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Preliminary study of nitrite content in South Sulawesi uncleaned edible bird nest

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Abstract. Edible bird nest is a nest made from saliva of swiftlet (*Aerodramus fuciphagus*). In term of food safety, nitrite is one of chemical hazards that should be checked in edible bird nest (EBN) which can affect human health. Occurance of nitrite in edible bird nest is determined from its level in uncleaned EBN and cleaning process. This preliminary study was aimed to check level of nitrite in edible bird nest taken from swiftlet houses in South Sulawesi. The nitrite analysis using spectrophotometer showed that nitrite content is varied among swiftlet houses ranged from 4.7 µg/g to 164.4 µg/g. This result showed that most EBN sample in uncleaned South Sulawesi edible bird nest is below the limit of nitrite content regulated by Indonesian government which is 125 µg/g. This finding also indicates that good farming practice should be introduced into swiftlet farmers.

1. Introduction 1

Edible nest swiftlet (*Aerodramus fuciphagus*) is a special bird produces high value of edible bird nest (EBN) and sometimes called as Caviar of the East and Indonesia is one of the main edible bird nest producer countries in the world. Several studies have proven that EBN has a variety of effects that are good for health[1] . Swiftlet nest is believed to cure various diseases such as lung, heartburn, cancer, ageless medicine, blood circulation and respiratory tract, even Aquired Immuno Deficiency Syndrome (AIDS)[2].

The swiftlet makes its nest from thick secretions of saliva by the male swiftlet's salivary glands. The nest serves as a breeding ground, laying eggs and caring for birds until they can fly [3]. Swiftly, birds make nests in many caves in the chalky area. In addition to the cave, swiftlet also places its nest in houses that have a micro habitat condition resembling a cave. These houses have dark rooms with temperatures of 26-28°C and humidity of 85-98% [4]. Edible bird nests require several stages of the production process before consumption. But, before consuming the bird nest, it must be washed separately to reduce levels of nitrites found in the swiftlet's nest originating from swiftlet's saliva and contamination from the environment [4].

Nitrites are compounds that contain nitrogen atoms that combine with oxygen atoms[5]. Nitrites are naturally present in EBN and nitrites combine with sodium to form inorganic salts which are used as antimicrobial agents, preservatives and color fixatives[5]. Nitrite as a preservative is permitted, in food products as long as it does not exceed the limits in accordance with the Regulation of the Minister of Agriculture of the Republic of Indonesia No.41 / Permentan / OT.140 / 3/2013 Regarding Animal Quarantine Actions on the importation or removal of edible bird nest into and from within the Territory of the Republic of Indonesia that the maximum limit of nitrite levels in swiftlet's nests is 125 mg / kg[6]. Excessive consumption of nitrites can cause harm to the wearer, both direct, poisoning and indirect, namely nitrites are carcinogenic [7]. Nitrites are harmful to human health when reacting with secondary amines and amides in the digestive tract to form carcinogenic N-nitrosamines which can cause gastric cancer [8]. Pregnant women who consume large amounts of nitrite are exposed to a high risk of having a baby with blue baby syndrome or infant methemoglobinemia because Nitrite can oxidize hemoglobin in the blood and make it unable to carry oxygen to body tissues, causing methaemoglobinaemia so that the baby's skin becomes blue[9]. Considering its effect in human health, preliminary study of nitrite content should be conducted in emerging EBN producers, South Sulawesi swiftlet houses.

2. Materials and methods

2.1. Edible bird nest preparation

Nine uncleaned edible bird net were collected from three swiftlet houses in three regency (Wajo, Pinrang and Pare-pare) in South Sulawesi. In each swiftlet house, three uncleaned EBNs were collected. The EBN samples were stored 4°C refrigerator before analysis. A piece of uncleaned edible bird was ground gently until the small tiny fragment of EBN obtained prior to nitrite analysis.

2.2. Standard curve

Standard curve was obtained from dilution of nitrite standard solution (certifur nitrite standard solution) into 7 level concentration which were 0.005 $\mu g.L^{-1}$, 0.01 $\mu g.L^{-1}$, 0.02 $\mu g.L^{-1}$, 0.05 $\mu g.L^{-1}$, 0.1 $\mu g.L^{-1}$, 0.15 $\mu g.L^{-1}$, 0.20 $\mu g.L^{-1}$. Diluted standard solution was added with 1 mL of sulfanilamide (Merck KGaA, Germany) then the solution was added with 1 mL of the naphtyl ethylene diamin (Merck KGaA, Germany). The standard solution was allowed to stand for 20 minutes then putted into a cuvette and measured its absorbance spectrophotometer (UV-1800, Shimadzu, China) at 540 nm wavelength [10].

2.3. Nitrite content determination

One gram of uncleaned EBN sample was ground. Eight mL of sterile water was added to uncleaned ground EBN. The EBN mixture solution was heated in magnetic stirrer hot plate (CAPP CRS-22H, CAPP, Germany) in 80°C for 5 minutes. The EBN mixture solution was added with 60 mL sterile water then was heated in water bath (Memmert WNB, Germany) in 80°C for 2 hours while stirred occasionally. Sterile water was added into the EBN mixture solution to reach 100 ml. The EBN mixture solution was filtered using Whatman filter paper no. 40 (GE Healrhcare, Germany). 45 mL of EBN filtrate was added with 2,5 ml sulfanilamide and 2,5 mL naphtyl etilene diamin. Around 1 ml of the aliquot was measured using spectrophotometer at 540 nm wavelength. Concentration of nitrite content in edible bird nest using this equation:

$$\text{Nitrite content } (\mu g/g) = \frac{C(mg/L)\ x\ FP\ x\ V\ (mL)}{W\ (g)}$$

where:

Nitrite content	= amount of nitrite in the sample (µg/g)
C	= amount of nitrite in the sample obtained from the calibrated curve (µg/L)
V	= volume of sample solution, (mL)
FP	= dilution factor of sample solution
W	= weight of sample (g)

3. Results and discussion

Uncleaned edible bird nests used for preliminary study of nitrite content were South Sulawesi EBN. Each sample was taken from swiftlet houses which have high production of edible bird nest. The swiftlet houses used in this study in located in three region: Wajo regency, Pare-Pare city and Pinrang regency. South Sulawesi is one of emerging producer of EBN in Indonesia and number of new swiftlet houses is increasing every year. Thus, quality of South Sulawesi EBN should be checked regularly.

Standard curve was used to determine the level of nitrite content in edible bird nest. Validation of test was checked by calculating its linearity. Linearity of standard curve was measured using an equation, y = Ax + B. Liniear regression equation of nitrite was y = 1.0904x + 0.0004. Standard curve of nitrite standard solution shown in Figure 1 was constructed using 7 level concentration which were 0.0 µg.L^{-1}, 0.01 µg.L^{-1}, 0.02 µg.L^{-1}, 0.05 µg.L^{-1}, 0.1 µg.L^{-1}, 0.15 µg.L^{-1}, 0.20 µg.L^{-1}. The standard curve was linear in the range of 0.0 µg.L^{-1} to 0.20 µg.L^{-1} . Coefficient of linear correlation of nitrite was 0.99995. The coefficient of linear correlation of this study was higher than the study of nitrite in Malaysia edible bird nest [8].

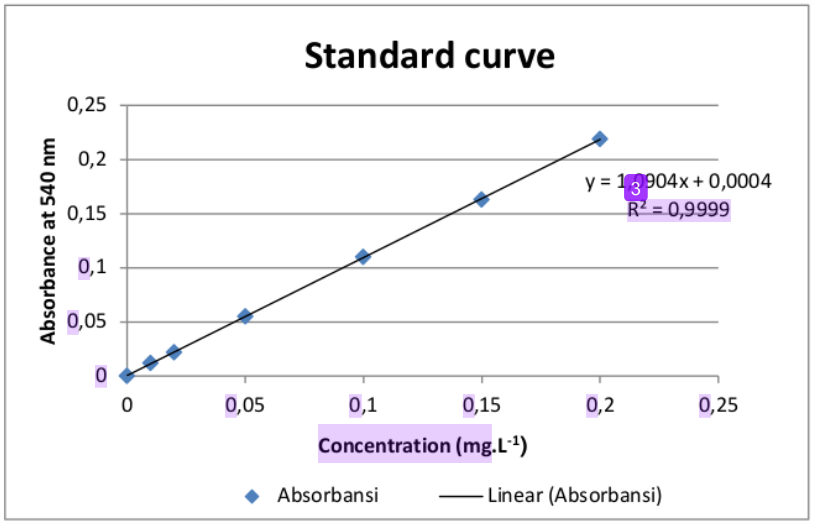

Figure 1. Linearity of standard curve of nitrite standard solution

Analysis of uncleaned South Sulawesi edible bird nest showed that each region has different level of nitrite content. Nitrite content in Wajo regency is ranged from 32.4 µg/g to 65.1 µg/g. Nitrite content in Pare-Pare city is ranged from 4.7 µg/g to 23.6 µg/g. Nitrite content in Pinrang regency is ranged from 48.4 µg/g to 164.4 µg/g. These result showed that Pinrang swiftlet houses produce edible bird nest with high level of nitrite and followed by Wajo regency and Pare-Pare city. The reason of this high content of nitrite is still unclear whether it was caused by micro and macro-environment situation or farming management edible nest swiftlet (*Aerodramus fuciphagus*). These results also showed that

two EBN samples which were obtained from Pinrang regency exceed limit of allowable nitrite content in EBN. The limit of allowable nitrite content in EBN is 125 µg/g. Even though, the nitrite content in Pare-Pare is high, the nitrite content in those EBNs can be reduced using proper cleaning system.

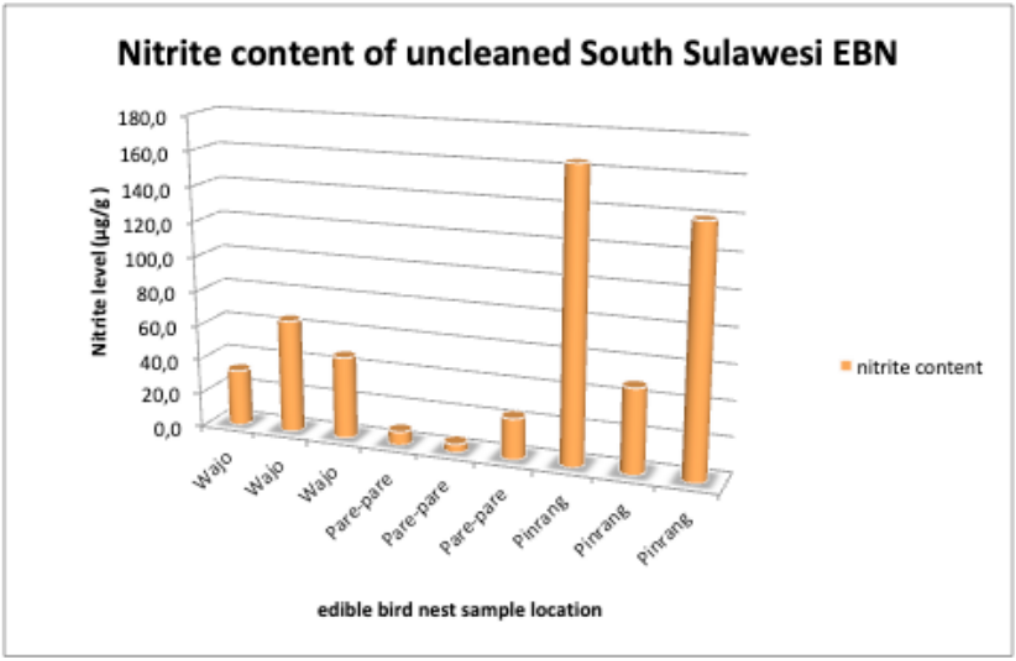

Figure 2. Nitrite content of uncleaned South Sulawesi edible bird nest

High level of nitrite content in Pinrang regency probably due to harvesting time of edible bird nest and cleaning time of swiftlet houses. There are many type of harvesting system of EBN conducted by edible nest swiftlet farmers which are two weeks harvesting, one month harvesting, two months harvesting, three months and six months harvesting. Harvesting system is selected by farmers based on EBN density and reproduction cycle of the swiftlet. The EBN obtained from Pinrang regency was nest which contain egg or could be called as laying nest, thus it influence harvesting time of the EBN. The longer time the bird stay in the nest, the higher nitrite content will be. In another part, EBN samples obtained from Pare-Pare city has lower amount of nitrite content. It was associated with its farming practice which introduce two weeks cleaning system. Swiftlet farmer in Pare-Pare cleaned his swiftlet houses by removing its feces once in two week. Other factors that may influence nitrite content in EBN was nitrite content in feces and water in the swiftlet house. On going study related to nitrite content in water and feces will be reported separately.

Nitrite content is generally found in edible bird nest, and nitrite analysis in edible bird nest is conducted as a part of food safety analysis to guarantee its safety to consumer. To assure food safety aspect of edible bird nest in Indonesia, as the main producer of EBN in the world, Indonesia government has regulated that each edible bird nest product should have sanitation certificate from Indonesia Agriculture Quarantine Agency. The sanitation certificate at least should contain a statement that free from listed quarantine diseases, acquired veterinary public health standard, type and quantity, name and address of shipper and consignee, place and date of loading, place of loading[6]. To meet the requirement of food safety of main importer country, China, Indonesia also regulated that each

EBN that would be exported to China should have a traceability system, free from avian influenza, and its nitrite content should less than 30 ppm.

This study recommends that good farming practices in swiftlet houses should be conducted and checked regularly to maintain its quality. New and safe farming practice should introduce to swiftlet farmers, thus they can enhance the quantity of the EBN they produce as well as the safety of the EBN product. Moreover, due to variability of nitrite content in uncleaned edible bird nests, EBN processor which clean EBNs should ensure the method they use to clean EBN was effective to decrease nitrite level to acceptable amount required by the government.

4. Conclusion

Nitrite analysis of South Sulawesi edible bird nests showed two sampling sites which are Wajo regency and Pare-Pare regency had lower nitrite content and below the limit of nitrite regulated by Indonesia government comparing to Pinrang regency. Nitrite content in Wajo regency was 32,4 µg/g, 66,5 µg/g, 47,9 µg/g. Nitrite content in Pare-pare regency was 7,6 µg/g, 4,8 µg/g, 23,6 µg/g. Nitrite content in Pinrang regency was 164,9 µg/g, 48,8 µg/g, 136,8 µg/g. Nitrite level variance was assumed due to variability of farming practice and harvesting time.

5. Acknowledgements

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