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Evaluation of Nitrite Concentration in Edible Bird's Nest (White, Yellow, Orange, and Red Blood)

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Abstract

The color of edible bird's nest is associated with its nitrite concentration, but this relationship remains inconclusive. This investigation aimed to evaluate the nitrite content in edible bird's nest of four different colors: white, yellow, orange, and red blood. Fifty-eight edible bird's nest samples were obtained from five swiftlet farmhouses in Borneo Island, Indonesia and analyzed for nitrite content using Genesys 30 visible spectrophotometer. Results showed that the dark-colored edible bird's nests (yellow, orange, and red blood) had higher nitrite concentrations of 304, 317, and 309 ppm, respectively, compared with the white-colored one (15 ppm). Therefore, the color of edible bird's nest was associated with its nitrite concentration. This study provided updated information about the nitrite concentration in edible bird's nest of various colors.

Keywords: contamination, public health, food safety

Introduction

During the COVID-19 outbreak, edible bird's nest (EBN) has been considered to boost immunity [1]. The main components of EBN are amino acids, carbohydrates, mineral salts, and glycoproteins [2]. Consumer demand for EBN has expanded significantly in recent years [3]. Initially, EBN is consumed only by the Chinese. After some time, the Dayaks people in Borneo Island, Indonesia have started to consume EBN. As a result, EBN has become an essential commodity in the export food trade from Indonesia to China.

In trade, red blood, yellow, and orange EBNs are pricier than white EBN [4] because of their positive influence on the health of a particular community. Different explanations have been suggested; one of which is that red blood EBN represents swiftlet bleeding diluted with saliva [5]. A previous work [6] reported that the color of EBN is related to its nitrite concentration.

The risk of carcinogenic nitrosamine formation is one of the adverse effects of high nitrite content in EBN [7]. Increased nitrite absorption was observed in patients with bladder cancer [8], pancreatic cancer, and gastric cancer [9]. Therefore, the maximum acceptable levels for this compound have been proposed. In food trading, China has set the allowable nitrite content value in EBN to be less than 30 ppm to enter the country; this number is lower than the 80 ppm Indonesian National Standard No. 8998:2021 [10].

Most EBNs are commonly produced from Borneo Island in Indonesia [5], where one swiftlet house could produce 111 kg/year [11]. However, almost no data are available on the nitrite levels of various EBNs from Borneo swiftlet farmhouses. Therefore, this study aimed to evaluate and compare the nitrite concentration in white, yellow, orange, and red blood EBNs of Borneo origin to provide information to consumers, healthcare professionals, and food manufacturers. This report may improve the future trading of EBNs.

Methods

Sample preparation. All samples including white, yellow, orange, and red blood uncleaned EBNs were collected from five swiftlet farmhouses in South Borneo (Table 1). The representative EBNs are depicted in Figure 1. All feathers in the samples were first removed using tweezers. In brief, 1 g from each cleaned EBN was gently grinded on a mortar until fragments were obtained. Afterward, 0.5 mg of each sample was added with a 3 mL of saturated NaCl (Merck, Germany) solution, followed by aquadest to reach a final volume of 50 mL [12].

Standard curve. A standard curve was constructed by diluting the nitrite standard solution (1 ppm) (Merck,

Table 1. Sample Collection of Edible Bird Nest from Five Swiftlet Farmhouses in South Borneo

Swiftlet Farmhouses	N	White	Yellow	Orange	Red blood
1	15	4	4	4	3
2	7	4	0	0	3
3	16	4	4	4	4
4	13	4	3	3	3
5	7	4	1	1	1
Total n	58	16	12	12	14

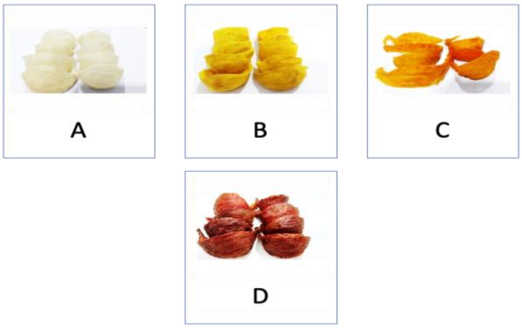

Figure 1. Representative Picture of the EBNs: A. White Nest, B. Yellow Nest, C. Orange Nest, D. Blood Nest

Germany) with 0.6 mL of saturated NaCl (Merck, Germany) solution, 0.5 mL of sulfanilamide (Merck, Germany) solution, 0.5 mL of naphthyl ethylene diamine (Merck, Germany) solution, and aquadest in six concentration levels (0, 0.2, 0.3, 0.4, 0.5, and 0.6 µg/L). The standard solutions were allowed to stand for 15 min and then placed in a cuvette to determine their absorbance using Genesys 30 visible spectrophotometer (Thermo Scientific, USA) at 541 nm wavelength [13].

Nitrite extraction and spectrophotometry. All samples were tested using Elmasonic S 30 H (Elma, Germany) at 40 °C for 30 min with occasionally stirring [14]. The samples were then removed from the sonicator and cooled down to room temperature. Sample extracts were prepared using Whatman filter sheets no. 42 (GE Healthcare, Germany). Nitrite concentration was measured in 1 mL of each extract using Genesys 30 visible spectrophotometer (Thermo Scientific, USA) at 541 nm wavelength [15].

Data analysis. Differences in nitrate levels among various farms and EBN colors were statistically analyzed using ANOVA, followed by Tukey HSD test. All statistical data were processed using SPSS for Windows 23.0.

Results and Discussion

Nitrite is of great concern because its high content in food can cause bladder cancer [8], pancreatic cancer, and gastric cancer [9] in humans. Indonesia is the most important supplier of EBN and the source of around 80% of the global nest supply to various countries, including China [15]. Borneo Island, which belongs to Indonesia Island, has become the most crucial EBN producer because of its many nesting sites [5, 16]. For EBN export trading to the People's Republic of China (PRC), the hazardous substance content must be below a certain level. The PRC government has set a limit of not more than 30 ppm nitrite. With this regulation, the EBN industry must naturally reduce the nitrite concentration in EBNs [17].

Nitrite is normally present in any swiftlet nest or cave and could be synthesized from ammonia by bacteria through anaerobic fermentation. Nitrite is created in the nest and absorbed by the swiftlet nesting habitat, particularly from the decomposing organic debris on the floor [6]. Bird dropping fermentation and natural ecological elements, such as air, water, and soil, have caused the penetration of nitrite into EBNs [7]. The lack of good farming practices in swiftlet farmers can be a factor in the high nitrite concentration of raw uncleaned (RUC) EBNs, which are collected from caves and farms (swiftlet home) without any cleaning technique, and raw cleaned (RC) EBNs, which are cleaned by sorting, soaking, feather and impurity removal, molding, drying, grading, and packaging [4]. The variations in nitrite levels may be attributed to a variety of factors, including differences in the environmental factors of cave and swiftlet farm, such as humidity, pH, and climate; age of harvested EBNs (harvesting time); contamination during harvest; and cleaning processes for the collected EBNs [16]. Appropriate management of swiftlet housing, such as regularly removing the swiftlet guano while leaving the cave guano uncleaned, may contribute to the decrease nitrite content in house nesting. In addition, the proper ventilation of swiftlet housing contributes to reducing bacterial anaerobic fermentation and lowering the nitrite levels [7].

In this work, 58 EBN samples were collected from five swiftlet farmhouses (Table 1). The linear regression equation for nitrite was $y = 0.9963x–0.0024$, and the linear correlation coefficient for nitrite was 0.99999. The highest mean of clean white EBN was 19.9400 ± 4.96674 ppm (Table 2). This value is still below the maximum limit for nitrite levels set by the PRC government. The nitrite level in this study was lower than the previous results of 100 ppm for RC EBNs from Hong Kong market [17] and 93 ppm for RUC EBNs from South Borneo, Indonesia [18]. By contrast, the nitrite level in the present work agreed with the previous values of 7.9–22 ppm for RC EBN from three houses-EBN in Malaysia [6]. The current data also showed that white EBN had the lowest nitrite concentration among the groups (Figure 2), indicating that all the investigated swiftlet farmhouses have applied good management practices.

Table 2. Multiple Comparisons of Nitrite Levels of Swiftlet Farmhouses-EBN (White) Derived from Different Sources using Tukey's HSD test. N = 4 for Each Group

EBN Samples (I)	EBN Samples (J)	Nitrite Concentration (ppm per g of Sample)			
		Mean	Mean Difference (I-J)	SD	*P* Value
SF 1		19.9400		4.96674	
	SF 2		4.60750	2.56890	0.412
	SF 3		4.81500	2.56890	0.371
	SF 4		6.82250	2.56890	0.109
	SF 5		6.36500	2.56890	0.148
SF 2		15.3325		2.25229	
	SF 1		−4.60750	2.56890	0.412
	SF 3		.20750	2.56890	1.000
	SF 4		2.21500	2.56890	0.906
	SF 5		1.75750	2.56890	0.957
SF 3		15.1250		4.20100	
	SF 1		−4.81500	2.56890	0.371
	SF 2		−.20750	2.56890	1.000
	SF 4		2.00750	2.56890	0.932
	SF 5		1.55000	2.56890	0.972
SF 4		13.1175		3.57852	
	SF 1		−6.82250	2.56890	0.109
	SF 2		−2.21500	2.56890	0.906
	SF 3		−2.00750	2.56890	0.932
	SF 5		−.45750	2.56890	1.000
SF 5		13.5750		2.40772	
	SF 1		−6.36500	2.56890	0.148
	SF 2		−1.75750	2.56890	0.957
	SF 3		−1.55000	2.56890	0.972
	SF 4		.45750	2.56890	1.000

SF: swiftlet farmhouse; HSD: honestly significant difference

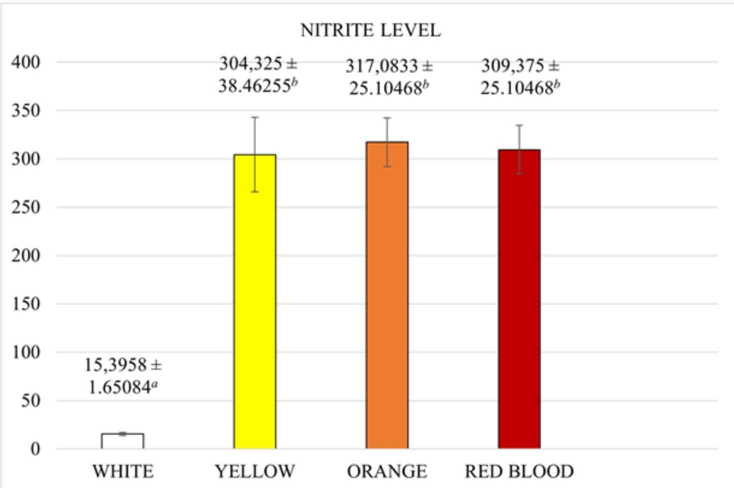

Figure 2. Multiple Comparisons of the Nitrite Levels of Swiftlet Farmhouses-EBN (White, Yellow, Red Blood, and Orange) Derived from Different Sources using Tukey's HSD Test

Some commercial EBNs generally have a white or yellowish–white color [19]. EBNs also exhibit a variety of colors such as bright yellow, red blood, and orange, which have more expensive market price than those with white color [20–22]. In the food market, red blood EBN is the most expensive type with cost ranging from US$ 1000 to US$ 15 000 per kilogram [4]. According to historical records from 1700 (Qing dynasty in China), red blood EBN provides more health benefits than white EBN [23]. However, this special EBN has a hidden threat.

The nitrite content in EBNs of various colors was also examined. Figure 2 shows the nitrite levels from the lowest to the highest as follows: white, yellow, red, and orange EBN. Tukey HSD test revealed that the nitrite content in the yellow, red blood, and orange EBNs was significantly higher by 30 folds compared with that in white EBN. Moreover, orange EBN contained the highest nitrite level for this group. The nitrite level in the orange EBN group was not significantly different from that in the yellow and red blood EBN groups.

This study compared nitrite concentrations in EBNs of different colors obtained from five swiftlet farmhouses. Results showed that orange EBN had higher nitrite concentration than yellow and red blood EBNs; however, the difference was not statistically significant. This work also found that the yellow, orange, or red blood EBN had significantly higher nitrite yield than white EBN. This finding agreed with previous works [6, 7, 17, 20]. Therefore, yellow, orange, or red blood EBN should not be traded to China because of their high nitrite concentrations. In 2011, Chinese authorities reported nitrite contamination in Zhejiang Province, thus raising public concern about the safety of EBN consumption. The highest reported nitrite concentration in red blood EBN has reached 11 000 ppm, which led to the immediate ban on importing EBNs [23].

The color of cave EBN is associated with its nitrite and nitrate contents [6]. Several studies have conducted tests in these types of EBN, particularly red blood EBN. Another work [22] reported that the Fe ion oxidation in AMCase-like protein plays remarkably in EBN color change. The other factor affecting the color change of EBN is the nitration of tyrosyl residue to the 3-nitrotyrosyl (3-NTyr) residue in the glycoprotein [21]. 3-Ntyr acts as an indicator of the color change from yellow to red in acid to red in alkali occurring at around pH 7. As a result of nitrite accumulation, EBN changes its color from white to yellow to orange to red [19]. Thus, environmental conditions play a significant role in the dark color formation of EBN. The elevated nitrite levels on EBN may be due to the contaminating nitrate and microbial nitrate reductase from the environment.

This study suggested that color is an indicator of the nitrite level in EBNs. The high nitrite concentrations in dark EBNs (yellow, orange, and red blood EBN) can be a hidden threat to human health. In addition, this work successfully proved that dark EBNs from Indonesia have high nitrite levels, and not all EBNs are good for human consumption due to their nitrite levels. Therefore, nitrite concentration for each batch must be controlled at the industrial level. The results highlight the concerns over nitrite concentration in EBNs as a novel food for human consumption and provide information for future research in the food industry.

Conclusion

Yellow, orange, and red blood EBNs have higher nitrite concentrations than white EBN and thus require additional treatment to reach the quality standards. Despite the nutrition advantages of these types of EBN, the toxication risk of their high nitrite content can threaten public health. Good nitrite control in the EBN industry may mitigate this risk.

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