

Detection of Edible Bird's Nest Using Fourier Transform Infrared Spectroscopy (FTIR) Combined with Principle Component Analysis (PCA)

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Abstract: Edible bird's nest (EBN) is rich in nutrients and health benefits; making it one of the Chinese delicacies over the centuries. However, due to the overpriced and limited supply of EBN, it is being adulterated with other cheaper versions. Therefore, the aim of this study is to establish a method of detecting adulterants in EBN using Fourier transform infrared spectroscopy (FTIR) as the spectrum fingerprinting analysis together with principal component analysis (PCA). Spiked samples have been developed for *Tremella* fungus and porcine gelatine at the concentrations of 1%, 5%, 10%, 20% and 30% (w/w). The FTIR method combined with PCA analysis was able to detect the adulteration of porcine gelatine and *Tremella* fungus in the sample of adulterated EBNs at low concentration of 1% (w/w). The simple approach employing FTIR combined with PCA may provide a useful tool for EBN detection.

Keywords: Edible bird's nest (EBN), authentication, adulteration, Fourier transform infrared spectroscopy (FTIR), principal component analysis (PCA)

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1. Introduction

Edible bird's nest (EBN) is a delicacy and known to provide essential health benefits including enhancing beauty of a person. It has been used as a traditional medicine for the past centuries. EBN are mainly from the species of *Collocalia* and *Aerodramus* which can be found in South East Asian countries such as Indonesia, Malaysia, Philippines, Thailand, Vietnam and small part of southern China (Marcone, 2005). The three most common cave nests are *A. fuciphagus*, *A. maximus* and *C. esculent* (Looi & Omar, 2016). The swiftlet birds

specifically the male partners produce saliva to build the nest during the breeding season. The feather (also seaweed and mosses) and vegetation were bonded together and cemented using their saliva secreted from their salivary gland. When exposed to air, the sticky secretion hardens forming the bird's nest (Kang *et al.*, 1991). The nest is built in a half-bowl shape from the strands of the solidified saliva thus, enhancing the capabilities of withstanding the bird's eggs and hatchlings (Sidq Ramji *et al.*, 2013).

Known as one of the health-promoting foods in China, which contributes to delicious and scrumptious taste, the composition of EBN has been studied making it among the world's expensive foods. Norhayati *et al.* (2010), stated that the main nutrients in EBN by *A. fuciphagus* including crude protein, sialic acid and high level of minerals such as calcium, sodium, magnesium, potassium phosphorus, iron, zinc and copper. The potential health effects of EBN have been studied over the years to have biological effects other than anti-ageing properties. One of the earliest discoveries is an epidermal growth factor (EGF)-like activity, which helps in proliferation, differentiation and target cell's survival (Herbst, 2004; Kong *et al.*, 1987).

The price of the EBN skyrocketed because of the high demand especially from China. According to The Star newspaper in 2017, the processed EBN has soared until RM 10,000/kg from RM 7,000/kg depending on the grade, type and origin. As for the unprocessed bird's nest, the price has increased from RM 1,300/kg to RM 2,700/kg (Wong, 2017). This explained the authentication issues that stirred up among the consumers. In an article by Wong (2013), the authentication of EBN can be of the fake ones, with adulterants and contaminated with bird droppings or any harmful materials to consumers. The common adulterants in EBN can be classified into Type-I and Type-II. Type-I are solid polysaccharides and polypeptides, whereas Type-II are water-soluble saccharides, polypeptides and salts (Shim *et al.*, 2016).

Various studies have been conducted in detecting adulterants in EBN using different methods with different percentage limit detections. These include polymerase chain reaction (PCR) (Guo *et al.*, 2014; Quek *et al.*, 2018), enzyme-linked immunosorbent assays (ELISAs) (Nur Azira *et al.*, 2016), infrared (IR) spectroscopy (Guo *et al.*, 2014; Shi *et al.*, 2017) and gas chromatography/liquid chromatography-mass spectrometry (GC/LC-MS) (Yang *et al.*, 2014). Fourier transform infrared spectroscopy (FTIR) is one of the applications of mid-IR spectroscopy, which can obtain spectra from solid, liquid or gas samples (Paul & Genesca, 2013). The IR beam was directed onto an optically dense crystal with high refractive index at certain angle. With minimum sample preparations needed, it is known as one of the fastest sampling, higher reproducibility and can minimize user to user spectral variations (Sousa *et al.*, 2018). However, FTIR produces a complex spectrum, which contains various variables. Therefore, a chemometric methods of principle component analysis (PCA) could allow the characteristics of the sample relationships and classified as a group of same characteristics (Granato *et al.*, 2018). Many studies done have reported the excellent combination of FTIR

and PCA in food analysis due to feasibility of the results. For instance, detection of lard in “rambak” crackers and determination of thermal effect on oxidation of extra virgin olive oil was successfully determined by PCA (Erwanto *et al.*, 2016; Selaimia *et al.*, 2017). Thus, the integration approach has become a powerful tool and reliable for the meaningful results. This study aims to use FTIR combined with PCA in the detection of adulteration in EBN.

2. Materials and Methods

Materials

Two types of EBN house nest (*Collocalia sp.*) and cave nest (*Aerodramus sp.*) were purchased from Malaysia and Indonesia respectively. *Tremella* fungus (TRE) (dried) was purchased from the local supermarket in Genting Highlands, Pahang, Malaysia. Porcine skin gelatine (P) (G2500-100G, #058K0109) and bovine skin gelatine (B) (G9391-100G, #126K0051) were purchased from Sigma-Aldrich (St. Louis, USA). Monosodium glutamate (MSG) powder was purchased from the local supermarket in Selangor, Malaysia. Five different EBN samples are tabulated in Table 1.

Table 1. Samples of EBN

No.	Sample name	Origin	Species	Type	Abbreviation
1	Cave nest	Indonesia	<i>Aerodramus sp.</i>	Cave nest	CN
2	Blood cave nest	Indonesia	<i>Aerodramus sp.</i>	Cave nest	BC
3	Orange nest	Malaysia	<i>Collocalia sp.</i>	House nest	ON
4	White nest 1	Malaysia	<i>Collocalia sp.</i>	House nest	HOUSE
5	White nest 2	Malaysia	<i>Collocalia sp.</i>	House nest	WW

Spiked Sample Preparation

The spiked samples were prepared following the procedure described by Jamalludin and Tukiran (2018), with a slight modification. The EBN samples were grinded using pestle and mortar. TRE was blended using Panasonic MX-SM1031 (240V~50Hz) until it turned into fine powders, while the P powder was directly taken from the bottle. The spiked samples of TRE and P were prepared in five concentrations which were 1%, 5%, 10%, 20% and 30% (w/w). After that, ON, BC, TRE and P were sieved into finer powders using a 1 mm sieve to make a constant particle size. The ON powders were weighed using a precision analytical balance. The ON samples were transferred into Eppendorf tube and were mixed using Advanced IR Vortex Mixer (Velp Scientifica, Italy) with the speed of 20x100 rpm for 1 minute. The ON samples were kept in an airtight bag for further analysis. These steps were also applied for BC, TRE and P.

FTIR

The FTIR was performed according to Jamalludin and Tukiran (2018). The infrared spectra were analysed in the range between 4000–650 cm^{-1} with Thermo Scientific Nicolet iS50 FT-IR (ThermoFisher Scientific, Waltham, USA). The OMNIC™ Spectra Software (Version 9.2.106) was used to perform FTIR analysis, which gives clear visual data including spectral identification tools, interpretation algorithms and scientific documentation. The

sample background was run before placing samples into the crystals. Accurately weighed amounts of sample powder were placed on the crystal diamond and making sure the crystal was all covered. All spectra were recorded in absorbance with the resolution of 4 cm^{-1} at an average of 32 scans. Each sample was analyzed individually in duplicate. The sample was removed using a dry tissue and the surface of FTIR spectrometer was cleaned with methanol before running the sample background.

Statistical Analysis

The quantitative data were analyzed using Unscrambler[®] v9.7 CAMO Software AS and the classification of data were performed using PCA. Score plot and loading plot were the outcomes produce by PCA that shows the variability of FTIR data. Fifteen sample variables of wavenumber (cm^{-1}) of 1633 cm^{-1} , 1602 cm^{-1} , 1518 cm^{-1} , 1516 cm^{-1} , 1444 cm^{-1} , 1442 cm^{-1} , 1434 cm^{-1} , 1415 cm^{-1} , 1336 cm^{-1} , 1247 cm^{-1} , 1248 cm^{-1} , 1241 cm^{-1} , 1029 cm^{-1} , 875 cm^{-1} , and 704 cm^{-1} were chosen for the PCA, since these wavenumbers (cm^{-1}) have either similarities or differences in spectrum between each sample.

3. Results

FTIR Spectra

Pure EBN

Five types of EBN spectral were compared as shown in Figure 1. The region between 3600-3100 cm^{-1} can be compensated for the water (O-H stretching) like those reported by Hamzah *et al.* (2013). This indicated that water molecules were present in EBN although the samples were in powdered form. Between the region of 2100 and 2160 cm^{-1} , only ON and HOUSE samples produced a significant peak at that region. Region 876-875 cm^{-1} was significant for all CN and almost disappeared in HOUSE (Guo *et al.*, 2017). Different wavenumber produced from the absorbance peaks might be contributed from asymmetric stretching vibrations of CH_2 (2934 cm^{-1}), CH_2 stretching lipids (2855 cm^{-1}), C=O triglycerides (1745 cm^{-1}), stretching vibration of C=O bond of amide I (1654 cm^{-1}) and N-H bending vibration for amide II band (1545 cm^{-1}), bending vibration CH_2 in polysaccharides (1430 cm^{-1}), symmetric stretching vibration of COO- groups of fatty acids and amino acids (1400–1300 cm^{-1}), phosphate vibration of nucleic acids (1235 cm^{-1}), C-O stretching vibration coupled with C-O bending of the C-OH groups of carbohydrates (1045 cm^{-1}), CH out-of-plane bending vibrations (1000–700 cm^{-1}) (Guo *et al.*, 2017; Hamzah *et al.*, 2013; Movasaghi *et al.*, 2008). All samples exhibit almost similar spectrum in the fingerprint region >1500 cm^{-1} thus showed the presence of similar compounds.

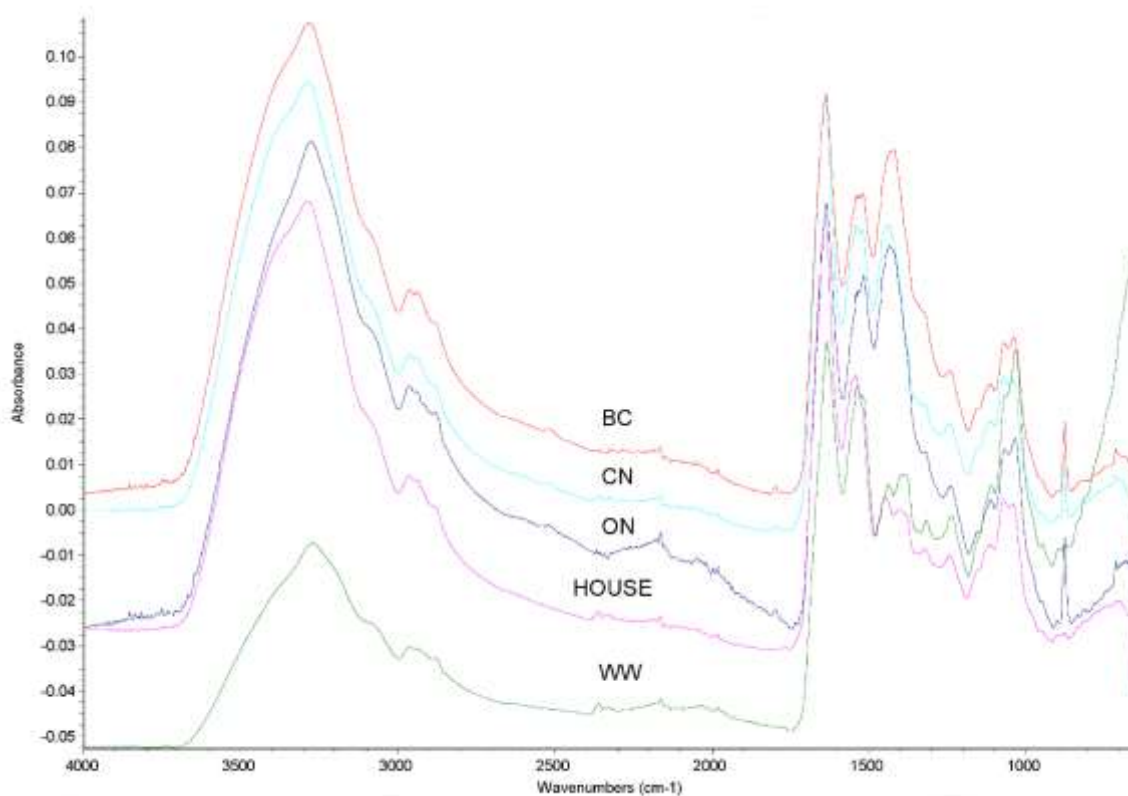


Figure 1. Overlapped spectrum of cave nest (*Aerodramus* sp.) and house nest (*Collocalia* sp.). BC: blood cave nest, CN: cave nest; ON: orange nest; HOUSE: white nest 1; and WW: white nest 2

Based on Figure 1, the peaks between $1635\text{--}1634\text{ cm}^{-1}$ were accounted for amide I and $1539\text{--}1515\text{ cm}^{-1}$ were accounted for amide II. The peaks $1445\text{--}1434\text{ cm}^{-1}$ and $1417\text{--}1393\text{ cm}^{-1}$ might indicate the presence of carboxylic (COOH) and aldehydes (CH=O), respectively. The peak at $1070\text{--}1030\text{ cm}^{-1}$ were the resulting vibrations of polysaccharides (C-O bonds), which has proven the presence of carbohydrates in EBN as reported by Hamzah *et al.* (2013) and Set (2012). As shown, there were no obvious spectral features that can be recorded, except for the varieties of different spectrum in the region between $1800\text{--}700\text{ cm}^{-1}$. The results for amide I and amide II were similar from the reported value $1600\text{--}1700\text{ cm}^{-1}$ and $1560\text{--}1335\text{ cm}^{-1}$ (Hamzah *et al.*, 2013; Shi *et al.*, 2017).

Adulterants

Four adulterants namely, TRE, MSG, P and B were compared. Figure 2 exhibits that TRE was significant at 1415 cm^{-1} (COOH bend or stretch), while MSG produced the highest number of spectra, which was easily being distinguished at 2050 cm^{-1} (C=C stretch), 857 cm^{-1} and 655 cm^{-1} among other adulterants. The exhibited spectrum was similar with the reported spectrum in National Institute of Standards and Technology (NIST) Chemistry WebBook (2018). P and B can be distinguished at $1634\text{--}1633\text{ cm}^{-1}$ (C=O stretch), $1337\text{--}1336\text{ cm}^{-1}$ (C-N bond) and 1081 cm^{-1} (C-O bond), which were not shown in other adulterants. Hence, it can be concluded that, P and B showed similar spectrum with different absorbance values. Hashim *et al.* (2010) and Eryilmaz *et al.* (2017) also mentioned that P and B can be found

similar in region $1656\text{--}1644\text{ cm}^{-1}$ (amide I), $1560\text{--}1335\text{ cm}^{-1}$ (amide II) and $1240\text{--}670\text{ cm}^{-1}$ (amide III).

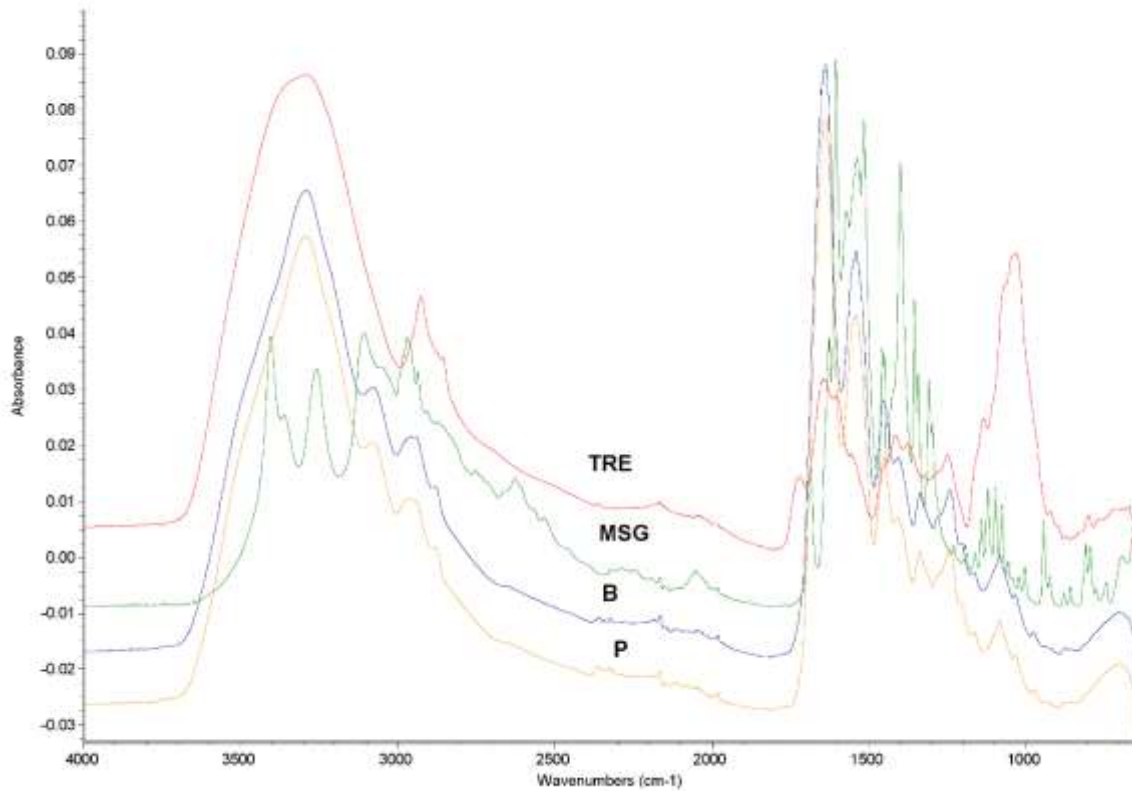
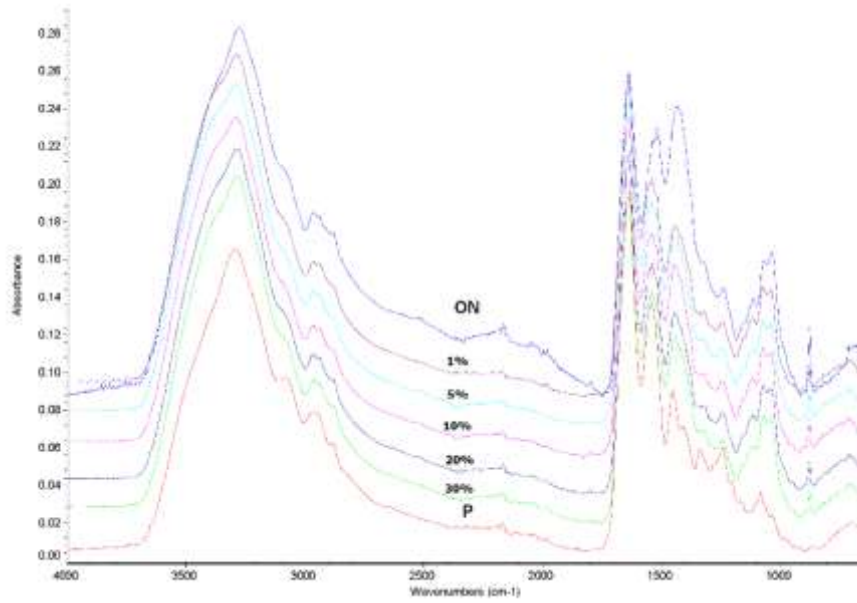


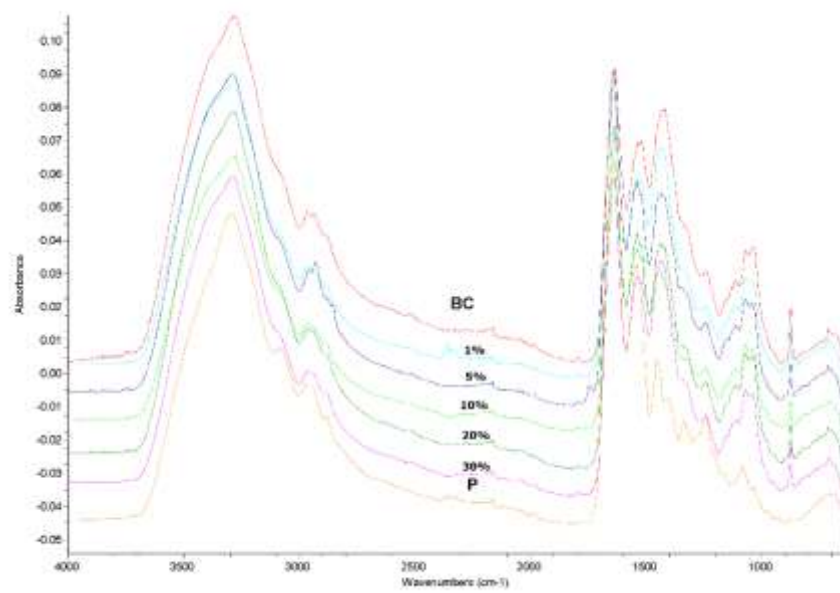
Figure 2. The overlapped spectrum of different types of adulterants. TRE: *Tremella* fungus; MSG: monosodium glutamate; B: bovine gelatine; and P: porcine gelatine.

Adulterated spiked samples

Figure 3 (a) and (b) represent the P adulterant that can be denoted at 1539 cm^{-1} , $1453\text{--}1444\text{ cm}^{-1}$ and $1081\text{--}1070\text{ cm}^{-1}$ for all P percentages in ON and BC. Meanwhile, the adulteration of TRE in ON and BC exhibited similar spectrum with the samples of the pure BC and ON illustrated by Figure 4 (a) and (b). Thus, further analysis using PCA is required to identify the adulteration of P and TRE in ON and BC.

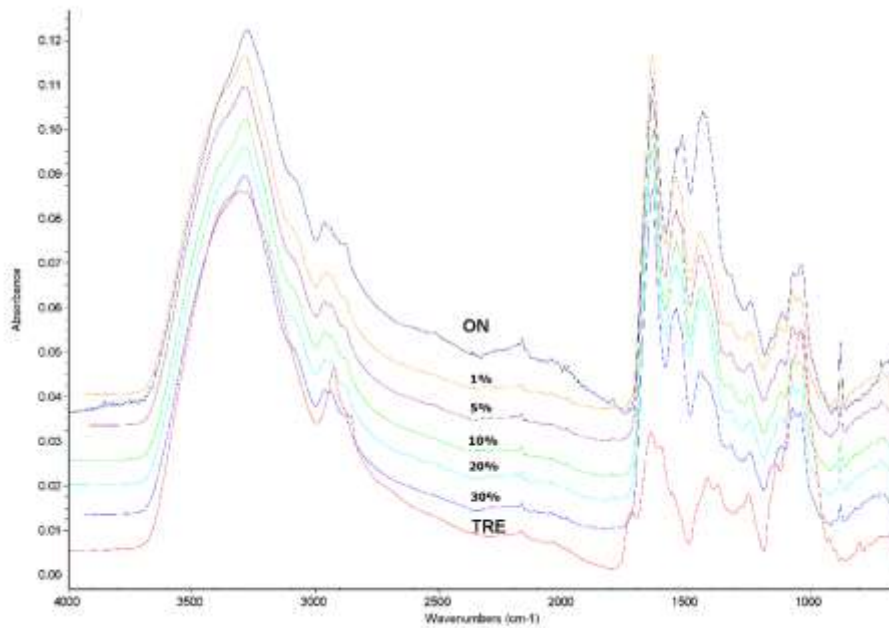


(a)

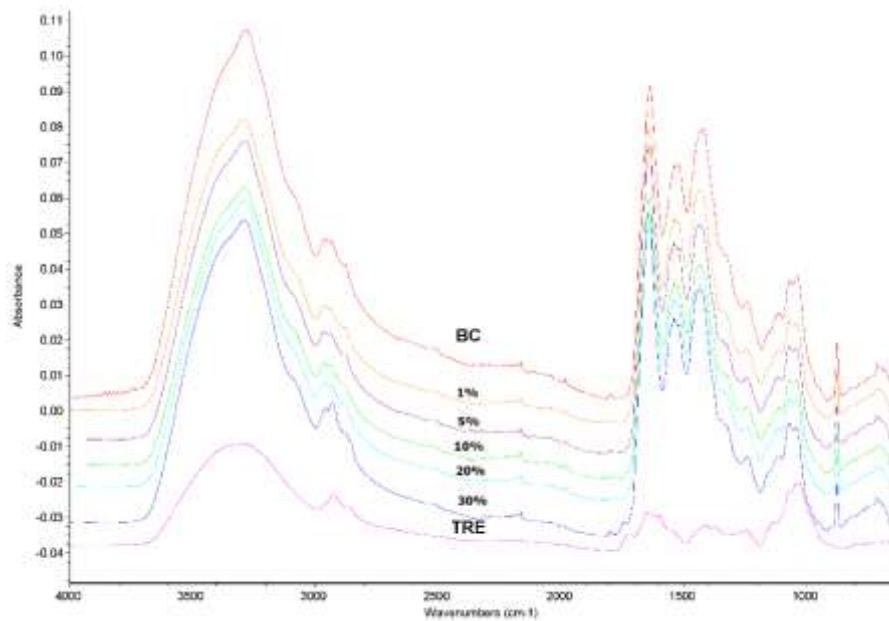


(b)

Figure 3. The overlapped spectrums of edible bird's nest (EBN) adulterated with different percentage of porcine gelatine (P). (a) Orange nest (ON); and (b) blood cave nest (BC).



(a)



(b)

Figure 4. The overlapped spectrums of edible bird’s nest (EBN) adulterated with different percentage of *Tremella fungus* (TRE). (a) Orange nest (ON); and (b) blood cave nest (BC).

Principle Component Analysis (PCA)

The PCA is one of the chemometric analysis, which utilizes low dimensional space (principle components) of the new latent variables from a larger dimensional data. The first

principle components (PC1) represented the highest variance in a data set and the second highest data set was grouped in the second principle components (PC2). The number of variables can be reduced by this method, which gives the graphical representation of the data. The results were presented in terms of loading plots, which showed the contribution of the variables to a given PC and the score plots, which showed the projections of the objects and the similarities can be measured among them (Massart *et al.*, 1997; Norhayati *et al.*, 2010). Fifteen variables were chosen to represent significance for each sample. Those wavenumber variables of 1633 cm^{-1} , 1602 cm^{-1} , 1518 cm^{-1} , 1516 cm^{-1} , 1444 cm^{-1} , 1442 cm^{-1} , 1434 cm^{-1} , 1415 cm^{-1} , 1336 cm^{-1} , 1247 cm^{-1} , 1248 cm^{-1} , 1241 cm^{-1} , 1029 cm^{-1} , 875 cm^{-1} , and 704 cm^{-1} had shown either significant similarities or differences between each sample.

In Figure 5 and Figure 6, the PCA could differentiate between the samples when ON and BC adulterated with P and TRE. The samples were classified into three groups in the PCA, where the group I represents the pure samples of ON or BC, group II represents either P or TRE and group III represents the adulterated samples with 1%, 5%, 10%, 20% and 30% of P or TRE. Figure 5 (a) and (c) of ON samples adulterated with P and TRE show that 97% and 99% of the variation were accounted for PC1 and 3% and 1% of the variation were accounted for PC2, respectively. Meanwhile, based on the Figure 6 (a) and (c), the results of BC samples adulterated with P and TRE show that PC1 accounted for about 95% and 100% of the variation and PC2 accounted for about 3% and 0% of the variation, respectively. Both score plots of P and TRE samples were located at the positive side of X-axis, however, P was located below X-axis, while TRE was located above X-axis. Hence, the PCA in both ON and BC samples was able to detect the grouping, similarities and differences of the input data even at low adulterants concentration at 1%.

In addition, the variables that were located farther from the origin of PCA loading plots have strong contributions for the PCA model (Marina *et al.*, 2010). Figure 5 (b), 6 (b) and 8 (d) showed that the wavenumbers of 1602 cm^{-1} and 1633 cm^{-1} have high contributions, indicating the separation of the samples between the adulterants and EBN samples. However, the loading plot of ON adulterated with TRE in Figure 5 (d) showed that 1633 cm^{-1} weakly contributed to the model, however, 1602 cm^{-1} contributes a strong influence on the variation of the samples. This might suggest that both adulteration in ON and BC with P and TRE can be differentiated through the variables that have strong contribution for the PCA model.

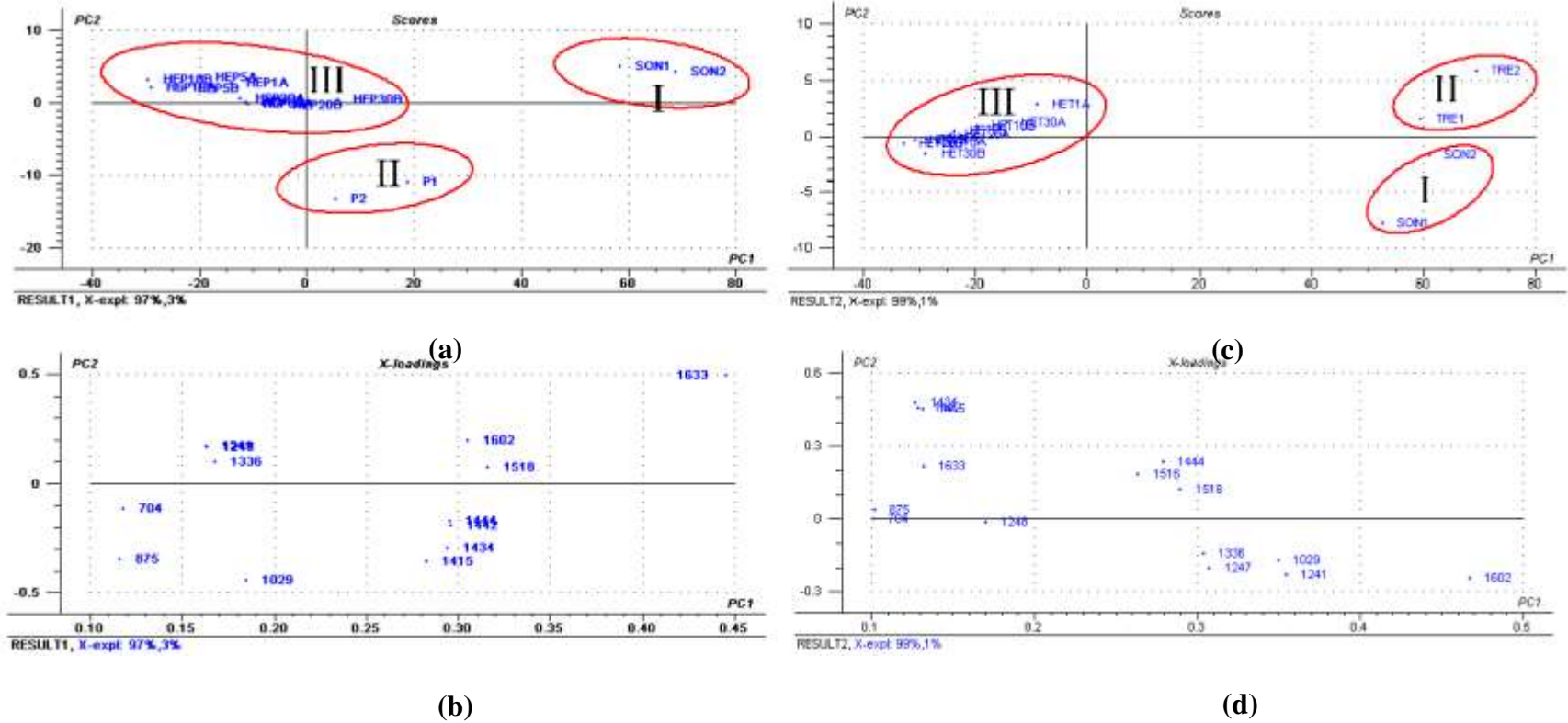


Figure 5. PCA grouping of orange nest (ON) adulterated with porcine gelatine (P) and *Tremella* fungus (TRE). **(a and b)** Score plots and loading plots for PCA grouping of orange nest (ON) adulterated with porcine gelatine (P) **(c and d)** Score plots and loading plots for PCA grouping of orange nest (ON) adulterated with *Tremella* fungus (TRE). Group I: orange nest; group II: porcine gelatine or *Tremella* fungus; group III: adulterated orange nest with porcine gelatine or *Tremella* fungus percentage.

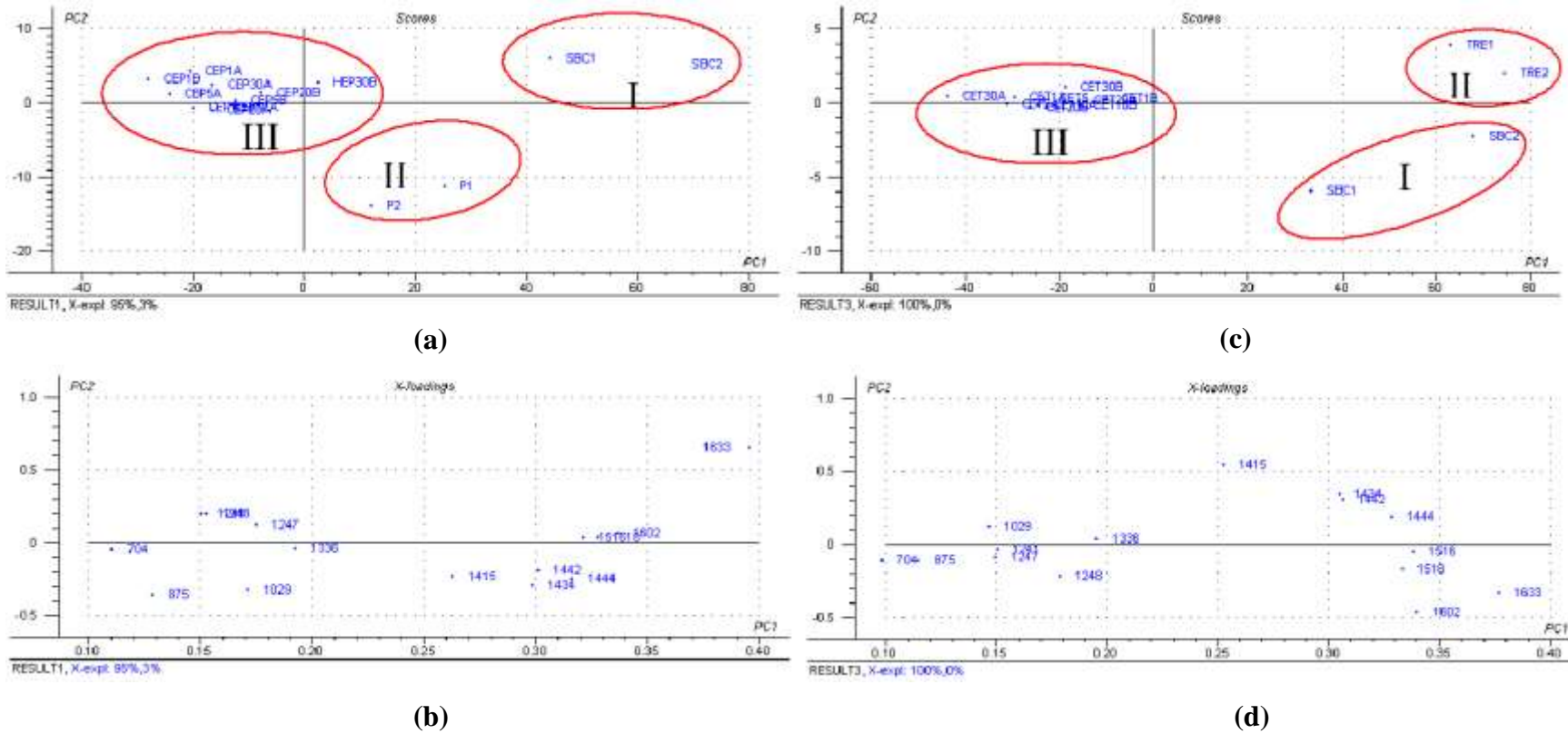


Figure 6. PCA grouping of blood cave nest (BC) adulterated with porcine gelatine (P) and *Tremella* fungus (TRE). (a and b) Score plots and loading plots for PCA grouping of blood cave nest (BC) adulterated with porcine gelatine (P) (c and d) Score plots and loading plots for PCA grouping of blood cave nest (BC) adulterated with *Tremella* fungus (TRE). Group I: blood cave nest; group II: porcine gelatine or Tremella fungus; group III: adulterated blood cave nest with porcine gelatine or *Tremella* fungus percentage

4. Conclusion

Fourier transform infrared spectroscopy (FTIR) is a reliable, fast and easy to use fingerprinting technique that can be applied for food authentication. The Principal Component Analysis (PCA) analysis can further concluded and differentiated EBN, adulterants and different concentration of adulterations for porcine (P) and *Tremella* fungus (TRE) in the edible bird's nest (EBN). This study showed that the presence of P and TRE adulterants in orange nest (ON) and blood cave nest (BC) can be detected as low as 1% (w/w) concentration through the significant distinction of the grouping in the PCA. The wavenumbers of 1602 cm^{-1} and 1633 cm^{-1} show high contribution to the separation between the adulterants and EBN samples. To conclude, FTIR with the combination of PCA is a reliable tool in authenticating EBN.

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Conflicts of Interest: The authors declare no conflict of interest.

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