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Using Amino Acids Composition Combined with Principle Component Analysisto Differentiate House and Cave Bird's Nests

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***Abstract* - The amino acid compositions of house and cave bird's nest were determined. Seventeen amino acids were identified, and hydroxyproline was not found. Principle component analysis (PCA) was applied to differentiate the birds' nests according to their origin and to assess the correlations between the bird's nest and their amino acid compositions. Studies on the score and loading plots indicated that cave bird's nest had the greatest concentrations ofarginine, serine, threonine, alanine, isoleucine, proline, leucine and aspartic acid. Thehouse bird's nest had the greatest concentrations of phenylalanine, glycine, tyrosine, methionine, lysine and valine. The bird's nest was also differentiated from other constituents such as gelatins and egg. This study indicated that the application of PCA was useful in differentiating bird's nest from other animal sources withsimilar amino acid profiles.**

***Keywords* - Bird's nest, Correlation loadings plot, Principle Component Analysis (PCA), Scores plot.**

INTRODUCTION

Edible bird's nest (EBN) is a nest that produced by several different swiftlet species. The nests are built by male swiftlet within the 35 days using a glutinous material found in saliva that secreted from the swiftlet's two sublingual salivary glands [1]. It is traded worldwide from two species mainly the White-nest swiflet (*Collocaliafuciphaga, C.germani*) and the Black-nest swiflet (*Collocalia maxima, C. unicolor*). Their habitats are commonly in the sea caves on the islands and along the coastline of Southeast Asian countries such as Thailand, Indonesia, Malaysia, Vietnam and Philippines [2]. Bird's nest is well-consumed for its high nutritional value that offer good effects for treating some diseases, enhances the immune systems, increase the energy and metabolism, regulate blood circulation and promote the growth. In recent years, a very high demand to the bird's nest has lead to the establishment of house farming swiftlet.

Many studies had reported the nutritional compositions of the edible bird's nest. It was stated that the major content of edible bird's nest is glycoprotein [2], [3]. However, there is no study was done to differentiate the nutritional compositions between the cave and the house farming bird' nest. There is also a lacking in scientific research regarding the authentication of the genuineness of bird's nest. The situation has giving the opportunity for an adulteration of edible bird's nest during processing stage with cheaper materials such as gelatin, white egg, seaweed and etc.

Recently, chemometric analysis has become a potential method for developing a discriminant analysis using multivariate data. Principle component analysis (PCA) helps to reduce the dimensionality in a data set and retain as much variation as possible [4]. This study was made with an attempt to determine the amino acid compositions in both cave and house birds' nests. Data was treated with PCA to differentiate the bird's nest source based on their amino acid composition. It also describes the potential use of PCA to discriminate the bird's nest from other constituents such as gelatin and egg which have similar amino acids profile.

MATERIALS AND METHOD

House and cave birds' nests were collected from various locations in Malaysia (Selangor and the island of Borneo). The birds' nests were crushed using mortar before used. It was weighed approximately within the range of 0.1-0.2g and mixed with 5 ml of 6 N HCl. The mixture was incubated in an oven at 110 °C for 24 hr [5]. An internal standard (AABA) was added to the resulting mixture prior to further dilution with distilled water. The solution was filtered and derivatised with AccQ borate buffer and AccQ Fluor reagent. The derivatised compounds were heated in an oven for 10 min prior to injection into HPLC. The chromatographic system was a

Waters® Alliance System (2695 separations module) supported with Waters® 2475 Multi-λ Fluorescence Detector at 250 nm excitation and 395 nm emissions. The column type was a Waters AccQ-Tag (3.9 x 150) mm with holding temperature at 36 °C. The eluent system consisted of three components: AccQ-Tag™ Eluent A concentrates deionised water and acetonitrile. The injection volume was 10 µl and the flow rate was set at 1 ml/min. The gradient flow was performed within 50 min of the running time analysis. Waters Empower™Pro software was used for data acquisition.

The results were analysed using principle component analysis (PCA) to project the origin of the test samples. The data were subjected to discriminant analysis using Unscrambler®X software version 9.7. The pattern recognition technique was used to transform the data matrix into a number of principle components (PCs) and the size of each component was measured to indicate the number of significant components in the data set.

RESULTS

Table 1 shows the amino acids composition in bird's nest that indicates the absence of hydroxyproline. Methionine and cystine werepresent in a low concentration compared to other amino acids.The spectral chromatogram in Fig. 1 exhibited two unknown peaks in the front row which were separated from the entire amino acids. The unknown peaks could be an indication to differentiate the bird nest from other animals based on the amino acids' spectral chromatogram pattern.

The scores plot in Fig. 2 revealed that both bird nests weredistributed symmetrically on the left and right represented the cave and house bird's nest, respectively. It covers the 88% of the total variances. The data were located inside the Hotelling T^2 ellipse was accepted at 95% confidence limit.

Fig. 3 shows the corresponding loading plots that indicates the correlations between the amino acids and both birds' nests. Glu that plotted in the inner circle (less than 50% correlation) is less important for describing both birds' nests. Two groups were identified in which the amino acids located symmetrically at left (Arg, Ser, Thr, Ala, Ile, Pro, Leu and Asp) and right (Phe, Gly, Tyr, Met, Lys and Val) sides were correlated to the cave and house bird's nest, respectively. The result shows that the bird's nest collected from the cave contain a high amount of amino acids compared to house bird's nest. The PCA results were used as database for both birds' nests. The accuracy of the results was tested against unknown birds' nests. The result indicates 5 unknown birds' nests were originated from the house birds' nest (Fig. 4).

A lot of fraud issue exists involving the adulteration of bird nest with other constituents such as gelatin and egg. As revealed in Fig. 5, both birds' nests were pooled in one group that significantly could be separated from the fish, bovine and porcine gelatin. The scores plot revealed that the animals with different habitats were grouped according to difference in food intake that contributes to the amino acids composition among them. Furthermore, the bird's nest could also be differentiated with egg as shown in Fig. 6. The low content of amino acids in egg compared to the bird's nest and gelatin have isolated the egg group from the Hotelling T^2 ellipse. In this case, bovine, porcine and fish gelatins were pooled into one group because of a similarity in amino acids profiles and the presence of hydoxyproline in them.

Figures and Tables

Table 1. Amino acids composition in house and cave bird's nest

Amino acids	Mean and standard deviation (expressed in % w/w)	
	House Bird's Nest	Cave Bird's Nest
Aspartic acid	4.64 ± 0.57	4.94 ± 0.22
Serine	4.16 ± 0.39	4.57 ± 0.63
Glutamic acid	3.75 ± 0.52	3.83 ± 0.25
Glycine	1.80 ± 0.18	1.83 ± 0.15
Histidine	1.82 ± 0.14	1.59 ± 0.24
Arginine	3.27 ± 0.28	3.56 ± 0.44
Threonine	3.15 ± 0.30	3.34 ± 0.44
Alanine	1.34 ± 0.16	1.68 ± 0.07
Proline	3.39 ± 0.35	3.57 ± 0.36
Cystiene	0.73 ± 0.06	0.46 ± 0.02
Tyrosine	2.49 ± 0.19	2.41 ± 0.32
Valine	3.51 ± 0.35	3.53 ± 0.40
Methionine	0.27 ± 0.02	0.20 ± 0.01
Lysine	2.30 ± 0.30	1.79 ± 0.24
Isoleucine	1.62 ± 0.17	1.72 ± 0.18
Leucine	3.32 ± 0.34	3.48 ± 0.29
Phenylalanine	2.68 ± 0.21	2.67 ± 0.30

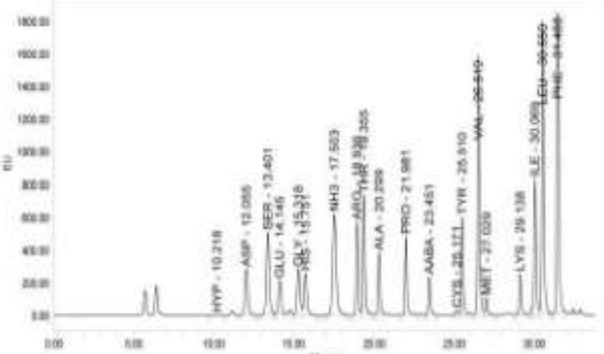

Fig.1 Spectral chromatogram of amino acid composition in bird's nest

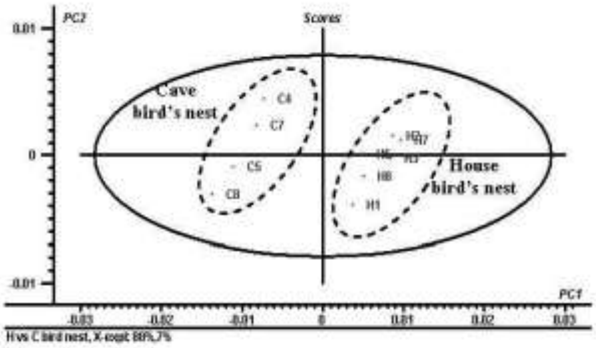

Fig. 2 Differentiation between cave and house bird's nest

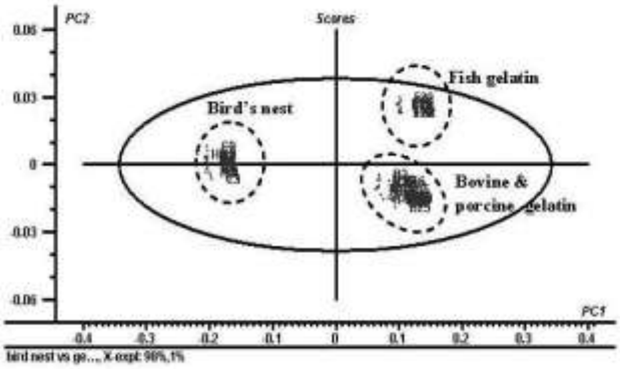

Fig. 5 Differentiation based on habitats (air, land and water)

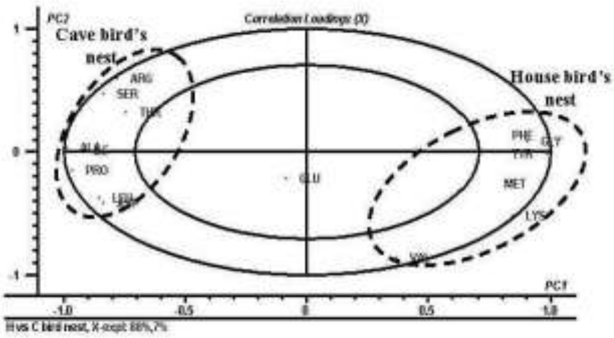

Fig. 3 The correlation between amino acids with cave and house bird's nest

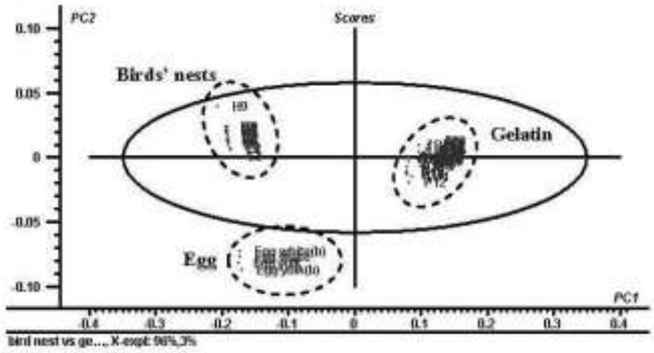

Fig. 6 Differentiation based on nutrition

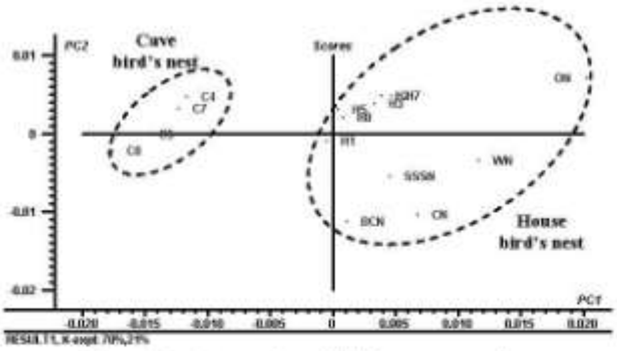

Fig. 4 Testing against bird's nest sample (BCN, SSSN, CN, WN and ON)

CONCLUSIONS

This study has showed the application of chemometrics analysis via PCA will provide the pattern recognition with mathematical and statistical operations, which was a beneficial tool that helps to distinguish between data sets with similar profiles. Amino acids composition could be used to provide data information in the PCA method. By this means, the different sources of birds' nests and its adulteration with other constituents could be determined.

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