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A Rapid Technique to Determine Purity of Edible Bird Nest

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ABSTRACT

Edible bird nest (EBN) is a gelatinous substance produced by different species of swiflets during the breeding season. It is widely consumed as a health food product for its high beneficial effects to human being. In recent years, adulteration of EBN is quite common, due to the high economic value and limited supply of natural authentic EBN. The compositional properties such as protein, carbohydrates, fat content and moisture were determined. The samples used were raw unprocessed EBN and of different grades (2A, 3A, 4A, 5A and biscuit white) processed EBN samples from the swiftlet species *Aerodramus fuciphagus*. Similarly, adulterants commonly used in EBN, namely agar, starch, sodium alginate, carrageenan, pork skin and egg white were also characterized. In both the raw and processed EBN samples the presence of similar compounds such as hydroxy, carboxyl, carbonyl, aryl, amines, alkynes, and nitro groups were detected. FTIR spectrum of the raw unprocessed EBN was identical to that of the spectra of processed EBN samples. The spectra of the pure adulterants were different from that obtained in EBN samples. The spectra of adulterated EBN samples with addition of the adulterants were apparently different from that of processed EBN. The fingerprint region of the spectra of pure edible bird and adulterated edible bird nest samples were different at <1700 cm^{-1}. The NH group was absent in all the adulterants except in pork skin and egg white. Interestingly, only pork skin has ester C=O stretch bond and C=CH group which were not present in EBN itself and in other adulterants. Hence, the EBN samples could be proven authentic and labeled as *halal* products using FTIR technique. The highest component found in EBN samples was protein (58.31-63.88%), followed by carbohydrate in the range (11.3-12.9%). The amount of moisture content is within the range of 7.0-9.34% and fat of 0.05-0.09%. The protein and carbohydrate contents of EBN are normally high. The FTIR technique is a useful tool and rapid technique for the determination of purity of EBN and thus as a deterrent to the commercial adulteration of EBN based products.

INTRODUCTION

Edible bird nest (EBN) is one of the best nourishing and comprehensive health food products [1]. It is produced by several different swiftlet species in the genus of *Aerodramus* and *Collocalia* and the nest is mainly built by male swiftlets [2]. The nests are constructed from the saliva of swiftlet, which has been secreted from the pair of sublingual salivary gland of swiftlets during nesting and breeding season. EBN consists of high valued glycoprotein rich with amino acids, carbohydrate, calcium, sodium and potassium [3]. It is commonly referred to as the „Caviar of the East' because of it is highly esteemed priced food product in the East [4]. Normally, people consumed these nests for wealth, power and prestige. As a rich source of amino acids, carbohydrates and mineral salts, bird nests have also been used for hundreds of years as an important health supplement in traditional chinese medicines. EBN was used for the treatment of malnutrition, a boost to the immune system, and to enhance the body's metabolism. More recently bird nests have also been used in cosmetic products [5, 6].

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Due to its high demand, especially from China, Hong Kong and Taiwan and undoubtedly one of the most expensive food ingredients in the world as well as rare product and limited supply of natural authentic EBN, this in turn has led to upsurge of a lot of fake and shoddy commodities edible bird nest products in global market. Unethical suppliers mixed the original EBN with additives in order just to increase the size of the nest and market value. The usual substances used to imitate EBN include edible plants, fish skin, mushroom, algae, agar, red seaweed, karaya gum, sodium alginate, *Tremella fungus*, egg white and even *non-halal* adulterant like pork skin are quite widespread. The natural colorant, namely, karayagum, red seaweed or *tremella fungus* are widely used to the adulteration of more expensive „Red Blood' bird nest, helping to turn the white colour of white EBN to the red colour. Many processes involve the addition of preservatives such as boric acid, potassium sulfite or sulfur dioxide (according to country regulations). Sugar, salt, and monosodium glutamate (MSG) are added to improve the taste. Gluten, white fungus, jelly, animal skin or synthetic rubber is often used to improve the shape and appearance of the nests [5].

The first comprehensive report on authentication of EBN can be traced back to the early 1990s [7]. A number of methods had since been explored in the past to determine the purity of EBN. The method can be categorized into two categories, such as empirical identification method and chemical method [8, 1]. Empirical test involved techniques such as visual examination (touching and smelling), burning tests, and coloring checks. However, a team of experienced people having certain ability to identify the genuine EBN and specialized in chemistry are needed for this method. The chemical method involved the use of various laboratory equipments, such as chromatography, electrophoresis and UV spectroscopy to find out some of the specific compounds present in EBN. These methods required pretreatment of EBN before analyzing. Both the methods are time consuming and laborious. Besides, the methods can useful for the identification of specific properties of EBN only. Therefore a simple and rapid method is required to reduce and eliminate the inferior quality of EBN being traded globally in the future.

FTIR spectroscopy is becoming an attractive alternative to the existing analytical techniques in food analysis because it is simple and rapid, low in cost, environmental friendly and non-invasive. IR spectroscopy measures the covalent chemical bonds, creating a molecular „fingerprint' of the chemicals present. This fingerprint can be used to identify and quantify chemicals present in a sample. The FTIR spectroscopy region 4000 – 600 cm $^{-1}$ in particular, is able to identify a large number of components and the absorption bands are sensitive to the physical and chemical states of individual constituents. A micro-diamond, ATR FTIR spectroscopy for the first time was used for rapid identification of original characters of EBN [1]. Recently, a handheld FTIR was invented to determine and evaluate the presence of additives present in EBN product like, salt, sugar and mono sodium glutamate (MSG) [5]. In addition, the conventional FTIR method was used in past to identify the quality of EBN from *Collocalia esculenta* of swiftlet species [1].

Therefore, there is an urgent need to identify the purity of edible bird nest. The increasing trend in fake and adulterated EBN products in the world market is disturbing. Therefore, this study was undertaken to determine the purity of EBN products using the Fourier Transform Infrared (FTIR) spectroscopy technique.

MATERIALS AND METHOD

Samples:

Samples used in this study are raw unprocessed bird nest, processed EBN of different grades (2A, 3A, 4A, 5A and white biscuit) from the swiftlet species *Aerodramus fuciphagus* and the commonly used adulterants such as carrageenan, agar, starch, sodium alginate, pork skin and egg white. The EBN samples were washed with distilled water to remove the impurities attached to the surface of EBN samples. The samples are then dried in the oven at 60 ^{0}C for 24 hours. The dried samples were ground to fine powder form using food grinder at a meshed of 600 μm. The ground samples were kept in an air tight container for analysis. All the adulterants were purchased from the local market, dried and ground to powder form before mixing with EBN and analyzed.

Experimental procedure and data processing:

About 1 mg of the solid samples of EBN was added after grinding 100 mg of the solid potassium bromide (KBr) in the ratio of 1:100. The mixture was milled to homogenize the mixture. For the adulterated samples, about 1mg of the solid samples of EBN and 1mg adulterants were added to the ground 100 mg of the solid KBr in the ratio of 1:1:100. The mixture was milled to homogenize it. An interferogram for the background correction was determined before the sample analysis. Then, the samples were compressed into thin layer pellets in the shape of a disc under high pressure of 10 tons for 5 minutes. All samples were subjected to ordinary FTIR spectrometry (Perkin-Elmer Model Spectrum 65, USA) under a spectral scanning range of 4000 ~ 600 cm $^{-1}$ for data collection and spectral processing.

Proximate analysis:

Nitrogen Content Determination (crude protein) was determined using the CHNS analyzer. The total carbohydrate content was determined using phenol sulphuric acid reaction as described by *Dubois, Gilles, Hamilton, Rebers and smith* [9]. Fat content was determined using Solvent Extraction (Submersion) Method using FOSS Soxtec^{TM} 2043 extraction equipment. The moisture content was determined by using the moisture analyzer (Sartorius MA 35, Germany). 0.5g samples were used to determine the moisture content.

Statistical analysis:

Each sample was analyzed individually in triplicate. Data were reported as mean ± standard deviation and showed in the graph.

RESULTS AND DISCUSSION

The KBr disc of EBN samples and EBN with adulterants was analyzed to determine the purity of EBN using the Fourier transform infrared spectroscopy (FTIR) technique.

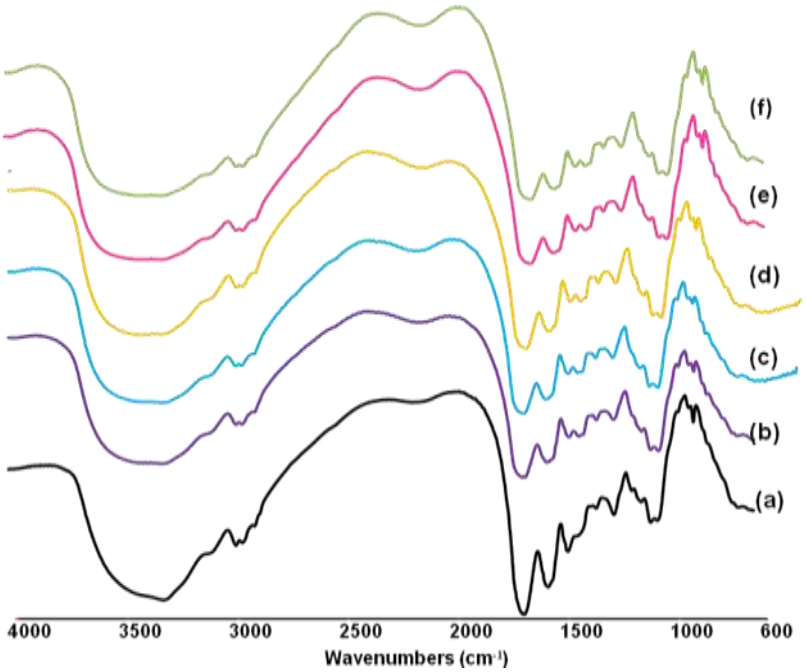

Fig. 1: FTIR spectra of EBN samples, Raw EBN (a) and different grades of EBN, (b) 2A grade EBN, (c) 3A grade EBN, (d) 4A grade EBN, (e) 5A grade EBN, (f) White biscuit

Figure 1 shows the FTIR spectra of raw and processed EBN samples of different grades (2A, 3A, 4A, 5A and white biscuit). The shape and pattern of the raw and processed EBN spectra in the fingerprint region (<1700 cm-^{1}) were similar. From the spectra, the raw and processed samples showed the presence of similar compounds. The compounds identified are as shown in Table 1, such as hydroxy, carboxyl, carbonyl, aryl, amines, alkynes, and nitro groups with their absorption ranges. The spectra also indicated that the properties and quality of EBN in the processed and unprocessed samples were similar.

Visual examination of the FTIR spectrum (Figure 1) showed that the functional group present in the region 3600-3100cm^{-1} is due to the O-H stretching of water. This indicated that water molecules are present in EBN sample in the form of moisture. This absorption band is seen at 3330.3 cm^{-1} for raw EBN, 3298.6 cm^{-1} for EBN graded 2A, 3287.1 cm^{-1} for EBN graded 3A, 3393.9 cm^{-1} for EBN graded 4A, 3381.77 cm^{-1} for EBN graded 5A and 3299.9 cm^{-1} for white biscuit. The water may be residual from the washing process of EBN. The band near 2100 cm^{-1} is due to the stretching vibration of C≡C of alkynes. The absorption peaks at 3100-2800 cm^{-1} is attributed to the H-C-H asymmetric and symmetric stretching vibration of lipids [5]. These symmetric absorption bands were observed at 2931.4 cm^{-1} for raw EBN and 2931.5 cm^{-1}, 2931.3 cm^{-1}, 2931.5 cm^{-1}, 2931.4 cm^{-1} and 2931.1 cm^{-1} for EBN grades 2A, 3A, 4A, 5A and white biscuit. The absorption band around 1654 and 1545 are assigned to the C=O Stretching vibration (amide I) and N-H bending vibration (amide II) respectively. From Figure 1, the bands at 16552.9 cm^{1},1654.4 cm^{-1}, 1651.8 cm^{-1}, 1652.1 cm^{-1}, 1643.32 cm^{-1} and 1644.0 cm^{-1} for raw and five grades of processed EBN samples (2A, 3A, 4A, 5A and white biscuit) respectively indicated the presence of Stretching vibration (amide I) of C=O bond. While, N-H bending vibration (amide II) for raw

EBN,EBN grades 2A, 3A, 4A, 5A and white biscuit was observed at the band 1543.7 cm^{-1}, 1545.3 cm^{-1}, 1538.5 cm^{-1}, 1544.8 cm^{-1}, 1544.5 cm^{-1} and 1536.6 cm^{-1} respectively. The two major peaks indicated the presence of protein [5]. Besides that, both raw and five processed EBN have absorption peak around 1444.0 cm^{-1}, 1400.0 cm^{-1} and 1318.0 cm^{-1} indicating the presence of carboxylic (COOH), aldehydes (CH=O) and amine group (C-N) respectively. The absorption band around 1030 cm^{-1} is the resulting vibrations of polysaccharides, C-O bonds. This peak denotes for the presence of carbohydrates in the EBN samples. Thus, the main compounds present in the EBN samples were carbohydrates and protein. It was noted that the fingerprint region is almost similar, around wavenumber of 1700 cm^{-1} to 600 cm^{-1} was similar.

The FTIR analysis of adulterants such as agar, starch, carrageenan, sodium alginate, pork skin and egg white were compared to the pure edible bird nest. Figure 2 shows the spectra for the pure sample and the adulterants. Although, the spectra looked almost similar to the EBN spectrum, the compounds present differed as shown in Table 2. Two additional absorption peaks are observed in pork skin, which were not present in other adulterants and EBN itself. The FTIR spectrum of pork skin (Figure 2 (g)), exhibits one sharp absorption band near 3009 cm^{-1} and another peak at 1742 cm^{-1}. The band near 3009 cm^{-1} is characteristic for CH of stretching vibration mode of C=CH groups. This is an indication that the pork skin contains a large amount of unsaturated fats [10]. The band at 1742 cm^{-1} is assigned for C=O of ester of fatty acids [10]. N – H bond is not present in all the other tested adulterants except egg white and pork skin. Thus, a comparison of pure EBN sample and adulterants can be easily distinguished by analyzing the compounds present around the fingerprint region.

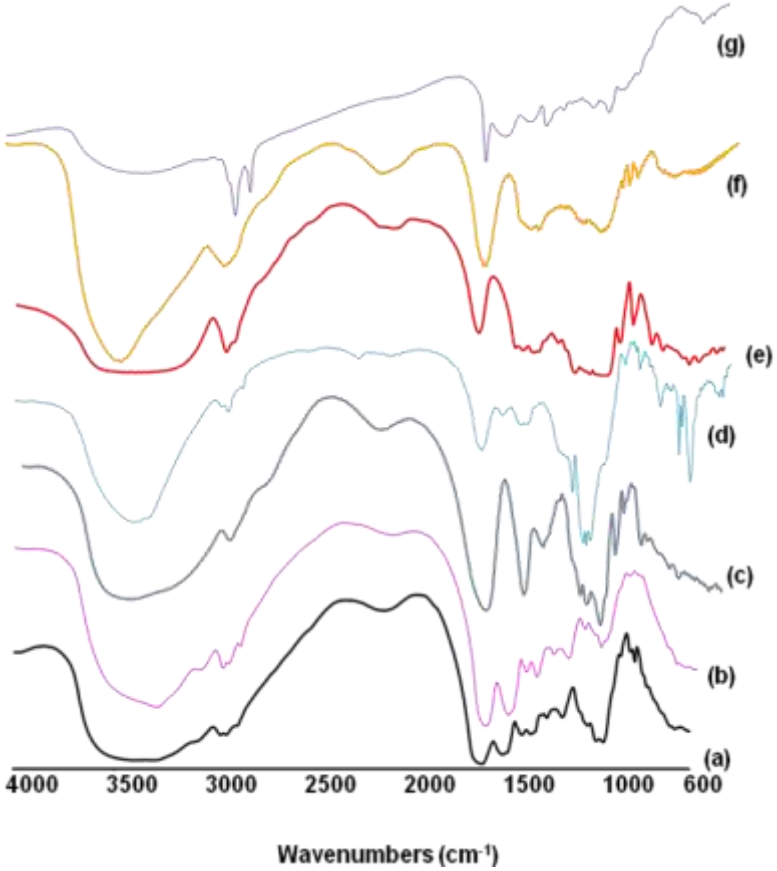

Fig. 2: FTIR spectra of samples, (a) 5A grade EBN, (b) Egg white, (c) Sodium alginate, (d) Carrageenan, (e) Starch, (f) Agar, (g) Pork skin

The KBr samples of EBN mixed with different adulterants were similarly analyzed using the FTIR. Grade 5A which is the highest quality grade edible bird nest was used. One milligram of EBN was mixed with one milligram adulterant. Figure 3 shows the spectra of pure and adulterated edible bird nest. The Figures 3(b), 3(c), 3(d), 3(e), 3(f) and 3(g) shows a comparison of spectrum obtained from pure EBN and adulterated samples. FTIR spectrum can easily be used as a simple and rapid method to differentiate the pure and the adulterated EBN. Comparing the bands and the compounds present especially around the fingerprint regions can be an effective and rapid method to identify the pure and adulterated edible bird nest. The FTIR technique showed good potential to detect adulteration of EBN if coupled together with the quantitative analysis technique. FTIR is a rapid and effective tool to identify adulterants in EBN samples.

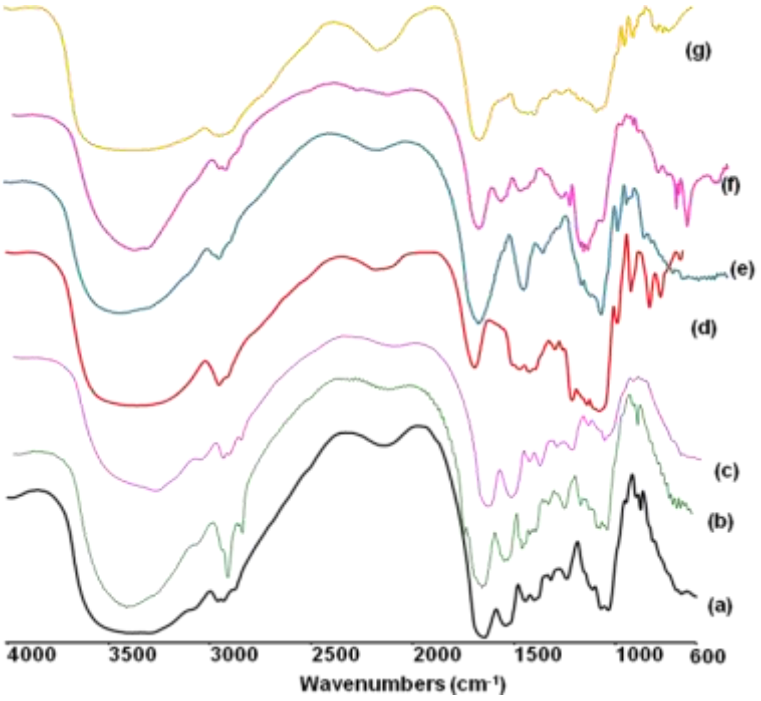

Fig. 3: FTIR spectra of samples, (a) 5A grade EBN, (b) Pork skin + 5A grade EBN, (c) Egg white + 5A grade EBN, (d) Starch + 5A grade EBN, (e) Sodium alginate + 5A grade EBN, (f) Carrageenan + 5A grade EBN, (g) Agar + 5A grade EBN

Table 1: Functional groups of raw (unprocessed) and processed EBN samples

No	Functional groups	Absorption ranges (cm^{-1})					
		Unprocessed EBN	Processed EBN				
			Grade 2A	Grade 3A	Grade 4A	Grade 5A	White Biscuit
1	OH stretch	3330.3	3298.6	3287.1	3393.9	3381.7	3299.9
2	CH_3 stretch	2931.4	2931.5	2931.3	2931.5	2931.4	2931.1
3	C≡C stretch	2114.2	2114.9	2147.0	2122.9	2131.1	2128.0
4	C=O Stretch	1652.9	1654.4	1651.8	1652.1	1643.3	1644.0
5	N-H Bend	1543.7	1545.3	1538.5	1544.8	1544.5	1536.6
6	COOH bend / stretch	1443.6	1442.7	1443.0	1444.9	1443.9	1444.5
7	CH=O	1399.5	1399.0	1400.0	1399.4	1399.6	1396.5
8	C-N	1317.1	1318.0	1317.6	1318.3	1317.8	1318.0
9	C-O	1034.3	1034.1	1032.5	1034.2	1034.8	1034.2

Table 2: Absorption range (cm^{-1}) and functional groups of EBN sample and adulterants

No	Functional groups	Absorption ranges (cm^{-1})						
		EBN	Adulterants					
			Starch	Agar	Carrageenan	Sodium alginate	Egg White	Pork skin
1	O-H Stretch	3381.7	3367.9	3435.6	3391.4	3434.7	3299.2	3431.1
2	C=CH	-	-	-	-	-	-	3009.9
3	CH_3 Stretch	2931.4	2932.2	2921.3	2966.4 2925.0	2931.4	2962.4	2924.5
4	C≡C Stretch	2131.1	2151.1	2143.4	2270.0 2111.0	2159.2	2130.5	-
5	Ester C=O Stretch	-	-	-	-	-	-	1745.1
6	C=O Stretch	1643.3	1649.9	1639.1	1653.2	1610.4	1650.0	1652.1
7	N-H (bend)	1544.5	-	-	-	-	1536.2	1531.0
8	COOH bend/stretch	1443.9	1459.5	1425.0	1457.7	1419.4	1452.5	1463.9
9	CH=O	1399.6	-	-	-	-	1397.9	-
10	C-N	1317.8	1369.0	1381.70	-	1319.9	1312.5	1378.0
11	C-O	1034.8	1015.4	1079.1	1125.7	1024.3	1076.9	1164.5

Table 3: Absorption range (cm^{-1}) and functional groups of EBN mixed with adulterants

No	Functional groups	Absorption ranges (cm^{-1})						
		EBN	EBN mixed with Adulterants					
			Starch + 5A Grade EBN	Agar + 5A Grade EBN	Carrageenan + 5A Grade EBN	Sodium alginate + 5A Grade EBN	Egg white + 5A Grade EBN	Pork skin + 5A Grade EBN
1	O-H Stretch	3381.7	3349.6	3390.7	3390.4	3431.0	3295.1	3434.9
2	C=CH	-	-	-	-	-	-	3009.3
3	CH_3 Stretch	2931.4	2931.6	2934.0	2963.3	2933.6	2961.5	2926.6
4	C≡C Stretch	2131.1	2139.1	2154.5	2114.1	2134.8	2131.3	2131.2
5	Ester C=O Stretch	-	-	-	-	-	-	1742.4
6	C=O Stretch	1643.3	1646.4	1647.1	1654.5	1639.7	1649.8	1652.1
7	N-H (bend)	1544.5	-	-	1535.7	-	1535.4	1540.0
8	COOH bend/stretch	1443.9	1419.9	1437.3	1441.0	1414.8	1448.6	1451.5
9	CH=O	1399.6	-	-	-	-	1395.5	1399.1
10	C-N	1317.8	1371.5	1377.0	-	1319.4	1314.7	1323.4
11	C-O	1034.80	1019.7	1071.8	1125.6	1028.5	1072.8	1027.1

Quantitative analysis for moisture, fat, protein and carbohydrates were done. Protein is one of the integral components in EBN. Proteins are constituents of cells and play a crucial role in most of the biological process. The crude nitrogen content in the EBN samples was analyzed by using CHNS analyzer. Around 2 ~ 3 mg of powdered samples were combusted in a CHNS elemental analyzer (Perkin-Elmer model 2400). Helium was used as a carrier gas. Cystine, organic analytical standard (C = 29.99%, N = 11.67%, H = 5.03%, S = 26.69%) was used to calibrate the instrument. Crude protein contents were calculated by multiplying the total nitrogen content by factor 6.25 [11]. The test result indicates that, protein was the highest constituents of EBN samples with having the range of 58.0 to 63.0 % as shown in Figure 4.

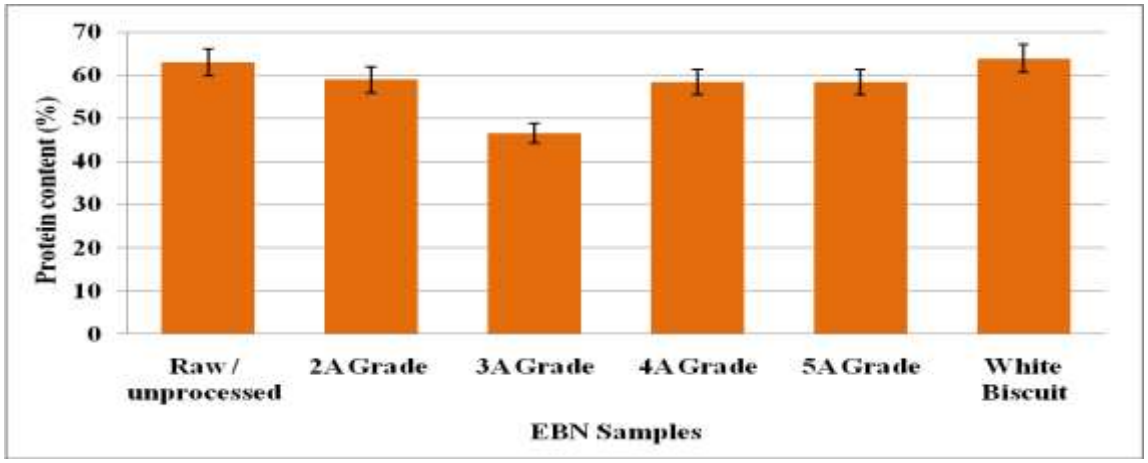

Fig. 4: Protein content of raw and graded EBN samples of *Aerodramus fuciphagus sp*

The results of the carbohydrate analysis of the raw and graded EBN samples are shown expressly in Figure 5. The test results indicate that this was the second highest occurring component in all the EBN samples. The carbohydrates content in all the EBN samples were virtually identical from 11 to 12 %. Total carbohydrate content was determined using phenol sulphuric acid method. Glucose was used in preparing the standard curve.

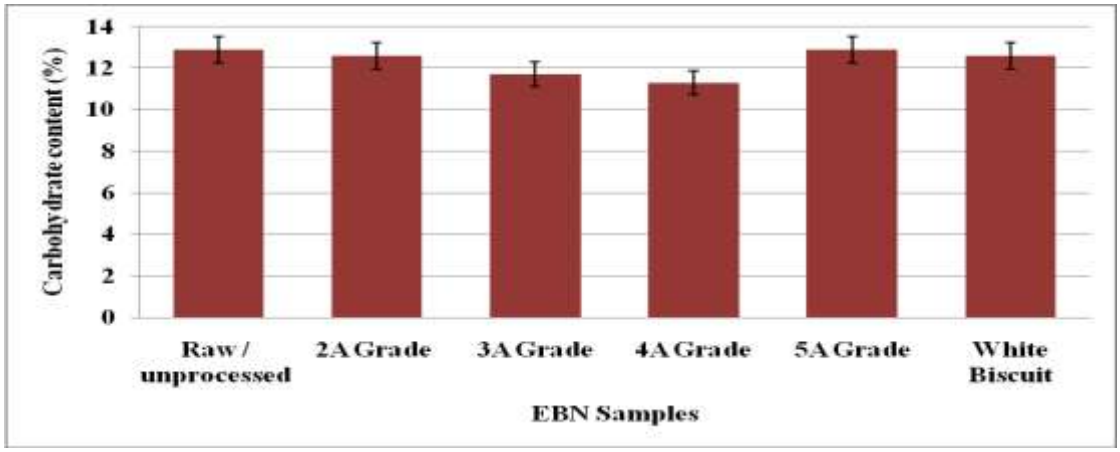

Fig. 5: Carbohydrate content of raw and graded EBN samples of *Aerodramus fuciphagus sp*

Besides that, the result of moisture content of the EBN samples was reported in Figure 6. As shown by Figure 6, the moisture content was detected from 7 % to 9.34 % in the EBN samples with white biscuit having lowest moisture content than other EBN samples. The water is may come from the washing process. The moisture content is frequently used as an index of stability and quality of bird nest. It is the most important and widely used analytical measurements in processing and testing of food products [12]. The moisture content of the samples was analyzed using a moisture analyzer model Ma 35, Sartorius.

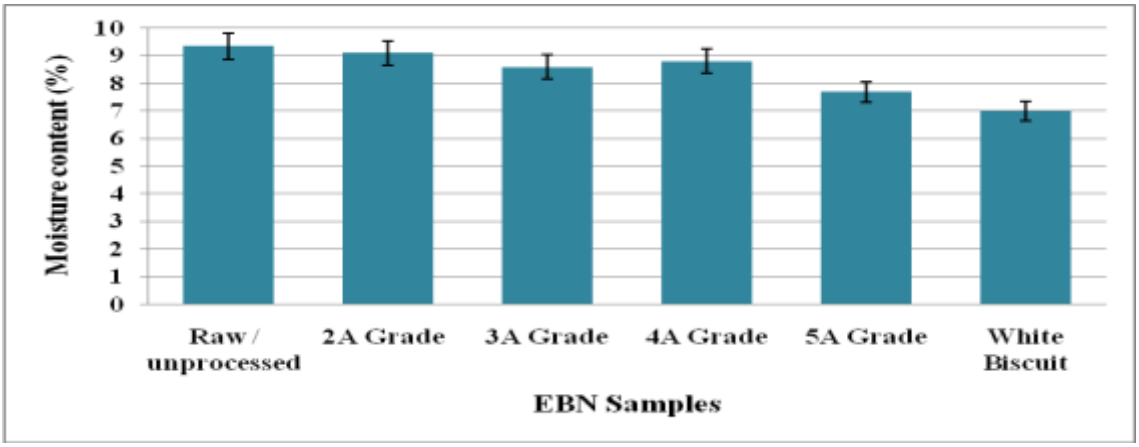

Fig. 6: Moisture content of raw and graded EBN samples of *Aerodramus fuciphagus sp*

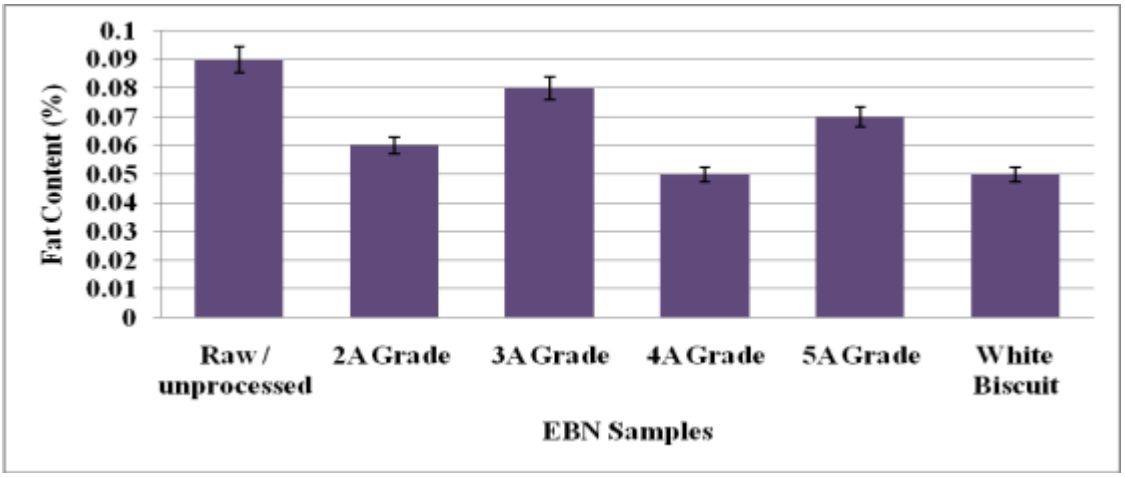

Fig. 7: Fat content of raw and graded EBN samples of *Aerodramus fuciphagus sp*

Fat is the lowest constituents of EBN. It was detected from 0.05 to 0.09 % as shown in Figure 7. Fat content was determined using Solvent Extraction (Submersion) Method using FOSS Soxtec^{TM} 2043 extraction equipment.

The main compounds present in the EBN samples were carbohydrates and protein, higher constituents. Furthermore, the proximate analysis showed that there was no significant differences were found in the compositional contents of the graded EBN.

Conclusion:

Fourier Transform Infrared (FTIR) spectroscopy is a simple and rapid method to identify the purity of edible bird nest products. The compounds present and the spectra at the fingerprint region were different for the pure and adulterated samples. The FTIR spectroscopy is able to identify the purity of EBN by comparing the bands and the compound present especially around the fingerprint regions. Moreover, FTIR technique is a non-destructive method of analysis and environmental friendly because no harmful chemicals are used.

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