LWT - Food Science and Technology 65 (2016) 428–435

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Differentiation between house and cave edible bird's nests by chemometric analysis of amino acid composition data

Eng-Keng Seow [a], Baharudin Ibrahim [b], Syahidah Akmal Muhammad [c, d], Lam Hong Lee [e], Lai-Hoong Cheng [a, *]

[a] Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800, USM, Penang, Malaysia
[b] Discipline of Clinical Pharmacy, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800, USM, Penang, Malaysia
[c] Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800, USM, Penang, Malaysia
[d] Doping Control Centre, Universiti Sains Malaysia, 11800, USM, Penang, Malaysia
[e] Faculty of Integrative Science & Technology, Quest International University Perak, No. 227, Plaza Teh Teng Seng (Level2), Jalan Raja Permaisuri Bainon, 30250 Ipoh, Perak, Malaysia

ARTICLE INFO

Article history:
Received 28 November 2014
Received in revised form
14 July 2015
Accepted 17 August 2015
Available online 20 August 2015

Keywords:
Amino acid
Edible bird's nest
Food authenticity
Principal component analysis
Orthogonal partial least square-discriminant analysis

ABSTRACT

A total of 60 pieces of house and cave edible bird's nest (EBN) samples were randomly collected from different locations in Malaysia and Indonesia. Amino acid compositions of the EBN samples were determined by gas chromatography-mass spectrometry (GC-MS). Data obtained was analyzed by Pearson Correlation analysis, Principal Component analysis (PCA) and Orthogonal Partial Least Square-Discriminant analysis (OPLS-DA). There were highly significant different correlations seen among amino acids in house- and cave-EBN samples. The model constructed by OPLS-DA was found to be a promising tool with high predictive power of 76.1%. Robustness of the model was validated and blind test samples were correctly assigned to their respective cluster. Tyrosine (TYR) and glutamic acid (GLU) were proposed as the promising markers for differentiating between house and cave EBN.

1. Introduction

Edible bird's nest (EBN) is formed by swiftlets using salivary secretions from sublingual salivary glands. The nest built serves as a shelter for swiftlets to breed and roost. Two types of swiftlets are responsible for producing edible nest of commercial interest, they are White nest swiftlet (*Aerodramus fuciphagus*) and Black nest swiftlet (*Aerodramus maximus*) (Koon & Cranbrook, 2002, chap. 1). Edible nests produced by the former are favored by processors and traders as they are almost purely hardened nest cement with traces of feathers and impurities.

According to Lim (2006, chap. 6), consumption of EBN could be traced back to Tang Dynasty (618–907 A.D.). To date, this tonic food is still popular among and savored by the Chinese community all over the world. Generally, the Chinese believe that EBN has potential therapeutic effects as Traditional Chinese medicine. There were research studies which examined the cell proliferative effect of EBN using Caco-2 cells (Aswir & Wan Nazaimoon, 2010), corneal keratocytes (Zainal Abidin et al., 2011) and human adipose-derived stem cells (Roh et al., 2012). All these studies confirmed the previous study which reported that EBN contains epidermal growth factor-like substances which could help to trigger cell division that results in skin rejuvenation (Kong et al., 1987). Apart from this, EBN extract was found to exhibit versatile medical potentials such as enhancing bone strength and dermal thickness (Matsukawa et al., 2011), treating erectile dysfunction (Ma, Liu, & Dai, 2012) as well as serving as an alternative chondro-protective agent in curing osteoarthritis (Chua et al., 2013).

Edible-nest swiftlets are only found in Southeast Asia (Chantler & Driessens, 1999). Malaysia has emerged as the third largest EBN producing country after Thailand and Indonesia. Constant demand of EBN by the Chinese community from China, Taiwan, Singapore and North America has boosted the local bird's nest industry as well as the establishment of swiftlet house farming. Driven by the lucrative return, some unscrupulous bird's nest processors were found to indulge in unethical practices such as adulterating EBN

* Corresponding author.
E-mail address: lhcheng@usm.my (L.-H. Cheng).

http://dx.doi.org/10.1016/j.lwt.2015.08.047
0023-6438/

with karaya gum, red seaweed, *Tremella* fungus, fried porcine skin and low quality bird's nest which are almost indistinguishable from the genuine samples by visual inspection (Ma & Liu, 2012; Marcone, 2005). Besides, consumers have been counterfeited into buying lower priced house-farmed EBN at premium price of cave EBN.

Numerous authentication methods on molecular level have been proposed, such as genetic identification based on cytochrome *b* sequence in mitochondrial DNA (Lin et al., 2009), combination methods of SYBRgreen PCR and 2DGE (Wu et al., 2010) and TaqMan-based real time PCR (Guo et al., 2014). Yang, Cheung, Li, and Cheung (2014) have also proposed a three-step protocol, using gas chromatography-mass spectrometry (GC-MS), environmental scanning electron microscopy and immunoblotting assay to identify genuine sample as well as monitoring the quality of EBN. However, these methods are rather expensive, tedious and time-consuming methods.

In the present study, a reliable, accurate, rapid and inexpensive method was proposed to differentiate between EBN of house-farmed and cave-harvested origin. It was hypothesized that house-farmed and cave EBN samples can be differentiated based on their amino acid profiles, due to the significant macro- and micro-environment that prevails in the swiftlet house farm and the cave. Amino acid profiles of both house and cave EBN were obtained using GC-MS. Data was analyzed critically by Pearson Correlation analysis, followed by a two-step chemometric analysis using Principal Component analysis (PCA) and Orthogonal Partial Least Square-Discriminant analysis (OPLS-DA).

2. Materials and methods

2.1. Sample preparation

Both house and cave processed EBN was obtained from reliable local suppliers and sponsors. An amount of 30 pieces of raw house nests were randomly collected from Alor Setar, Baling, Bukit Mertajam, Kota Bharu, Kota Kinabalu, Mengkarak, Sarikei, Segamat, Taiping and Teluk Intan house farms in Malaysia; while another 30 pieces of raw cave nests were sampled from Bau, Miri and Sandakan (Malaysia) and Aceh, Medan and Pasir Panjang (Indonesia). Classification of locations based on states in Malaysia is as follows: Alor Setar, Baling, Bukit Mertajam, Taiping and Teluk Intan are located in the northern region; Kota Bharu and Mengkarak are in the east coast region; Segamat is in the southern region of Peninsular Malaysia; whereas Kota Kinabalu and Sarikei are located in the East Malaysia. Location of each sampling point is shown in Fig. 1. Each raw nest was treated as an individual sample and was processed under the same cleaning and air-drying process. House nests and cave nests were soaked in water for 3 h and 4 h, respectively prior to the removal of feathers and impurities using tweezer. The nests were dried in oven at 35–40 °C for 16–20 h. Processed nests were dipped into liquid nitrogen for 10 s before being ground into powder. Samples were labeled according to location and kept in air-tight bottles prior to analysis.

2.2. Moisture content

Sample moisture content was determined using 784 KFP Titrino with a 703 Ti Stand pump unit (Metrohm, Riverview, USA) following the AOAC Official Method 2001.12.

2.3. Crude protein content

Crude protein content was determined using Vapodest 50S equipped with Vapodest Manager Version 1.2 (20) (Gerhardt, Brackley, UK) following the AOAC Official Method 2001.11. The equation used to calculate the amount of protein in sample is as follows, with 6.25 as conversion factor:

$$\text{Protein content} = \frac{(\text{mL HCl of sample} - \text{mL HCl of blank}) \times \text{Normality} \times 14.007 \times 6.25}{\text{Sample weight in miligram}}$$

2.4. Amino acids analysis

Determination of amino acid profile was done with the aid of a commercial test kit, EZ:faast™ GC-MS Protein Hydrolyzate Kit KG0-7168 (Phenomenex, Torrance, CA, USA). Zebron EZ-AAA Amino-Acid GC column (10 m × 0.25 mm) was provided in the test kit. Prior to the determination of amino acid content, samples were hydrolyzed following the AOAC Official Method 994.12. There is a limitation of this acid hydrolysis method, whereby cystine (C–C), methionine (MET) and tryptophan (TRP) could not be accurately quantified due to partial oxidation of C–C and MET, and complete destruction of TRP. Apart from that, deamidation of asparagine (ASN) and glutamine (GLN) to aspartic acid (ASP) and glutamic acid (GLU), respectively, leads to a mixture quantification. Sample analysis was performed using Shimadzu GCMS-QP 2010 equipped with AOC-20S autosampler and data was analyzed using LabSolutions GCMS solution Version 2.70 software (Shimadzu, Nakagyo-ku, Kyoto, Japan). GC settings were as follows: split mode injections at 1:15 ratio were performed at 300 °C with injection volume of 1.5 μL; flow rate of carrier gas (helium) was set at 1.1 mL min^{-1}; oven temperature was increased from 110 °C to 320 °C at a rate of 30 °C min^{-1}. MS detector was run at SIM mode (selected ion monitoring) for quantitative analysis at scan range of 45–450 *m*/*z* with sampling rate at 2^2 (3.5 scans/s). Temperature of MS source, MS quad and auxiliary were set at 240 °C, 180 °C and 310 °C, respectively.

Concentration points at 50, 100 and 200 nmol/mL were prepared using pure amino acid standards provided by the test-kit to construct calibration curves. Concentrations of amino acids in the samples were calculated based on the linear equations obtained. Internal standard (norvaline) method based on peak areas ratio was applied for quantification. The amino acid contents were expressed in mg 100 g^{-1} of dry protein weight.

2.5. Method verification

Sensitivity of instrument was performed using pure amino acid standards prior to determining amino acid contents in the samples. Calibration curves of amino acid were constructed using concentrations at 50, 100 and 200 nmol/mL. Six independent replicates of the standard at 50 nmol/mL were injected and the standard deviation, σ was obtained. Limit of Detection (LOD) was determined by