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Red vs. White Edible Bird's Nests: Oxidative Processing Drives Nitrite Disparity and Food Safety Risks

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ABSTRACT

This study investigates nitrite concentration disparities between red and white edible bird's nests (EBNs), driven by oxidative processes linked to post-harvest washing protocols. Utilizing a randomized block design, ten EBN samples (five red, five white) were collected from a processing facility in Sidoarjo, Indonesia. Red EBNs were sourced from discontinued stock, while white EBNs were obtained from current production batches. Nitrite levels were quantified using UV-Vis spectrophotometry (541 nm), with statistical analysis performed through two-way ANOVA ($\alpha = 0.05$) and effect size calculations (Cohen's *d*). Results revealed a 5.7-fold higher nitrite concentration in red EBNs (88.87 ± 12.42 ppm) compared to white EBNs (15.48 ± 4.44 ppm; *p* < 0.05), with a very large effect size (Cohen's *d* = 6.24) and 93% variance explained by nest type ($\eta^2 = 0.93$). The oxidative degradation of tyrosine residues during intensive washing, coupled with iron-mediated catalysis and environmental factors, was identified as the primary driver of nitrite accumulation in red nests. Despite compliance with Indonesia's safety threshold (200 ppm), the elevated nitrite levels raise concerns about nitrosamine formation in acidic gastric environments, necessitating mitigation strategies such as polyphenol integration and optimized drying protocols. This study highlights the critical influence of processing practices on nitrite dynamics and underscores the need for standardized protocols to align with stringent international standards. Future research should address endogenous nitrite sources, microbial contributions, and environmental parameter impacts to enhance food safety and industry sustainability.

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Introduction

Edible bird's nest (EBN) derived from *Collocalia fuciphaga* represents a high-value commodity in Asia, particularly in Indonesia which contributes approximately 80% of global production (Widyastuti et al., 2024). These nests are widely utilized in traditional Chinese medicine and as functional food ingredients due to their rich protein content, antioxidants, and essential amino acids (Chua et al., 2021; Rahmawati et al., 2022). International market demand has shown a consistent upward trajectory, increasing from 405.3 tons in 2012 to 1,335.0 tons in 2023, with a market value of USD 474.34 per ton (BPS, 2023). However, this growing demand has led to processing practices that may introduce chemical contaminants, particularly nitrite compounds (NO_2^-) (Quek et al., 2015).

Nitrite represents a hazardous compound that can form naturally or through chemical processes during drying, cleaning, or storage of EBN. This substance has the potential to form nitrosamines - classified as Group 1 carcinogens by IARC - when reacting with secondary amines in acidic environments such as the stomach (pH 1.5-5). The acidic conditions convert nitrite into nitrosating agents (NO^+ and N_2O_3) that catalytically form N-N=O bonds with amines (Breider et al., 2018; Cioc et al., 2023). This process is accelerated by natural catalysts in the digestive tract, including gastric micelles that reduce amine pKa values, thereby increasing nitrosation rates by up to 300% (Gonta, 2020). Furthermore, sunlight exposure during EBN processing may trigger similar reactions between nitrite and dimethylamine compounds (Yang et al., 2024). The Indonesian National Agency of Drug and Food Control (BPOM) has established a maximum nitrite limit of 200 mg/kg for food products (BPOM Regulation, 2012), as excessive consumption may lead to methemoglobinemia, digestive disorders, and increased risk of colorectal cancer (Green, 2023; Robles, 2024). Despite these concerns, studies on nitrite levels in EBN remain limited, particularly for red nests which undergo more intensive oxidation and environmental exposure during processing.

Previous EBN research has primarily focused on nutritional analysis, including protein content (38-63%) and minerals using Kjeldahl methods (Lestari & Pratama, 2021), as well as microbial contaminants (Ibrahim et al., 2021). However, studies on chemical contaminants, particularly nitrite, remain scarce and

have been limited to either white or red nests without comparative analysis of contamination levels. The distinct cleaning and processing methods between red and white nests may significantly influence nitrite accumulation, necessitating further investigation using more accurate UV-Vis methods to ensure compliance with food safety standards (Aminullah et al., 2023; Yeo et al., 2023).

This study aims to analyze nitrite content in both nest types using UV-Vis spectrophotometry. Beyond addressing the research gap regarding comparative nitrite levels between red and white nests, the findings are expected to provide valuable references for producers to optimize processing methods and ensure product safety. The novelty of this research lies in its comparative approach between two nest types - previously unexplored - and the application of UV-Vis methodology which offers superior precision compared to prior studies.

Method

Study Design

This study utilized a randomized block design (RBD) to systematically evaluate nitrite concentration disparities between red and white edible bird's nests (EBNs). The experimental framework was structured to control for confounding variables inherent to nest heterogeneity, such as geographical origin, harvesting time, and post-harvest handling. Samples were grouped into homogeneous blocks based on processing batches (n = 5 red and n = 5 white EBNs per block), with randomization applied within each block to minimize bias and isolate the effect of nest type (red vs. white) on nitrite accumulation.

Sample Population

Ten EBN samples (five red and five white) were collected from a processing company in Sidoarjo Regency, East Java, Indonesia. Red EBNs (25 g each) were sourced from discontinued stock, while white EBNs (5–6 g per piece) were obtained from current production batches. All samples originated from swiftlet houses in Kalimantan and underwent a standardized single-wash protocol at PT.XYZ. Inclusion criteria required moderate feather cleanliness and uniform weight. Samples were randomized using simple random sampling to ensure representativeness and stored at 4°C in laboratory refrigerators (Thipwimonmas et al., 2021).

Data Collection and Instrumentation

Nitrite content was quantified via spectrophotometry following AOAC Method 993.30 (AOAC, 1990). Samples were homogenized, dissolved in 50 mL volumetric flasks, and ultrasonicated at 40°C for 30 minutes after adding saturated NaCl (3 mL). The filtered solution was diluted to 10 mL, followed by sequential addition of 0.5 mL sulfanilamide (5-minute incubation) and 0.5 mL N-(1-naphthyl) ethylenediamine dihydrochloride (NED; 15-minute incubation). Absorbance was measured at 541 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). Calibration curves were constructed from nitrite standard solutions (0–1.0 ppm), and concentrations were calculated using the formula:

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

where C = nitrite concentration from the calibration curve ($\mu g \cdot L^{-1} \mu g \cdot L^{-1}$), V = solvent volume (mL), and W = sample weight (g).

Data Analysis

Statistical differences in nitrite levels between red and white EBNs were assessed using two-way ANOVA (α=0.05α=0.05) in RStudio (Version 2025.05.0+496, Posit, Boston, USA). Effect sizes (Cohen's *d*) and variance proportions ($\eta 2 \eta 2$) were computed to evaluate the magnitude of nest type influence.

Results

The visual comparison of red and white edible bird's nests (EBNs) highlights a distinct oxidative color disparity, primarily attributed to variations in post-harvest washing protocols. **Figure 1** demonstrates the pronounced reddish hue of processed red EBNs, which arises from oxidative degradation of tyrosine residues in mucin glycoproteins during prolonged or intensive washing

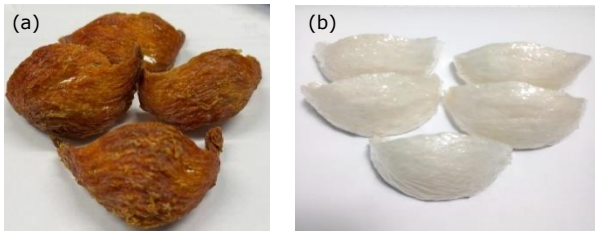

Figure 1. Visual comparison between red edible bird's nests (a) and white (b)

This study revealed (Table 1) a significant difference (*p* < 0.05) in nitrite levels between red and white edible bird's nests (EBNs), with red nests exhibiting markedly higher mean nitrite concentrations (88.87 ± 12.42 ppm) compared to white nests (15.48 ± 4.44 ppm). The Cohen's *d* value of 6.24 (95% CI [5.12, 7.36]) confirmed a very large effect size, far exceeding Cohen's threshold for a "large effect" (*d* ≥ 0.8). This indicates that 93% of the variance in nitrite levels ($\eta^2 = 0.93$) can be attributed to nest type, underscoring the dominant role of intrinsic or extrinsic factors associated with red nests in nitrite accumulation.

Table 1. Nitrite Concentrations and Cohen's d Effect Size in Red vs. White Edible Bird's Nests (EBNs)

Nest Type	Nitrite (ppm)	Cohen's d [95% CI]
Red	88.87 ± 12.42a	6.24 [5.12, 7.36]
White	15.48 ± 4.44b	-

Note: Means with different superscript letters indicate statistically significant differences ($p < 0.05$)

Discussion

The elevated nitrite levels in red nests may be explained by interconnected mechanisms. The nitration of tyrosine residues in mucin glycoproteins to form 3-nitrotyrosine not only contributes to the characteristic red pigmentation but also correlates with nitrite accumulation (Shim & Lee, 2018). Recent findings reporting nitrite concentrations up to 309 ppm in natural red nests (Ningrum et al., 2022) suggest that this biochemical process may be more intensive than observed in the current study.

Environmental factors also play a critical role. Cave conditions, characterized by high humidity (85–95%) and specific mineral content, create an environment conducive to natural nitrification (Kumpook et al., 2023). However, artificial processing practices, such as exposing white nests to nitric acid vapor to enhance market value, further elevate nitrite levels, explaining the greater data variability in red nests (Kumpook et al., 2023). Additionally, the higher iron content in red nests acts as a catalyst, facilitating the conversion of nitrate to nitrite (Kumpook et al., 2023). The synergistic interaction of biochemical processes, environmental conditions, and mineral composition collectively accounts for the observed nitrite levels and variability in red nests.

Although red nest nitrite levels remain below Indonesia's BPOM safety threshold (200 ppm), the substantial effect size (*d* = 6.24) and 5.7-fold mean difference raise concerns about the cumulative risk of nitrosamine formation. Nitrite can react with secondary amines in the acidic gastric environment (pH 1.5–5) to form N-nitroso compounds—classified as Group 1 carcinogens by the IARC (Niklas et al., 2023; Ren et al., 2024; Shi, 2022). This process is exacerbated by heat exposure during food processing, which promotes pre-ingestive nitrosamine formation (Bonifacie et al., 2021; Niklas et al., 2023; Xie et al., 2023), and salivary nitrite contributions during digestion (Niklas et al., 2023).

Mitigation strategies, such as incorporating polyphenols (e.g., catechin, gallic acid) to neutralize nitrite or inhibit nitrosation (Ren et al., 2024), highlight the urgency of integrating natural antioxidants into red nest production. Optimizing drying parameters to minimize nitrite accumulation and standardizing processing protocols, particularly given the catalytic role of iron in red nests (Kumpook et al., 2023), are critical. Future standards must address not only nitrite limits but also potential nitrite-amine interactions during storage and consumption.

Study Limitations and Future Directions

This study did not comprehensively analyze nitrite sources (endogenous vs. environmental) and focused solely on two nest types. Endogenous nitrite, produced via nitrifying bacterial activity (*Nitrosomonas* and *Nitrobacter*) in nest environments and swiftlet salivary glands, significantly contributes to nitrite accumulation (Widiyani et al., 2021). Environmental factors, including humidity, sanitation, and nitrogen compound exposure, further enhance substrate availability for bacterial conversion (Widiyani et al., 2021; Afani et al., 2024). The extreme variability in nitrite levels (5.7–843.8 µg/g) between cave and house nests (Quek et al., 2015), coupled with the positive correlation between darker/redder hues and nitrite content (Quek et al., 2015; Hudaya et al., 2023), necessitates further investigation. Future research should: (1) explore endogenous nitrite biosynthesis mechanisms, including the role of commensal microbial nitrate reductase (*Nitrosomonas* spp.); (2) assess the impact of environmental parameters (pH, temperature, humidity) on nitrite accumulation, building on Afani et al., (2024)'s findings linking sanitation to nitrification substrates; and (3) map correlations among nitrite levels, nitrate reductase-producing microbial activity, and nest color as a visual contamination indicator (Hudaya et al., 2023). Addressing these gaps is essential to meet export safety standards (Widiyani et al., 2021; Kusumawati & Kurniawan, 2024) and enhance product competitiveness through targeted risk mitigation.This section is also a major part of the research articles and is also usually the longest part of an article. Discussion of the research presented in this section are the result. The process of data analysis such as statistical calculations or other processes for the achievement of its research. Please present the discussion narratively.

Conclusion

Red edible bird's nests (EBNs) exhibit 5.7-fold higher nitrite levels than white EBNs (88.87 ± 12.42 vs. 15.48 ± 4.44 ppm; *p* < 0.05), driven by tyrosine nitration, cave conditions, processing practices, and iron catalysis. Despite meeting Indonesia's 200 ppm safety limit, elevated nitrite raises nitrosamine risks via gastric interactions and heat exposure, necessitating mitigation strategies like polyphenol additives and optimized drying. Future research should clarify endogenous nitrite sources, environmental influences (pH, humidity), and microbial links to color. Addressing these gaps is critical to meet stricter international standards (e.g., China's 30 ppm) and ensure industry sustainability through revised safety protocols and collaborative policy action.

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