Pertanika J. Trop. Agric. Sci. 39 (2): 181 - 195 (2016)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Discrimination between Cave and House-Farmed Edible Bird's Nest Based on Major Mineral Profiles

Seow, E. K.[1], Ibrahim, B.[2], Muhammad, S. A.[3,4], Lee, L. H.[5], Lalung, J.[3] and Cheng, L. H.[1]*

[1]Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia
[2]Discipline of Clinical Pharmacy, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia
[3]Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia
[4]Doping Control Centre, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia
[5]Faculty of Integrative Science & Technology, Quest International University Perak, No. 227, Plaza Teh Teng Seng (Level 2), Jalan Raja Permaisuri Bainon, 30250 Ipoh, Perak, Malaysia

ABSTRACT

The high priced cave edible bird's nest (EBN) has attracted unscrupulous EBN producers to adulterate EBN with lower priced house-farmed EBN due to the fact that both are almost indistinguishable by visual inspection. In the present study, major mineral contents such as calcium, sodium, magnesium and potassium of both EBN types were analysed using inductively coupled plasma-optical emission spectrometry (ICP-OES). Three pattern recognition techniques namely hierarchical cluster analysis (HCA), principal component analysis (PCA) and linear discriminant analysis (LDA) were employed to determine the influence of harvesting origins on mineral profiles. With the use of HCA and PCA, EBN samples have successfully been grouped into two distinct clusters. From the PCA score plot, principal component 1 (49.53 %) and principal component 2 (41.11%) accounted for 90.64% of the total variability. In addition, LDA presented excellent performance in discriminating and predicting membership of the two EBN sample types with classification rate of 100%.

Keywords: Edible bird's nest, hierarchical cluster analysis, inductively coupled plasma-optical emission spectrometry, linear discriminant analysis, mineral content, principal component analysis

ARTICLE INFO

Article history:
Received: 29 January 2015
Accepted: 26 January 2016

E-mail addresses:
ekseow@hotmail.com (Seow, E. K.),
baharudin.ibrahim@usm.my (Ibrahim, B.),
syahidah.muhammad@usm.my (Muhammad, S. A.),
lamhong.lee@qiup.edu.my (Lee, L. H.),
japareng@usm.my (Lalung, J.),
lhcheng@usm.my (Cheng, L. H.)
* Corresponding author

ISSN: 1511-3701

INTRODUCTION

Edible bird's nest (EBN) is highly consumed by the Chinese community because they uphold the belief handed down based on anecdotal evidences that EBN is beneficial to relief respiratory ailments and enhance body energy. The work by Kong et al. (1987), which suggests the presence of epidermal growth factor (EGF)-like substance in EBN, has drawn the attention of consumers as well as researchers. Since then, extensive research activities have been conducted to confirm the presence of EGF-like substance in EBN and its potential use in medical field and cosmetic industry for cell proliferative effect. This idea was substantiated by positive results reported in studies using human adipose-derived stem cells (Roh et al., 2012), corneal keratocytes (Zainal Abidin et al., 2011) and Caco-2 cells (Aswir & Wan Nazaimoon, 2010). Apart from that, EBN extract has been found effective in curing erectile dysfunction (Ma et al., 2012), improving bone strength and dermal thickness (Matsukawa et al., 2011) and inhibiting influenza virus infection (Guo et al., 2006).

Generally, EBN is built by gelatinous strand of nest cement secreted by swiftlets, namely, White nest swiftlet (*Aerodramus fuchipagus*) and Black nest swiftlet (*Aerodramus maximus*) during breeding seasons (Koon & Cranbrook, 2002). These swiftlets are found in the South-East Asia region and inherently inhabit the caves (Chantler & Driessen, 1999). Comparatively, EBN produced by the White nest swiftlet is of higher economic value as it is entirely made of pure salivary nest cement with only traces of impurities. On the other hand, though the nest of Black nest swiftlet is full with feathers and requires tedious cleaning process, it is still heavily harvested as the exploitation is worthwhile due to the fact that the price of the nest is extremely high.

With the increasing demand for EBN, the price of this product is expected to increase as the stock available in the market could not fulfil the growing needs. A recent survey reported by Manan and Othman (2012) revealed that the raw pre-processed EBN was sold at RM 3000/kg to RM 4500/kg in the market in year 2010 to 2011. The market price of EBN is always doubled after the laborious and time consuming cleaning process (Lim, 2006). Therefore, many investors are lured by the lucrative revenue and venture into EBN house-farming. Efforts have been done by the house farmers to ensure that only the pure breed of White nest swiftlet, which could produce EBN of high commercial value, would inhabit and breed in the farm (Lim, 2006). Unfortunately, EBN harvested from the house farm is much lower priced in the market than those harvested from the cave.

Driven by the unscrupulous desire, unethical EBN manufacturers tend to adulterate cave EBN with lower priced house EBN; some even make intentional false claims by selling house nest as cave nest. Besides, adulteration of EBN with addition or substitution with less expensive materials such as egg white, *Tremella* fungus, gelatin, karaya gum, fried porcine skin, starch, soybean and red seaweed

(Marcone, 2005; Ma & Liu, 2012), is commonplace.

Authentication methods at molecular level using Taqman-based real-time PCR (Guo et al., 2014), combination of DNA based PCR and protein based two dimensional gel electrophoresis methods (Wu et al., 2010), DNA sequencing-based method (Lin et al., 2009) and SDS-PAGE electrophoresis (Marcone, 2005) have been proposed. However, these techniques are rather tedious, time-consuming and costly.

EBN was built by swiftlets inhabiting in the caves and house farms and it was hypothesised that the minerals profile of EBN would be affected by the environments, as well as the supporting materials it attached to. The objective of this study is to distinguish EBN samples harvested from the cave and the house farm based on simple minerals profile analysed using inductively coupled plasma-optical emission spectrometry (ICP-OES). Correlation of mineral pairs within each group of sample was analysed using Pearson correlation analysis and pattern recognition techniques, namely, hierarchical cluster analysis (HCA), principal component analysis (PCA) and linear discriminant analysis (LDA) were employed to investigate the relationship between elemental concentration and the type of EBN samples studied.

MATERIALS AND METHODS

Materials

In this study, forty eight EBN samples were analysed. Twenty four of these were house nests harvested from different locations in West Malaysia, namely, Alor Setar, Bukit Mertajam, Kota Bharu, Segamat, Taiping and Teluk Intan. The twenty four cave nests were harvested from the caves located in East Malaysia (Bau and Sandakan) and Indonesia (Aceh and Medan). All EBN samples used in this study were raw genuine samples collected from different locations (see Figure 1) with the assistance of reliable suppliers and sponsors. All pre-processed samples were cleaned and air-dried under the same process. EBN samples were soaked in water and the feathers and impurities were removed using tweezers until the nests were devoid of visible feathers and impurities and followed by air-drying. Then, cleaned nests were dipped into liquid nitrogen for 10 seconds prior to grinding them into powder form. The samples were kept in air-tight bottles and stored at room temperature until further analysis.

Moisture Content

Moisture content of the samples was determined by volumetric Karl Fischer titration (784 KFP Titrino, Metrohm, Switzerland) following AOAC Official Method 2001.12.

Elemental Analysis

About 0.25 g of EBN powder was digested in a mixture of 3 mL H_2O + 2 mL HNO_3 + 1 mL H_2O_2 with a microwave digester (MARSXpress, CEM Corporation, Matthews, NC), following the method described in Saengkrajang et al. (2013). The digestion was carried out at 220ºC for 45 minutes until a clear transparent solution

was obtained. The digest was then made up to 50 mL with 2% HNO_3 solution and kept chilled in plastic bottles prior to mineral determination.

The concentrations of sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES), Perkin Elmer optima 7000DV equipped with S10 autosampler and WinLab32™ for ICP V5.1 (Perkin Elmer, Waltham, MA). The calibration was performed with standard mixture from Perkin Elmer (Waltham MA) and all elements were determined at axial plasma view. The instrumental settings of the ICP-OES were as follows: the source equilibration delay was 15 seconds, plasma parameters were set at plasma 15 L/min, auxiliary 0.2 L/min, nebulizer 0.8 L/min and power 1300 W. Flow rate of sample was 1.5 mL/min with Argon as carrier gas. There was a washing step between the samples at the rate of 1.5 mL/min for 30 seconds. The wavelengths for each element were: Ca, 317.933 nm; Na, 589.592; Mg, 285.213 and K, 766.490.

Method Verification

The raw data were pre-processed and the concentration of each element was expressed in unit of mg/100 g dry matter basis to minimise data fluctuation. Calibration curves for Ca, Na, Mg and K were constructed using external standards method. Coefficient of determination, r^2 of calibration curves for the elements were all above 0.9900. Repeatability was determined by intra- and inter-day variation studies, while reproducibility was determined by two different analysts that conduct the same method. This method showed a very good precision in repeatability and reproducibility, with relative standard deviation (RSD) of elements determined ranged from 0.80 to 5.69%.

Statistical Analysis

Experimental data obtained were analysed using the statistical package SPSS version 22 for Windows (SPSS Inc., Chicago, IL). Independent samples t-test was conducted to determine significant difference between mean values. Pearson correlation analysis was used to study the direction (positive/negative) and strength (weak/moderate/strong) of the correlation between elements within each type of nest samples. Three pattern recognition techniques: hierarchical cluster analysis (HCA), principal component analysis (PCA) and linear discriminant analysis (LDA) were used to observe the possible pattern and trend in classification.

RESULTS AND DISCUSSION

Elemental Composition of the EBN Samples

Calcium (Ca), sodium (Na), magnesium (Mg) and potassium (K) composition of both house EBN and cave EBN from different locations and descriptive statistics of both types of EBN are tabulated in Tables 1 and 2, respectively. Based on the independent samples t-test result, it is evident that Ca content in cave EBN is significantly higher than house EBN but the Mg and

Na contents are significantly lower in cave EBN. Nonetheless, there is no significant difference observed in the K content in both types of EBN.

Since K is not significantly different for the two types of samples, mineral composition could better or more accurately be compared by its ratio after being normalised to K content. Generally, the average major minerals contents determined in this study were arranged in the decreasing order of Ca > Na > Mg > K, which is in accordance with the research findings of Norhayati et al. (2010). For cave samples, the ratio of Ca:Na:Mg: K is 101:13:6:1, whereas for the house samples the ratio is 46:33:8:1. Obviously, calcium content in the cave EBN samples was slightly more than double of those found in the house EBN samples, and the reverse is true for Na content. The discrepancy in the element contents of both samples could largely be contributed by the inherent different environmental conditions prevailing in the cave and in the house farm (Sia & Tan, 2014).

Cave EBN is normally found as self-supporting nests that attached to vertical or

Table 1
Major minerals profile of house nests and cave nests.

Location	Type	Sample size	Ca	Mg	Na	K
Alor Setar	House Nest	5	706±32	122±11	632±78	18±2
Bukit Mertajam	House Nest	5	780±62	123±6	625±66	12±3
Kota Bharu	House Nest	5	665±51	127±8	633±100	14±1
Segamat	House Nest	3	777±98	138±11	548±150	20±1
Teluk Intan	House Nest	3	787±13	130±7	358±13	18±0.4
Taiping	House Nest	3	750±19	112±7	228±22	18±1
Medan	Cave Nest	4	1741±314	93±30	94±50	8±2
Aceh	Cave Nest	4	1389±334	102±19	112±69	13±4
Sandakan	Cave Nest	8	2263±207	130±23	294±166	33±10
Bau	Cave Nest	8	1203±42	90±3	274±24	6±1

Values are mean±standard deviation reported in mg/100g dry matter.

Table 2
Descriptive statistics for house and cave edible bird's nests.

	Minerals content (mg/100g dry matter)							
Element	House nests (n=24)				Cave nests (n=24)			
	Min.	Max.	Mean	STDEV	Min.	Max.	Mean	STDEV
Ca*	586	891	737	67	1141	2542	1677	504
Na*	203	795	536	167	38	630	224	131
Mg*	106	149	125	10	60	159	106	25
K	8	21	16	3	5	51	17	14

*Mean values are significantly different ($P<0.05$).

concave surface of a cave wall. Therefore, it is easy to rationalise high Ca content found in cave EBN. According to Northup and Lavoie (2010), mineral dissolution and precipitation processes in caves are microbially mediated reactions. Cave dissolution process involves iron-, sulfur- and manganese- oxidising bacteria, through which activities considerable acidity is being generated and subsequently used to dissolve cave wall that is rich in calcium carbonate. Meanwhile, the mineral precipitation process was reported to be either passive where microbial cells acts as nucleation sites or active, where bacterially produced enzymes control mineralisation. In passive mineralisation, dissolved metal (Ca^{2+}) was found to sorb onto amphoteric functional groups (such as carboxyl, phosphoryl and amino constituents) found on negatively charged cell walls, sheaths or capsules, following which carbonate (HCO_3^-) precipitates and in turn serves as nucleation site for calcium carbonate precipitation (Lowenstem & Weiner, 1989; Konhouser, 1997, 1998). It is believed that similar mechanism could have occurred by mineralisation on salivary strands (which is high in proteins) of a cave EBN.

On the other hand, Na content in the house EBN was found to be significantly higher than those cave EBN samples (Table 2). Interestingly, Na was also reported to be the predominant element in processed house-farmed EBN harvested from different locations in Thailand (Saengkrajang et al., 2013), pre-processed house-farmed EBN in Penang, Malaysia and pre-processed cave EBN in Sumatra Indonesia (Nurul Huda et al., 2008). Our raw data showed that Na content was extremely high in EBN harvested from Alor Setar, Bukit Mertajam and Kota Bharu house farms (Table 1 & Figure 1), which are located at the coastal locations facing the Malacca Straits. The Na content recorded was 2-3 folds higher when compared to the other samples harvested from other locations. Based on the report of Norhayati et al. (2013), this high Na content could be attributed to the accumulation of Na from marine aerosols through atmospheric deposition into the EBN. It is believed that sea salt concentration in the air could be high at these locations as a result of the persistent on-shore winds which generate sea water droplets and marine aerosols (sea sprays). The speculation was made based on the unique drinking behaviour of swiftlets, which capture the water droplets in the air. Therefore, the Na content in marine aerosol (swiftlet's saliva) is assumed to contribute to the nest Na content.

Besides the environmental factor, swiftlet diets could contribute partly to the difference in elemental profile of both types of samples. According to Lourie and Tompkins (2000), swiftlet's diets vary and are very much dependent on their foraging regions and food availability. Apart from this, White nest swiftlet's diet was discovered to be diverse and this species was predicted to survive and adapt well in urban areas (shop lot house farms). This could be a factor that yields the different minerals composition patterns in EBN harvested from different origins.

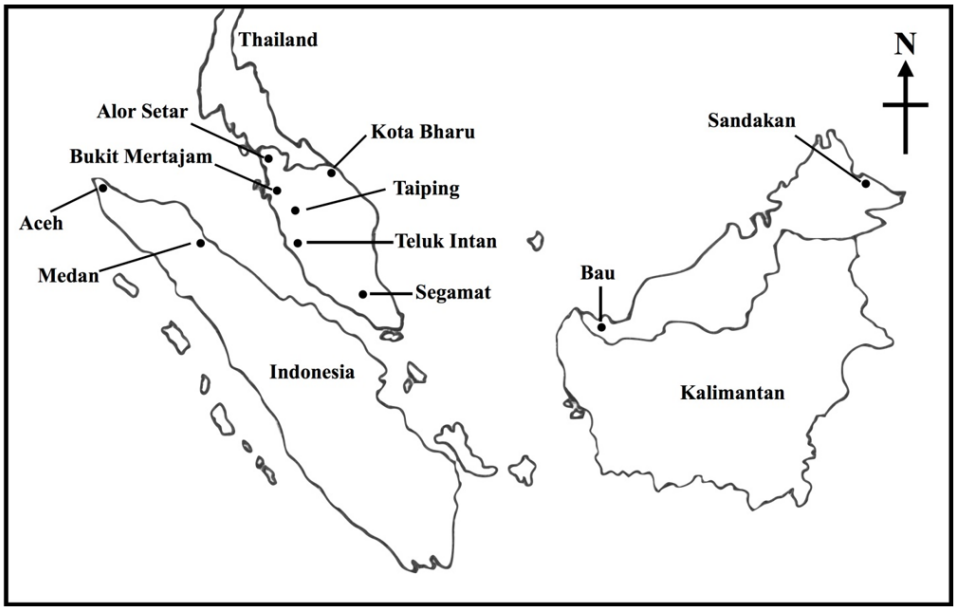

Figure 1. Edible bird's nest sampling points.

Pearson Correlation Analysis

The correlation matrix between mineral pairs of both types of EBN is presented in Table 3. Ca was found to demonstrate moderate positive correlation with Mg and it was significantly different at $r = 0.450$ ($P < 0.05$) for cave EBN samples. The Na content correlated significantly with K content at moderate values with $r = -0.477$ and 0.505 ($P < 0.05$) for house EBN and cave EBN, respectively. Interestingly, there were strong positive correlations between the mineral pairs in the cave EBN samples such as Ca and K ($r = 0.776$, $P < 0.01$), Na and Mg ($r = 0.609$, $P < 0.01$) and Mg and K ($r = 0.832$, $P < 0.01$). The significant relationship between the minerals leads us to further analyse the influence of macro- and micro-environmental factors on minerals composition of EBN.

Hierarchical Cluster Analysis (HCA)

Hierarchical cluster analysis (HCA) is an unsupervised classification method that discerns objects into groups based on the level of similarity between them based on the relative contribution of the variables. The clustering method used was the nearest neighbour (single linkage) method, measured based on squared Euclidean distance. A dendrogram was an easy visualisation aid produced with the samples of the same similarity level being grouped together. The use of HCA has successfully assigned the EBN samples into two main clusters, *i.e.* house EBN (n = 24) and cave EBN (n = 24), based on the dendrogram cut at a distance of 17.5 as presented in Figure 2. All the EBN samples were accurately classified into their own clusters which indicated that the elemental

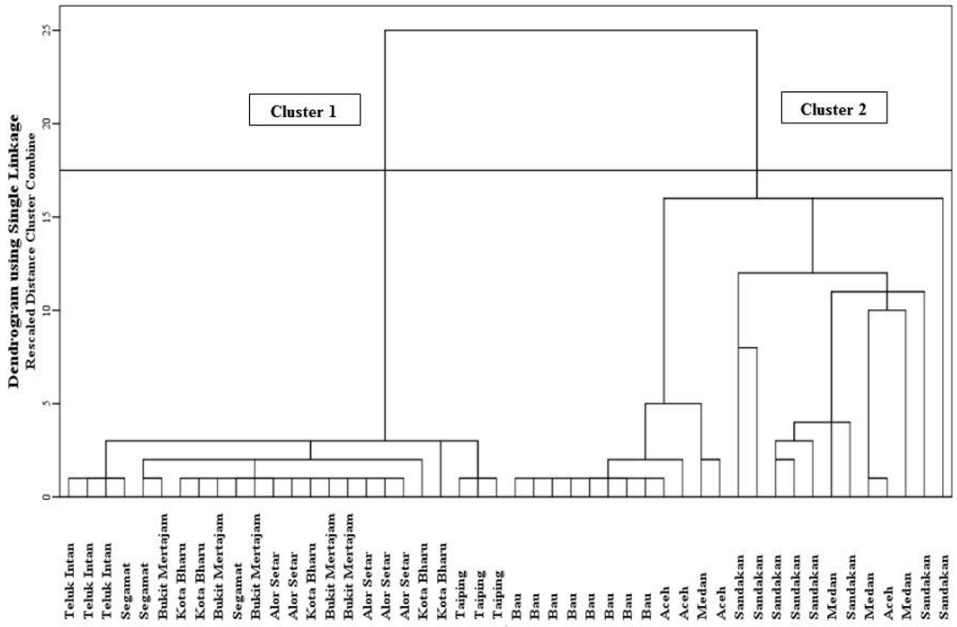

Figure 2. Dendogram of hierarchical cluster analysis. Cluster 1: house nests; cluster 2: cave nests

Table 3
Pearson correlation of minerals content in house and cave edible bird's nest.

House nests				
Element	Ca	Na	Mg	K
Ca	1			
Na	-0.069	1		
Mg	0.338	0.275	1	
K	0.077	**-0.477***	0.208	1

Cave nests				
Element	Ca	Na	Mg	K
Ca	1			
Na	0.151	1		
Mg	**0.450***	**0.609****	1	
K	**0.776****	**0.505***	**0.832****	1

** and * correspond to significance of correlation at the 0.01 level and 0.05 level (2-tailed), respectively.

composition could be appropriately used in classification of the type of EBN sample.

Principal Component Analysis (PCA)

Principal component analysis (PCA) is a chemometric tool used for dimension reduction of data set through which the most significant and important data would be extracted for further analysis (Abdi & William, 2010). Basically, PCA demonstrates primary evaluation and visualisation of between-class similarity based on the contributing variables variation direction in a multivariate space. PCA was carried out on a data matrix consists of EBN elemental profiles. The principal component (PC) scores and possible clustering results are illustrated in Figure 3A. Only two PC were extracted from the dataset to explain the total variability up to 90.64%. Two clusters

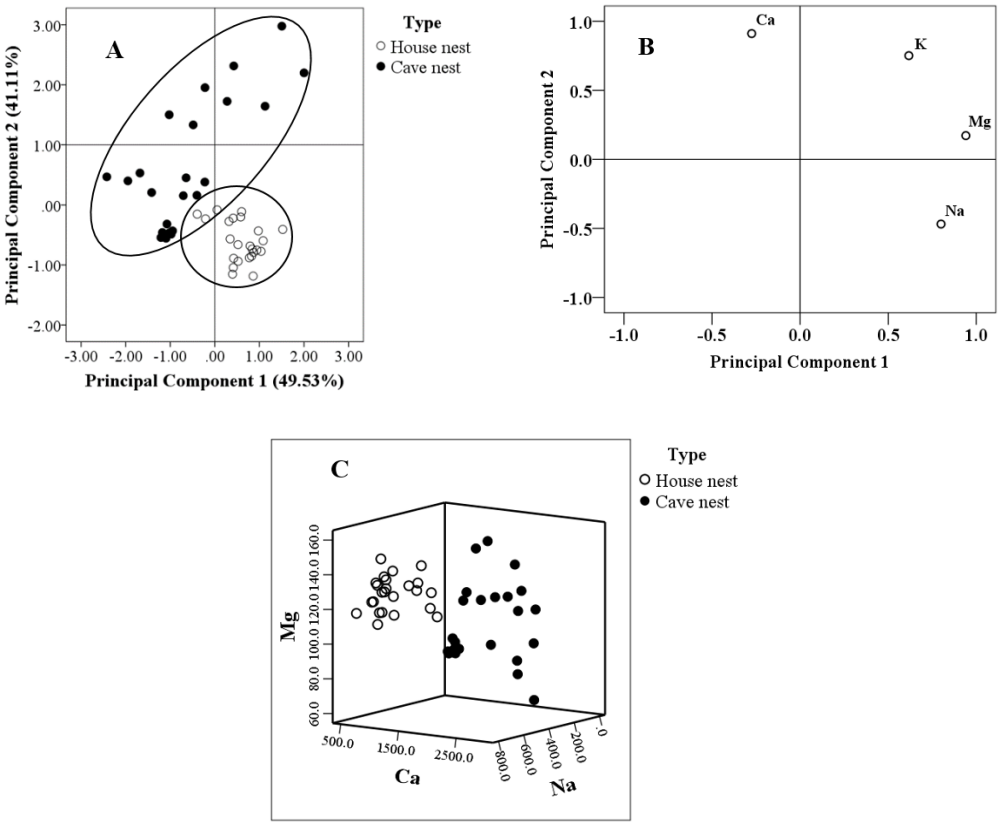

Figure 3. Principal component analysis was applied to study possible clustering between (A) house nest and cave nest, and their respective influential variables loaded as shown in (B). A simple 3-D plot of Ca vs. Na vs. Mg as illustrated in (C) gives a simple view of clustering potential

were identified and separated successfully at the diagonal by PC1 (explaining 49.53% of the variability) and PC2 (explaining 41.11%). Mg and Na, and Ca and K were the highly loading variables in PC1 and PC2, respectively, as shown in Figure 3B. In particular, the loading scores for Mg, Na, Ca and K were 0.941, 0.800, 0.911 and 0.751, respectively. A simple 3D-plot of Ca vs. Na vs. Mg concentrations was constructed to give a simple view of sample distribution or clustering potential as shown in Figure 3C, as these three variables contribute most of the variance. This 3D-plot is in good agreement with the PCA result (Figure 3A) that it provides a good discrimination pattern whereby house nest and cave nest are separated. As illustrated in Figure 3A, house EBN was observed to distribute more closely as compared to cave EBN. Geographical origin with different environmental conditions could be the key factor that contributes to the differences in EBN collected from different locations. Recently, authenticity assessment of commodities such as cabbages (Bong et

al., 2013), Croatian wines (Kruzlicova et al., 2013), Spanish cherries (Matos-Reyes et al., 2013) and Brazilian honey (Batista et al., 2012) through determination of mineral profiles analysed by chemometric analysis was found to be a valuable tool in classification according to geographical origins. Hence, PCA was further applied to investigate the possible groupings within class for both house and cave EBN.

As shown in Figure 4A, house EBN samples collected from the northern region (Alor Setar, Bukit Mertajam and Kota Bharu), whereas the remaining samples obtained from the central (Taiping and Teluk Intan) and the southern region (Segamat) in Peninsular Malaysia were separated into two clusters by PC1 which accounts for only 37.54% of the total variability. Na and K were the variables highly loaded in PC1, with the loading scores of -0.776 and 0.863, respectively, as illustrated in Figure 4B. Likewise, PC1 with the highly loading K, Mg and Ca variables, which explained 67.82% of variability (Figure 4C) categorised Sandakan cave EBN samples as one cluster and the other samples from Aceh, Bau and Medan as another cluster. As shown in Figure 4D, K, Mg and Ca were positively loaded in PC1 with the scores at 0.963, 0.897 and 0.731, respectively. The minerals profile of the cave EBN is associated to the cave wall the nest adheres to. The mineral profile of Sandakan cave EBN, which differs from the other locations may be attributed by the unique materials of the cave wall in Sandakan. This is evidenced by the geological survey that Sandakan rocks, which consist predominantly of mudstone, sandstone and siltstone with minor coal seams and conglomerate (Lee, 1970), are different in composition from the caves in Bau which are composed of fossiliferous limestone (Wolfenden, 1965). The lithological variations between different locations are due to facies changes (Lee, 1970). The results are in good agreement with the findings discovered by Saengkrajang et al. (2013), Norhayati et al. (2010) and Nurul Huda et al. (2008) that nutritional composition of EBN could be distinguishable by breeding sites. However, it could be observed that even for the samples collected from the same location and within the same breeding season, the distributions were scattered. Hence, other contributing factors should be taken into considerations for future research studies. A better distinct separation between the samples harvested from different locations could be achieved by increasing the sample size.

Linear Discriminant Analysis (LDA)

Linear discriminant analysis (LDA) is a supervised pattern recognition approach which separates classes based on their dissimilarities by maximising the variance between classes and minimising the variance within classes (Roggo et al., 2003; Liu et al., 2012). A stepwise method was used to investigate if cave and house EBN could be differentiated by their elemental composition. Cross-validation procedure was carried out by employing the leave-one-out technique to evaluate the robustness

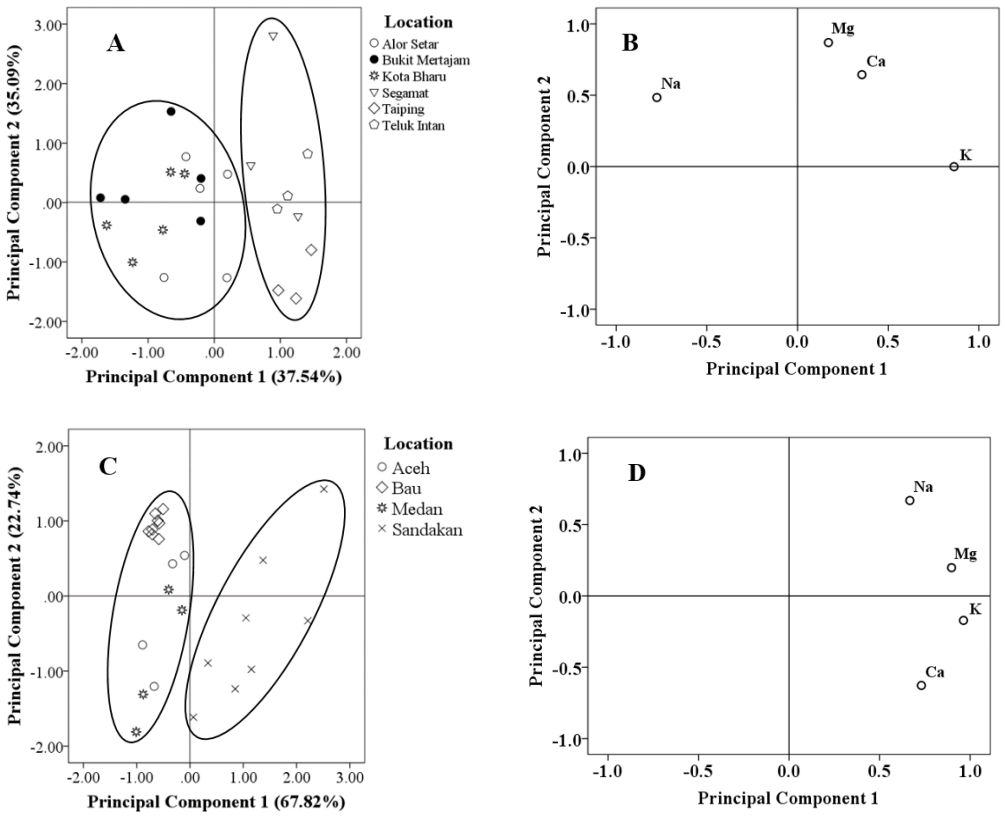

Figure 4. Principal component analysis was applied to study sample distribution within (A) house nest and (C) cave nest for geographical origins, and their respective influential variables loaded as shown in (B) and (D)

of the classification model. Each sample was classified based on the discriminant functions generated from the remaining samples and the accuracy of the classification was calculated as rate of cross-validation (Lachenbruch, 2006). LDA is used to assess the EBN samples with respect to the type based on the elemental composition. Four major elements (Ca, Na, Mg and K) were evaluated through LDA and only one linear discriminant function (DF) responsible in elucidating the differences between cave and house-farmed EBNs was derived. This DF explained 100% of the total variability between two types of EBN and the relative contribution of each parameter identified is as depicted in Eq. (1).

$$Z = 0.616\,Ca - 0.489\,Na - 0.290\,Mg - 0.012K \quad [1]$$

Ca and Na exhibited strong contribution in discriminating cave EBN from house EBN, whereas Mg showed relatively lower contribution in explaining the variation between the cave EBN and house EBN. Scores of DF for EBN samples of different types correspond to the behaviour of the parameters in the DF as depicted in Figure 5.

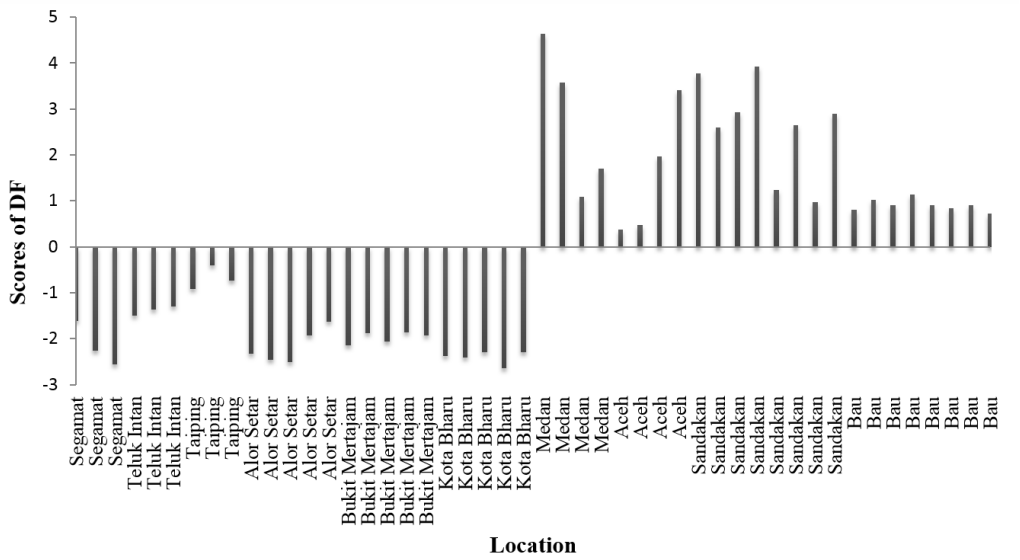

Figure 5. Scores for the discriminant function

Overall, all the house EBN samples showed negative contribution to the DF, whereas all the cave EBN samples demonstrated positive contribution. The sources of the samples were correctly identified in accordance to their own origins.

The membership of the EBN samples was predicted by employing a stepwise discriminant procedure, as shown in Table 4. A leave-one-out cross validation method was used to evaluate the robustness of this prediction model. Both overall classification and cross-validation classification were 100%, which implied that all samples, were correctly assigned to their own cluster. The results exhibited that this classification model was a very promising tool in discrimination of the EBN samples according to the types.

CONCLUSION

The use of elemental composition determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) combined with chemometric approach is verified to be a powerful tool in discriminating edible bird's nest based on types. Ca and Na were the elements which demonstrated strong contributions in the differentiation of two types of EBN samples. The robustness of the classification model has been validated and found to possess great predicting power at a classification rate and cross-validation rate of 100%.

ACKNOWLEDGEMENTS

This work was funded by Universiti Sains Malaysia Short Term Research Grant (Grant No: 304.PTEKIND.6313033). Seow acknowledges the Fellowship awarded by Universiti Sains Malaysia. The authors would like to thank Mr. George Ng Aun

Table 4
Classification of edible bird's nest samples and percentage of classification according to types through cross-validation method.

Type			Predicted Group Membership		Total
			House nest	Cave nest	
Original	Count	House nest	24	0	24
		Cave nest	0	24	24
	%		100	100	100[b]
Cross-validated[a]	Count	House nest	24	0	24
		Cave nest	0	24	24
	%		100	100	100[c]

[a]Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.
[b]100% of original grouped cases correctly classified.
[c]100% of cross-validated grouped cases correctly classified.

Heng, Dato Feasa, Mr. S.D. Hng, Mr. L.C. Ling, Mr. Chaw Seow Peoh, Mr. Sia Meu Seng, Mr. Tan Yoke Tian, Mr. Thomas Lee, Mr. Tan Sooi Huat and Mr. Peter Lau for sponsoring edible bird's nest samples to the project. The authors have declared no conflict of interest.

REFERENCES

Abdi, H., & William, L. J. (2010). Principal component analysis. *Wiley Interdisciplinary Reviews: Computational Statistic*s, *2*(4), 433-459.

Association of Official Analytical Chemists (AOAC). (2005). *AOAC Official Methods of Analysis* (18th ed.). Washington DC: Association of Official Analytical Chemists.

Aswir, A. R., & Wan Nazaimoon, W. M. (2010). Effect of edible bird's nest on Caco-2 cell proliferation. *Journal of Food Technology*, *8*(3), 126-130.

Batista, B. L., Da Silva, L. R. S., Rocha, B. A., Rodrigues, J. L., Berretta-Silva, A. A., Bonates, T. O., ... & Barbosa, F. (2012). Multi-element determination in Brazilian honey samples by inductively coupled plasma mass spectrometry and estimation of geographic origin with data mining techniques. *Food Research International, 49*(1), 209-215.

Bong, Y. S., Song, B. Y., Gautam, M. K., Jang, C. S., An, H. J., & Lee, K. S. (2013). Discrimination of the geographic origin of cabbages. *Food Control, 30*(2), 626-630.

Chantler, P., & Driessens, G. (1999). *Swifts, a guide to the swifts and treeswifts of the world*. (2nd ed.). UK: Pica Press.

Guo, C. T., Takahashi, T., Bukawa, W., Takahashi, N., Yagi, H., Kato, K., ... & Suzuki, Y. (2006). Edible bird's nest extract inhibits influenza virus infection. *Antiviral Research, 70*(3), 140-146.

Guo, L., Wu, Y., Liu, M., Wang, B., Ge, Y., & Chen, Y. (2014). Authentication of edible's bird nest by TaqMan-based real-time PCR. *Food Control*, *44*, 220-226.

Kong, Y. C., Keung, W. M., Yip, T. T., Ko, K. M., Tsao, S. W. & Ng, M. H. (1987). Evidence that epidermal growth factor is present in swiftlet's (*Collocalia*) nest. *Comparative Biochemistry and Physiology*, *87*(2), 221-226.

Konhauser, K. O. (1997). Bacterial iron mineralization in nature. *FEMS Microbiology Reviews, 20*(3-4), 315-326.

Konhauser, K. O. (1998). Diversity of bacterial iron mineralization. *Earth-Science Reviews, 43*(3), 91-121.

Koon, L. C., & Cranbrook, E. (2002). *Swiftlets of Borneo: Builders of Edible Nest*. Malaysia: Natural History Publications (Borneo) (Chapter 1).

Kruzlicova, D., Fiket, Ž., & Kniewald, G. (2013). Classification of Croatian wine varieties using multivariate analysis of data obtained by high resolution ICP-MS analysis. *Food Research International*, *54*(1), 621-626.

Lachenbruch, P. A. (2006). Discriminant analysis. *Encyclopedia of Statistical Sciences* (p.3). New Jersey: John Wiley and Sons.

Lee, D. T. C. (1970). *Sandakan peninsula, eastern Sabah East Malaysia*. Malaysia: Government Printing Office, Kuching, Sarawak, (Chapter III).

Lim, C. (2006). *Make millions from swiftlet farming: A definite guide*. Malaysia: Truewealth (Chapters 1 & 6).

Lin, J. R., Zhou, H., Lai, X. P., Hou, Y., Xian, X. M., Chen, J. N., Wang, P. X., Zhou, L., & Dong, Y. (2009). Genetic identification of edible bird's nest based on mitochondrial DNA sequences. *Food Research International*, *42*(8), 1053-1061.

Liu, X., Xue, C., Wang, Y., Li, Z., Xue, Y., & Xu, J. (2012). The classification of sea cucumber (*Apostichopus japonicas*) according to region of origin using multi-element analysis and pattern recognition techniques. *Food Control*, *23*(2), 522-527.

Lourie, S. A., & Tompkins, D. M. (2000). The diets of Malaysian swiftlets. *Ibis*, *142*(4), 596-602.

Lowenstam, H. S. & Weiner, S. (1989). *On biomineralization.* (p.324). New York: Oxford University Press.

Ma, F., & Liu, D. (2012). Sketch of the edible bird's nest and its important bioactivities. *Food Research International*, *48*(2), 559-567.

Ma, F. C., Liu, D. C., & Dai, M. X. (2012). The effects of the edible bird's nest on sexual function of male castrated rats. *African Journal of Pharmacy and Pharmacology*, *6*(41), 2875-2879.

Malaysian Food Act 1983 (Act 281) and Regulations. (2014). *Malaysia: International Law Book Services* (Twelfth Schedule).

Manan, Z., & Othman, M. S. H. (2012). *An economic analysis of swiftlet edible bird's nest in industry.* Malaysia: Forestry Department Peninsular Malaysia (Chapter 4).

Marcone, M. F. (2005). Characterization of the edible bird's nest the "Caviar of the East". *Food Research International*, *38*(10), 1125-1134.

Matos-Reyes, M. N., Simonot, J., López-Salazar, O., Cervera, M. L., & de la Guardia, M. (2013). Authentication of Alicante's Mountain cherries protected designation of origin by their mineral profile. *Food Chemistry, 141*(3), 2191-2197.

Matsukawa, N., Matsumoto, M., Bukawa, W., Chiji, H., Nakayama, K., Hara, H., & Tsukahara, T. (2011). Improvement of bone strength and dermal thickness due to dietary edible bird's nest extract in ovariectomized rats. *Bioscience, Biotechnology and Biochemistry*, *75*(3), 590-592.

Norhayati, M. K., Azman, O., & Wan Nazaimoon, W. M. (2010). Preliminary study of the nutritional content of Malaysian edible bird's nest. *Malaysian Journal of Nutrition*, *16*(3), 389-396.

Norhayati, M. T., Suhaimi, S., Foo, T. F., M. Suhaimi, M., & M. Talib, L. (2013). Temporal distribution and chemical characterization of atmospheric particulate matter in the Estern Coast of Peninsular Malaysia. *Aerosol and Air Quality Research*, *13*(2), 584-595.

Northup, D. E. & Lavoie, K. H. (2010). Geomicrobiology of cave: A Review. *Geomicrobiology Journal, 18*(3), 199-222.

Nurul Huda, M. Z., Zuki, A. B. Z., Azhar, K., Goh, Y. M., Suhaimi, H., Awang Hazmi, A. J., & Zairi, M. S. (2008). Proximate, elemental and fatty acid analysis of pre-processed edible bird's nest (*Aerodramus fuciphagus*): A comparison between regions and type of nest. *Journal of Food Technology*, *6*(1), 39-44.

Pettersen, R. C. (1984). The Chemical Composition of Wood. In R. Rowell), *The Chemistry of Solid Wood* (p. 119). American Chemical Society.

Roggo, Y., Duponchel, L., & Huvenne (2003). Comparison of supervised pattern recognition methods with McNemar's statistical test Application to qualitative analysis of sugar beet by near-infrared spectroscopy. *Analytica Chimica Acta*, *477*(2), 187-200.

Roh, K. B., Lee, J., Kim, Y. S., Park, J., Kim, J. H., Lee, J., & Park D. (2012). Mechanism of edible bird's nest extract-induced proliferation of human adipose-derived stem cells. *Evidence-based Complementary and Alternative Medicine, 2012*.

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.

Sia, Y. H., & Tan, J. H. (2014). Swiftlets farming for production of edible bird's nests. *Patents US 8651061 B2.*

Wolfenden, E. B. (1965). *Bau mining district, West Sarawak, Malaysia Part I: Bau*. Malaysia: Government Printing Office, Kuching, Sarawak, (Chapter II).

Wu, Y., Chen, Y., Wang, B., Bai, L., Wu, R. H., Ge, Y., & Yuan, F. (2010). Application of SYBRgreen PCR and 2DGE methods to authenticate edible bird's nest food. *Food Research International*, *43*(8), 2020-2026.

Zainal Abidin, F., Chua, K. H., Ng, S. L., Mohd Ramli, E. S., Lee, T. H., & Abd Ghafar, N. (2011). Effects of edible bird's nest (BN) on cultured rabbit corneal keratocytes. *BMC Complementary and Alternative Medicine*, *11*(1), 94.