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THE EFFECT OF ASCORBIC ACID ON NITRITE AND PEROXIDE LEVELS AND NUTRITIONAL CONTENT OF EDIBLE SWIFTLET'S NEST

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ABSTRACT

Edible Swiftlet's Nest (ESN) is one of the highest-value export commodities. However, these commodities often contain the chemical compounds nitrite and H_2O_2, which are toxic and dangerous. This fatal impact made Indonesia create a standard, as stated in SNI 8998:2021, which requires a maximum nitrite content of 80 mg/kg. To achieve this goal, it is important to standardize specific washing procedures that can significantly reduce nitrite and H_2O_2 levels. In this research, the effect of the washing method with the addition of ascorbic acid additives with various concentrations on the levels of nitrite, H_2O_2, and ESN nutrients was studied. The use of ascorbic acid 5% (w/v) was detected to produce the most significant reduction in nitrite (from 331.40 to 75.85 ppm). The use of ascorbic acid also succeeded in ensuring the absence of H_2O_2 and maintaining the nutritional content of ESN.

Keywords: Edible Swiftlet's Nest, Ascorbic Acid, Nitrite, Peroxide, Nutrition.

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INTRODUCTION

Indonesia is the largest exporter of Edible Swiftlet's Nest (ESN) in the world, which in 2020 exported 1312.5 tons of ESN worth USD 540.4 million.[1,2] There is a large demand for ESN exports because it is an herbal medicine and nutritious food with important benefits such as immunoregulation, antiviral, anti-inflammatory, antioxidant, and neuroprotective. These advantages make ESNs have high economic value.[3] ESN has a variety of colors, from white to black. However, brown or black ESNs are rarely in demand due to the high level of foreign matter contamination.[4] To improve its quality, ESN is generally treated with hydrogen peroxide (H_2O_2). Even though this material is toxic and is suspected to be the main source of reactive free radicals that cause neurodegenerative diseases.[5,6] In addition to color, ESN producers are faced with food safety demands regarding nitrite levels in ESN. In this regard, Indonesia requires a maximum nitrite content of 80 mg/kg, as stated in SNI 8998:2021. Reducing nitrite levels using running water accompanied by drying with cold air has a weakness in that this technology requires a long time to process and still leaves water vapor, which has the potential to store dissolved nitrite in the ESN. Instead, treatment using ascorbic acid appears to be a more promising alternative. Ascorbic acid is a nitrite-reducing agent because it can convert nitrites to nitric oxide. This research uses an alternative method of washing ESN using ascorbic acid, which aims to reduce nitrite and peroxide levels in ESN. Washing ESN with ascorbic acid can reduce nitrite levels because ascorbic acid is a nitrite-reducing agent because it can convert nitrite into nitric oxide. Apart from nitrites, ascorbic acid can also trigger the decomposition of hydrogen peroxide into compounds that are more environmentally friendly.[7]

EXPERIMENTAL

Materials

The ESN used was obtained from CV. Dwi Anugrah Surabaya, Indonesia. The materials used were N-1-naphthylenediamine dihydrochloride (NED), sulphanilamide solution, ascorbic acid (Merck, Germany), CH_3COOH (≥99%; Sigma Aldrich, USA), sodium nitrite (Sigma Aldrich, USA), and deionized water (Hach, Singapore). The equipment and instruments used were a Shimadzu UV-1700 UV-Vis spectrophotometer as well as the Quantofix® 91311 nitrite test kit and 91319 Peroxide test kit (Macherey-Nagel, Germany).

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ESN Preparation

ESN washing follows the "Dwi Anugrah Surabaya" procedure, which includes wetting and cleaning. ESN is immersed in water for 2 minutes to make it bloom. ESN was cleaned of attached feathers and shell fragments. Clean ESN samples were printed like bowls and dried to a moisture content of 6%. The same procedure was applied for washing with ascorbic acid additives 1; 2; 3; 4; and 5% (w/v).

Nitrite Analysis

The nitrite content was analyzed using the spectrophotometric method[8], following the procedure of Ratnarathorn and Dungchai (2020).[9] The sample solution was prepared by dissolving nitrite in 0.5 g of ESN in 20 mL of distilled water (ratio 1:40 (w/v)). The solution was heated at 80 °C for 30 minutes, centrifuged at 3500 rpm for 15 minutes, and then filtered. 2.5 mL of sulfanilamide and NED were added to it. The solution is stirred until homogeneous. The solution was analyzed at the same wavelength and compared with the results of the Quantofix® 91311 Nitrite Test Strip.

Peroxide Analysis

Peroxide analysis using the spectrophotometric method with regard to the procedure Santana *et al.* (2021).[10] For sample analysis, a 100 mL volumetric flask was filled with 80 mL of ESN H_2O_2 solution. Successively, 1 mL of sodium hexametaphosphate, 1 mL of cobalt, and 1 mL of saturated sodium bicarbonate solution were added until 100 mL of the mixture was obtained. The sample H_2O_2 concentration was calculated using the regression curve equation and multiplied by the five-fourth dilution factor (1.25). The results of the spectrophotometric analysis were compared with the results of the analysis using Quantofix® 91319.

Nutrient Analysis

Analysis of the nutritional content of ESN, without and with ascorbic acid additives, was carried out according to the procedures of Ibrahim *et al.* (2021).[11] Moisture and ash content, protein, carbohydrate, and ESN fat were analyzed using gravimetric, kjeldahl, iodometric, and soxhlet extraction methods respectively.

RESULTS AND DISCUSSION

Nitrite Levels

The results of the analysis using UV-Vis spectrophotometry showed a decrease in the ESN nitrite from 331.40 ppm to 223.38 ppm after treatment with 1% (w/v) ascorbic acid. Use of 2% ascorbic acid, 3%, 4%, and 5% (w/v) respectively also showed a decrease in nitrite to 184.99, 164.70, 84.70, and 75.85 ppm. The same pattern of decreasing ESN nitrite levels was also shown from the results of the analysis using the kit, with a total reduction of up to 75% (see Table-1). Specifically, the low limit of detection (LOD) kit causes nitrite levels above 80 ppm to go undetected.

Table-1: Nitrite Levels in ESN Obtained Using Ascorbic Acid as Washing Solutions in Various Concentrations

Sample	Nitrite Concentration (ppm)		Sample	Nitrite Concentration (ppm)	
	UV-Vis	Strip Test		UV-Vis	Strip Test
ESN	331.40	Undetected	ESN + AA 3%	164.70	Undetected
ESN + AA 1%	223.38	Undetected	ESN + AA 4%	84.70	>80
ESN + AA 2%	184.99	Undetected	ESN + AA 5%	75.85	40 – 80

In this case, ascorbic acid acts as a reducing agent capable of donating electrons to nitrites to form nitric oxide.[12]

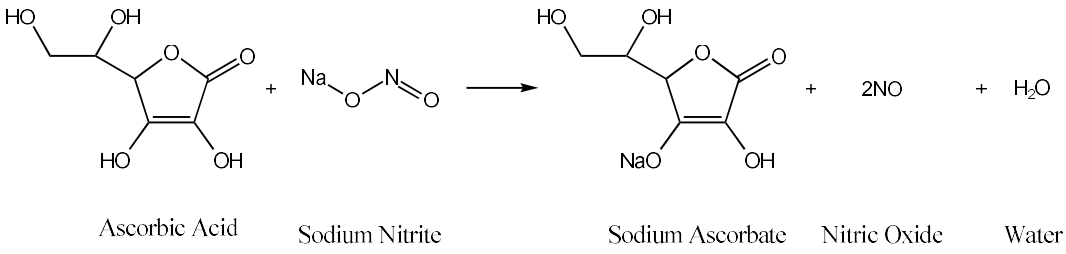

Fig.-1: The Chemical Reactions of Ascorbic Acid and Nitrite

H_2O_2 Levels

The results of the UV-Vis spectrophotometry analysis showed that the ESN H_2O_2 content was 0.51 ppm. In addition to nitrites, ESN washing with the addition of ascorbic acid additives also reduced ESN H_2O_2 levels. Successively, 1% and 2% (w/v) ascorbic acid treatments reduced H_2O_2 to 0.43 ppm and 0.15 ppm. The absence of H_2O_2 in the ESN was detected after treatment of the ESN with 3% to 5% (w/v) ascorbic acid (see Table-2).

Table-2: H_2O_2 Levels in ESN Obtained Using Ascorbic Acid as Washing Solutions in Various Concentrations

Sample	H_2O_2 Level (ppm)		Sample	H_2O_2 Level (ppm)	
	UV-Vis	Strip Test		UV-Vis	Strip Test
ESN	0.51	0 - 0.5	ESN + AA 3%	0	0
ESN + AA 1%	0.43	0 - 0.5	ESN + AA 4%	0	0
ESN + AA 2%	0.15	0 - 0.5	ESN + AA 5%	0	0

Ascorbic acid can reduce not only nitrites but also H_2O_2 in ESNs by forming the metabolite diketogulonate (see Fig.-2). The less stable dehydroascorbic acid reacts with H_2O to produce this metabolite.[13]

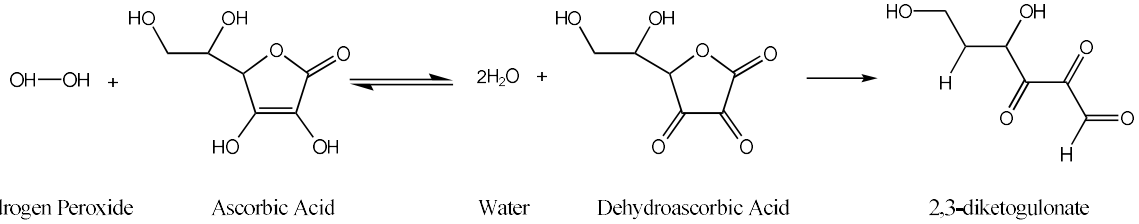

Fig.-2: The Chemical Reactions of Hydrogen Peroxide and Ascorbic Acid

Nutrients Levels

The results of the analysis of the ESN nutritional content, without and with ascorbic acid additives, are shown in Table-3. The water content of ESN without ascorbic acid treatment was 14.54%. This result is in line with that reported by Chu *et al.* (2023), namely 13.29±5.58%.[14] A significant difference was detected in the ash content of the ESNs in this study, i.e., 1.78%, which was lower than that reported by Cheng et al. (2021), which is 5.0%.[15] ESN carbohydrate content was also found to be different, which was much higher (59.96%) than reported by Zulkifli *et al.* (2019), which is 47.7±2.39%.[16] This result is thought to be closely related to sialic acid metabolism, which produces carbohydrates in the form of glycoproteins.[17] The protein and fat content of ESNs showed no significant differences from those reported by other researchers.[18] Most of the proteins in the ESN bind to glycans as glycoproteins in sialic acid metabolism, thus providing a correlation between carbohydrate and protein composition.[19] ESN protein content is strongly influenced by the type of feed involved in sialic acid metabolism.[20]

Table-3: Results of Analysis of Nutrient Levels on ESN

Test Parameters	ESN	ESN + Ascorbic Acid 5%
Moisture (%)	14.54	13.96
Ash (%)	3.22	3.55
Carbohydrate (%)	59.96	58.75
Protein (%)	22.08	23.30
Fat (%)	0.00	0.00

Analysis of the nutritional content of ESN samples after 5% ascorbic acid treatment showed results that were not much different. This shows that the ascorbic acid treatment had no significant effect on the nutritional content of ESN. The antioxidant properties of ascorbic acid provide protection against the sensory and nutritional properties of ESNs.[21,22]

CONCLUSION

Ascorbic acid treatment has been carried out to reduce nitrite and H_2O_2 ESN levels. Immersing in a 5% ascorbic acid solution lowers the nitrite level to 75.85 ppm and ensures the absence of H_2O_2 in the ESN, as

required by SNI 8998-2021. Furthermore, ESN treatment using ascorbic acid additives also did not affect the nutritional content of the product. These results show great potential for the use of ascorbic acid additives to improve the quality of Indonesian ESNs in the future.

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CONFLICT OF INTERESTS

There is no conflict of interest, according to the authors.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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