Research Article

Utilization of Ultrasonic Waves to Reduce Nitrite Levels in Edible Bird Nest

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Abstract | Indonesia is the world's largest producer and exporter of edible bird nest (EBN), with the leading destination being the People's Republic of China (PRC). The provision of nitrite levels is less than 30 ppm for raw cleaned EBN, so the EBN is washed to reduce it. The industry generally washes EBN with reverse osmosis (RO) water, but this method does not significantly reduce nitrite levels. Therefore, an alternative method is needed. This study aimed to analyze the effect of washing using RO water combined with ultrasonic waves to reduce nitrite level of EBN. The result of this study illustrated the effectiveness of reducing nitrite levels in EBN from two different methods. Nitrite levels in the samples were measured using a UV-Vis spectrophotometer. Utilizing an ultrasonic wave frequency of 40 kHz for 20 seconds could reduce nearly 80% of nitrite levels. It was considered more effective than standard methods, which were only able to reduce nitrite levels by up to 60%.

Keywords | Edible bird nest, Nitrite, Ultrasonic wave

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INTRODUCTION

Edible bird nest (EBN) is an export commodity with high economic value. Indonesia is the largest EBN producer country in the world (Kementan, 2020, 2021). IQFast system of the Agricultural Quarantine Agency showed that exported EBN commodities of Indonesia reached 1,500 tons in 2022, mainly to the People's Republic of China/PRC (Barantan, 2023). EBN export requirements to China are explained in the Hygiene, Quarantine, and Inspection Requirements Protocol for EBN Importation from Indonesia to PRC between the Ministry of Agriculture of the Republic of Indonesia and the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (Kementan, 2014b). The three main requirements include traceability, heating until the internal temperature of EBN reaches 70 °C for 3.5 sec, and a nitrite level of less than 30 ppm. Traceability intends to facilitate tracing if there is a discrepancy with the food safety guarantee. Heating intends to kill the Avian Influenza virus (Kementan, 2013).

Edible bird nest comes from the salivary secretions of swiftlets which contain mucins such as glycoproteins (Marcone, 2005). The saliva hardens, forming a half bowl that sticks to the wall (Utomo et al., 2015). Edible bird nest contains high-quality food substances that are believed to be able to overcome stomach problems, improve kidney function, increase libido, relieve asthma, cure tuberculosis, boost the body's immune system, speed up wound healing and surgery, increase metabolism, and improve concentration (Aswir and Wan, 2010; Hobbs, 2004; Lim et al., 2021; Ningrum, 2021; Roh et al., 2012; Saimah et al., 2016). The EBN has many benefits, which is the reason for China's high consumption rate.

Edible bird nest can contain nitrite from decaying organic matter on the floor. Nitrite is synthesized from ammonia by bacteria through anaerobic fermentation. Nitrate that enters the adult body, nearly 5-7%, can turn into nitrite. The conversion of nitrates to nitrites and nitrosamines is toxic to humans. The impact that is often caused is methemoglobinemia. International Agency for Research on Cancer confirms that nitrite toxicity is ten times higher than nitrate's and potentially triggers cancer in humans (Chamandoost et al., 2016; Nur and Suryani, 2012).

The nitrite requirements export to PRC as the main destination for the Indonesian EBN market needs to be responded to by adjusting the EBN harvesting method, which can threaten the sustainability of swiftlets and additional treatment in the washing process. Reverse Osmosis (RO) water is commonly used to wash the EBN, which is the standard method. Hydration technology processes RO water using a semipermeable membrane to remove ions, molecules, and particles (Ahuchaogu et al., 2018). A standard method is considered unable to reduce EBN nitrite levels effectively because the EBN nitrite levels are slightly above the requirements of the Government of PRC (Susilo et al., 2016).

Ultrasonic waves are sound waves with a frequency greater than 20 kHz. Its utilization is commonly used in medicine, marine, biology, physics, chemistry, pharmaceuticals, industry, etc. Ultrasonic waves can be used in sonar or cavitation principles. The EBN washing treatment utilizes ultrasonic waves using the cavitation principle (Pirngadi, 2017; Vernes et al., 2020). Some industries used ultrasonic only to clean EBN from dirt that sticks to the surface, but the nitrite reducing effect is still unknown. A suitable time and frequency for Ultrasonic wave utilization are expected to be an alternative treatment to effectively reduce EBN nitrite levels without reducing the quality and the safety guarantee. This study aimed to analyze the effect of washing using RO water combined with ultrasonic waves to reduce EBN nitrite levels.

MATERIALS AND METHODS

Sample preparation

EBN sampling was carried out by purposive sampling from the swiftlet farmhouses (SFHs) in West Sumatra Province, Indonesia. The characteristics of the selected sample are white swiftlet nests, bowl-shaped, weighing approximately 6-8 grams/sampel, light feather composition, originating from SFHs with the same harvest period and season. Samples were divided into two treatment groups. Group 1 was washed using the general method (M1), while Group 2 was washed using the ultrasonic wave method, referred to as the ultrasonic method (M2). Each washing method used seven samples from the same SFHs. The edges of the EBN attached to the fins are scraped using an iron grinder to remove coarse dirt such as wood chips or other adhering material. Each EBN chip was weighed and divided into two parts labeled with an identity. Furthermore, each part is washed by standard and ultrasonic methods.

Standard washing method

The procedure for standard washing method was adopted from previous study (Wahyuni et al., 2022). One part of the EBN sample was washed with running RO water for 10+2 seconds to remove more minor impurities and soften the texture of the EBN. The sample is then dried using a particular tissue for food processing (hygiene food processing) and air dried for 150+5 minutes. The next step is to clean the EBN from fine feathers and dirt using food-grade stainless steel tweezers and assisted with a soft brush. Samples were rinsed with running RO water for 15+2 seconds. Samples were dried again by aerating at room temperature for 120 minutes. Samples were soaked in 5.75 liters of RO water for 20 seconds and then samples were re-formed. The samples were air-dried again until the moisture content ranged from 10-12%. Samples were tested by spectrophotometric method to see their nitrite levels. Each sample at each stage is processed using different equipment to avoid cross-contamination.

Ultrasonic washing method

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Tabel 1: EBN nitrite levels in standard methods and ultrasonic methods.

Standard method				Ultrasonic method			
Sample code	Nitrite level before washed (ppm)	Nitrite level after washed (ppm)	Nitrite level reduction percentage (%)	Sample code	Nitrite level before washed (ppm)	Nitrite level after washed (ppm)	Nitrite level reduction percentage (%)
1	22.58	8.88	60.67	8	4.77	0.86	81.97
2	7.01	2.99	57.35	9	14.76	0.11	99.26
3	14.03	5.08	63.79	10	4.48	0.78	82.59
4	5.17	3.31	35.98	11	15.77	8.53	45.91
5	10.55	7.08	32.89	12	3.24	0.43	86.73
6	11.81	1.90	83.92	13	16.40	1.36	91.71
7	14.27	1.49	89.56	14	16.80	3.97	76.37
Mean±SD	12.20±5.70	4.39±2.76	60.59±19.90[a]	Mean±SD	10.89±6.34	2.29±3.03	80.65±15.74[b]

Note: [a, b] different superscripts in the same row indicate there was a significant difference between treatments (P<0.05).

Testing nitrite levels with a spectrophotometer

The nitrite testing method for EBN was adopted from the AOAC 973.31 method regarding determining nitrite in meat. The BBUSKP Animal Biosafety Laboratory carried out method validation for EBN testing and has been accredited ISO/IEC 17025:2017 by Komite Akreditasi Nasional (National Accreditation Committee). The preparation for EBN nitrite levels test with a spectrophotometer was started with preparing a standard nitrite solution. A total of 0.1 mL of 1000 µg/mL nitrite standard stock solution was pipetted and put into a 10 mL measuring flask. Then, deionized water was added to the tera mark and homogenized to obtain a 10 µg/mL standard nitrite solution. Standard working nitrite solution was prepared by taking 0; 0.2; 0.3; 0.4; 0.5; 0.6, and 0.7 mL of 10 µg/mL nitrite standard solution, then each was put into a 10 mL measuring flask. A total of 0.6 mL of saturated sodium chloride solution was added to each measuring flask, then deionized water was added until the tera mark and homogenized, then a standard 0 nitrite solution was obtained; 0.2; 0.3; 0.4; 0.5; 0.6 and 0.7 µg/mL. For each standard solution was added 0.5 mL of sulfanilamide solution. After 5 minutes, 0.5 mL of naphthyl-ethylenediamine solution was added. After 15 minutes, the absorption was measured with a visible spectrophotometer at the maximum wavelength (541 nm).

The sample was prepared by adding deionized water to a vial filled with 0.500±0.001 gram of EBN sample. The solution was transferred to a 50 mL measuring flask using a glass funnel. Ion-free water was added to 40 mL with 3 mL of saturated sodium chloride solution, and then deionized water was added to the tera mark. The solution was put into an ultrasonic device at 40 °C for 30 minutes, then cooled to room temperature and filtered with Whatman paper no. 42. The filtrate was transferred to a 10 mL measuring flask until the tera mark and added 0.5 mL of sulfanilamide solution. After 5 minutes, 0.5 mL of NED solution was added and homogenized. After 15 minutes, the absorbance value was measured with a visible spectrophotometer at 541 nm. A spiked sample (nitrite standard solution) was always used in each test. Seven repetitions were applied in each group in this study.

Data analysis

Research using a one-way test. The normality test and variance test were performed first. Normality test to see the data distribution and variance test to see the same variance using Levene's test. The effectiveness of these methods was compared using an independent sample t-test (P<0.05).

RESULTS AND DISCUSSION

All EBN samples from both groups showed nitrite levels decreased after washing. The group that was washed with the standard method (M1) showed an average difference in the reduction of nitrite levels of 7.8 ppm with a reduction percentage of 60.59%. In comparison, the group washed with the ultrasonic method (M2) showed an average difference of 8.6 ppm with decreasing percentage of 80.65%. The decrease in nitrite levels in the M2 group was more significant than in the M1 group. The data obtained are presented in Table 1.

Previous research using ascorbic acid resulted reduce 87% of EBN nitrite levels (Utomo et al., 2015), and the use of citrus and sea salt solution combination resulted reduce 86% of EBN nitrite levels (Ningrum et al., 2023). Both methods utilize additional sustances to reduce nitrite levels. Research related to the physical reduction of nitrites that does not change the taste of EBN has never been done. This study's use of ultrasonic waves is expected to reduce EBN nitrite levels without changing the product's taste and maintaining consumer acceptance.

The EBN nitrite content varies. EBN harvested from caves have higher nitrite levels than EBN harvested from SFHs. The color of EBN is an essential indicator of the presence of nitrite (Quek et al., 2015). Yellow, orange, and red swiftlet nests have higher nitrite concentrations than white EBN (Ningrum et al., 2022). Several factors affect EBN nitrite levels, including humidity, pH, climate, EBN's harvested age, and the cleaning process (Yeo et al., 2021). The EBN nitrite content can be controlled by monitoring each upstream and downstream processing chain. The nitrite content below 30 ppm guarantee can be done with good maintenance at the EBN, transportation from the swiftlet house to the processing site, and supervision during processing (Kementan, 2014a).

Nitrite is naturally found in the environment, such as in water and soil. EBN harvested from caves or SFHs often contain nitrites. Nitrite formed in EBN comes from organic material that decomposes on the floor. Nitrite is synthesized from ammonia by bacteria through anaerobic fermentation. Swiftlets also drop elements such as water and soil, which can cause nitrite penetration into the EBN (Ningrum et al., 2022). In addition to their natural presence, nitrites can be found in food due to their use as preservatives to prevent spoilage and maintain color in meat and meat products such as smoked beef, ham, corned beef, hamburgers, and smoked fish (Quek et al., 2015).

The nitrite levels decreased in all treatment groups and had different distributions. The M1 group has a broader or more varied distribution than the M2 group. The distribution of decline in the M1 group was 30-80%, while the distribution of reduction in the M2 group was narrower in a range close to the average, namely 80%. The average decrease in the M2 group was more significant than M1. The data can be seen in Figure 1. The data distribution indicated that the consistency of the decline M2 group was better than M1.

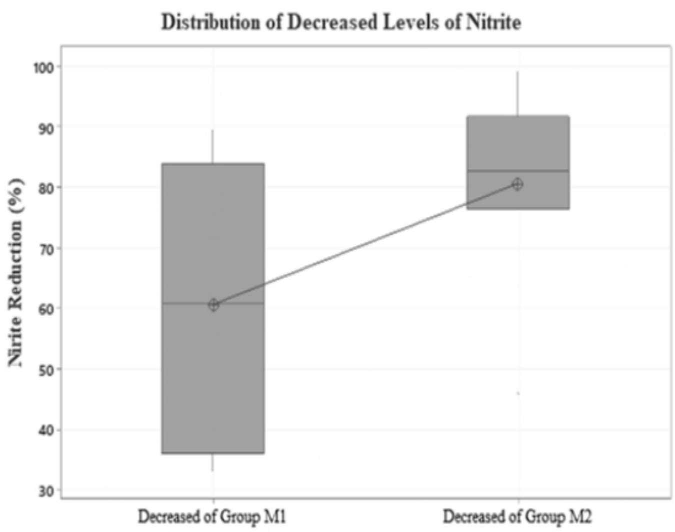

Figure 1: EBN decreased nitrite levels distribution of groups M1 and M2.

In addition to its content, the quality of EBN is also determined by the visual appearance, especially the fiber structure. Washing using ultrasonic waves in this study maintained the structure of the EBN. Similar to the general method (Figure 2).

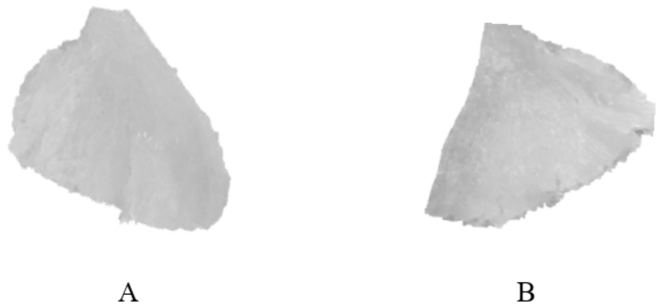

Figure 2: EBN structure. A: standard washing methods; B: Ultrasonic washing method.

The ultrasonic washing method (M2) used the principle of cavitation. Frequency plays an essential role in the cavitation process. The high frequency caused the wave to propagate longitudinally in the liquid medium towards the EBN. This process caused stretching and density. The wave stretching breaks the molecular bonds between solutions, so the gases dissolved in the solution are trapped, causing the appearance of bubbles filled with trapped gas, known as the cavitation effect. Cavitation bubbles hit the surface of the EBN and released nitrite (Pirngadi, 2017; Vernes et al., 2020).The wave frequency used in this study was modified from the method used to clean EBN, 40 KHz (Seenivasan and Sin, 2022; Thida et al., 2017). The pre-research results were obtained by comparing several time durations, at 10, 15, 20, and 25 seconds with frequencies of 37 and 40 kHz. The optimum time for a wave frequency of 40 kHz without reducing the EBN quality is 20 seconds, used in this study.

Environmental management in SHFs plays a vital role in upstream supervision. Nitrite and nitrate contamination are obtained from the fermentation process of dirt, soil, and the environment. One of the considerations is the presence of ventilation in SFHs, which is expected to suppress the fermentation process and reduce nitrite levels (Quek et al., 2015). The condition of the SHFs must be considered to ensure that it does not become the leading cause of high nitrite levels in the product. The washing stage is the critical point downstream for guaranteeing EBN nitrite levels. Washing treatment with the proper method can reduce EBN nitrite levels. Full attention to the nitrite content of EBN, which will be exported to the PRC, requires the EBN industry in Indonesia to provide optimal treatment to reduce nitrite content.

The People's Republic of China has the highest EBN consumption rate in the world. Nearly 80% of the world's

EBN production goes to the PRC market (Fan et al., 2022). Southeast Asian countries are the main suppliers. The three countries with the highest supply of EBN are Indonesia, Thailand, and Malaysia. Setting a maximum nitrite threshold of 30 ppm requires Indonesia continuously improve to maintain the EBN exported quality and quantity. The standard washing treatment used by industries so far uses RO water. In this study, the results showed a decrease in nitrite levels of more than 60%. The method developed using ultrasonic waves can suppress nitrite levels by up to 80%. The second method is considered more effective than the first method.

CONCLUSIONS AND RECOMMENDATIONS

EBN was washed using ultrasonic, more effectively decreasing nitrite levels than the standard method, which only uses RO water. The decreased consistency shows that implementing the ultrasonic method gives better results and should be an alternative method for business actors.

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NOVELTY STATEMENT

The findings of this study provide novel insights into the potency of ultrasonic waves to reduce nitrite levels of EBN. Overall, this study emphasizes the physical method that does not change the taste, maintained structure, and reduced nitrite levels of EBN significantly. These results suggest that ultrasonic method could be alternative washing method for EBN washing industries

AUTHOR'S CONTRIBUTION

DJ, HL, and TP design the research. DJ sample collection and testing. HL and TP supervision of the research process. DJ composing the script. HL and TP reviewed and corrected the manuscript. ZS head of animal quarantine installation in Batam city. All authors have contributed significantly, and all authors agree with the manuscript's final content. The Manuscript has not been published or submitted to other journals previously.

Conflict of interest

The authors have declared no conflict of interest.

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