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CHEMICAL COMPOSITION AND AUTHENTICITY OF SELECTED EDIBLE BIRD NEST IN MALAYSIA AND INDONESIA

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UNIVERSITI MALAYSIA PERLIS

2015

Chemical composition and authenticity of selected Edible Bird Nest in Malaysia and Indonesia

by

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LIST OF SYMBOLS

°C	Celsius
g	Gram
min	Minute
N	Normality
mg	Miligram
mg/L	Miligram per litre
ppm	Part per million
v/v	Volume to volume ratio
rpm	rotation per minute
α	Significance level
Ho	Hypothesis null
σ	Standard deviation

LIST OF ABBREVIATIONS

EBN	Edible Bird Nest
UL	Unclean Langkawi
UP	Unclean Perlis
UJ	Unclean Java
UB	Unclean Balikpapan
UK	Unclean Kalimantan
CL	Clean Langkawi
CP	Clean Perlis
CJ	Clean Java
CB	Clean Balikpapan
CK	Clean Kalimantan
ANOVA	Analysis of variance
Avg	Average
NO_2^-	Nitrite
NO_3^-	Nitrate
Na_2CO_3	Sodium carbonate
NaOH	Sodium hydroxide
$CuSO_4$	Copper sulphate
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
H_2O_2	Hydrogen peroxide
HNO_3	Nitric acid

TFA	Trifluoroacetic acid
BSA	Bovine Serum Albumin
FTIR	Fourier Transform Infrared Spectroscopy
UV-Vis	Ultraviolet Visible Spectrophotometer
AAS	Atomic Absorption Spectroscopy
JMP software	JMP software from SAS Institute, USA (Version 10)
ISE	Ion selective sensor
E-tongue	Electronic tongue
AOAC	Association of Official Analytical Chemists
K	Potassium
Fe	Iron
Zn	Zinc
K	Potassium
Fe	Iron
Zn	Zinc
Cu	Copper
Pb	Lead

Komposisi kimia dan ketulenan sarang burung yang boleh dimakan terpilih daripada Malaysia dan Indonesia

ABSTRAK

Kajian ini dijalankan untuk menentukan keaslian EBN dan komposisi EBN yang bersih dan tidak bersih dan juga untuk membangunkan satu teknik pantas menggunakan teknologi sensor untuk menentukan kandungan nitrit dan nitrat. Sampel yang digunakan adalah EBN yang tidak bersih dan bersih daripada dua negara utama pengeksport: Malaysia (Langkawi dan Perlis) dan Indonesia (Java, Balikpapan dan Kalimantan) daripada spesis *Aerodramus fuciphagus* dan beberapa bahan tiruan iaitu agar, kanji, natrium alginat, rumpai laut, putih telur dan kulit babi. Pengenalpastian kumpulan berfungsi telah dikaji menggunakan teknik FTIR. Dalam kajian ini, kedua-dua EBN tulen yang tidak bersih dan bersih memberikan corak FTIR spektrum yang sama. Kehadiran kumpulan berfungsi seperti hidroksil (OH^-), karboksil ($COOH^-$), amina (NH) alkina (C-C), alkena (C≡C), karbonil (C=O) dan nitro (N=O) telah dikenal pasti. Manakala, spektra bahan tiruan tulen adalah berbeza dengan EBN tulen. Spektra EBN tidak tulen dengan penambahan bahan tiruan menunjukkan persamaan dengan EBN tulen tetapi kehadiran kumpulan berfungsi adalah berbeza. Kawasan cap jari pada gelombang <1500 cm^{-1} untuk spektrum EBN tulen dan EBN yang tidak tulen dengan penambahan bahan tiruan adalah berbeza dimana Kumpulan NH tidak hadir dalam semua bahan tiruan tulen kecuali kulit babi dan putih telur. Selain itu, hanya kulit babi mempunyai ikatan ester C=O berbanding EBN tulen dan bahan tiruan yang lain. Ikatan ester C=O ini adalah getaran daripada asid lemak pada gelombang 1745.1 cm^{-1}. Walaubagaimanapun, kedua-dua EBN dan bahan tiruan mempunyai ikatan C=O amide iaitu ikatan untuk protein pada gelombang 1670-1600 cm^{-1}. Oleh itu, FTIR boleh digunakan sebagai kaedah yang mudah dan cepat untuk mengenalpasti ketulenan EBN dan bahan tidak halal. Komponen terbesar dalam EBN adalah protein dengan 22.4% hingga 32.7%. Kemudian, diikuti dengan kandungan karbohidrat dengan 9% hingga 12%. Kelembapan (6.92% hingga 13.88%), abu (1.1% hingga 8.8%) dan yang paling rendah adalah lemak mengandungi 0.01% hingga 0.07%. Nitrit dan nitrat dalam EBN telah ditentukan dengan menggunakan teknik sensor elektrod ion terpilih (ISE) juga dipanggil E-tongue. Teknik sensor adalah lebih cepat berbanding dengan teknik kromatografi ion biasa. Kepekatan nitrit dan nitrat dalam EBN tidak bersih, bersih dan air buangan EBN adalah berbeza dari 0.2 hingga 2.9 ppm, lebih rendah daripada bacaan standard iaitu 30 ppm dan selamat untuk dimakan oleh manusia. Ujian ketepatan yang telah dijalankan ke atas sarang burung yang boleh dimakan daripada Perlis menggunakan teknik sensor memberikan 100.2 hingga 100.8% ketepatan dengan nilai RSD kurang daripada 1%, manakala menggunakan teknik ion kromatografi memberikan 96.9 hingga 102.8% ketepatan dengan nilai RSD 9 hingga 13%. Justeru itu, teknik sensor mempunyai sensitiviti yang tinggi dan boleh digunakan sebagai teknik piawai dalam menentukan kandungan nitrit dan nitrat dalam sampel sarang burung yang boleh dimakan selain mengurangkan masa analisis daripada 2 jam kepada 10 minit. Unsur mineral seperti kalium dan besi adalah tinggi dalam EBN manakala kandungan plumbum adalah sangat rendah oleh itu ianya tidak beracun.

Chemical composition and authenticity of selected Edible Bird Nest in Malaysia and Indonesia

ABSTRACT

This study was conducted to determine the authenticity of EBN and the compositional properties of unclean raw and clean EBN as well as to develop a rapid technique to determine the nitrite and nitrate content using sensor technology. The samples used were raw unclean and clean EBN from two major exporting countries: Malaysia (Langkawi and Perlis) and Indonesia (Java, Balikpapan and Kalimantan) from species *Aerodramus fuciphagus* and some common adulterants namely agar, starch, sodium alginate, carrageenan, egg white and pork skin. Functional groups identification study was employed using FTIR technique. In this study, both unclean and clean EBN gave similar pattern of FTIR spectra. The presence of similar compounds such as hydroxyl (OH), carboxyl (COOH), amines (NH), alkanes (C-C), alkynes (C≡C), carbonyl (C=O) and nitro (N=O) groups were identified. The spectra of pure adulterant were apparently different with pure EBN while the spectra of adulterated EBN samples with addition of the adulterants were quite similar from that of pure EBN but the compounds present were different. The fingerprint region of the spectra of pure and adulterated edible bird nest samples were quite similar at wavenumber <1500 cm^{-1}. The NH group was absent in all the adulterants except in pork skin and egg white. Pork skin has ester C=O stretch bond which was not present in EBN itself and in other adulterants. The C=O stretch is an ester vibration band of fatty acids at wavenumber 1745.1 cm^{-1}. However, both EBN and adulterants have C=O stretch (amide) around 1670-1600 cm^{-1} for protein. Hence, FTIR can be used as a simple and rapid method to identify the authenticity of EBN and the non-halal component as adulterants. The largest component in EBN was protein with 22.4% to 32.7% respectively followed by carbohydrate ranging from 9% to 12%. Moisture (6.92% to 13.88%), ash (1.1% to 8.8%) and the lowest was fat content 0.01% to 0.07% respectively. Nitrite and nitrate in EBN was determined using sensor techniques using ISE electrode called E-tongue. This sensor technique is faster compared to the normal ion chromatography technique. Concentration of nitrite and nitrate in wastewater, unclean and clean EBN varies from 0.2 to 2.9 ppm which is lower than the standard set by China for export purposes. In this study, nitrite was less than 30 ppm and therefore safe for human consumption. The accuracy test carried out using Perlis EBN using sensor technique gave 100.2 to 100.8% recovery with RSD value less than 1% where as using ion chromatography gave 96.9 to 102.8% with RSD value 9 to 13%. Thus, sensor technique is highly sensitive and can be used as a standard method in determination of nitrite and nitrate in EBN samples as well as reduce the analysis time from 2 hour to 10 minutes. Mineral elements such as potassium and iron were high but lead content is low hence non-toxic.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Edible bird nest (EBN) refers to the nest produced by several different swiftlet species (Marcone, 2005). The common EBN traded around the world derived from two popular species which are white nest swiftlet (*Aerodramus fuciphagus*) and black nest swiftlet (*Aerodramus maximus*) (Babji et al., 2011). These nests were built from a glutinous material found in saliva secreted from the swiftlet's two sublingual salivary glands and may fuse with different materials like foliage and plumes (Chan, 2006).

EBN is generally mentioned as the 'Caviar of the East' as it is viewed as the most lavish creature nourishment item in the world. EBN from *Aerodramus* comprises of fat (0.14-1.28%), ash (2.1%), carbohydrate (25.62-27.26%) and protein (62.0-63.0%) (Marcone, 2005). EBN has been regarded as an esteemed food tonic for the Chinese people due to its highly evaluated function of being nutritious and medical benefit such as anti-aging, anti-cancer and immunity-enhancing. In Traditional Chinese Medicine (TCM), EBN has been utilized for a long time as a vital wellbeing supplement. EBN are also used for the treatment of malnutrition, a boost to the immune system and enhance body metabolism.

EBN comprises of high esteemed glycoprotein rich with amino acids, sugar, calcium, sodium and potassium (Norhayati et al., 2010). Sialic acid is the major component of carbohydrate (Colombo et al., 2003; Kathan and Weeks, 1969). Sialic acids have beneficial effect on neurological and scholarly focal points in babies (Chau et al., 2003). As

a superb insusceptible framework arbitrator, sialic acid influences the stream resistance in bodily fluid which thus repulses microorganisms, infections and other destructive organisms.

EBN is also rich in mineral salts where they contain high amount of sodium and calcium. It has been testified that the processed EBN contain calcium extended from 503.6 to 2071.3 mg/g and sodium content went from 39.8 to 509.6 mg/g which are higher than other mineral elements such as magnesium, potassium and others (Norhayati et al., 2010).

1.2 Statement of Problem

1.2.1 Authenticity of EBN

The high demand for EBN especially from China, Hong Kong and Taiwan and with limited supply has led to production of fake and adulterated EBN products in the global market (Set, 2012). The pure EBN has been adulterated with less expensive materials such as karaya gum, red seaweed, tremella fungus, sodium alginate, agar, natural plant gum, egg white and even the non-halal ingredient such as pork skin usually incorporated during the processing stages to increase the net weight or nutrient content prior to sale (Marcone, 2005).

Due to its high market value and indeed one of the most lavish animal food product in the world, the rate of adulteration of EBN is increasing (Koon and Cranbrook, 2002). Therefore, there is an urgent need to identify the authenticity of EBN. In this study, Fourier Transform Infrared Spectroscopy (FTIR) has been used to determine the authenticity of

EBN because it is a very simple and rapid technique. Another advantage is that no chemical pretreatment are used for the analysis.

1.2.2 Nitrite and Nitrate Content in EBN

The Malaysian EBN industry is facing problem of rejection from the importer especially China due to the high nitrite and nitrate content in EBN samples. The Chinese government demanded that nitrite must not be presented in EBN or its product in spite of the fact that the presence of natural nitrite in bird nest is being perceived. The presence of nitrite and nitrate in EBN have to comply with standards set by China and should not be more than 30 ppm (DSM, 2011).

In this study, the cleaning process of EBN is assessed for its effect to reduce nitrite and nitrate content in the wastewater, unclean and clean EBN. Isa (2012) reported that, cleaning process help to reduce nitrite and nitrate content in EBN by soaking them in the water. In conventional technique, the EBN was clean by soaking in the water until the nest is softened and then the small feathers and plumage were physically detached with tweezers.

1.3 Objective of the Study

The objectives of this study are:

1. To determine the authenticity of EBN from different locations in Malaysia and Indonesia.

2. To determine the compositional properties of raw unclean and clean EBN from different locations in Malaysia and Indonesia.

3. To develop a rapid technique to determine nitrite and nitrate in raw unclean, clean and wastewater EBN using sensor technology.

1.4 Scope of the study

This study was conducted to evaluate the quality of EBN that were collected from Malaysia: Perlis and Langkawi and Indonesia: Java, Balikpapan and Kalimantan. The authenticity of EBN were also determined to identify the pure EBN and faked or adulterated EBN. The compositional properties of EBN such as moisture, ash, protein, carbohydrate, fat, sialic acid and elemental contents were evaluated for quality, food value energy booster and health related properties. Since, one of the exportation parameter is nitrite and nitrate, the content of nitrite and nitrate in wastewater, unclean and clean EBN were also determined.

1.5 Chapter Summary

This chapter provided a brief background about the study, problem statement, objective and scope of the research. Edible bird nest which was constructed from the saliva of swiftlet consists of high amount of protein, carbohydrate and mineral which are good for human consumption as health supplement. However, the high demand of EBN has led to the production of fake EBN. So, there is an urgent need to identify the authenticity of EBN

using a rapid and environmentally friendly FTIR technique which is a rapid technique. The exportation of EBN to China has to comply with the standard set by China. The content of nitrite should not be more than 30 ppm. Therefore, a rapid technique using sensor was developed and evaluated for the determination of nitrite and nitrate in EBN.