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The diversity in nutritional profile of farmed edible bird's nests from several regions in Indonesia

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Abstract. *Elfita L, Wientarsih I, Sajuthi D, Bachtiar I, Darusman HS. 2020. The diversity in nutritional profile of farmed edible bird's nests from several regions in Indonesia. Biodiversitas 21: 2362-2368.* Edible bird's nest (EBN) is produced by certain swiftlet species mainly *Aerodramus fuciphagus*. This study aimed to compare the composition of proximate, nitrite, nitrate, amino acid and protein profiles of EBNs collected from different regions in Indonesia (West Sumatra, South Sumatra, West Java, West Kalimantan, Central Sulawesi, and Southeast Sulawesi). The results showed that the order of proximate composition was protein (53.09-56.25%) > carbohydrate (19.57-23.04%) > moisture (17.08-21.50%) > ash (5.44-6.25%) > fat (0.07-0.57%). Nitrite and nitrate contents were 3.11-18.28 ppm and 650.11-1051.06 ppm, respectively. Amino acid analysis found that EBNs contained 18 amino acids, composed of ten essential amino acids and eight non-essential amino acids. Aspartic acid content of EBNs from West Sumatra and West Kalimantan (4.21 and 3.27%, respectively) were much higher than the one found in other regions, which was on the range of 0.32-0.37%. SDS-PAGE analysis demonstrated that majority of EBNs possessed seven protein bands with molecular weight range of 19.6 to 82.7 kDa. However, EBNs from West Sumatra and West Kalimantan showed abundant of protein with molecular weight of approximately 34.0 kDa. Thus, EBNs collected from different regions in Indonesia showed different nutritional profiles.

Keywords: Amino acid, edible bird's nest, Indonesia, nutritional profile, proximate, SDS-PAGE

INTRODUCTION

Edible bird's nest (EBN) is a nest made of salivary secretions from male swiftlets of genera *Aerodramus*/ *Collocalia*, when female swiftlets spawn. EBN is often consumed as healthy and luxury food particularly by Chinese community due to their suggested health benefits. It has also been used for traditional Chinese medicine in China and Southeast Asia, because it is believed that EBN can cure many diseases.

Study of EBN chemical composition is important for understanding its biological activity both as medicine and as functional food. Many studies on chemical composition of EBN, including physicochemical, proximate, amino acid, protein, hormone, sialic acids, and mineral content have been published (Marcone et al. 2005; Huda et al. 2008; Norhayati et al. 2010; Liu et al. 2012; Hamzah et al. 2013; Paydar et al. 2013; Saengkrajang et al. 2013; Helmi et al. 2018; Quek et al. 2018). Those studies found that the composition of EBN consists mainly of protein and carbohydrate, followed by moistures and ash. In addition, EBN also contains fat and trace amount of minerals including sodium, calcium, and magnesium. However, It is well known that the nutrient content of EBN is different, influenced by seasonal variation and breeding sites (Norhayati et al. 2010). This is because different locations have different vegetation and insect population, resulting in variation in macro- and micronutrient composition of EBNs.

Presently, EBN-producing countries are in South-East Asia, with Indonesia, Malaysia, and Thailand producing more than 95% of the world's supply (swiftletecopark.com.my). As one of mega-biodiversity countries, Indonesia has a diverse avifauna including swiftlets (*Collocalia fuciphaga*) that in consequent produce a lot of EBNs, and in fact, Indonesia supplies approximately 75% of the world's EBN market. Nevertheless, studies on the properties and characteristics of EBN from Indonesia are still limited. So far, few studies on nutritional analysis of EBN have been reported, however, the samples were sporadically collected from one or two islands in Indonesia (Huda et al. 2008; Hamzah et al. 2013; Helmi et al. 2018).

The comparison of the nutritional composition of EBN from different regions in Indonesia has important reference value for the EBN commercial development and application. Therefore, the objective of this study was to analyze and compare, in a more systematic manner, the

chemical composition of EBNs collected from four main islands in Indonesia, which are Sumatra, Java, Kalimantan, and Sulawesi. In addition to the nutritional composition, nitrite, and nitrate contents also become a big concern due to their toxicity. Nitrite has been used as a food preservative and antibotulinal agent in the food processing industry and its level is strictly controlled to prevent food toxicity (DSM 2011). The source of nitrite and nitrate could have been derived from ammonia through anaerobic fermentation by the bacteria in bird soil (Langham 1980). It is also suspected that these variations of nitrite level among EBNs derived from different sources with subjected to different environments, humidity, and climate of the habitat (Jong et al. 2013). Therefore, in this study, we also analyzed nitrite and nitrate composition in EBNs and compared those contents with those in EBNs from surrounding countries.

MATERIALS AND METHODS

Sample collection and preparation

EBNs were collected from six EBN farms in four main Islands in Indonesia, which were Sumatra, Java, Kalimantan, and Sulawesi. The location of farms was in Painan in West Sumatra (WS), Sekayu in South Sumatra (SS), Bogor in West Java (WJ), Ketapang in West Kalimantan (WK), Palu in Central Sulawesi (CS) and Bombana in Southeast Sulawesi (SES) (Figure 1). The EBN samples were purchased directly from farmers (20 pieces, approximately 200 g from each farmer). The EBNs were verified by Research Center for Biology, Indonesian Institute of Sciences (LIPI), and confirmed that all EBNs were from *Aerodramus fuciphagus* Thunberg, 1812. The EBNs were washed with distilled water, and cleaned to remove dirt and feathers manually by using forceps and scissors. After drying at 60°C for 24 hours, the samples were finely ground using a grinder, placed in airtight containers, labeled according to the regions, and kept at room temperature until used for further analysis.

Proximate analysis

The content of moisture, ash, crude protein, and fat of EBN samples was determined by the official methods of The Association of Official Analytical Chemistry (AOAC). Moisture content was determined by drying 1 g of EBN samples in an oven at 105°C until a constant weight was obtained (AOAC Method 934.01). Ash content was determined by dry ashing 2 g of EBN samples in a furnace at 550°C for 4 hours (AOAC Method 942.05). Semimicro-Kjeldhal method was used to determine the protein content of EBN by using 1 g of samples (AOAC Method 2001.11). The Soxtec system was used to determine the fat content. The carbohydrate content was determined by the difference method (subtracting the percent of crude protein, fat, and ash from 100% of dry EBN samples).

Nitrite and nitrate analysis

Nitrite and nitrate content were measured by using UV-VIS spectrophotometer. $NaNO_2$ and KNO_3 and were used as nitrite and nitrate standard, respectively. The nitrite was determined by diazotizing 50 ml water-dissolved EBN samples with 1 mL of sulfanilamide and coupling with 1 mL of *N*-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye. The resulting color was measured at a wavelength of 540 nm. The nitrate content was determined by reacting 50 mL of water-dissolved EBN samples were with 1 mL of 1 N HCl. The absorbance and reference were read at 275 nm and 220 nm, respectively. Concentration of nitrite and nitrate was calculated by using each standard curve.

Figure 1. Six locations in where the EBNs were collected (modified from https://asiapacific.anu.edu.au/mapsonline/base-maps/indonesia). WS: West Sumatra; SS: South Sumatra; WJ: West Java; WK: West Kalimantan; CS: Central Sulawesi; SES: Southeast Sulawesi

Amino acid analysis

Amino acid analysis was performed by High-Performance Liquid Chromatography (HPLC) using AccQ-Tag column (3.9 x 150 mm, Waters, USA) with Acetonitrile 60% as mobile phase and flow rate 1 $ml.min^{-1}$. Dried EBN samples (0.1 g) were mixed with 5 mL of 6 M HCl at 110°C for 22 hours to hydrolyse the protein, diluted into 50 mL of water and filtered with 0.45 µm membrane filter. Hydrolyzed samples (500 µL) were mixed with 40 µL of alpha-Aminobutyric acid (AABA) and 460 µL water. AccQ-Fluor Borat (70 µL) and Fluor A reagent (20 µL) were then added into the mixture (10 µL), mixed by vortex and kept at 55°C for 10 minutes. Five microliters of this sample were loaded into the column. The UV detector was operated at 248 nm for peak identification, while the excitation and emission wavelengths for fluorescence detector were 250 nm and 395 nm, respectively, for amino acid quantification. The amount of amino acid was calculated based on the peak area in comparison with standard. Alkaline hydrolysis was done for determination of tryptophan content.

SDS-PAGE

Ground EBN samples (1.0 g) were dissolved with 30 mL of ddH_2O were treated by sonication for 45 minutes, and centrifuged at 10.000 rpm for 30 minutes. The supernatants were dialyzed overnight against ddH_2O in a dialysis bag with a 3500 cutoff molecular weight. The protein solutions were finally freeze-dried and stored in dark bottles until use. EBN samples were analyzed by 10% SDS-PAGE, followed by Coomassie brilliant blue R-250 staining. Molecular weight of EBN proteins was calculated from standard curve of relative migrant distance (Rf) versus log of molecular weight of protein markers.

Statistical analysis

All data were expressed as mean ± standard deviation. The data were statistically treated by one-way ANOVA, followed by Duncan's test with $p<0.05$ considered to be statistically significant. The statistical analysis was performed by using SPSS-22 software. Principal Component Analysis (PCA) was carried out for separating EBN samples according to the concentrations of amino acids.

RESULTS AND DISCUSSION

Proximate analysis

The proximate composition of EBN collected from various regions in Indonesia is shown in Table 1. The order of the highest to the lowest proximate composition was in the order of protein (53.09-56.25%) > carbohydrate (19.57-23.04%) > moisture (17.08-21.50% > ash (5.44-6.25%) > fat (0.07-0.57%), which was in concordance with previous findings (Marcone 2005; Huda et al. 2008; Saengkrajang et al.2013; Halimi et al. 2014).

Protein, the largest constituent, was found to be in the range of 53.09-56.25% (average 54.58%), with the highest and the lowest protein content was found in EBNs from WS (56.25%) and SS (53.09%), respectively. The protein content of EBN from WS differed significantly from protein value of EBNs from other regions ($P<0.05$). These findings, as expected, indicated that geographical location affects the protein content of EBN. The protein content in this study, however, was little bit lower than those of EBNs collected from Thailand, which was 60.9-66.9% (Saengkrajang et al. 2013), EBNs from Malaysia (Perlis and Langkawi) and Indonesia (Java, Kalimantan and Balikpapan), which was 59.8-65.8% (Hamzah et al. 2013), but similar to those of EBNs from Malaysia (Pahang and Terengganu), which was 55.48-58.55% (Halimi et al. 2014), and higher than those EBNs from Malaysia (Penang), which was 24.36-49.30% (Huda et al. 2008). Protein value is closely related to availability of good feeding environment and abundance of feed in the area (Huda et al. 2008). Protein is a major component in EBN accounting for 50-65% of dried weight EBN and it plays a key role in nutritious and pharmacological effects, and it also may have important biological functions (Ma and Liu 2012). It has been reported that protein in EBN, lactoferrin, and ovotransferrin attenuated H_2O_2-induced cytotoxicity, and decreased radical oxygen species in human SH-SY5Y cells through increased scavenging activity (Hou et al. 2015). Although the protein content of EBNs from WS was significantly different from protein contents from other regions, the protein contents in this study were still in the range of protein content of EBNs from surrounding countries. A slight difference in the content may be due to the difference in the environment, but not due to the difference in climate or seasons.

Table 1. Proximate composition of EBNs from several regions in Indonesia

Proximate composition (%)	EBN samples*					
	WS	SS	WJ	WK	CS	SES
Protein	56.25 ± 0.06^{a}	55.66 ± 0.11^{b}	53.38 ± 0.04^{c}	53.45 ± 0.04^{c}	55.67 ± 0.04^{b}	53.09 ± 0.07^{d}
Carbohydrate	20.29 ± 0.08^{d}	19.84 ± 0.06^{e}	20.70 ± 0.11^{c}	23.04 ± 0.10^{b}	20.80 ± 0.06^{c}	19.57 ± 0.03^{f}
Moisture	17.08 ± 0.04^{e}	18.33 ± 0.06^{c}	19.55 ± 0.01^{b}	17.65 ± 0.06^{d}	17.74 ± 0.04^{d}	21.50 ± 0.11^{a}
Ash	6.25 ± 0.06^{a}	5.79 ± 0.08^{b}	5.80 ± 0.01^{b}	5.46 ± 0.06^{cd}	5.73 ± 0.01^{b}	5.44 ± 0.00^{d}
Fat	0.14 ± 0.00^{d}	0.39 ± 0.02^{c}	0.57 ± 0.07^{b}	0.41 ± 0.02^{c}	0.07 ± 0.01^{d}	0.41 ± 0.01^{c}

Note: *WS: West Sumatra; SS: South Sumatra; WJ: West Java; WK: West Kalimantan; CS: Central Sulawesi; SES: Southeast Sulawesi. a-f: same letter in each category raw are not significantly different ($P \geq 0.05$)

Carbohydrate contents of EBNs examined in this study were between 19.57-23.04% with an average value of 20.71%. The highest carbohydrate content was found in EBN from WK (23.04%), while the lowest carbohydrate content was found in EBN from SES (19.57%). The carbohydrate values of EBNs from WK differed significantly from carbohydrate values of EBNs from other regions ($P<0.05$). These carbohydrate contents were lower than those of EBNs from Malaysia (Penang), which were 27.57-58.21% (average 35.44%) (Huda et al. 2008), from Thailand, which was 25.4-31.4% (average 29.66%) (Saengkrajang et al. 2013), but similar to those of EBNs from Malaysia (Pahang and Terengganu), which were 22.28-25.79% (average 24.04%) (Halimi et al. 2014). These carbohydrate values, however, were higher than those of EBNs from Malaysia (Perlis and Langkawi) and previous reports from Indonesia (Java, Kalimantan, and Balikpapan), which were 8.5-16.4% (Hamzah et al. 2013). Carbohydrates in the EBNs were shown to be composed of 9% sialic acid, 7.2% galactosamine, 5.3% glucosamine, 16.9% galactose, and 0.7% fucose (Ma and Liu 2012). Similar to the protein content, the carbohydrate content of EBNs from WK was significantly different from that of other regions, however overall carbohydrate composition in this study was also in the range of carbohydrate content of EBNs from surrounding countries, perhaps due to similar climate and environment.

Moisture content was in the range of 17.08-21.50% (average 18.64%), with the lowest and highest content was in EBN sample from WK and SES, respectively. Moisture content was found to be significantly different among EBNs ($P<0.05$). The content of moisture was much higher than those of EBNs from Malaysia (Perlis and Langkawi) and Indonesia (Java, Kalimantan, and Balikpapan), which was 10.87-13.88% (average 12.94%) (Hamzah et al. 2013) and EBNs from Malaysia (Pahang and Terengganu), which was 15.87-15.90% (average 15.89%) (Halimi et al. 2014), slightly higher than those of EBNs from Malaysia (Penang), which was 13.77-20.20% (average 16.15%) (Huda et al. 2008), but slightly lower than to those of EBNs collected from Thailand, which was 17.8-24.3% (average 19.82%) (Saengkrajang et al. 2013). The moisture content is frequently used as an index of stability and quality of edible bird's nest (Hamzah et al. 2013). Interestingly, moisture content was significantly different among almost all EBNs used in this study. It is suggested that moisture is sensitive parameter that might be influenced by the environment of the farm, however, further investigation is needed.

The content of ash was in the range of 5.44-6.25% (average 5.75%). Statistical analysis showed that only ash content of EBN from WS was significantly different with ash content of EBNs from other regions, The ash content in this study was lower than to those of EBNs collected from Thailand, which was 5.9-7.4% (average 6.72%) (Saengkrajang et al. 2013), but much higher than those of EBNs Malaysia (Pahang and Terengganu), which was 2.57-2.60% (average 2.59%) (Halimi et al. 2014). It is similar to those of EBNs from Malaysia (Penang), which was in average of 5.35%, although EBNs from Penang contains wide range of ash content (2.75-7.53%) (Huda et al. 2008). In opposite to moisture content, the ash content was almost the same within all EBNs used in this study, different from the ash content in EBNs from Malaysia which had quite wide range.

Fat, the smallest constituent, was found to be significantly different among the EBNs from different locations. The fat content was in between 0.07-0.57% (average 0.33%), with the highest and the lowest contents were in EBNs from West Java and Central Sulawesi, respectively. Statistical analysis categorized fat content in this study into 3 groups, i.e. group 1 which was WJ, group 2 which were SS, WK and SES, and group 3 which included WS and CS. This percentage of fat content in this study was similar to those of EBNs Malaysia (Pahang and Terengganu), which was 0.29-0.67% (average 0.48%) (Halimi et al. 2014), but much lower than to those of EBNs collected from Thailand, which was 0.4-1.3% (average 0.96%) (Saengkrajang et al. 2013) and EBNs from Malaysia (Penang), which was 0.47-2.00% (average 0.80%) (Huda et al. 2008). However, all those reports have shown much high fat content compared to those which reported by Hamzah et al (2013), where the fat content was extremely low (0.04-0.07% for unclean EBNs and 0.01-0.05% for clean EBNs). Fat content is one concern of customers when they choose EBNs to consume and low-fat content is preferable.

Nitrite and nitrate concentration

Nitrite and nitrate contents of EBNs used in this study were shown in Table 2. Nitrite contents were found to be in the range of 3.11-18.28 ppm (average 8.40 ppm), with the highest and lowest nitrite content in EBNs from CS and WJ, respectively. On the other hand, the content of nitrate was in between 650.11-1,051.06 ppm (average 881.52 ppm), with the highest and lowest nitrate content in EBNs from WK and SES, respectively. The results showed that in average nitrate content was 105-fold higher than nitrite content. This is likely because nitrate is a more stable ion and can be generated from nitrite oxidation. According to Malaysian Food Regulation 1985 and Malaysia Standard MS2334:2011, raw EBNs which have undergone a cleaning process should contain no more than 30 ppm of nitrite contents. All EBNs collected in this study have satisfied the requirement.

In previous study, Hamzah et al. (2013) reported that clean EBNs collected from Kalimantan and Perlis were devoid of nitrite and nitrate. Although they found nitrite in clean EBNs from Java, Balikpapan, and Langkawi, the concentration was extremely low (≤0.5 ppm). In addition, Paydar et al. (2013) reported that the nitrite and nitrate concentration of house EBNs from Malaysia was in the range of 7.9-22.0 ppm (average 14.3 ppm) and 20.4-87.4 ppm (average 43.9 ppm), respectively. Thus, the nitrite and nitrate concentration of EBNs used in this study were much higher compared to that of Malaysia. Since the variation of nitrite and nitrate level of EBNs from different sources is associated with the difference in environment, humidity, and climate of the habitat (Jong et al. 2013), it is suggested that this difference was due to difference in environment of

the EBN farms, however, more research is required in order to elucidate the reasons of high content of nitrite and nitrate of EBNs used in this study.

Amino acid composition

Amino acid analysis showed that EBNs collected from different locations in Indonesia contained 18 amino acids, including ten essential amino acids and eight nonessential amino acids (Table 3). The highest and lowest total amino acid content were found in EBNs from WS and WK, respectively. This is in line with the protein content where EBN from WS showed the highest content (Table 1). The content of essential amino acids ranged between 16.15 and 20.88%. EBN collected from WK contained the lowest essential amino acids (16.15%) compared to those from other locations. On the other hand, EBN from WS showed the highest amount of essential amino acid (20.54%). Saengkrajang et al. (2013) reported that methionine (an essential amino acid) and cysteine (a nonessential amino acid) were the major amino acids in EBNs from Thailand, however, in this study we found that those amino acids were among the lowest percentage in EBNs. Study from Malaysia also reported that methionine was the minor amino acids in house bird's nest from Selangor and Borneo (Ismail et al. 2013). From EBNs examined our this study, it was found that leucine, phenylalanine, threonine, and valine were major essential amino acids, similar to another report from Malaysia, in which valine, lysine, and leucine were the major essential amino acid in EBNs collected from Pahang and Terengganu (Halimi et al. 2014).

On the other hand, nonessential amino acid content was higher than essential amino acids, ranged between 21.28 and 27.84%. EBN collected from WK also contained a smaller amount of nonessential amino acid compared to those from other locations. Similar to essential amino acids, EBN from WS showed the highest amount of nonessential amino acid (27.84%). The highest content of nonessential amino acid found in EBNs examined was serine, however arginine, glutamic acid, proline, and tyrosine were also high. These results are different from EBNs of Thailand, in which glutamine was most abundant (Saengkrajang et al. 2013) and EBNs from Malaysia, in which glutamic acid and aspartic acid were most abundant (Halimi et al. 2014). In our study, aspartic acid was found extremely high in EBNs from WS (4.21%) and WK (3.27%), while in EBNs from other locations was very low (0.32-0.37%).

All EBNs examined showed various concentrations of amino acids. Serine (4.57%) demonstrated the highest concentration and methionine (0.12%) demonstrated the lowest contents. EBN from WS showed the highest content of amino acid content (48.38%). In addition, it was also demonstrated a unique characteristic in terms of content of several amino acids, such as serine (5.02%), aspartic acid (4.21%) and histidine (2.13%), with higher concentration than that EBNs from other locations (Table 3).

Furthermore, principal component analysis (PCA) was able to separate EBN samples from each location (Figure 2). The two first principal components explained 94.84% of the total variance in the dataset: F1 accounted for 77.71% and F2 for 17.13%. PCA revealed SS, CS, and WJ have similar amino acid composition compared to other EBNs and were characterized by high isoleucine and methionine. EBN from WK was characterized by low concentration of leucine, valine, and lysine compared to EBNs from other locations. EBN from WS was characterized by high histidine content, however, EBN from SES has very low histidine content.

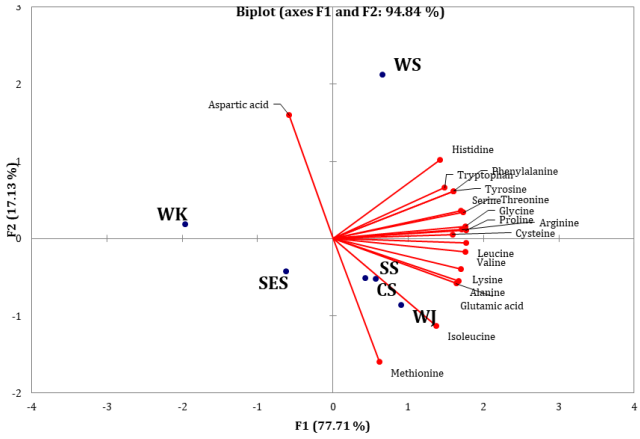

Figure 2. Principal component analysis (PCA) of amino acid data from Table 3. The biplot shows amino acid data and location as vectors. Vectors that are close together are correlated in terms of amino acid composition for each location. *Note: WS: West Sumatra, SS: South Sumatra, WJ: West Java, WK: West Kalimantan, CS: Central Sulawesi, SES: Southeast Sulawesi.

Table 2. Composition of nitrite and nitrate of EBNs from several regions in Indonesia

Proximate composition (%)	EBN samples*					
	WS	SS	WJ	WK	CS	SES
Nitrite	13.58 ± 4.83^{b}	3.98 ± 0.89^{e}	3.11 ± 0.83^{e}	6.31 ± 1.87^{c}	18.28 ± 1.07^{a}	5.13 ± 4.74^{d}
Nitrate	1,049.32 ± 1.52^{a}	713.22 ± 2.64^{c}	797.99 ± 2.80^{b}	1,051.06 ± 2.60^{a}	1,027.39 ± 1.23^{a}	650.11 ± 2.83^{d}

Note: *WS: West Sumatra, SS: South Sumatra, WJ: West Java, WK: West Kalimantan, CS: Central Sulawesi, SES: Southeast Sulawesi. a-f: same letter in each category column are not significantly different ($P \geq 0.05$)

Table 3. Amino acid composition of EBNs from several regions in Indonesia

Group	Amino acid	AA composition (%) of EBN samples					
		WS*	SS*	WJ*	WK*	CS*	SES*
Essential	Histidine	2.13	1.85	1.86	1.56	1.83	1.61
	Isoleucine	1.44	1.60	1.68	1.36	1.61	1.50
	Leucine	3.63	3.59	3.77	2.80	3.58	3.21
	Lysine	2.14	2.29	2.34	1.70	2.28	1.91
	Methionine	0.06	0.15	0.16	0.10	0.13	0.10
	Phenylalanine	3.52	3.24	3.36	2.78	3.20	3.16
	Threonine	3.62	3.37	3.54	2.73	3.42	3.14
	Tryptophan	0.55	0.54	0.53	0.51	0.54	0.52
	Valine	3.45	3.46	3.64	2.61	3.44	3.14
	Subtotal	20.54	20.09	20.88	16.15	20.03	18.29
Non-essential	Alanine	1.32	1.43	1.53	0.96	1.40	1.29
	Arginine	3.42	3.39	3.45	2.62	3.16	3.11
	Aspartic acid	4.21	0.37	0.32	3.27	0.37	0.34
	Cysteine	0.65	0.69	0.64	0.50	0.60	0.57
	Glutamic acid	3.51	3.68	4.15	2.72	3.90	3.31
	Glycine	2.06	2.01	2.03	1.54	2.01	1.77
	Proline	3.90	3.77	3.94	2.94	3.79	3.36
	Serine	5.02	4.64	4.89	3.83	4.85	4.19
	Tyrosine	3.75	3.53	3.56	2.96	3.36	3.34
	Subtotal	27.84	23.51	24.51	21.34	23.44	21.28
Total		48.38	43.60	45.39	37.49	43.47	39.57
Essential/total		0.42	0.46	0.46	0.43	0.46	0.46

Note: *WS: West Sumatra, SS: South Sumatra, WJ: West Java, WK: West Kalimantan, CS: Central Sulawesi, SES: Southeast Sulawesi

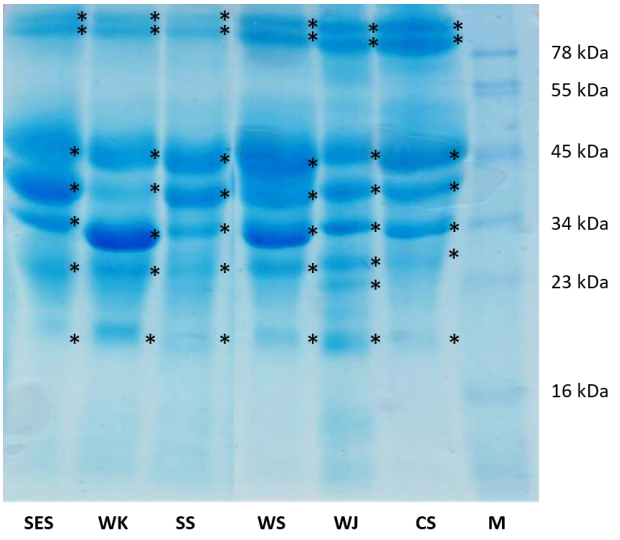

Figure 3. Protein profile of six EBNs collected from different regions in Indonesia. WS: West Sumatra, SS: South Sumatra,WJ: West Java, WK: West Kalimantan, CS: Central Sulawesi, SES: Southeast Sulawesi

SDS-PAGE analysis

SDS-PAGE analysis showed that in general, all EBNs had similar protein profiles (Figure 3). Most EBNs consisted of seven proteins with molecular weight of approximately 19.6 kDa, 26.8 kDa, 34.0 kDa, 37.5 kDa, 45.0 kDa, 76.9 kDa, and 82.7 kDa, respectively. However, EBN from WS showed extremely high intensity of protein band with molecular weight of approximately 34.0 kDa. EBN from WK also possessed higher amounts of 34.0 kDa protein, instead, it had smaller amounts of 37.5 kDa protein. EBN from SS, on the other hand, has shown less 31.7 kDa protein. Furthermore, EBN from WJ showed a slightly different pattern; it possessed an extra abundant protein with molecular weight of approximately 23.0 kDa in addition to seven protein bands shown by majority of EBNs. Based on the intensity of the total protein bands it seems that EBN from WS and WK showed the strongest and the weakest intensity, respectively, suggesting that the SDS-PAGE results were in line with amino acid results (Table 3). Although EBN from WK showed strong intensity in protein with molecular weight of 34.0 kDa, the intensity of proteins with other molecular weight is very weak. The result of SDS-PAGE analysis of EBNs used in this study is different with previous study, in which wider ranges of molecular weight of proteins (18-552 kDa) were detected in EBNs from Kalimantan and Java (Helmi et al. 2018). These results imply that EBNs from different locations have different protein profiles.

In conclusion, all EBNs collected from WS, SS, WJ, WK, CS, and SES showed different nutritional profiles. Furthermore, their nutritional profiles were also different from that of EBNs of Malaysia and Thailand. Based on our knowledge, this study was the first comprehensive investigation on nutritional profile of EBNs collected from several regions in Indonesia. So far, only proximate analysis and protein profile of EBNs from Kalimantan and Java have been reported in separate investigations. Furthermore, no report on amino acid composition of

EBNs from Indonesia has been published. Thus, we believe that this comprehensive analysis will be an important reference and will be beneficial for the commercial development and application of EBN in Indonesia. However, since this study only investigated nutritional profile of EBNs collected from few farms in each Island, more extensive study on nutritional profile of EBNs from other locations in Indonesia is needed in order to obtain a complete picture of EBNs character in Indonesia.

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