

# **A PRELIMINARILY STUDY OF ELEMENTAL CHARACTERIZATION FOR GEOCHEMICAL MARKERS OF HOUSE AND CAVE EDIBLE BIRD'S NEST USING NAA TECHNIQUE**

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## **ABSTRACT**

Edible bird's nest (EBN) is one the most highly valued food products of South East Asia. The nest is made by certain species of swiftlets with a high protein glutinous secretion produced by their salivary glands. Malaysia is situated right at the heart of the "golden triangle" of swiftlet bird nest production, making it a strong producer in this lucrative agriculture industry. Issues facing the EBN industry include unknown source of origin with various quality grades, thus affecting export market and production subjected to fraud such as adulteration and counterfeiting. This study aims to characterize the elemental profiles as geochemical markers and evaluate the relationships between these markers which could be used for the verification of the geographical origin of EBN in Malaysia. EBN samples from house and cave nests that represent a range of geographical and environmental characteristics were analysed using Neutron Activation Analysis (NAA) technique. The method was validated by analysing the Standard Reference Material SRM-1515 (Apple Leaves) and SRM-1573a (Tomato Leaves) of NIST. A total of 15 elements were determined. The results showed significant different in elemental profile of EBN between the nest types on Al, As, Ba, Br, Ca, Cl, Na, Sc, Sm, Th and Rb content.

Keywords: edible bird's nest, geochemical markers, neutron activation analysis

## **INTRODUCTION**

The capability to certify food origin is becoming significant economic importance beside the issue in quality control and safety of food. In several countries, the use of geographical indications allows producers to obtain market recognition and often a premium price. For example, some food products such as wines, cheeses, oils, honey, beers, meats, pistachios and potatoes are marketed using labels (e.g. GI, Geographic Indication) that are based on standards of identity or composition related to a very specific production area. Europe has developed a system which provides legal protection to regional foods through the Protected Designation of Origin (PDO) labels (EU Regulation, 1982) to ensure the safety and quality of the products protecting them against fraud and imitation [1].

The edible bird's nest (EBN) industry has been identified as one of the National Key Economic Area (NKEA) for the agricultural sector under the Economic Transformation Programme (ETP) of Malaysia. EBN is a very lucrative agricultural industry in Malaysia, fetching over US \$6 billion worth of global sales. The majority of EBN traded worldwide comes from two heavily exploited species, the White-nest swiftlet (*Aerodramus fuciphagus*) and the Black-nest swiftlet (*Aerodramus maximus*). Their habitats range from the Nicobar Islands in the Indian Ocean to sea caves in the coastal regions of Thailand, Vietnam, Indonesia, Borneo and the Palawan Islands in the Philippines. Malaysia is situated right at the heart of the "golden triangle" of swiftlet bird nest production, making it a strong producer in this industry [2]. EBN is a dried glutinous secretion from the salivary glands of several different swiftlet species that mostly harvested in wild or farmed in special house. It is widely consumed as a health food due to its high beneficial effect on human health.

Questions concerning origin are among the first to be asked during grading the quality of EBN. In addition, consumers in key markets are increasingly concerned with the origin of their food and are willing to pay more if there can be assured of its origin. Thus, development of appropriate analytical tools to ensure geographical traceability is indeed essential. In addressing food safety issues in Malaysia, the Food Hygiene Regulations 2009 has provision for traceability but mechanism on how traceability can be achieved is not prescribed. Present mechanism is mainly paper-based system that pass information along with the commodity. However, such system is subject to failure either inadvertently or deliberately (fraud). Thus there is a need for an independent system to verify the origin of food and hence audit the traceability control systems.

Nuclear techniques involving elemental and stable isotopes fingerprinting provide a robust analytical tool for use in conjunction with food safety surveillance programmes to provide independent verification of food traceability systems [3]. Techniques such as Neutron Activation Analysis (NAA) for determining elemental composition and isotope ratio mass spectrometry (IRMS) to measure stable isotope ratio (SIR) eg. It is well known that the content of selected minerals and trace elements in foods clearly reflects the soil type and the environmental growing conditions thus can be used to assure the geographical origin of food samples [4].

This study aims to characterize the elemental profiles as geochemical markers and evaluate the relationships between these markers which could later be used for the verification of the geographical origin of EBN in Malaysia. Hypothesis of this study is that the trace-element of the EBN from two sources will provide unique and representative fingerprints that make it possible to differentiate between samples of different origins.

## **METHODOLOGY**

Samples were received from Ministry of Health in total of 17 samples consisted of 7 samples from house nest and 10 samples from cave nest of unknown specific location. Approximately 500mg of powdered EBN samples were weighted into polyethylene irradiation vials. Standard reference materials (SRMs) were co-irradiated with the sample, standard and blank to ensure accuracy and precision of data. The irradiations for NAA were performed in the TRIGA Mark II reactor at Malaysian Nuclear Agency that operated at 750kW with a thermal neutron flux of about  $2.0 \times 10^{12}$  n/cm<sup>2</sup>/s. For short irradiation, all samples, standard, blank and SRMs were irradiated

for a period of 60 seconds, and the cooling time for 10 min for first counting, and the second counting conducted after 24 hrs. In long irradiation, each batch containing the EBN samples, standard, blank and were irradiated for 6 hrs. The cooling times took 3 days for first counting, and 3 weeks for the second counting. A high resolution coaxial Ortec hyper-pure germanium detector (HPGe) with a resolution of 1.9 keV at 1332 keV gamma-rays line of Co-60 was used for counting. The spectral data were processed using the spectrum analysis software namely Gamma Visions. Quantification of major, minor and trace elemental concentrations were based on relative method and data were reported in dry weight (d.w.).

### *Statistical analysis*

The elemental values were analyzed using one way analysis variance (ANOVA) at  $P < 0.05$  followed by Kruskal-Wallis H test using Past3.12 software (folk.uio.no/ohammer/past) to compare the values between the cave and the house nest. Kruskal-Wallis H test was chosen because it does not require the assumption of data normality.

## **RESULTS AND DISCUSSION**

### *Quality assessment*

Standard reference material namely Tomato Leave 1573a and Apple Leaves 1515 provided by National Institute of Standard and Technology (NIST) were applied as quality control material of each analytical regiment implemented. The accuracy of the analytical technique was evaluated by analyzing the SRM using a Z-score calculation [5]. The Z-score of an element concentration was computed based on the following equation:

$$Z - score = \frac{x - c}{\sqrt{u_x^2 + u_c^2}}$$

Where  $x$  is the analytical results;  $c$  is the certified value,  $u_x$  is the uncertainty of analytical results, and  $u_c$  is the uncertainty of certified value. The uncertainty of the analytical results were obtained after estimating the contribution from the sources in example errors in weighing, spectral peak counts, background counts and uncertainties in standard value whilst the uncertainty of the certified value was based on the certificate. For acceptance of results:  $-2 < z < 2$  is anticipated. However, if  $z < -3$  or  $z > 3$ , it is consider that the result is “out-of-control” and corrective action will be taken. Whilst the precision simply described by the relative standard deviation of the measurement of the proposed SRMs.

Results obtained for the SRMs are presented in Table 1. Data obtained indicate good precision and good agreement with the certified values. Analytical results disclosed that the precision of the analysis is satisfactory where percentage relative standard deviations (RSD) of the SRMs results were lower than 20% and relative errors varied from 0 to 19.2%. The Z-score values obtained for most of the elements analyzed from both SRMs were  $|Z\text{-score}| \leq 2$ , indicating that the results are satisfactory and are within the ranges of certified data at the 95% confidence level. However, some elements were not indicated any Z-score value as in the certificate they are given only information value of concentrations.

**Table 1:** Results of analysis of Standard Reference Materials (in mg/kg).

Elements	Measurement value	Certified or recommended value	Error (%)	RSD (%)	Z-score
Tomato Leave 1573a					
Al	600 ± 11	598 ± 12	0.33	1.83	-0.12
Ba	59 ± 6	63	-6.35	10.17	
Br	1200 ± 50	1300	-7.69	4.17	2.00
Ca	50800 ± 762	50500 ± 900	0.59	1.50	-0.25
Co	0.67 ± 0.05	0.57 ± 0.02	17.5	7.46	-1.86
Cr	1.91 ± 0.1	1.99 ± 0.06	-4.02	5.24	0.69
K	26000 ± 540	27000 ± 500	-1.48	2.03	0.54
Mg	11930 ± 850	12000	-0.58	7.12	
Mn	238 ± 11	246 ± 8	-3.25	4.62	0.59
Na	144 ± 10	136 ± 4	5.88	6.94	-0.74
Rb	13.5 ± 0.7	14.89 ± 0.27	-9.34	5.19	1.85
Sc	0.101 ± 0.01	0.1	1.04	9.90	
Sm	0.17 ± 0.02	0.19	-10.5	11.8	
Th	0.118 ± 0.01	0.12	-1.67	8.47	
V	0.766 ± 0.05	0.835 ± 0.01	-8.26	6.53	1.35
Zn	28.1 ± 2.0	30.9 ± 0.7	-9.06	7.12	1.32
Apple Leaves 1515					
Al	288 ± 15	286 ± 9	0.70	5.21	-0.11
Ba	19 ± 5.5	49	0	11.2	
Br	1.67 ± 0.2	1.8	-7.22	12.0	
Ca	15100 ± 230	15260 ± 150	-1.05	1.52	0.58
Cl	562 ± 28	579	-2.94	4.98	0.61
K	16500 ± 260	16100 ± 200	2.48	1.58	-1.22
Mg	2880 ± 120	2710 ± 80	6.27	4.17	-1.18
Mn	54 ± 6	54 ± 3	0	11.1	0.00
Na	28.4 ± 3	24.4 ± 1.2	16.4	10.6	-1.24
Rb	10.2 ± 1.9	10.2 ± 1.5	0	18.6	0.00
Sc	0.030 ± 0.005	0.03	0	16.7	
Sm	3.27 ± 0.20	3	9.00	6.12	
V	0.21 ± 0.04	0.26 ± 0.03	-19.2	19.0	1.00
Zn	12.9 ± 0.8	12.5 ± 0.3	3.20	6.20	-0.47

***Elements in edible bird's nest sample***

The mean, maximum and minimum values observed for 18 elements in EBN samples are presented in Table 2. Both type of EBN showed relatively high amount of essential macronutrient particularly Al, Ca, Cl, K, Mg and Na. The EBN from cave had highest concentrations of Al, Ca and Mg. Meanwhile concentrations of Cl, K and Na in house type are more abundance. The Ca levels of the EBN are among the highest of all the elements analyzed. The cave nests have significantly higher levels ( $P < 0.05$ ) Ca compared to house nests. Calcium in form of calcium carbonate is the major component of cave natural structure. This might explain

on high concentrations of Ca from the cave nest. The element of Al, Cl, K and Na were significantly different ( $p < 0.05$ ) among the type of EBN except for Mg.

**Table 2: Elemental concentrations of EBN**

Elements	House EBN (n=7)			Cave EBN (n=10)		
	Average	Min	Max	Average	Min	Max
Al (mg/kg)	33.7	5.58	100	3240	689	4366
Ca (%)	0.69	0.59	0.75	1.36	0.77	2.59
Cl (mg/kg)	2392	912	3915	42.1	19.5	58.8
K (mg/kg)	311.6	231	431	187	147	506
Mg (%)	0.12	0.10	0.15	0.17	0.09	0.21
Mn (mg/kg)	1.34	0.44	3.43	4.52	1.33	11.0
Na (%)	0.61	0.37	0.81	0.03	0.02	0.22
V (mg/kg)	<0.03			0.19	0.07	0.33
As (mg/kg)	<0.05			0.18	0.10	0.30
Ba (mg/kg)	<1.0			8.41	3.86	11.1
Br (mg/kg)	3.53	1.63	5.33	0.36	0.19	0.99
Co (mg/kg)	0.06	0.04	0.08	0.07	0.05	0.11
Cr (mg/kg)	0.55	0.46	0.68	0.40	0.17	1.17
Rb (mg/kg)	1.05	0.78	1.23	<0.1		
Sc (mg/kg)	<0.001			0.005	0.004	0.012
Sm (mg/kg)	<0.002			0.010	0.004	0.015
Th (mg/kg)	<0.01			0.02	0.01	0.04
Zn (mg/kg)	9.05	2.28	23.0	6.49	2.80	12.8

The cave nests demonstrated significant amount of minor and trace elements such as Mn, V, As, Ba, Br, Co, Cr, Sc, Sm, Th and Zn. Only few elements showed detectable amount of concentration in house EBN samples. Therefore, elements of V, As, Ba, Sc, Sm and Th can be identified as the key element for geochemical marker that differentiate both type of EBN. Those elements in cave nests signified their presence in the sample but not in the house nests. This may be due to the natural environment of the cave that promote some form of nutrient absorption into the birds' nest. The surrounding habitats, availability and abundance of food source could be associated to the assortment of elements found in this study [6]. There was no statistically significant difference ( $P > 0.05$ ) of Mn, Co, Cr and Zn between both type of EBN except for Br.

## CONCLUSION

Several elements demonstrated significant contributions in the differentiation of two types of EBN samples. However, it is recommended that a more comprehensive study be conducted involving more samples from different region or locations in order to confirm the present findings.

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