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## The Potency of *Citrus aurantiifolia* Swingle and Sea Salt Solution as a Cleansing Agent for Edible Bird's Nests

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### Abstract

The present study aimed to evaluate the potency of *Citrus aurantiifolia* Swingle and sea salt solution as a promising cleansing agent for edible bird's nests (EBNs). Ascorbic acid, sodium, and chloride analyses of the *C. aurantiifolia* Swingle and sea salt solution were conducted using HPLC, ICP-OES, and titrimetry, respectively. The characteristics of physical samples and hydrogen peroxide detection were examined in this study. The reduction activity of the *Citrus aurantiifolia* Swingle and sea salt solution on the nitrite of 120 white EBNs was studied. This nitrite reduction activity was compared with the available method (standard), drinking water method, 6% *C. aurantiifolia* Swingle extraction method, and sea salt water. An organoleptic test was performed by three trained panelists to determine the color of the samples before and after treatments. The results showed that the *C. aurantiifolia* Swingle and sea salt solution contained 0.27 mg/100 mL of ascorbic acid, 76370.93 mg/L of sodium, and 7555.61 mg/100 mL of chloride. In comparison results, the *C. aurantiifolia* Swingle and sea salt solution can reduce nitrite levels up to 86%, remove hydrogen peroxide, and improve the natural color of EBNs. The present study provides the first potential future cleansing agent for EBNs.

**Keywords:** animal food origin, food safety, veterinary public health

### Introduction

Indonesia is well known as a leading country in edible bird's nest (EBN) production [1]. Despite the high price of EBN products, demand for this animal product is increasing in the international market because of public trust in its efficacy [2]. In general, the Chinese community believes that EBN can preserve immunity against Covid-19 [3]. To meet the high demand, Indonesian trading must meet the bare minimum requirements of EBN importing countries. The nitrite content of EBNs shipped to China, in particular, must not exceed 30 ppm [4]. This requirement is in place because a high nitrite content of animal-derived food products can endanger consumer health. High nitrite levels in humans can cause toxic reactions, such as nausea and vomiting, and long-term nitrite consumption can cause cancer [5–7].

The primary issue in the EBN sector is that uncleaned EBNs are of poor quality and include high nitrite levels [8]. Nitrite can be found in EBNs because of bird droppings and contamination from the swiftlet house's high humidity. To meet export demands for EBNs, EBN industries implemented a hazard analysis and critical control points (HACCP) plan to lower nitrite levels in EBNs. The previous procedure can reduce the nitrite

level of EBNs by no more than 29.93% [9]. However, this technique is still applied at the critical control point in the HACCP plan for the EBNs' cleaning process. In addition, to eliminate dirt and brighten the color of EBNs, hazardous agents such as hydrogen peroxide are increasing in EBN cleaning processing [10].

Ascorbic acid has been shown to lower nitrite levels in EBNs by up to 88.89% in white EBNs [11]. Furthermore, salt is effective in washing vegetables and fruits [12] and removing pesticide residues from tomatoes [13]. *Citrus aurantiifolia* Swingle is high in vitamin C, with ascorbic acid levels reaching 35.02 mg/100 g [14]. Using a combination of *Citrus aurantiifolia* Swingle and sea salt, the current study refines the EBN cleaning method to provide an effective solution for the EBN industry. Thus, this study evaluates the nitrite reduction potential of these natural ingredients. We propose that the abovementioned combination could be developed as a food-grade cleanser for EBNs, thereby avoiding hazardous agents in its processing.

### Materials and Methods

**Sample preparation.** This study's sample size was determined by Slovin's formula (Table S1). The total number of samples utilized in this study was 120 white EBNs

with heavy feathers. All samples were gathered at a warehouse in Indonesia where the EBN was sourced from swiftlet houses. Initially, all feathers from the samples were removed using tweezers. Following the standard SNI 8998:2021 procedure, the characteristics of physical samples were determined based on their color, size, and moisture content (Table S2) [15].

**Hydrogen peroxide contamination test.** The procedure for detecting hydrogen peroxide was adapted from a previous study [16]. The presence of hydrogen peroxide in the samples was detected using Quantofix® Peroxide 25 (Macherey-Nagel, Germany). One Quantofix® Peroxide test strip was dipped in the sample solution for 1 s before being air-dried for 15 s. The results were read by comparing the test paper color to the indicator color (0–25 mg/L hydrogen peroxide).

***Citrus aurantiifolia* Swingle and sea salt solution and its composition.** A *Citrus aurantiifolia* Swingle and sea salt solution was formulated in the Veterinary Medicine Laboratory, Universitas Wijaya Kusuma Surabaya. According to a previous study [17], ascorbic acid was extracted from the fruit of *C. aurantiifolia* Swingle. Bali sea salt was dissolved in an aquadest until it reached saturation. The fruit extractant of *C. aurantiifolia* was added to the saturated sea salt solution until it reached a maximum of 6% final concentration (pH 1.96). A total of 9 mL metaphosphoric acid was added to 1 mL *C. aurantiifolia* Swingle–sea salt solution, and this mixture was sonicated for 15 min. The solution was extracted in an Eppendorf tube and centrifuged at  $4500 \times g$  for 10 min. An aliquot

was passed through a 0.45  $\mu$ L syringe filter and injected into the HPLC system to analyze ascorbic acid [18]. For sodium analysis, a volume of 10 mL concentrated nitric acid ( $\text{HNO}_3$ ) was added to 1 mL samples and heated at  $150^\circ\text{C}$  for 15 min in microwave digestion. All samples were transferred to a 50 mL flask after digestion, and 0.5 mL yttrium internal standard (100 mg/L) was added. Samples were diluted to the final volume with deionized  $\text{H}_2\text{O}$ . The solutions were filtered using filter paper and analyzed at a wavelength of 568.8 nm using inductively coupled plasma optical emission spectroscopy (ICP-OES) [19]. Finally, the determination of chloride in *C. aurantiifolia* Swingle–sea salt solutions was performed through the titrimetric method according to SNI 3556:2016 [20].

**Comparison with the available method.** The *Citrus aurantiifolia* Swingle and sea salt solution technique was compared to the available washing method using 30 s of running water (8). In this work, additional methods were used to determine the nitrite reduction activity of water or *C. aurantiifolia* Swingle extract. The designed cleaning protocols are listed in Table 1. A total of 24 EBNs without cleaning treatment were used as the negative control. Three repetitions were applied in this study.

**Organoleptic test.** Each sample was put on a cleaned, dried glass and examined with the naked eye. Three panelists trained for organoleptic testing visually observed the samples. Changes in sample color after treatment were observed according to the standard SNI 8998:2021 method [15].

**Table 1. Designed Cleaning Protocols for Edible Bird's Nests (EBN)**

Methods	Protocol	Duration	References
Running water (positive control)	A total of 24 EBNs is washed under running water	30 s	[9]
Drinking water	A total of 24 EBNs is soaked in drinking water and rinsed under running drinking water	Soaking: 10 min Rinsing: 15 s	The present study
6% of <i>C. aurantiifolia</i> Swingle extract	A total of 24 EBNs is soaked in <i>C. aurantiifolia</i> Swingle extract and rinsed under running drinking water	Soaking: 10 min Rinsing: 15 s	The present study
Sea salt water	A total of 24 EBNs is soaked in sea salt water and rinsed under running drinking water	Soaking: 10 min Rinsing: 15 s	The present study
<i>C. aurantiifolia</i> Swingle and sea salt solution	A total of 24 EBNs is soaked in <i>C. aurantiifolia</i> Swingle and sea salt solution and rinsed under running drinking water	Soaking: 10 min Rinsing: 15 s	The present study

**Nitrite measurement.** According to a previous study [21–25], nitrite measurement was performed using spectrophotometry. The standard curves were prepared by dissolving standard nitrite solution (1 ppm) in 0.6 mL saturated sodium chloride solution (Merck, Germany), 0.5 mL sulfanilamide solution (Merck, Germany), 0.5 mL naphthyl ethylene diamine solution (Merck, Germany), and distilled water to six concentration levels (0 g/L, 0.2 g/L, 0.3 g/L, 0.4 g/L, 0.5 g/L, and 0.6 g/L). The standard solution was allowed to stand for 15 min and then put into a cuvette. Nitrite levels were evaluated using Genesys 30 visible spectrophotometer (Thermo Scientific, USA) with a wavelength of 541 nm. All samples were checked for nitrite before and after treatment using the spectrophotometer. A total 1 g of each sample was mortally ground until the sample size became fine. As much as 0.5 mg of each sample was added to 3 mL of saturated NaCl solution (Merck, Germany). Then, up to 50 mL distilled water was added to the solution for. All samples were sonicated using Elmasonic S 30 H (Elma, Germany) at 40 °C for 30 minutes. The samples were homogenized every 5 min to avoid sedimentation. Then, the mixture was removed from the sonicator and placed at room temperature until it cools down. The solution was filtered with Whatman filter paper No. 42 (GE Healthcare, Germany). A total of 1 mL of each extracted sample was measured for nitrite content using a Genesys 30 visible spectrophotometer (Thermo Scientific, USA) at a wavelength of 541 nm.

**Statistical analysis.** The ANOVA test was used to compare nitrite levels across treatments statistically, and Duncan's test was used to determine if there was a significant difference ( $p < 0.05$ ). The Tukey test analyzed data comparing nitrite levels before and after treatment. All statistical analysis data in this study were processed using SPSS 23.0.

## Results and Discussion

Nitrite is naturally present in EBNs. However, inadequate swiftlet house management practices generally produce EBNs with high nitrite contents [26]. Because nitrite is known to cause cancer if consumed long-term, a proper nitrite reduction method should be applied in the EBN industry. This study's newly designed cleaning method provides a food-grade cleanser to reduce the nitrite level of white EBNs. This simple approach provides for the hygiene and safety of related products, making it an effective and reasonable cleanser of EBNs. Heavy feather white EBN is deemed to have a greater level of nitrite, the most important component that could provide a risk in EBNs [8]. In this study, 120 samples of EBNs were evaluated for their nitrite concentration and the presence of hydrogen peroxide. The results showed nitrite levels above 100 ppm in all samples (Table 2 and Figure 1). The previous method's highest capacity is only

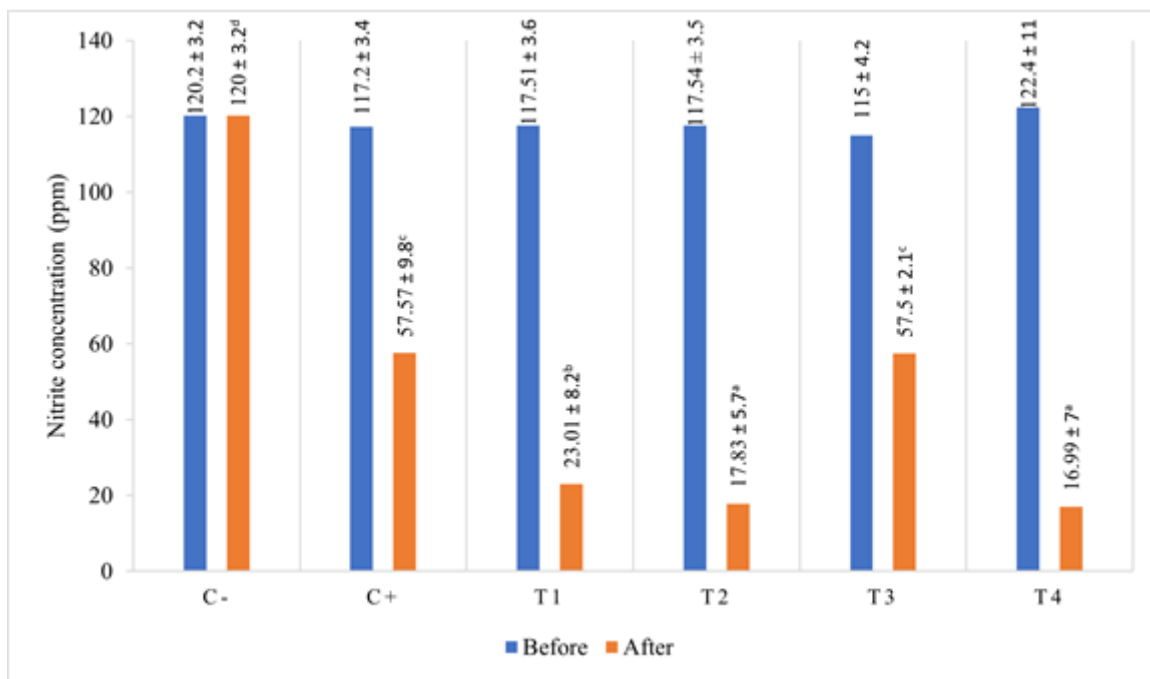
a 29.93% reduction in nitrite concentration in EBNs. This result indicates that the nitrite content is still high, 70.07 ppm, after cleaning with the available approach. This result is still far above the intended value of 30 ppm set by Indonesian regulations. We propose a cleaning method using a *C. aurantiifolia* Swingle and sea salt solution in this study. We find a substantial reduction of up to 86% in nitrite levels once *C. aurantiifolia* is applied to the treatments (Table 2 and Figure 1). Ascorbic acid from *C. aurantiifolia* Swingle in the solution (Table 3) substantially contributes to nitrite reduction by converting nitrite acid in EBNs to nitrite oxide. Ascorbic acid has been reported to decrease nitrite activity in EBNs [11] and meat products [27].

In addition, nitrite can cause discoloration in EBNs. The nitrite concentration of white EBNs is often lower than that of darker-colored EBNs [21]. In the EBN industry, a quality controller considers a product's color in determining whether it will be accepted. The most common color sold on the market is white [28]. Some manufacturers also sell bright yellow, orange, and red; however, the most popular color is white. An EBN is more expensive the whiter it is [29]. The adulteration of EBNs, including bleaching, poses a rising hazard to human health. Using bleaching agents such as hydrogen peroxide on an EBN has been prohibited. Although an earlier study [16] found no occurrences of hydrogen peroxide in cleaned EBNs, the present study detected hydrogen peroxide in 40 samples of uncleaned EBNs or raw materials (Table S2). Surprisingly, the proposed cleanser successfully removed all hydrogen peroxide in the samples (Table 4). The process for removing hydrogen peroxide can be performed in the presence of inorganic cations such as sodium, potassium, magnesium, and calcium [30]. Additionally, we added sea salt to the proposed liquid. We suggest that the *C. aurantiifolia* Swingle and sea salt solution could be a promising cleanser agent for hydrogen peroxide removal.

Because discoloration is an important issue in the EBN market, we performed an organoleptic test to compare the performance of the *C. aurantiifolia* Swingle and sea salt solution to those of other procedures. The best outcome was obtained by the *C. aurantiifolia* Swingle and sea salt solution. This treatment resulted in a brighter color for the EBN than other treatments (Figure 2). In this work, the reduction nitrite activity of *C. aurantiifolia* Swingle's ascorbic acid improved the natural color of EBNs. We suggest that using sea salt brings about unsubstantial nitrite reduction (Figure 2). Nonetheless, the use of sea salt can enhance the effectiveness of this solution for cleaning the dirt and sand that usually adheres to EBNs. Salt is used as a cleaning agent in vegetables and fruits [12] and tomatoes [13] but never in EBNs. We proposed combining *C. aurantiifolia* Swingle and sea salt to improve the natural color of white EBNs.

**Table 2. Comparison of Reduction Activity for Each Treatment of White Edible Bird’s Nests (N = 120)**

Treatments	Average Nitrite Concentration Before Treatment (ppm per g of Sample)	Average Nitrite Concentration After Treatment (ppm per g of Sample)	Reduction Activity (%)
Running water (Positive control)	117.12	57.57	51
Drinking water	117.51	23.01	80
6% <i>C. aurantiifolia</i> Swingle extract	117.54	17.83	85
Sea salt water	115.00	57.50	50
<i>C. aurantiifolia</i> Swingle and sea salt solution	122.39	16.99	86



**Figure 1. Results of the Nitrite Reduction of Edible Bird’s Nest Samples (N = 120) Before and After Treatments. C-: Negative Control (No Washing Treatment), C+: Standard Method, T1: Modified Method With Drinking Water, T2: Modified Method with *C. aurantiifolia* Swingle, T3: Modified Method with Sea Salt Water, T4: Modified Method with the *C. aurantiifolia* Swingle and Sea Salt Solution**

**Table 3. Composition of the *C. aurantiifolia* Swingle and Sea Salt Solution)**

Compound	Average Concentration
Ascorbic acid	0.27 mg/100 mL
Chloride	7555.61 mg/100 mL
Sodium	76370.93 mg/L

**Table 4. Hydrogen Peroxide Test Results (N = 40)**

Treatments	Positive Results	
	Before	After
6% <i>C. aurantiifolia</i> Swingle extract	20	20
<i>C. aurantiifolia</i> Swingle and sea salt solution	20	0



**Figure 2. Color Observation Results of Tested EBNS. A: Negative Control (No Washing Treatment), B: Standard Method, C: Modified Method with Drinking Water, D: Modified Method with *C. aurantiifolia* Swingle, E: Modified Method with Sea Salt Water, F: Modified Method with the *C. aurantiifolia* Swingle and Sea Salt Solution**

## Conclusion

The *C. aurantiifolia* Swingle and sea salt solution can reduce nitrite levels in the white EBNS by up to 86%, remove hydrogen peroxide, and improve the natural color of this animal product origin. The *C. aurantiifolia* Swingle and sea salt solution is the first promising cleansing agent for EBNS.

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## Data Availability Statements

Data are available upon request from the corresponding author. The data are not publicly available because of privacy from the EBN processing partner, which allowed the project to be conducted within their facility.

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