ASIA-PACIFIC JOURNAL OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY

AsPac J. Mol. Biol. Biotechnol. 2025
ISSN: 0128-7451, eISSN: 2672-7277
Vol. 33 (3): 202-212
https://doi.org/10.35118/apjmbb.2025.033.3.20

RESEARCH ARTICLE

Metabolomic characterization of different types of Malaysian edible bird's nest

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Abstract

Edible Bird's Nest (EBN), produced by *Aerodramus fuciphagus* and *Aerodramus maximus*, has been widely consumed for its nutritional and medicinal properties. Despite its increasing global demand, variations in its metabolite composition due to geographical origin and processing methods remain inadequately explored. The current study investigates the metabolite profiles of farmed and commercialized EBN to determine the influence of environmental factors, diet availability, and processing on its biochemical composition. It is hypothesized that EBN from different Malaysian regions exhibits distinct metabolite compositions, potentially impacting its functional properties. Farmed EBN samples were obtained from Perak, Kelantan, Johor, and Sarawak, while commercialized EBN was sourced from a local pharmacy. The metabolite extraction process followed standardized protocols, and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy was used for analysis. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to evaluate metabolite variations, while antioxidant properties were assessed using DPPH and nitric oxide scavenging assays. Findings revealed significant differences in metabolite composition between farmed and commercialized EBN. Higher acetone and 4-hydroxyproline levels were detected in commercialized EBN, suggesting potential alterations due to processing. Among farmed samples, Johor's EBN exhibited the highest nitric oxide scavenging activity, while Kelantan's EBN demonstrated the strongest DPPH radical inhibition. These variations highlight the impact of geographical and processing factors on EBN's biochemical properties. Establishing a comprehensive metabolite profile may contribute to industry-wide standardization, ensuring product quality and reinforcing EBN's potential as a functional food.

Keywords: antioxidant, Edible Bird's Nest, geographical variation, metabolomics, processing methods, scavenging activity

INTRODUCTION

Edible Bird's Nest (EBN) is primarily produced by two species of swiftlets. *Aerodramus fuciphagus*, commonly referred as the white-nest swiftlet, and *Aerodramus maximus*, known as the black-nest swiftlet (Kong et al., 2022). Specific environmental factors such as humidity levels (80 - 90%), temperature (26 - 35°C), and adequate food availability determine the survival and reproductive success of these birds

Received: 10 March 2025
Accepted: 10 June 2025
Published: 30 September 2025
Published by: Malaysian Society for Molecular Biology and Biotechnology (MSMBB)

(Chok et al., 2021). EBN mainly composed of saliva, originated from the sublingual glands of the bird. Male swiftlets are responsible to construct the nest using this saliva which act as fixative to bind other materials for instance, feathers and plant components. These nests are found attached to the surface of cave walls in natural set-up or wooden beams in artificial structures. Various physical characteristics including dry mass, size, colour, impurity levels, and presence of feathers determined the grading of the nests (Lee et al., 2021). Additionally, EBN-producing swiftlets are commonly distributed across Southeast Asia, from Malaysia, up to southern region of China (Chok et al., 2021).

The use of EBN is common in traditional Chinese cuisine and medicine which can be traced back to the Tang dynasty between 618 to 907 AD (Yeo et al., 2021). For ages, EBN has been highly prized for its countless health benefits, largely contributing to the rich content of glycoproteins, sialic acid, and several others bioactive compounds. Documented research revealed that EBN demonstrated anti-aging, anti-inflammatory, antiviral, immunomodulatory, antioxidant, and neuroprotective properties (Chua et al., 2021; Fan et al., 2022). Sialic acid, one of the predominant key bioactive compounds in EBN, has been linked to improved brain function, strengthened immune response, and exerting anti-inflammatory responses (Loh et al., 2022; Li et al., 2023). Furthermore, presence of proteins, carbohydrates, and other essential nutrients in EBN, further emphasizing its role as a functional food with significant health-enhancing properties (Chok et al., 2021). Exceeding its nutritional value, EBN is also believed to facilitate cell regeneration and skin whitening, making it a sought-after ingredient in both health as well as cosmetic industries (Lai et al., 2021).

The growing demand for edible bird's nest (EBN) as a dietary product has introduced notable challenges in maintaining consistent quality across the industry. EBN is broadly classified into raw uncleaned (RUC) and raw cleaned (RC) types. RUC EBN is sourced directly from natural habitats such as caves and swiftlet farms, whereas RC EBN undergoes a detailed cleaning process involving soaking, impurity removal, bleaching, shaping, and packaging to ensure its suitability for consumption (Yeo et al., 2021; Zulkefle et al., 2024). It is noteworthy that the global EBN continues to grow, largely due to its perceived health benefits. However, despite its high demand, in-depth scientific research to address its metabolite composition remains limited. While previous studies have documented EBN's nutritional content and bioactive components (Yeo et al., 2021; Wang et al., 2023), there are still gaps in understanding factors such as farming conditions, geographical attribution, and processing methods affect its biochemical composition.

Grasping these knowledge gaps is exceptionally important as issues related to authenticity, quality control, and adulteration have become more pressing in the industry. Hence, this study is set to explore the varieties of metabolites present in farmed and commercially available EBN through metabolomic techniques while evaluating the influence of geographical, diet availability, and processing. Gaining insights into these aspects is essential for establishing reliable markers and promoting industry-wide standardization. Furthermore, profiling EBN's bioactive metabolites can expand its potential applications in nutraceuticals and functional foods. By integrating analytical chemistry, food science, and biotechnology, this research contributes to maintaining product integrity while further exploring EBN's therapeutic value.

MATERIALS AND METHODS

EBN preparation

In this study, farmed EBN samples (Aerodramus fuciphagus) were randomly collected from multiple locations across different regions of Malaysia, including Perak (north), Kelantan (east), Johor (south), and Sarawak (East Malaysia), and subsequently pooled to represent each region. Whereas commercialized EBN refers to cleaned and processed nests (powder) sold for consumption was obtained from a local pharmacy in Sarawak. Good Manufacturing Practice (GMP) of industrial cleaning method (MS 2333:2010) was adapted and standardized for all farmed EBN samples. All samples were properly labelled and stored in airtight containers at room temperature, within a cool and dry cabinet until further analysis. Raw samples were also routinely inspected for any signs of mold or fungal growth.

EBN extraction

The extraction process was adapted from the method outlined by Goh et al. (2001), with some modifications. Initially, 2 g of each EBN sample was dissolved in 50 mL of distilled water and left to incubate overnight at 4°C. The solution was then heated to 65°C for an hour, after which the supernatant was carefully separated. To facilitate precipitation of the desired components, the collected supernatant was combined with chilled absolute acetone in a 1:2 ratio and incubated at 4°C for another hour. The precipitate was subsequently collected, freeze-dried, and stored at -80°C for later analysis.

EBN 1H-NMR analysis

The extraction process was conducted based on the method outlined by Kim et al. (2010), with slight modifications. A 30 mg sample was placed into a 2 mL Eppendorf tube, followed by the addition of 0.8 mL of KH_2PO_4 buffer (pH 6.0) prepared in deuterium oxide, containing 0.05% (w/w) trimethylsilyl propionic acid sodium salt (TSP), along with methanol-d_4 in a 1:1 ratio. The mixture was vortexed and subjected to ultrasonication for 15 minutes, then centrifuged at 10,000 rpm for 10 minutes to eliminate insoluble components. After centrifugation, 0.6 mL of the clear supernatant was carefully transferred into a 5 mm NMR tube for analysis. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were acquired at 26°C using a 500 MHz spectrometer, employing a PRESAT sequence to suppress residual water signals. Data acquisition involved 32 scans per sample, with an acquisition time of 1.3 seconds and a spectral width ranging from −1.00 to 8.00 ppm. A 0.1% TSP solution was used as the reference standard for calibration.

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay

EBN samples were prepared at a concentration of 2 mg/mL in DMSO and stored at 4°C until analysis. For the assay, 50 µL of the prepared sample was mixed with 100 µL of a 50 mg/L DPPH solution in ethanol and left to incubate at room temperature for 30 minutes. Following incubation, absorbance was measured at 517 nm. Quercetin was used as the positive control. The percentage of scavenging inhibition was calculated using Equation 1.

Nitric oxide assay

EBN samples (2 mg/mL in DMSO) were stored at 4°C until use. The Griess reagent was freshly prepared by mixing sulphanilamide and naphthyl ethylenediamine dihydrochloride in phosphoric acid. For the assay, equal volumes (60 µL) of a 10 mM sodium nitroprusside solution and the sample were incubated at room temperature for 180 minutes. After adding 60 µL of the Griess reagent, absorbance was measured at 550 nm. Quercetin served as the positive control, and nitrite radical scavenging activity was calculated according to Equation 2.

Statistical analysis

Metabolites identification was conducted using Chenomx NMR Suite software (version 5.1 Professional, Edmonton, Canada), while NMR spectra were analysed and visualized with MestReNova software. Multivariate analysis was conducted using SIMCA software (version 14.1, Umetrics, Umea, Sweden). To compare commercial and farmed EBN, multivariate statistical analyses were performed. Principal Component Analysis (PCA). Partial Least Squares Discriminant Analysis (PLS-DA) was used to refine the identification of key metabolites. Fold change analysis identified significant variations, and False Discovery Rate (FDR) correction was applied to minimize false positives in the results. For antioxidant properties of EBN (DPPH assay and nitric oxide assay), all data were expressed as mean ± standard deviation based on triplicate measurements. All dataset comparisons were performed using ANOVA, followed by Fisher's Least Significant Difference (LSD) test for post hoc analysis. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using Minitab statistical software (USA).

Equation 1:

$$Scavenging\ Inhibition\ (\%) = \left(\frac{Absorbance\ of\ Control - Absorbance\ of\ Sample\ or\ Standard}{Absorbance\ of\ Control}\right)$$

Equation 2:

$$Nitric\ Oxide\ Scavenging\ Activity\ (\%) = \left(\frac{Absorbance\ of\ Control - Absorbance\ of\ Sample\ or\ Standard}{Absorbance\ of\ Control}\right)$$

RESULTS AND DISCUSSION

Visualization of ^{1}H-NMR spectra and metabolite identification

The NMR spectra and the corresponding chemical shifts of the identified metabolites are presented in Figure 1 and Table 1, respectively. NMR profiling of EBN revealed 25 compounds, including essential primary metabolites such as amino acids and organic acids. Notable differences were observed in the NMR spectra (Figure 1) between commercialized and farmed EBN. The commercialized EBN exhibited fewer peaks than farmed EBN and contained several distinct, unidentified single peaks within the 1.0 to 1.5 ppm range.

The analysis identified four amino acids (4-hydroxyproline, alanine, glycine, and phenylalanine) exhibiting differential detection across all samples. Notably, alanine and glycine were detected in all samples, including commercial EBN, whereas 4-hydroxyproline was present only in farmed EBN samples from Kelantan and Johor. Kim et al. (2010) and Saengkrajang et al. (2013) reported a similar amino acid profile in their study on EBNs collected from various locations in Thailand and Malaysia. Additionally, other amino acids reported by Kim et al. (2010) and Saengkrajang et al. (2013) were not observed in the current study. Saengkrajang et al. (2013) concluded that the discrepancy of amino acids may be attributed to variations in the geographical origin of the EBN samples and differences in the cleaning methods applied.

The higher levels of acetone found in commercialized EBN, as compared to raw EBN, may account for some of the observed variations such as method and instrument (Daud et al., 2021; Mohamad Ibrahim et al., 2021). Moreover, the elevated acetone concentration in commercial EBN could be a result of ketosis in the swiftlets, a condition commonly linked to migrating birds (Mohamad Nasir et al., 2021). While swiftlets are not typically migratory, certain studies have suggested that they are highly sensitive to environmental fluctuations, and drastic changes in their habitat could potentially initiate migratory behaviour (Lindholm et al., 2019). Besides, the elevated acetone levels in commercialized EBN may be attributed to the processing methods employed, which could significantly alter its metabolite composition.

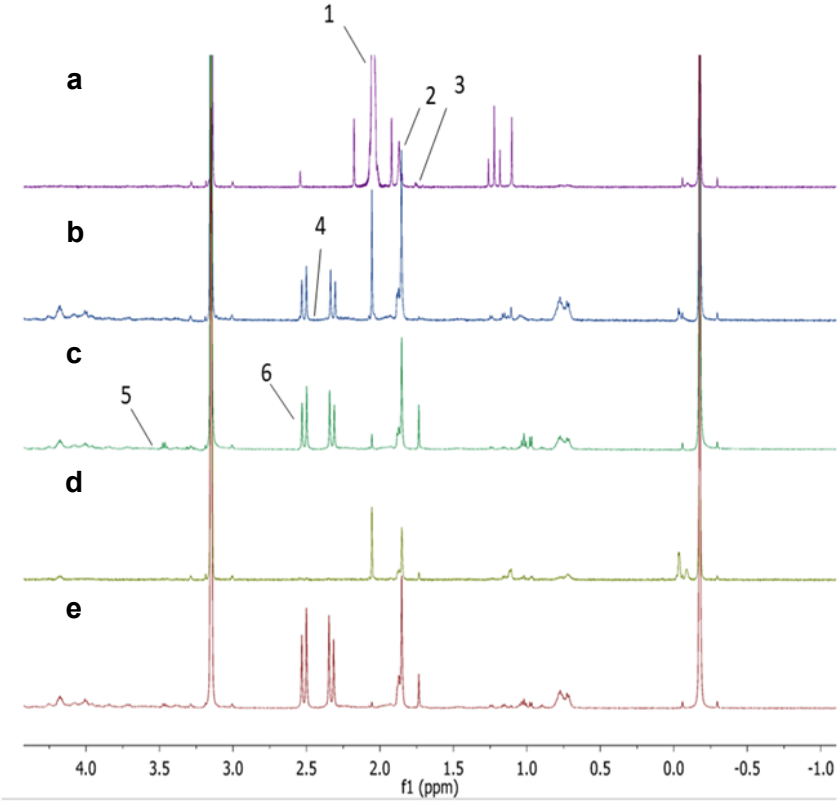


Figure 1. ^{1}H-NMR spectra comparison of EBN samples – (a) Commercialized vs. farmed EBN, (b) Sarawak, (c) Perak, (d) Kelantan, and (e) Johor. (1: methyl protons of aliphatic amino acids, 2: methylene groups, 3: acetyl group from N-acetylneuraminic acid, 4: sugar ring protons and amino acid side chains, 5: anomeric protons of sugar residues, and 6: aromatic protons).

Table 1. Identified metabolites in commercialized (C) EBN and farmed (S: Sarawak, P: Perak, K: Kelantan, and J: Johor) EBN.

Metabolites	**δH**	**Availability**				
		C	**S**	**P**	**K**	**J**
2-Hydroxybutyrate	4.00 (dd, J=6.5), 1.73 (m), 1.62 (m), 0.88(t)	+	+	+	+	+
3-Hydroxyisovaleric acid	1.26 (s), 2.33 (s)	+	-	-	-	-
3-Hydroxyisobutyrate	3.70 (m), 3.53 (dd), 2.48 (m), 1.06 (d, J=7.1)	+	-	-	-	-
4-Hydroxyproline (collagen)	4.66 (s), 4.33 (m), 3.46 (dd, J=12.6), 3.3 (m), 2.42 (m), 2.13 (m)	-	-	-	+	+
Acetamide	7.53 (s), 6.78 (s), 2.01 (s)	+	-	-	-	+
Acetate	1.89 (s)	+	+++	++	+	++
Acetone	2.21 (s)	+++	++	+	++	+
Alanine	3.77 (dd), 1.46 (d)	+	+	+	+	+
Betaine	3.88 (s), 3.24 (s)	+	+	+	+	+
Carnitine	3.91 (s), 3.02 (s)	+	+	+	+	+
Choline	4.05 (m), 3.50 (m), 3.17 (s)	-	++	+	+	+
Citric acid	2.67 (d, J=16 Hz), 2.48 (d, J=15.5 Hz)	-	+	+	-	+++
Dimethylamine	2.72 (s)	+	+	+	-	+
Ethanol	3.63 (m), 1.18 (d)	+	++	++	++	++
Formate/formic acid	8.46 (s)	-	+	-	-	+
Glycine	3.55 (s)	+	+	+	+	+
Hydroxyvaleric acid	4.02 (m), 1.66 (m), 1.59 (m), 1.33 (m), 1.29 (m), 0.88 (m)	-	-	-	++	-
Isobutyric acid	2.37 (m), 1.04 (d)	+	+	+	+	+
Lactic acid	4.09 (dd), 1.31 (s)	+	++	++	++	++
Phenylalanine	7.33 (m), 7.27 (m), 7.23 (d, J=7.0 Hz), 3.97 (m), 3.27 (m), 3.11 (m)	-	+++	++	+	+++
Phosphoglyceric acid	4.5 (m), 3.88 (s), 3.80 (s)	-	+	+	-	+
Propionic acid	2.15 (m), 1.05 (m)	-	-	+	-	+
Propylene glycol	3.87 (s), 3.54 (s), 3.43 (s), 1.13 (s)	-	-	+	+	+
Tartaric acid	4.30 (s)	-	++	+	+	++
Fatty acids	0.87 (m), 1.28 (m)	-	++	+	-	++

(-: not detected, +: low detection, ++: moderate detection, +++: high detection, and ++++: very high detection)

Despite some variations in the findings, the metabolites identified in this study such as betaine, carnitine, and lactic acid, have demonstrated antioxidant properties. Betaine has been shown to play a pivotal role in supporting liver health by improving hepatic function and safeguarding against liver damage (Veskovic et al., 2019). Furthermore, it demonstrates significant anti-inflammatory and antioxidant effects, which may provide therapeutic potential in mitigating the impact of various diseases and conditions. These attributes highlight its capacity to promote metabolic health and alleviate oxidative stress (Dobrijević et al., 2023). L-carnitine is a key molecule in the transport of long-chain fatty acids into the mitochondria, where they are metabolized for energy production. This compound has been associated with improved cardiovascular and neurological health, as well as enhanced muscle function and other vital physiological processes (Virmani and Cirulli, 2022). Once viewed primarily as a metabolic byproduct with potentially adverse effects over time, recent findings suggest that L-lactate may possess neuroprotective properties in numerous pathological conditions, including diabetic encephalopathy, Alzheimer's disease, stroke, traumatic brain injury, and epilepsy, thus supporting its potential role in brain health during stress or injury (Akter et al., 2023).

Metabolic profiling for differentiating EBN from various Malaysian regions

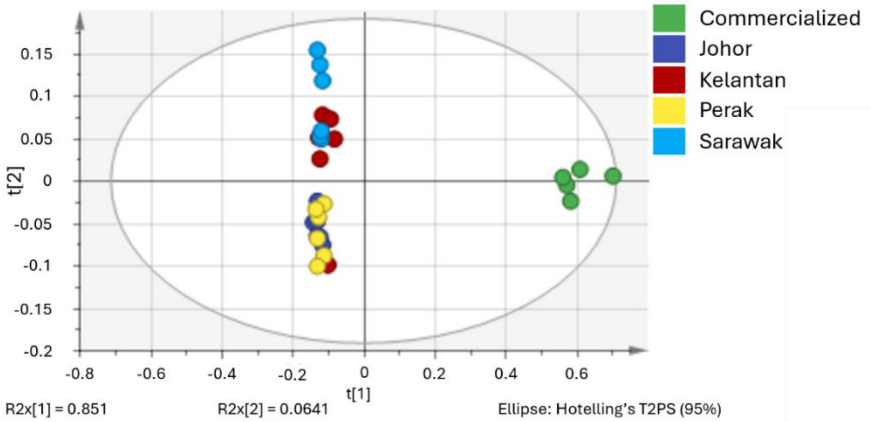
Metabolite levels in EBN were quantified by analysing characteristic peaks from the NMR spectra. Principal Component Analysis (PCA) in Figure 2 demonstrated a robust model fit ($R^2X = 0.985$) and high predictive accuracy ($Q^2 = 0.885$), confirming significant differences in metabolite composition between the EBN groups. The PCA score plot and loading plot revealed distinct clustering patterns, with farmed EBN from Perak and Johor grouped together, while Kelantan and Sarawak samples formed another distinct group, and commercialized EBN separated out. These findings were further validated by Hierarchical Cluster Analysis (HCA) in Figure 3, which showed a clear separation of samples based on their regional metabolic profiles, reinforcing the role of geographical origin in shaping EBN's metabolite composition.

The natural habitats of swiftlets vary with environmental factors, which significantly affect the properties of the EBN they produce (Prabhakar et al., 2015). The observed variation in metabolite composition may be attributed to the geographic origin of the edible bird's nest (EBN), as environmental factors influence the diet of swiftlets (Tong et al., 2021), ultimately affecting the biochemical makeup of their saliva. Notably, farmed EBN samples from Perak and Johor exhibited similar metabolite compositions, including 4-hydroxyproline, phosphoglyceric acid, propionic acid, and propylene glycol. Conversely, EBN samples from Sarawak and Kelantan shared metabolites such as choline, isobutyric acid, lactic acid, and tartaric acid. These findings collectively suggest that EBN sourced from different regions may vary in metabolite composition and likely influenced by the availability of food resources in the swiftlets' foraging environments (Tong et al., 2021).

Comparative metabolite profiling of farmed and commercialized EBN

The relative metabolite concentrations in farmed and commercialized EBN are illustrated in Figure 4, based on ^{1}H-NMR spectroscopy. Distinct differences in metabolite composition were observed, with certain compounds showing significant variation between the two EBN types. Acetone had the highest relative abundance in commercialized EBN (33.25 on average), whereas farmed EBN exhibited significantly lower levels (0.08 to 0.52). A similar trend was noted for 4-hydroxyproline, where commercialized EBN contained markedly higher levels, while farmed EBN maintained consistent concentrations across different regions. Conversely, hydroxyvaleric acid was significantly lower in commercialized EBN, whereas Sarawak EBN exhibited notably higher concentrations compared to other farmed samples. Additionally, lactic acid and phenylalanine were found in lower amounts in commercialized EBN than in farmed EBN. According to Seow et al. (2016), phenylalanine is typically present at higher levels than common adulterants in EBN. The lower phenylalanine content in commercialized EBN suggests a lower likelihood of adulteration compared to farmed samples. Meanwhile, choline, a key metabolite essential for lipid transport and neurotransmitter synthesis, was relatively consistent across all EBN types.

a)



b)

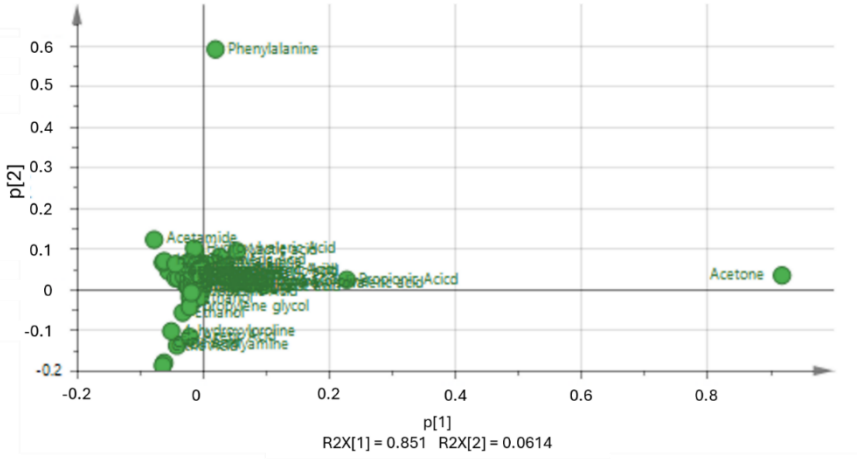


Figure 2. PCA-based classification of EBN from different regions – (a) PCA score plot and (b) loading plot derived from NMR data, illustrating metabolic variations across all EBN samples.

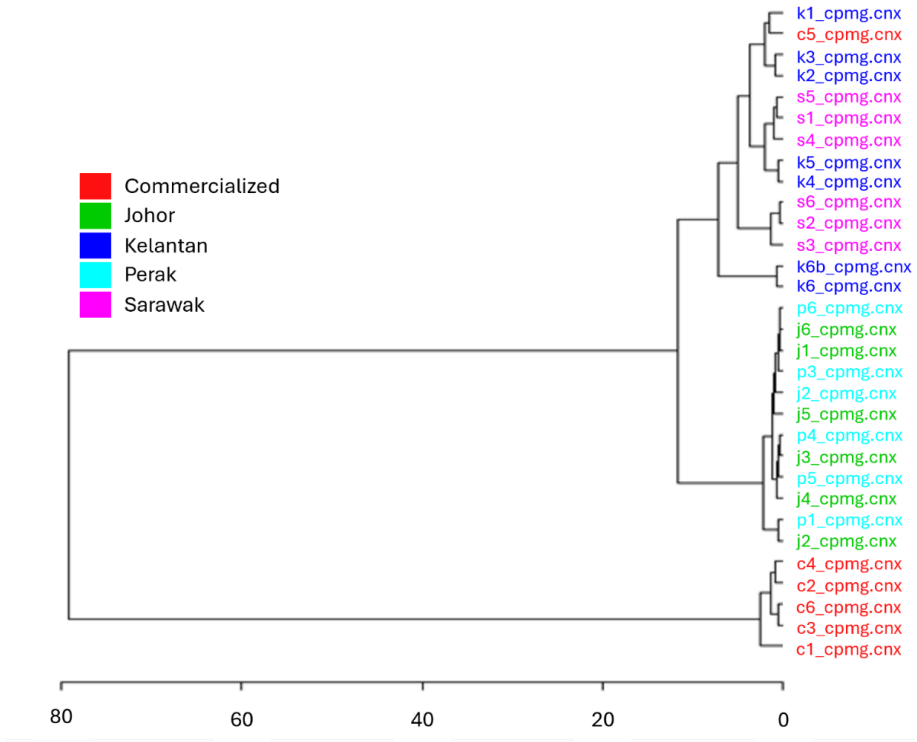


Figure 3. Dendrogram of similarity between farmed EBN and commercialized EBN.

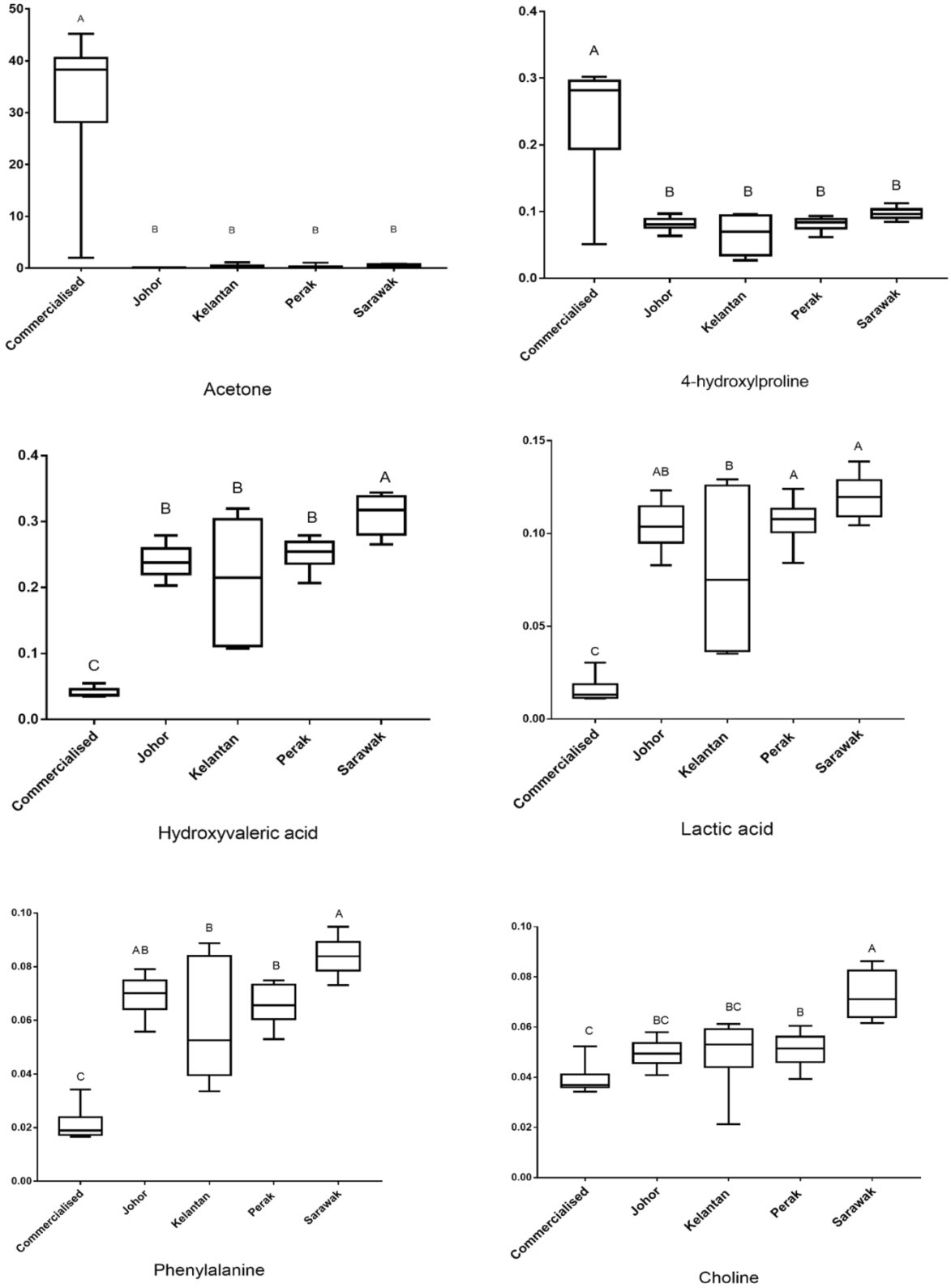


Figure 4. Boxplot of key metabolites detected using ^{1}H-NMR (500 MHz). Means that do not share a letter are significantly different. (The letters represent that there are significant differences among the groups).

Evaluation of antioxidant activity in Edible Bird's Nest (EBN)

In the nitric oxide scavenging assay, EBN exhibited antioxidant properties by competing with oxygen to neutralize nitrite radicals produced by sodium nitroprusside (SNP) under physiological pH in an aqueous environment. The free radical scavenging activity results are summarized in Table 2. Among the farmed EBN samples, Johor showed the highest antioxidant activity, followed by Perak, Kelantan, and Sarawak. The commercialized EBN demonstrated a 44% inhibition, surpassing all farmed EBN samples. In contrast, the DPPH assay, which assesses an antioxidant's ability to scavenge stable radicals or donate hydrogen, revealed lower antioxidant activity compared to the nitric oxide scavenging assay. The inhibition percentages recorded in Table 3 indicate that Kelantan had the highest activity, followed by Perak, Johor, and Sarawak. The commercialized EBN showed 15% inhibition, higher than Sarawak and Johor but lower than Kelantan.

The present findings demonstrate that EBNs sourced from Johor exhibited the highest antioxidant activity among the farmed samples, emphasizing regional variation in antioxidant potential. Analysis through NMR spectroscopy revealed the presence of bioactive metabolites, including betaine (Shim et al., 2016) and carnitine (Szkudelska and Szkudelski, 2022), which are known contributors to antioxidant mechanisms and may underlie the observed activity. Consistent with observations by Quek et al. (2018a), the current data also showed that commercialized EBNs possessed greater antioxidant capacity compared to farmed counterparts, suggesting that processing and production techniques may enhance or preserve antioxidant constituents. Supporting earlier findings by Sarzi-Puttini et al. (2021), which attributed the superior free radical scavenging efficiency of house nests to the abundance of sulfhydryl-containing amino acids and phenolic hydroxyl groups, our results similarly revealed enhanced antioxidant activity in commercialized samples.

Geographical influences on antioxidant capacity were further evidenced in this study. Tong et al. (2021) reported that EBNs originating from different Malaysian regions exhibited distinct metabolite compositions due to environmental variables such as urban development, availability of food and water sources, and topographical features. For example, the presence of 6-hydroxymelatonin (6-OHM)—a compound associated with antioxidant activity—was detected in nests from Clusters 2 (Perak, Sarawak), 3 (Perlis, Kelantan, Kedah, Penang), and 4 (Johor, Sabah, Pahang). Our findings reinforce this association, as Johor demonstrated the highest nitric oxide scavenging inhibition, while Kelantan showed the greatest inhibition in the DPPH assay. These results underscore the role of geographical and ecological factors in shaping the antioxidant potential of EBNs. Additionally, the variation in antioxidant activity is further influenced by species differences and nesting environments. Quek et al. (2018b) previously demonstrated that house-nest EBNs from *A. fuciphagus* in Peninsular Malaysia exhibited nearly double the scavenging efficiency of cave-nest EBNs from *A. maximus* in East Malaysia, a trend that aligns with the current observations and supports the significance of species and habitat in determining bioactivity profiles. Collectively, the results indicate that variations in antioxidant activity among EBNs may be attributed to differences in geographical origin and processing methods. The elevated activity in commercialized samples, alongside the observed regional disparities among farmed EBNs, align with earlier studies suggesting that environmental conditions and metabolite profiles play a role in modulating antioxidant capacity. These findings contribute to a broader understanding of the factors affecting EBN bioactivity and may inform future research on their potential functional applications.

Table 2. Percentage of nitric oxide scavenging inhibition in farmed and commercialized EBN.

EBN	Percentage inhibition (%)
Perak	39.28 ± 0.433
Kelantan	32.17 ± 5.89
Johor	41.05 ± 17.22
Sarawak	25.74 ± 2.87
Commercialized	44.28 ± 4.65

Table 3. Percentage of DPPH assay inhibition in farmed and commercialized EBN.

EBN	Percentage inhibition (%)
Perak	15.22 ± 0.006
Kelantan	19.46 ± 0.02
Johor	13.95 ± 0.01
Sarawak	11.82 ± 0.03
Commercialized	15.40 ± 0.01

CONCLUSION

This study provides a comprehensive metabolite profiling of different types of Malaysian Edible Bird's Nest (EBN), highlighting distinct biochemical compositions. The findings suggest that processing methods and geographical origins influence metabolite diversity, potentially affecting EBN's nutritional and medicinal properties. To ensure consistent quality of edible bird's nest, standardized processing methods, origin traceability, and advanced metabolomic profiling should be implemented. Regulatory oversight, producer training, and centralized quality monitoring are also crucial to minimize variability and preserve the nutritional and medicinal value of EBN. Also, metabolite variability in EBN is influenced by both its origin and processing techniques, which can impact its overall bioactivity. Analytical tools such as ^{1}H-NMR have proven valuable in identifying key constituents, including amino acids and sialic acid-related compounds. Consistent detection of these bioactive underscores the importance of standardized quality control to ensure product reliability. Key bioactive compounds identified such as betaine, carnitine, and lactic acid may contribute to its health benefits, supporting its role in functional food and therapeutic applications. Key bioactive compounds identified may contribute to its health benefits, supporting its role in functional food and therapeutic applications. Further research is needed to explore the biological significance of these metabolites and their potential implications for veterinary and human health.

ACKNOWLEDGEMENTS

The authors acknowledge the use of Grammarly for language editing in the preparation of this manuscript. Project grant was supported by Centre of Excellence on Swiftlet grant number 6371400-10301 (Q7).

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

REFERENCES

Akter, M., Ma, H., Hasan, M., Karim, A., Zhu, X., Zhang, L., & Li, Y. (2023). Exogenous L-lactate administration in rat hippocampus increases expression of key regulators of mitochondrial biogenesis and antioxidant defense. *Frontiers in Molecular Neuroscience*, *16*, 1117146. https://doi.org/10.3389/fnmol.2023.1117146

Chok, K. C., Ng, M. G., Ng, K. Y., Koh, R. Y., Tiong, Y. L., & Chye, S. M. (2021). Edible bird's nest: recent updates and industry insights based on laboratory findings. Frontiers in Pharmacology, 12, 746656. https://doi.org/10.3389/fphar.2021.746656

Chua, K. H., Mohamed, I. N., Mohd Yunus, M. H., Shafinaz Md Nor, N., Kamil, K., Ugusman, A., & Kumar, J. (2021). The anti-viral and anti-inflammatory properties of edible bird's nest in influenza and coronavirus infections: from pre-clinical to potential clinical application. Frontiers in Pharmacology, 12, 633292. https://doi.org/10.3389/fphar.2021.633292

Daud, N. A., Mohamad Yusop, S., Babji, A. S., Lim, S. J., Sarbini, S. R., & Hui Yan, T. (2021). Edible bird's nest: physicochemical properties, production, and application of bioactive extracts and glycopeptides. Food Reviews International, 37(2), 177-196. https://doi.org/10.1080/87559129.2019.1696359

Dobrijević, D., Pastor, K., Nastić, N., Özogul, F., Krulj, J., Kokić, B., ... & Kojić, J. (2023). Betaine as a functional ingredient: metabolism, health-promoting attributes, food sources, applications and analysis methods. *Molecules*, *28*(12), 4824. https://doi.org.10.3390/molecules28124824

Fan, Q., Liu, X., Wang, Y., Xu, D., & Guo, B. (2022). Recent advances in edible bird's nests and edible bird's nest hydrolysates. Food Science and Technology, 42, e67422. https://doi.org/10.1590/fst.67422

Goh, D. L. M., Chua, K. Y., Chew, F. T., Liang, R. C. M. Y., Seow, T. K., Ou, K. L., ... & Lee, B. W. (2001). Immunochemical characterization of edible bird's nest allergens. Journal of allergy and clinical immunology, 107(6), 1082-1088. https://doi.org/10.1067/mai.2001.114342

Kim, H. K., Choi, Y. H., & Verpoorte, R. (2010). NMR-based metabolomic analysis of plants. Nature protocols, 5(3), 536-549. https://doi:10.1038/nprot.2009.237

Kong, H. K., Chan, Z., Yan, S. W., Lo, P. Y., Wong, W. T., Wong, K. H., & Lo, C. L. (2022). Revealing the species-specific genotype of the edible bird's nest-producing swiftlet, Aerodramus fuciphagus and the proteome of edible bird's nest. Food Research International, 160, 111670. https://doi.org/10.1016/j.foodres.2022.111670

Lai, Q. W. S., Guo, M. S. S., Wu, K. Q., Liao, Z., Guan, D., Dong, T. T., ... & Tsim, K. W. K. (2021). Edible bird's nest, an Asian health food supplement, possesses moisturizing effect by regulating expression of filaggrin in skin keratinocyte. Frontiers in Pharmacology, 12, 685982. https://doi.org/10.3389/fphar.2021.685982

Lee, T. H., Wani, W. A., Lee, C. H., Cheng, K. K., Shreaz, S., Wong, S., ... & Azmi, N. A. (2021). Edible bird's nest: The

functional values of the prized animal-based bioproduct from Southeast Asia–A review. Frontiers in pharmacology, 12, 626233. https://doi.org/10.3389/fphar.2021.626233

Li, D., Xie, T., Guo, T., Hu, Z., Li, M., Tang, Y., ... & Wang, H. (2023). Sialic acid exerts anti-inflammatory effect through inhibiting MAPK-NF-κB/AP-1 pathway and apoptosis in ulcerative colitis. Journal of Functional Foods, 101, 105416. https://doi.org/10.1016/j.jff.2023.105416

Lindholm, C., Altimiras, J., & Lees, J. (2019). Measuring ketones in the field: rapid and reliable measures of β-hydroxybutyrate in birds. Ibis, 161(1), 205-210. https://doi.org/10.1111/ibi.12643

Loh, S. P., Cheng, S. H., & Mohamed, W. (2022). Edible bird's nest as a potential cognitive enhancer. Frontiers in Neurology, 13, 865671. https://doi.org/10.3389/fneur.2022.865671

Mohamad Ibrahim, R., Mohamad Nasir, N. N., Abu Bakar, M. Z., Mahmud, R., & Ab Razak, N. A. (2021). The authentication and grading of edible bird's nest by metabolite, nutritional, and mineral profiling. Foods, 10(7), 1574. https://doi.org/10.3390/foods10071574

Mohamad Nasir, N. N., Mohamad Ibrahim, R., Abu Bakar, M. Z., Mahmud, R., & Ab Razak, N. A. (2021). Characterization and extraction influence protein profiling of edible bird's nest. Foods, 10(10), 2248. https://doi.org/10.3390/foods10102248

Prabhakar, A., Quach, A., Zhang, H., Terrera, M., Jackemeyer, D., Xian, X., ... & Forzani, E. S. (2015). Acetone as biomarker for ketosis buildup capability-a study in healthy individuals under combined high fat and starvation diets. Nutrition journal, 14, 1-11. https://DOI 10.1186/s12937-015-0028-x

Quek, M. C., Chin, N. L., Yusof, Y. A., Law, C. L., & Tan, S. W. (2018a). Characterization of edible bird's nest of different production, species and geographical origins using nutritional composition, physicochemical properties and antioxidant activities. *Food Research International*, *109*, 35-43.

Quek, M. C., Chin, N. L., Tan, S. W., Yusof, Y. A., & Law, C. L. (2018b). Molecular identification of species and production origins of edible bird's nest using FINS and SYBR green I based real-time PCR. Food Control, 84, 118-127. https://doi.org/10.1016/j.foodcont.2017.07.027

Saengkrajang, W., Matan, N., & Matan, N. (2013). Nutritional composition of the farmed edible bird's nest (Collocalia fuciphaga) in Thailand. Journal of food composition and analysis, 31(1), 41-45. https://doi.org/10.1016/j.jfca.2013.05.001

Sarzi-Puttini, P., Giorgi, V., Di Lascio, S., & Fornasari, D. (2021). Acetyl-L-carnitine in chronic pain: A narrative review. Pharmacological Research, 173, 105874. https://doi.org/10.1016/j.phrs.2021.105874

Seow, E. K., Ibrahim, B., Muhammad, S. A., Lee, L. H., & Cheng, L. H. (2016). Differentiation between house and cave edible bird's nests by chemometric analysis of amino acid composition data. Lwt, 65, 428-435. https://doi.org/10.1016/j.lwt.2015.08.047

Shim, E. K., Chandra, G. F., Pedireddy, S., & Lee, S. Y. (2016). Characterization of swiftlet edible bird nest, a mucin glycoprotein, and its adulterants by Raman microspectroscopy. Journal of food science and technology, 53, 3602-3608. DOI 10.1007/s13197-016-2344-3

Shukri, N. N. H. M., Nawi, N. M., Abdullah, A. M., & Man, N. (2018). Consumer's perception on the quality of controversial contents in edible bird's nest products. Pertanika Journal of Scholarly Research Reviews, 4(1).

Szkudelska, K., & Szkudelski, T. (2022). The anti-diabetic potential of betaine. Mechanisms of action in rodent models of type 2 diabetes. Biomedicine & Pharmacotherapy, 150, 112946. https://doi.org/10.1016/j.biopha.2022.112946

Tong, S. R., Lee, T. H., Cheong, S. K., & Lim, Y. M. (2021). Geographical factor influences the metabolite distribution of house edible bird's nests in Malaysia. Frontiers in Nutrition, 8, 658634. https://doi.org/10.3389/fnut.2021.658634

Veskovic, M., Mladenovic, D., Milenkovic, M., Tosic, J., Borozan, S., Gopcevic, K., ... & Radosavljevic, T. (2019). Betaine modulates oxidative stress, inflammation, apoptosis, autophagy, and Akt/mTOR signaling in methionine-choline deficiency-induced fatty liver disease. *European journal of pharmacology*, *848*, 39-48.

Virmani, M. A., & Cirulli, M. (2022). The role of l-carnitine in mitochondria, prevention of metabolic inflexibility and disease initiation. *International journal of molecular sciences*, *23*(5), 2717. https://doi: 10.3390/ijms23052717

Wang, X., Hu, D., Liao, F., Chen, S., Meng, Y., Dai, J., ... & Li, G. (2023). Comparative proteomic analysis of edible bird's nest from different origins. Scientific Reports, 13(1), 15859. https://doi.org/10.1038/s41598-023-41851-6

Ya'acob, F. F., Isamail, M. Z., Ramli, M. F., Majid, M., Mokhtar, N. A., Badyalina, B., & Shaari, N. F. (2021). The Impact of Covid-19 on Malaysian Edible Birdnest (EBN) Industry. Journal of Academic Research in Business and Social Sciences, 11(12), 2566-2577. http://dx.doi.org/10.6007/IJARBSS/v11-i12/11756

Yeo, B. H., Tang, T. K., Wong, S. F., Tan, C. P., Wang, Y., Cheong, L. Z., & Lai, O. M. (2021). Potential residual contaminants in edible bird's nest. Frontiers in pharmacology, 12, 631136. https://doi.org/10.3389/fphar.2021.631136

Zulkefle, N. N., Ibrahim, M. A., Azuan, N. F., Ch'ng, S. E., Ismail, N., Abu Bakar, M. Z., & Chan, K. W. (2024). A Review on the Edible Bird's Nest Quality and Manufacturing Standards of the Three Largest Exporting Countries in the World. Journal of Food Quality, 2024(1), 5608357. https://doi.org/10.1155/2024/5608357