https://dx.doi.org/10.14334/Proc.Intsem.LPVT-2021-p.29

Detection of Nitrite in Cleaned Edible Bird Nest from Sumatra Island

Widiyani P[1,2], Latif H[1], Lukman DW[1], Sudarwanto MB[1]

[1]*Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia*
[2]*Indonesian Agriculture Quarantine Agency, Center for Animal Quarantine and Animal Biosafety, Jakarta, Indonesia*
e-mail: widiyaniplatika@gmail.com

ABSTRACT

Edible bird nest (EBN) is a bird's nest commonly made from the saliva of a swiftlet from the species Aerodramus fuciphagus. As the largest exporting country, Indonesia must comply with the nitrite content standard set by China, which is 30 ppm. The maximum limit of nitrite content in EBN exported to China currently refers to the regulation of nitrite content in processed baby food because nitrite can cause poisoning and harm human health. This project aims to study and analyze the nitrite content in EBN from Sumatra as a reference and levels of nitrite policy in Indonesia. The number of samples was calculated proportionally from the data on the swiftlet houses using OpenEpi software. A total of 18 samples cleaned EBN from swiftlet houses were obtained from various regions of Sumatra Island. Samples were washed with water one time. Samples were tested with the spectrophotometric method at The Center for Diagnostic of Agricultural Quarantine. The results showed that from the total 18 samples cleaned EBN; five samples were higher than the nitrite content standard (30 ppm). The average nitrite in cleaned EBN is 30.1913 ppm. Swiftlet farmers should be managing swiftlet houses and apply good farming practices.

Keywords: Edible bird nest, Nitrite, Sumatra

INTRODUCTION

Edible bird nest is made from the compressed saliva of swiftlet species, Aerodramus fuciphagus. Edible bird nest (EBN) needs about 35 days to complete one nest during the breeding season (Teh & Ma 2018).

Indonesia is currently the largest exporter and production country of edible bird nests globally (Hamzah Z et al. 2013; Looi & Omar 2016). The number of exports of EBN from Indonesia to China in 2015 was 14 tons. Subsequently, this number increased to 22 tons in 2016 and 52 tons in 2017. The conversion value of EBN exports to China totalled US$87.4 million or equivalent to Rp1.18 trillion. (Gumilar 2018).

The high demand for EBN in the international market is due to the belief that the properties contained in EBN, especially that of EBN, have medicinal properties. (Amriani et al. 2019), as food or health drink for gastronomy (But et al. 2013), and its abilityto boost the immune system (Chantakun & Benjakul 2020). Sialic acid in EBN has also known as an anti-influenza (Haghani et al. 2016) and anti-diarrhoea (Chua et al. 2014).

Edible bird nest from Indonesia has a high protein amount of 59.8-65.8%, and sialic acid as much as 10%. The potential quality of EBN from Indonesia is quite popular with foreign countries, so that the international market demand for EBN from Indonesia increases from year to year (Hamzah Z et al 2013). Variations in the composition and protein content of EBN are highly dependent on geographical, food sources and differences in the genus or gallus of swallows (Zukefli et al. 2017).

The swallow's nest industry is faced with various food safety for consumers demands, related to the quality of EBN and the fulfilment of nitrite level (Chan et al. 2013; Looi & Omar 2016). The maximum limit for nitrite levels in EBN exported to China is 30 ppm. The maximum limit for nitrite levels in EBN exported to China is 30 ppm. The maximum limit refers to the rules for nitrite levels in processed baby food. Therefore, it is crucial to study the nitrite levels in EBN because nitrite can cause poisoning and harm human health. Nitrite can be toxic and dangerous because it can cause methemoglobinemia conditions resulting in impaired oxygen flow, difficulties in breathing (Saputro et al. 2016) and an increased risk of colon cancer (Thorburn 2015).

MATERIALS AND METHODS

Research location

This study was conducted to determine the nitrite level in EBN by sampling swiftlet houses in various areas in Sumatra. There are six, eight and four samples from A, B, and C areas, respectively. Sampling was carried out from August 2020 to March 2021. A spectrophotometric method is used to test the nitrite content of EBN and carried out at the Center for Diagnostic of Agricultural Quarantine. Samples were washed at the EBN processing company in Tangerang Jakarta.

Sampling method

This research is a cross-sectional study. A total of 18 samples of cleaned EBN were from swiftlet houses in various regions in Sumatra. Sampling is taken randomly in groups (random cluster sampling) from A, B and C areas. The criteria for the EBN sample used were white EBN, measuring at least 6 grams per piece of the nest, ready to harvest, having a light to medium level of cleanliness and a weight of about 30-40 grams. The criteria for the swallow houses selected as the sampling location are the swiftlet houses located on the islands of Sumatra, which produce EBN regularly every month. Uncleaned EBN samples were washed with water at the EBN processing company for the washing process as declared in the washing process.

Data processing

Sample data processing

Data on nitrite level in EBN was processed using Microsoft Excel and presented descriptively.

Nitrite measurement stage

Sample selection

The criteria for EBN samples are white EBN, measuring at least 6 grams per piece of the nest, ready to harvest, has a light to moderate level of cleanliness and a weight of 30-40 grams. The uncleaned EBN was washed with water one time. The criteria for the swallow house selected as the sampling location is that the swallow house was located in various areas on Sumatra Island, which regularly produces EBN every month.

Washing process

Uncleaned EBN is pre-cleaned and scraped (approx. 5±2 seconds). Initial washing was for 10±2 seconds on each sample. The washing uses reverse osmosis (RO) water to remove dirt and soften (softening). The sample was dried in tissue paper and aerated for 150±5 minutes. The process of feather removal was conducted using tweezers made of stainless steel and brushed using a soft brush. The sample was rinsed using running water until the water touched the entire surface of the EBN with a time of 15±2 seconds. Samples were dried again for 24 hours (one night) and moulded. Then the sample was dried until completely dry (about 14 hours). Spectrophotometry methods tested samples of cleaned EBN weighing 0.5 grams.

Nitrite examination

The materials used for this study were: solutions of 1 ppm nitrite; nitrite working standard; sulfanilamide; saturated sodium chloride (NaCl); N-(1-naphthyl) ethylene dihydrochloride (NED, Merck KGaA, Germany); saturated NaCl, and ion free water.

The tools used are: a set of UV-visible spectrophotometer, analytical balance, blender, ultrasonic digester, timer, test tube, measuring flask, beaker, funnel, Erlenmeyer, micropipette (10, 100, 1000 µl), icebox, refrigerator, freezer (-40 C), sterile tweezers, label paper, microtip/pipette tip (10, 100, 1000 µl), computer, stationery, gloves, hygienic sample plastic, and sterile disposable spatula.

Stage of nitrite examination using spectrophotometry method

The first step is to determine the standard curve obtained from the dilution of the standard nitrite solution to 7 concentration levels, such as 0.0 µg.l-1, 0.2 µg.l-1, 0.3 µg.l-1, 0.4 µg.l-1, 0.5 µg.l-1, 0.6 µg.l-1, 0.7 µg.l-1, then the diluted standard solution was added with 0.6 ml of saturated sodium chloride (NaCl) and ionized water to reach 10 ml. After that, adding one ml of sulfanilamide. After 5 minutes, we added 1 ml of naphthyl ethylene diamine (NED).

The standard solution was allowed to settle for 15 minutes then put into a cuvette, and the absorbance spectrophotometer (UV-1800) was measured at a wavelength of 540 nm (Yusuf et al. 2020). The second step is 0.5 g of the blend and homogenized sample, then 40 ml of ion-free water is added until 50 ml. The next step is to add 3 ml of saturated sodium chloride (NaCl) solution. The mixed solution was heated in an ultrasonic digester at a temperature of 400C for 30 minutes. The hybrid solution was filtered using Whatman filter paper no. 41 (GE Heartcare, Germany). Transfer the supernatant into a 10 ml volumetric flask up to the mark of 10 ml, add 2.5 ml of sulfanilamide and after 5 minutes, add 2.5 ml of NED and homogenize. After 15 minutes, start measuring the absorption using a spectrophotometer at a wavelength of 540 nm (Yusuf et al. 2020). Furthermore, the concentration of nitrite level is calculated with the following formula:

$$\text{Nitrite level } (\mu g.g^{-1}) = \frac{C \times V \ solvent}{W}$$

Annotation:
C = the amount of nitrite in the sample is obtained from the calibrated curve (μgl^{-1})
V = sample solvent volume (ml)
W = sample weight (g)

RESULTS AND DISCUSSION

The results showed five samples containing nitrite higher than the standard nitrite level of 30 ppm. All samples are from Sumatra, as shown in Figure 1. The average of nitrite in the cleaned EBN is 30.1913 ppm. Data on the nitrite in cleaned EBN are presented as shown in Table 1.

Figure 1. Map of Sumatra island

Table 1. Data on nitrite level in cleaned edible bird nest from Sumatra Island

Origin	Code of sample	Average of nitrite level in each area (ppm)	Total average of nitrite Level (ppm)	Median (ppm)	Standard deviation
Area A	00-08	51.7743			
Area B	09-12	11.9623	30.1913	15.4212	33.9257
Area C	13-18	14.3686			

Research on the nitrite content in EBN has been carried out today. The research results on white EBN in the Hong Kong Market (all samples imported from Indonesia, Malaysia, Thailand and Vietnam) showed variations in nitrite content between 0 ppm to 6430 ppm. The median value of nitrite content in white EBN from the four countries is 100 ppm (Chan et al. 2013). The test results for the nitrite level were higher than 30 ppm because the EBN sample was a cleaned EBN washed one time, and there was lower than previous research. The EBN that was washed one time did not significantly reduce the nitrite level. The average nitrite content of EBN one time cleaned from South Kalimantan was 65.24±3.38 ppm and decreased during the washing process. The average nitrite level of EBN cleaned in one time and two times cleaning was not significantly distinct. The most significant decrease in nitrite levels was obtained in EBN washed three times. The nitrite level of EBN, cleaned three times, decreased to an average of 30.87±2.11 ppm (Susilo et al. 2016). Different washing frequencies give other nitrite reductions. In addition to cleaning the nest from feathers and dirt, washing can indirectly reduce the nitrite level of the EBN. The nitrite level in EBN is influenced by the length of time the EBN is exposed to water. The longer the EBN is exposed to water, the lower the nitrite level (Chan et al. 2013; Hamzah Z et al 2013). (Chan et al. 2013; Hamzah Z et al 2013). It is essential to know that nitrite on EBN is quickly reduced. EBN nitrite could be removed (98%) by soaking it in water (Chan et al. 2013). But it will reduce market interest for EBN.

Commonly, swiftlet constructs their nest in caves location, near the coastal region or tropical rainforest. The nest is built onto the smooth surface of the concave walls and located at least 2.5 m above the ground. Currently, urbanization has reduced nesting sites for swiftlets to seek out unoccupied buildings for new nesting sites. Dark, damp and cooling conditions in the building supported appropriate environmental conditions. Over the years, many activities have grown in swiftlet farming. Swiftlet houses imitate a cave-like environment with the purpose to provide nesting sites and attract swiftlet. Swiftlet farmers do not control the birds' movement, breeding or diets. Swiftlets can move freely to hunt for insects. The newly constructed swiftlet houses are primarily in rural or agricultural land (Chua & Zukefli 2016).

Nitrite contamination on EBN occurs from its habitat (Utomo et al. 2018). The environmental cleanliness of the swiftlet house is an important thing and has a strong correlation with the amount of nitrite in the EBN. Various studies have shown that the EBN can be contaminated with nitrite from the environment (Hamzah et al. 2013). Environmental conditions also influence nitrite levels in EBN. The environment affects mainly the floor of the swallow houses when there is a decay of organic material (Amriani et al. 2019). Generally, EBN exists swiftlet droplets that contain ammonia. The ammonia will be oxidized by oxygen to nitrite and then oxidized again to nitrate. The formation of nitrite in the swallow's nest results from a natural process of changing nitrogen in the swiftlet house environment (Chan et al. 2013). Nitrite in the EBN comes from the oxidation of swiftlet droplets or faeces in the air. The manure that remained in the EBN contains ammonia. Ammonia will be oxidized by oxygen to nitrite, then oxidized to nitrate (Amriani et al. 2019). Nitrite is formed naturally by the oxidation of sodium nitrate ($NaNO_3$) by nitrogen in the air (Leonanda & Zolanda 2018; Yenil & Yemiş 2018). Nitrogen in the atmosphere in large quantities cannot be directly utilized by living things. Nitrogen must be converted into ammonia to nitrite and then nitrite to nitrate (Kiding et al. 2015). The high nitrite levels may also be due to anaerobic fermentation by specific bacteria in the presence of ammonia in EBN (Paydar et al. 2013). Variations in nitrite are due to the variability of maintenance practices in the swiftlet house and harvest time in the EBN (Yusuf et al. 2020).

Nitrite has been used in processed foods since a long time ago, and its use is strictly limited to prevent poisoning in humans (Paydar et al. 2013). The positive effects of nitrite include colour development and a source of antioxidants to protect flavours from rancidity (Saputro et al. 2016). Nitrite was also used as an antimicrobial food additive, especially useful for killing *Clostridium botulinum* which produces the deadly botulism toxin. The properties of nitrite can support colour stability, enhance aroma, and inhibit the formation of oxidation products (Thorburn 2015). Nitrite is added to food as an additive and has been widely used in meat and meat products, smoked fish, cheese, fruit juices and mineral water. (Thorburn 2015; Saputro et al. 2016; Yenil & Yemiş 2018).

The use of very high nitrite can cause food poisoning. Large amounts of nitrite in humans can cause gastrointestinal disturbances, bloody diarrhoea and even death. Chronic poisoning from nitrites can cause generalized depression and headaches (Abdurrivai & Syamsinar 2019). Nitrite on food has been associated with methemoglobinemia in infants (Thorburn 2015). Methemoglobinemia is a condition where nitrite will bind to haemoglobin (Hb) in the blood and inhibit oxygen transport in the bloodstream (Rosita 2014; Amanati 2016). This condition will result in a lack of oxygen in the blood (hypoxia) and is characterized by blue skin (cyanosis), shortness of breath, vomiting and death (Samsuar et al. 2020). Patients with methemoglobinemia will have breathing difficulties due to an impaired oxygen respiration system. Nitrites react with strong acids in the stomach (Saputro et al. 2016). Nitrite can increase cancer risk factors due to the formation of N-nitroso compounds which are carcinogenic (Paydar et al. 2013). In addition, it will formated of the carcinogen N-nitrosamine as a high carcinogenic compound. N-Nitrosamines can cause colorectal cancer (Saputro et al. 2016). The maximum daily intake dose of nitrite is limited to 0-0.07 mg/kg body weight (Amriani et al. 2019). According to the Food and Agriculture Organization, the acceptable daily intake of nitrite in EBN is 30 μg/g (Chua & Zukefli 2016). The maximum limit for nitrite level in EBN exported to China has been set at 30 ppm (Keputusan Kepala Barantan 2013).

CONCLUSION

The average nitrite in cleaned EBN from Sumatra is 30.1913 ppm. The cleaned EBN one time washing with running water did not significantly reduce the nitrite level. Cleanliness of the swiftlet house environment is essential and strongly correlates with the amount of nitrite in the EBN; various studies have shown that the EBN can be contaminated with nitrite from the environment. Swiftlet farmers should be emphasized on swiftlet house management and apply good farming practices.

ACKNOWLEDGEMENT

Acknowledgement submitted to the Ministry of Agriculture for providing scholarships and research grants, for the Center of Diagnostic Agriculture Quarantine Agency, for swiftlet house owners who have provided samples, and for EBN processing that has helped conduct research.

AUTHORS CONTRIBUTION

Widiyani P, Sudarwanto MB, Latif H and Lukman DW were contributed equally to this work.

REFERENCES

Abdurrivai, Syamsinar N. 2019. Hubungan kandungan Nitrat (NO_3) dan Nitrit (NO_2) pada air lindi dengan kualitas air sumur gali di Kelurahan Bangkala Kecamatan Manggala Kota Makassar Tahun 2017. Jurnal Sulolipu Media Komunitas Sivitas Akad dan Masyarakat. 17:1-10.

Amanati L. 2016. Uji nitrit pada produk air minum dalam kemasan (AMDK) yang beredar di pasaran. Jurnal Teknologi Proses dan Inovasi Indonesia. 1.

Amriani A, Syam H, WIjaya M. 2019. Pembuatan teh fungsional berbahan dasar buah mahkota dewa (*Phaleria macrocarpa*) dengan penambahan daun stevia. Jurnal Pendidikan Teknologi Pertanian. 5:S251-S261.

But PPH, Jiang RW, Shaw PC. 2013. Edible bird's nests - How do the red ones get red?. J Ethnopharmacol. 145:378-380. doi: 10.1016/j.jep.2012.10.050

Chan GKL, Zhu KY, Chou DJY, Guo AJY, Dong TTX, Tsim KWK. 2013. Surveillance of nitrite level in cubilose: Evaluation of removal method and proposed origin of the contamination. Food Control. 34:637-644. doi: 10.1016/j.foodcont.2013.06.010

Chantakun K, Benjakul S. 2020. Effect of pretreatments and retort process on characteristics and sensory quality of edible bird's nest beverage. Int J Food Sci Technol. 55:2863-2871.

Chua LS, Zukefli SN. 2016. A comprehensive review of edible bird nests and swiftlet farming. J Integr Med. 14:415-428.

Chua YG, Bloodworth BC, Leong LP, Li SFY. 2014. Metabolite profiling of edible bird's nest using gas chromatography/mass spectrometry and liquid chromatography/mass spectrometry. Rapid Commun Mass Spectrom. 28:1387-1400.

Gumilar P. 2018. Ini deretan daerah potensial penghasil sarang walet [Internet]. Available from: https://ekonomi.bisnis.com/read/20180303/99/745469/ini-deretan-daerah-potensial-penghasil-sarang-walet

Haghani A, Mehrbod P, Safi N, Aminuddin NA, Bahadoran A, Omar AR, Ideris A. 2016. *In vitro* and *in vivo* mechanism of immunomodulatory and antiviral activity of Edible Bird's Nest (EBN) against influenza A virus (IAV) infection. J Ethnopharmacol. 185:327-340. doi: 10.1016/j.jep.2016.03.020

Hamzah Z et al. 2013. Nutritional properties of edible bird nest. Bioprocess Eng. 3:600-607.

Keputusan Kepala Barantan. 2013. Keputusan Kepala Badan Karantina Nomor 832/Kpts/OT.140/L/3/2013 tentang Pedoman Persyaratan dan Tindakan Karantina Hewan Terhadap Pengeluaran Sarang Walet dari Wilayah Negara Republik Indonesia ke Republik Rakyat China [Internet]. Available from: https://karantina.pertanian.go.id/page-72-pedoman-karantina-hewan.html

Kiding A, Khotimah S, Linda R. 2015. Karakterisasi dan Kepadatan bakteri nitrifikasi pada tingkat kematangan tanah gambut yang berbeda di kawasan hutan lindung gunung Ambawang Kabupaten Kubu Raya. Protobiont. 4:17-21.

Leonanda BD, Zolanda Y. 2018. Reaktor nitrifikasi biofilter untuk air limbah sisa makanan dan feses ikan. Met J Sist Mek Termal. 2:9.

Looi QH, Omar AR. 2016. Swiftlets and edible bird's nest industry in Asia. Pertanika J Sch Res Rev. 2:32-48.

Paydar M, Wong YL, Wong WF, Hamdi OAA, Kadir NA, Looi CY. 2013. Prevalence of nitrite and nitrate contents and its effect on edible bird nest's colour. J Food Sci. 78.

Rosita N. 2014. Analisis kualitas air minum isi ulang beberapa Depot Air Minum Isi Ulang (DAMIU) di Tangerang Selatan. J Kim Val. 4:134-141.

Samsuar, Ahmad R, Dodo H. 2020. Analisis kadar nitrit pada sosis sapi kemasan yang beredar di pasar tradisional Kota Bandar Lampung secara Spectrofotometri UV Vis. J Farm Lampung. 9:44-55.

Saputro E, Bintoro V, Pramono Y. 2016. Agen kyuring alami pengganti natrium nitrit sintetis pada kyuring daging sapi. Mediagro. 12:65-75.

Susilo H, Latif H, Ridwan Y. 2016. Penerapan metode pencucian dengan air mengalir untuk menurunkan kadar nitrit pada sarang burung walet. J Kedokt Hewan - Indones J Vet Sci. 10:95-97.

Teh S-S, Ma Z-F. 2018. Bioactive components and pharmacological properties of edible bird's nest. Int Proc Chem Biol Environ Eng. 103:29-34.

Thorburn C. 2015. The edible birds' nest boom in Indonesia and South-east Asia: A nested political ecology. Food Cult Soc. 17:535-553.

Utomo B, Widyaratri Y, Widyanto RM. 2018. Metode untuk mempertahankan kandungan nitrit sarang burung walet selama penyimpanan. Jurnal Gizi. 1:1-5.

Yenil N, Yemiş F. 2018. Nitrite in nature: Determination with polymeric materials. Pakistan J Anal Environ Chem. 19:104-114.

Yusuf B, Farahmida P, Jamaluddin AW, Amir MN, Maulany RI, Sari DK. 2020. Preliminary study of nitrite content in South Sulawesi uncleaned edible bird nest. IOP Conf Ser Earth Environ Sci. 486.

Zukefli SN, Chua LS, Rahmat Z. 2017. Protein extraction and identification by gel electrophoresis and mass spectrometry from edible bird's nest samples. Food Anal Methods. 10:387-398. doi: 10.1007/s12161-016-0590-7