and 55.2%, respectively except for commercialised EBN with 46.6%. However, no significant difference in protein composition was found between different types of farmed EBN. In contrast, crude protein levels found in this study were lower than other studies (Norhayati et al., 2010; Saengkrajang et al., 2013) but higher than local ones reported by Nurul Huda et al. (2008). The differences of protein content in the EBN could be caused by the variation in climate conditions (Hun et al., 2015). Proteins found in EBN have been proven to have many potential benefits such as epidermal growth (Ma & Liu, 2012) and inhibition of influenza viruses (Guo et al., 2006).

Carbohydrate contents of the farmed EBN were not significantly different from each other with an average value of 27 % but it was significantly higher in the commercialised EBN compared to the farmed EBN from different regions. The lower carbohydrate content from the farmed EBN could also be due to cleaning and washing processed used to clean EBN which is different from commercialised cleaning method for commercialised EBN. The carbohydrate content of farmed EBN was found to be similar (20.1-23.1%) with previous reports which ranged between 10.63-31.4% (Ma & Liu, 2012; Saengkrajang et al., 2013). The unusually high amount of carbohydrate in commercialised EBN could be due to the presence of adulterant such as gum karaya, which predominantly consists of carbohydrate (Marcone, 2005).

The fibre was found to be very minute in every EBN examined between 0.1 - 0.7 % while none was detected in Kelantan EBN. The presence of fibre was only reported in a study by Saengkrajang et al. (2013) and it was in accordance with farmed EBN (with an exception of Sarawak origin) in this study as fibre being the smallest constituents in EBN. Crude fat was found to be the highest in composition in Sarawak EBN followed by commercialized EBN. The crude fat content detected (0.3 – 1.9 %) was lower compared to the studies of EBN done in provinces in Indonesia (2.3 – 9 %) but in close margin to the other studies done in Penang, Malaysia (0.2 - 2.5 %) (Marcone, 2005; Nurul Huda et al., 2008) Crude fat was found to be the highest in Sarawak EBN which might be due to the differences in humidity level of Sarawak's caves which contributed to the hydrolytic cleavage of the triacylglycerol of EBN (Marcone, 2005).

The amount of ash found in commercialised EBN was lower than in farmed EBN but was within the range as reported by previous studies (2.1 - 7.4%) for farmed EBN (Ma & Liu, 2012; Saengkrajang et al., 2013).

Variations found in the proximate composition of EBN were contributed by the locations (regions) as good feeding environment and affluence of insects serve as indicator of sustainable swiftlet populations.

Amino Acid Analysis

The amino acid composition of farmed and commercialized Malaysian EBN is presented in Table 2 with the total collection of essential amino acids collected from the farmed EBN is between 156.81 – 236.6 mg amino acid/ g protein while the commercialised EBN has a total of 237.9 mg amino acid / g protein. Among all essential amino acids, valine was found to be the highest in all farmed EBN samples including commercialised EBN while methionine was the very least non-essential amino acid in all types of EBN samples. As for the non-essential amino acids, cysteine was found to be the highest in commercialized EBN but appeared to be the second least amino acid in all farmed EBNs. The most predominant amino acids found in farmed EBN collected from different regions of Malaysia is aspartic acid which was the highest in Kelantan (53.7 mg amino acid/g protein) compared to Johor, Perak, Sarawak with 49.7, 49.3 and 36.8 mg amino acid/g protein respectively.

The total collection of essential amino acids of farmed EBN in this study was far higher than what was found in Thailand (15.9 to 31.6 mg amino acid/g protein) (Saengkrajang et al., 2013). Aspartic acid was found to be the most predominant amino acids in farmed EBN collected from different regions of Malaysia and being one of the non-essential amino acids found in EBN, these findings were in agreement with Saengkrajang et al. (2013) in which glutamine, among non-essential amino acid which was the most predominant amino acids found in Thailand's EBN. Aspartic acid is made from glutamic acid and has a role in neurotransmission or neuromodulator (D'Aniello, 2007). Glutamic acid was also considered to be one of the markers to differentiate house from cave EBN (Seow et al., 2016).

Conversely, cystine was the most predominant essential amino acid in commercialised EBN and this sulphur-containing amino acid are known to be the most potent modulators in lipid metabolism with beneficial functions against arteriosclerosis and metabolic syndrome (Oda, 2006). Adulterated EBN (white) was said to have less phenylalanine and tyrosine in which it produced less intense yellow-red reaction during xanthoproteic acid test (Marcone, 2005). However, the level of both amino acids was almost similar between commercialized and farmed EBN in this study.

The amount and composition of the amino acid found in the EBN might directly relates to the diet and environment of the

Table 2
Amino acid composition (mg amino acids/g protein) of farmed EBN samples collected from various regions of Malaysia in comparison to commercialized EBN

Origin of EBN	Amino acid (mg/g protein;n=2)							
	Histidine	Threonine	Valine	Methionine	Isoleucine	Leucine	Phenylalanine	Total essential amino acid
Commercialized	24.10±2.20	25.64±1.16	39.69±1.09	9.23±3.18[a]	20.24±1.83	38.55±0.65	38.78±0.95	237.9
Johor	19.14±0.45	38.74±0.88	40.84±0.05	3.60±0.23[ab]	17.99±0.53	36.28±0.31	35.19±0.05	211.56
Kelantan	20.95±0.24	41.89±0.33	41.11±0.21	3.23±0.09[b]	18.21±0.07	37.88±0.09	36.96±0.34	219.46
Perak	18.66±1.07	39.65±0.74	40.80±0.11	3.68±0.1 [ab]	17.47±0.07	36.51±0.05	35.56±0.26	210.62
Sarawak	12.55±3.61	20.55±6.36	25.33±7.40	4.31±1.68[ab]	11.35±2.98	22.89±5.92	18.92±4.69	232.83

Origin of EBN	Amino acid (mg/g protein;n=2)								
	Aspartic Acid	Glutamic Acid	Serine	Glycine	Arginine	Alanine	Cystine	Total non-essential amino acid	Total amino acid
Commercialized	74.86±8.57	53.19±0.73	43.37±2.77	20.065±0.44	51.44±0.27	19.17±0.87	126.58±14.21[a]	388.69	626.59
Johor	49.78±0.20	41.17±1.97	38.79±0.92	18.43±0.77	33.92±0.42	13.91±0.51	5.41±0.68[b]	201.41	412.97
Kelantan	53.79±0.85	43.42±0.94	40.99±0.16	21.01±0.16	35.42±0.20	16.65±0.37	5.53±0.5 [b]	216.81	436.27
Perak	49.38±0.01	42.52±0.01	38.13±0.16	19.73±0.55	34.06±0.38	14.67±0.31	4.42±0.89 [b]	202.91	413.53
Sarawak	29.73±10.01	28.31±9.46	27.19±9.29	11.69±3.40	20.95±7.12	11.35±3.28	4.86±2.38 [b]	245.47	478.3

* Means in the category column that do not share a letter are significantly different
**Means in the category column that do not have a letter are not significantly different

swiftlets itself. Past research have shown that fatty acid content in the EBN relates to the diet of the insects that the swiftlet preys on (Chua et al., 2014). This diet would likely affect the amount and composition of the amino acid in the EBN itself. Nevertheless, EBN was found generally low in essential amino acids composition and not a good candidate as a sole source of protein in human diets (Seow et al., 2016).

Mineral Content

The mineral levels detected in different types of EBN is shown in Table 3 and expressed in mg / g of EBN. As the amount of ash content in the commercialised EBN and the farmed EBN is similar, the mineral content found in the samples are also similar. According to Table 3, the mean calcium content (Ca) was the highest in both commercialised and farmed EBN collected from Sarawak followed by iron (Fe) and potassium (K). Manganese (Mn) was the least mineral component found in all types of EBN in this study. In agreement with Saengkrajang et al. (2015), Ca content was the highest in both the commercialised EBN and farmed EBN and Mn was the least mineral compound found in all EBN in this study. This can be explained due to different locations where the EBN were collected as insects (food source for swiftlets) that live near the seashore may constitute high content of Ca and other minerals such as sodium (Na), magnesium (Mg) and potassium (K) (Lee et al., 2007). Differences in the environmental conditions in which EBN were collected in this study has contributed to the discrepancy in the Ca level as reported by Seow et al. (2016) that Ca content was slightly more in the cave EBN in comparison to farmed EBN collected from Johor, Kelantan and Perak. This finding was in agreement with Nurul Huda et al. (2008) and Saengkrajang et al. (2015) with its vital role in regulating nerve and muscle function (Soetan et al., 2010). It is believed that the Na content of their nest (swiftlet saliva) was correlated with the Na content in marine aerosol (water droplets in the air) through different drinking behaviour of swiftlets (capturing water droplets in the air) (Seow et al., 2016).

It can be concluded that differences in elemental profile of different types of EBN could be contributed to availability of food and types of swiftlets (white or red

Table 3
Elemental composition of farmed EBN samples collected from various regions of Malaysia in comparison to commercialised EBN

Origin of EBN	Content of elements (mg/ g dry matter; n = 1)							
	Copper	Magnesium	Manganese	Potassium	Sodium	Calcium	Iron	Selenium
Commercialised	1.6	46.8	1.4	140.8	125.8	412.8	148.6	38.6
Johor	10.8	48.2	2	10.92	127	ND	62.8	ND
Kelantan	7.2	47.6	1.6	81.2	120.6	ND	70.8	ND
Perak	11.2	47.4	24.6	80.8	119.6	ND	434.8	ND
Sarawak	6.6	55.8	1.3	205.1	149.1	2343.3	282.3	ND

*ND means not determined

nest swiftlets). Although these minerals are important micronutrients in human diets, excessive intake of minerals can be harmful to health (Norhayati et al., 2010).

CONCLUSION

Based on the results obtained from this study, farmed EBN collected from Malaysia (Perak, Kelantan, Johor, Sarawak) were found to have 55% of protein content in comparison to the commercialised EBN which had 46.6% of protein content. Both types of EBN in this study had a high composition of amino acids with cysteine being the predominant amino acid in commercialised EBN while aspartic acid was the main essential amino acid in all farmed EBNs. Calcium was the most abundant EBN mineral in all EBN samples while manganese was the least abundant EBN mineral found in all EBN. As the farmed EBN has a higher protein level it was believed to be more nutritional and beneficial to consume as compared to the commercialized EBN. The nutritional composition of EBN might be affected by the process of commercialisation of EBN for human production as well as the seasonal, breeding sites and diet of the swiftlets. (Saengkrajang et al., 2013).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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