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Reduction of Nitrite Levels in Edible Bird Nest on Lombok Island with Alkali Solution and Oxygen Water

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Abstract: In this study, sampling was carried out at 3 locations of swallow nest farmers, namely in East Lombok, Central Lombok and West Lombok. The type of swallow nest used in this study was the Linchi swallow nest. This study was a quantitative study, namely to find the best variation in soaking time with alkaline water and Oxygen Water to reduce nitrite levels. Sample measurement using a Uv-Vis spectrophotometer instrument and characterization using FTIR. The results of the study showed the highest reduction in nitrite levels using an alkaline solvent with a pH of 7.5, Central Lombok, East Lombok and West Lombok Edible Bird's Nest Sample, respectively, as follows: 87.88%; 96.26%; and 94.59% with an optimum contact time of 30 minutes. While the highest reduction in nitrite levels for Central Lombok, East Lombok and West Lombok, Edible Bird's Nest samples were as follows: 92.51%; 98.5%; and 97.85% with an optimum contact time of 15 minutes. This is also supported by FTIR spectra data from optimum conditions for reducing nitrite levels where the characteristics of the FTIR spectra in the 1400 cm-1 wavenumber region have a %T value after treatment> to %T before treatment. The results of the macronutrient test showed that the bird's nest contains carbohydrates, proteins and lipids. While the results of the phytochemical test showed that the bird's nest sample contained phenolic compounds. In this study, it has been successfully reduced nitrite levels in bird's nests using alkaline solvents pH 7.5 and Oxygen Water which is the latest method that is cheaper and more efficient.

Keywords: Alkali water; Edible bird nest; Nitrit; Oxygen water

Introduction

The linchi swiftlet *(Collocalia linchi)* is a type of bird that uses its saliva to make nests. Swiftlet nests or commonly known as Edible Bird's Nests (EBNs) have many benefits both in the industrial and health sectors such as being used as food ingredients, and as a treatment for skin regeneration. Swiftlet nests are one of the main export commodities in Indonesia, meeting 80% of the world's swiftlet nest needs and China is the largest consumer of swiftlet nests in the world (Yeo et al., 2021). China consumes almost 60% of the world's bird's nest market and currently China requires that the nitrite content in bird's nests entering China must not exceed 30 ppm and be free of peroxide content (Paydar et al., 2013). Health Risks Related to Nitrite: If consumed in excess, nitrite can be toxic to the body. Under certain conditions (such as high temperatures or acidic conditions), nitrite can react with amines to form nitrosamines, compounds known to be carcinogenic (Chamandoost et al., 2016; Moradi & Hosseini, 2016; Seyyedsalehi et al., 2023). Nitrites in high concentrations can also interfere with the function of hemoglobin in the blood, causing a condition called methemoglobinemia, which can reduce the blood's capacity to carry oxygen (Raubenheimer et al., 2019).

Reducing nitrite levels in swallow's nests is an important concern because nitrite is a chemical

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compound that can have a negative impact on human health if consumed in excessive amounts and can damage the cell structure of living things (Karwowska & Kononiuk, 2020). Bird's nest is highly valued in the food and health industries, especially in Asia, because it is considered to have various health benefits. However, the presence of nitrite in bird's nest can be a problem that affects the safety and quality of the product. Various processing and purification methods, such as washing with certain solutions, irradiation, or controlled fermentation, have been developed to reduce nitrite levels without damaging the quality of the bird's nest. One way to reduce nitrite content in bird's nest is by washing with ascorbic acid solution (Vitamin C). This method can reduce nitrite content by 87-88% in bird's nest, but the price of ascorbic acid is relatively expensive so it is less efficient (Utomo et al., 2016).

This study explains how the effect of washing using alkaline water and Oxygen Water on reducing nitrite levels in Linchi Edible Bird's Nest samples and macronutrient and phytochemical content qualitatively. The purpose of this study is to improve the quality of Linchi Edible Bird's Nest by reducing nitrite levels which is done by soaking in alkaline water and Oxygen Water at various times because the use of alkaline water and Oxygen Water is cheaper, more efficient and environmentally friendly. The benefits of this study are to find an alternative method that is efficient, cheap and environmentally friendly to improve the quality of Linchi swiftlet nests

Method

Appendix

Volume pipette, dropper, measuring cylinder, 100 mL and 50 mL measuring flask, 250 mL Erlenmeyer flask, 250 mL beaker glass, Whatman filter paper, separating funnel, analytical balance, watch glass, UV-vis spectrophotometry cuvette

Material

The nests of the Linchi type of swiftlet were taken from 3 different locations, NaNO2, Sulfanilamide, naphtyl ethylene diamine (NED), Alkali solution, Oxygen Water solution, Aquades (H2O).

Preparation of Edible Bird's Nest Sample

Edible Bird's Nest used was taken from the linchi swiftlet species. Samples were obtained from 3 different locations: Central Lombok, East Lombok and West Lombok. Then each bird's nest sample was put into an airtight plastic container. Weighed 20 grams of sample.

Nitrite Level Testing

Preparation of standard solutions containing NaNO2 as much as 0.0; 0.2; 0.4; 0.6; 0.8; and 1 mg/L, and blanks containing distilled water. As much as 0.0; 0.2; 0.4; 0.6; 0.8; and 1 mg/L, $NaNO_2$ solutions were each added with 1 mL of sulfanilamide reagent in a 50 ml flask, stirred and after five minutes added 1 mL of naphtyl ethylene diamine (NED) reagent stirred and placed up to the 100 ml mark and waited for 15 minutes, the absorption was measured at a wavelength of 543 nm. The prepared samples were then weighed each as much as 1 g then ground and added with 8 ml of H_2O. The mixed samples were then heated in a water bath at a temperature of 80 ^{0}C for approximately five minutes, then 60 ml of H2O was added. The mixture was then heated at 80 ^{0}C for two hours (while stirring occasionally).

The sample was removed from the water bath and then transferred while being rinsed with hot H2O. Dilution was carried out by adding H_2O solution to a 100 mL measuring flask to the boundary mark. The sample was then filtered and taken as much as 45 ml, placed in a 50 ml measuring flask. The sample was then added with 2.5 ml of sulfanilamide reagent, shaken, and added with 2.5 ml of NED. The sample was then left for 15 minutes and then its absorption was measured at a wavelength of 543 nm (Guimarães et al., 2014; Li et al., 2024).

Variation of Washing Time to Reduce Nitrite Levels with Alkali Solution and Oxygen Water

The prepared Edible Bird's Nest was then weighed 1 gram each and soaked for 5, 10, 15, 30, 45 and 60 minutes in 50 mL of Oxygen Water and alkali solution. After that, separate the swiftlet nest from the solution, then dry it at room temperature. The bird's nest sample was then tested for nitrite content. In the FTIR characterization data collection, the Edible Bird's Nest was taken before treatment with solvents and at optimum conditions for reducing nitrite levels in the sample. The results of the reduction in nitrite levels in Edible Bird's Nest were calculated using the following Formula 1.

$$\%\text{Nitrite: } \frac{N_1 - N_0}{N_0} x100\% \quad (1)$$

Information:
N_0 = Concentration Nitrite Before
N_1 = Concentration Nitrite After

Biochemical Test of Edible Bird's Nest
Macronutrient Test

Edible Bird's Nest of Central Lombok, Edible Bird's Nest of East Lombok and Edible Bird's Nest of West

Lombok, each dissolved in distilled water and heated. The solution of each Edible Bird's Nest obtained was allowed to cool. To be used for Carbohydrate, Protein, and Lipid tests.

Carbohydrate Test

Benedict's test

Prepare 3 test tubes, and put 3 mL of Benedict's reagent into each tube (Hernández-López et al., 2020). Next, each of the Edible Bird's Nest solutions was added a few drops into each tube containing Benedict's sea. Tube 1: Edible Bird's Nest Central Lombok. Tube 2: Edible Bird's Nest East Lombok. Tube 3: Edible Bird's Nest West Lombok. Then the three tubes were put into boiling water until the color of the solution in the tube changed. The color obtained was recorded

Molisch's test

Prepare 3 clean and dry test tubes. Insert 2 mL of Edible Bird's Nest solution into each test tube successively and label it. Add 3 drops of Molisch reagent into each tube using a clean dropper then shake gently (Nurprialdi et al., 2023). Tube 1: Edible Bird's Nest Central Lombok. Tube 2: Edible Bird's Nest East Lombok. Tube 3: Edible Bird's Nest West Lombok. Flowed as much as 1 mL of concentrated sulfuric acid solution through the tilted tube wall, it can be seen that the sulfuric acid is in the bottom layer. A positive reaction is formed when a purple ring is formed on the upper surface of both liquids.

Protein Test

Prepare 3 clean and dry test tubes. Put 2 mL of Edible Bird's Nest solution into each tube. Add 2 mL of 10% NaOH into each tube and mix. Slowly drip 0.5% $CuSO_4$ solution until a purple color appears (Lan et al., 2024).

Lipid Test

Dissolve a little each of the Edible Bird's Nest solutions of Central Lombok, Edible Bird's Nest of East Lombok and Edible Bird's Nest of West Lombok in 2 mL of chloroform. Add 2 mL of concentrated sulfuric acid to each tube. The sulfuric acid layer fluoresces green and the chloroform layer is bluish red to bright red and purple.

Phytochemical Test

Edible Bird's Nest of Central Lombok, Edible Bird's Nest of East Lombok and Edible Bird's Nest of West Lombok were each dissolved in ethanol. Macerated for 24 hours. Filtered to separate the filtrate (ethanol) and residue. The filtrate obtained was evaporated with an evaporator to obtain extracts from each Edible Bird's Nest (Oktavia & Sutoyo, 2021).

Phenolate test

Edible Bird's Nest is added with a few drops of $FeCl_3$ solution. The color indicator is blue, purple blue and brown precipitate.

Alkaloid Test

Edible Bird's Nest Extract Dragendorff's reagent is added, the color change is observed. The positive color indicator of alkaloids is dark orange to red.

Result and Discussion

Determination of the calibration curve by absorbance measurement was carried out at various nitrite concentrations, namely 0.0; 0.2; 0.4; 0.6; 0.8; and 1 mg/L, then the absorbance data was made into a calibration curve as in Figure 1, to connect the absorbance with the concentration so that the regression equation $y = 1.4617x + 0.0741$ was obtained, with a correlation (r) = 0.9977. As a general rule, a value of r> 0.9900 indicates a good curve (Artaya, 2019). In other words, the results of the linearity parameter testing in this study have met the existing requirements.

Table 1. Concentration of Nitrite Standard Solution

Concentration (mg/L)	Absorbance
0.0	0.041
0.2	0.375
0.4	0.685
0.6	0.973
0.8	1.253
1.0	1.503

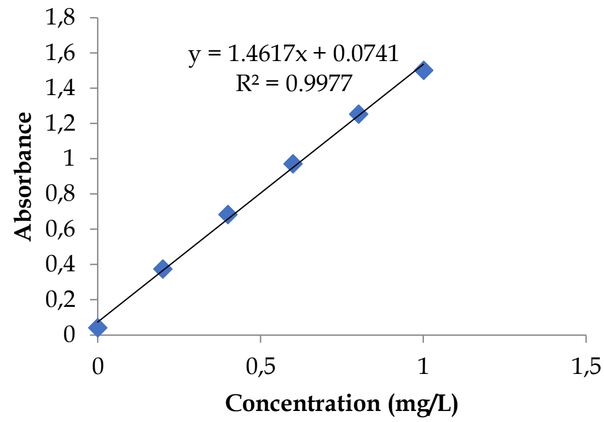
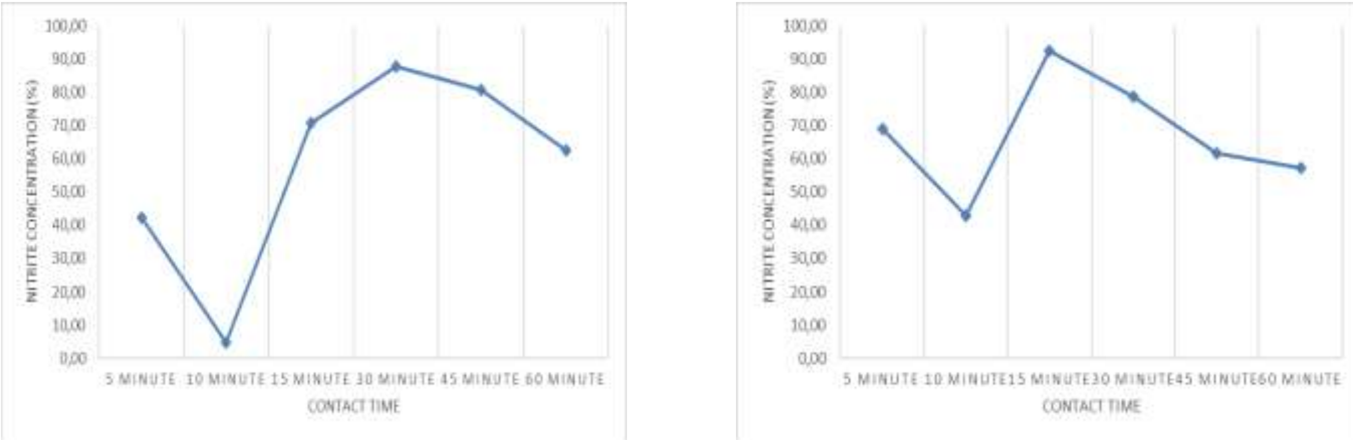


Figure 1. Nitrite standard curve

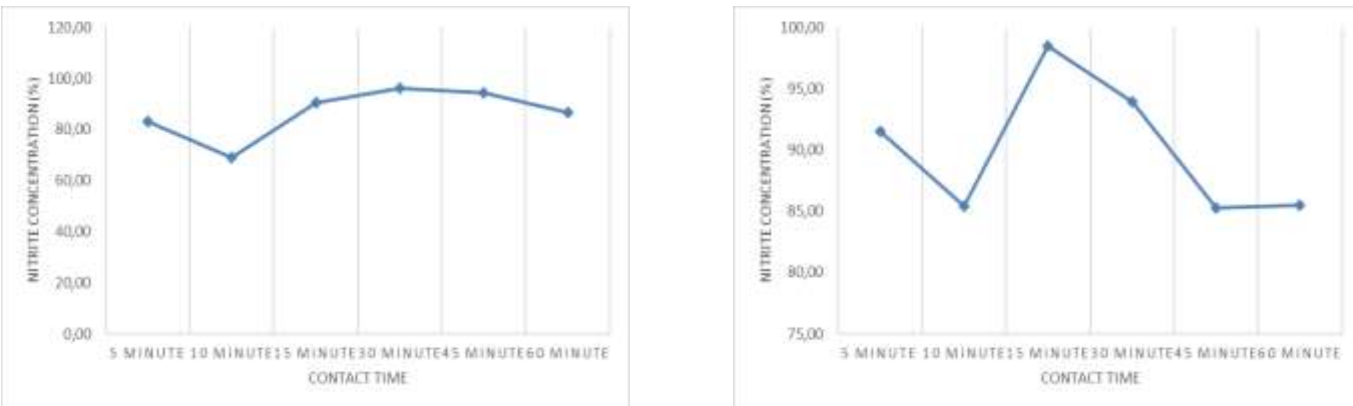
Results of Variation of Washing Time to Reduce Nitrite Levels with Alkali Solution and Oxygen Water

In Figure 2, it can be seen that the largest decrease in nitrite levels in Edible Bird's Nest from Central Lombok was at a contact time of 30 minutes for alkali solution and 15 minutes for Oxygen Water solution with the highest percentage decrease of 87.88% for alkali solution and 92.51% for Oxygen Water solution. Meanwhile, for Edible Bird's Nest from East Lombok, the largest percentage decrease was also obtained at a contact time of 30 minutes for alkali solution and 15 minutes for Oxygen Water solution with the highest percentage decrease of 96.26% for alkali solution and 98.50% for Oxygen Water solution.



(a) (b)

Figure 2. Graph of nitrite level decrease of Central Lombok samples: a) Alkali solution; b) Oxygen water solution



(a) (b)

Figure 3. Graph of nitrite level decrease of East Lombok samples: a) Alkali solution; b) Oxygen water solution

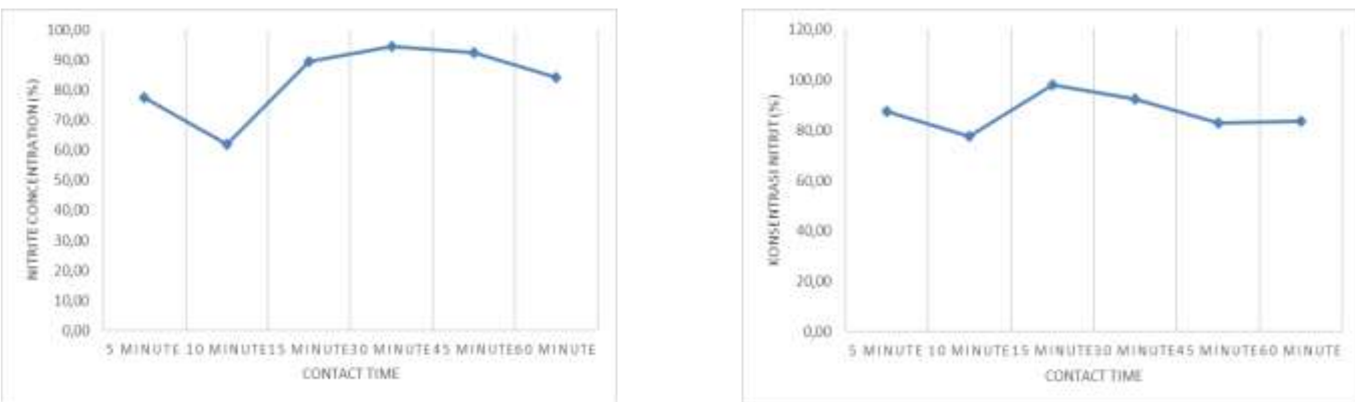
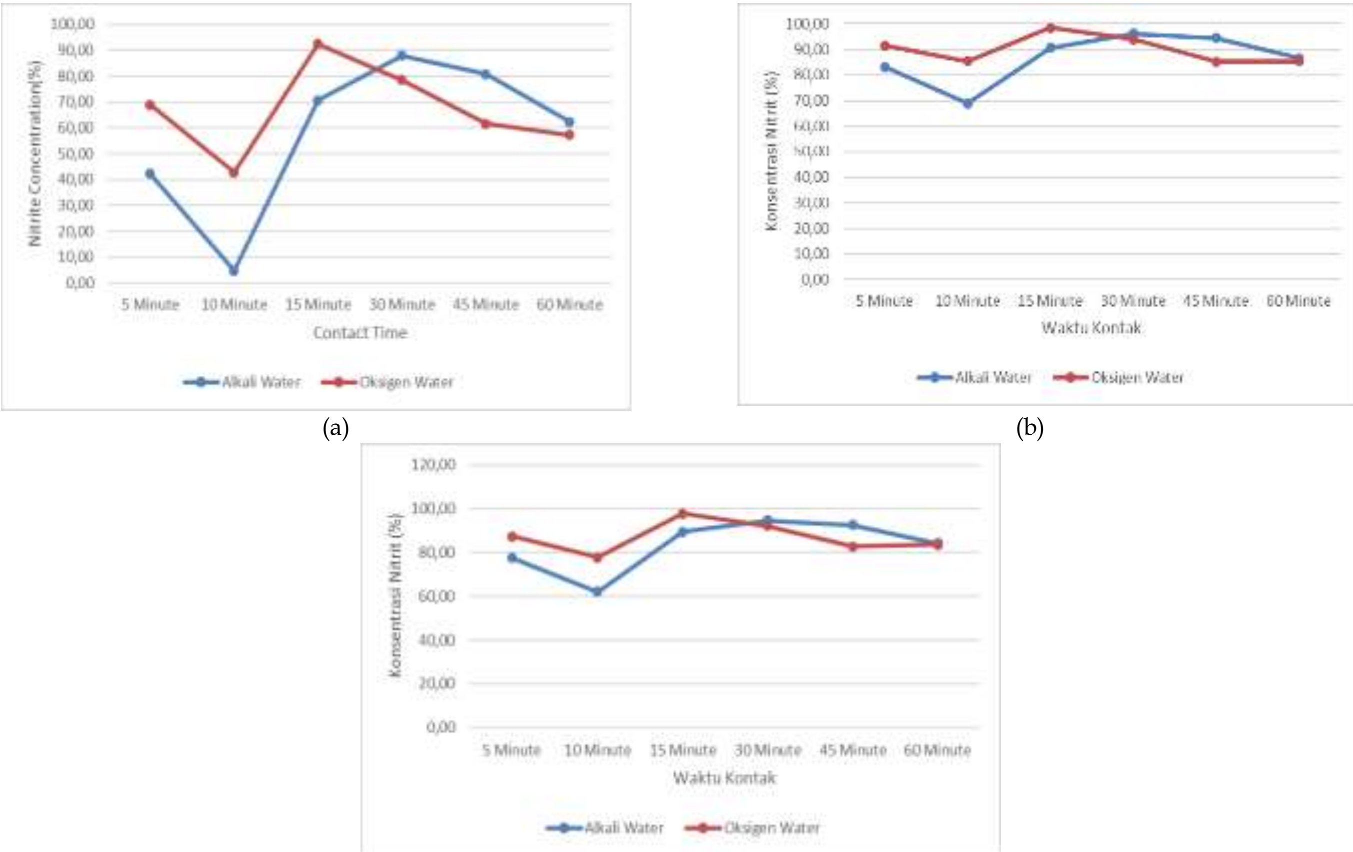


Figure 4. Graph of nitrite level decrease of West Lombok samples: a) Alkali solution; b) Oxygen water solution

The Edible Bird's Nest from West Lombok, the largest percentage decrease was also at a contact time of 30 minutes for alkali solution and 15 minutes for Oxygen Water solution with the highest percentage decrease of 94.59% for alkali solution and 97.85% for Oxygen Water solution. In Figures 2, 3 and 4 it can be seen that the higher the contact time, the Edible Bird's Nest will reach its optimum point which will then decrease over time.

This is because the nitrite that has dissolved in the solution is bound back into the sample, thus reducing the percentage of Nitrite anion (NO2) release into the solution (Zhi et al., 2023; Zhi et al., 2023).



(c)

Figure 5. Graph of nitrite level decrease against solvent: a) Edible bird's nest Central Lombok; b) Edible bird's nest East Lombok; c) Edible bird's nest West Lombok

In Figure 5, a graph of changes in the decrease in nitrite levels with variations in contact time of each solvent, namely alkali solution and Oxygen Water solution, is plotted. It is found that all Edible Bird's Nests have the highest percentage decrease, namely in the Oxygen Water solution with the highest percentage, namely 92.51% in the Edible Bird's Nest Sample of Central Lombok, 98.50% Edible Bird's Nest Sample of East Lombok, and 97.85% Edible Bird's Nest Sample of West Lombok. This is influenced by the fact that the Oxygen Water solution has a high oxygen content so that it can act as a reducing solution for nitrite into nitrate which is easily dissolved in water molecules in the Edible Bird's Nest Sample (Wang et al., 2020). Previous research using ascorbic acid resulted in an 87% reduction in EBN nitrite levels (Juniwati et al., 2023), and the use of a combination of citrus and sea salt solutions resulted in an 86% reduction in EBN nitrite levels (Ningrum, 2023).

The results obtained in this study are higher than previous studies. The Nitrite Reduction Process by Oxygen Molecules in Oxygen Water Solution as follows:

$NO2^-$ (Nitrite) + O2 → $NO3^-$ (Nitrate)

The alkaline solution used in this study has a pH of 7.5. At this pH, the nitrification process takes place well. If the pH is above this, the oxidation reaction of nitrite to nitrate is less efficient (Le et al., 2019).

FTIR Spectra Characteristics of Edible Bird's Nest Sample Lombok

In FTIR (Fourier Transform Infrared Spectroscopy) spectra, the nitro functional group ($-NO_2$) has a very specific characteristic absorption band, mainly due to the two N-O bonds that are in a unique molecular environment. The FTIR spectrum for the nitro group usually shows several strong absorption bands in a certain range, namely: N-O Asymmetric Band: The nitro group shows a strong absorption band in the region of 1520–1550 cm^{-1}, which is due to the asymmetric vibration of the N-O bond. This is usually the strongest absorption for the nitro group. N-O Symmetric Band: Another absorption band appears in the region of 1345–1385 cm^{-1}, which is due to the symmetric vibration of the

N-O bond. This band is generally slightly weaker than the asymmetric band.

Other Band Contributions: In the FTIR spectrum, the nitro group can also contribute to the band around 720–760 cm^{-1}. However, these bands tend to be weaker and are often difficult to distinguish if there is a lot of interference from other groups in the molecule. These bands are the main indicators of the presence of nitro groups in the molecule, and their intensity depends on the concentration and interaction of the molecular environment in the sample (Khan et al., 2018). Based on Figure 6. A strong spectrum is seen at the peak of 1520–1550 cm^{-1} which indicates the presence of the NO2 group in the edible bird's nest sample.

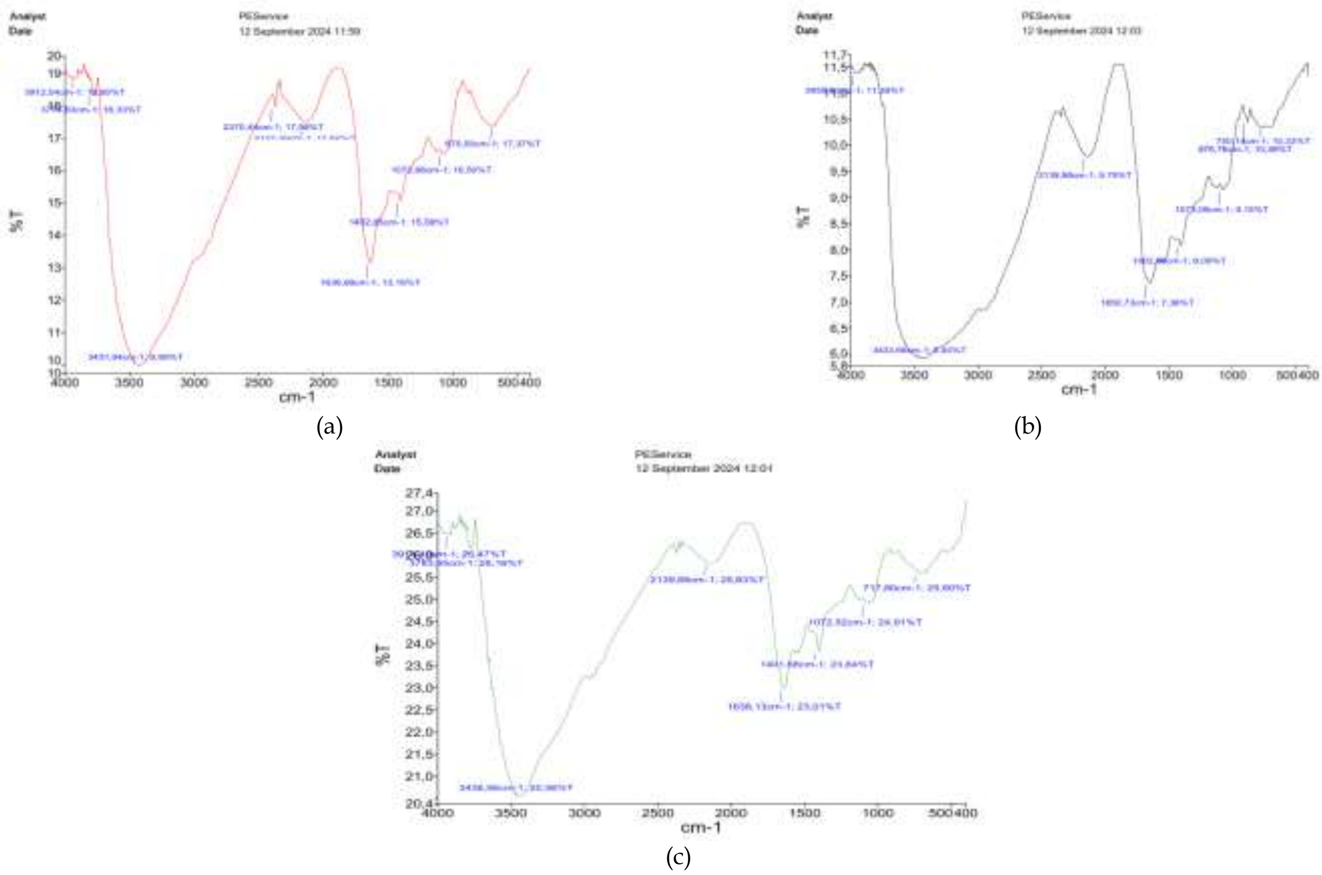


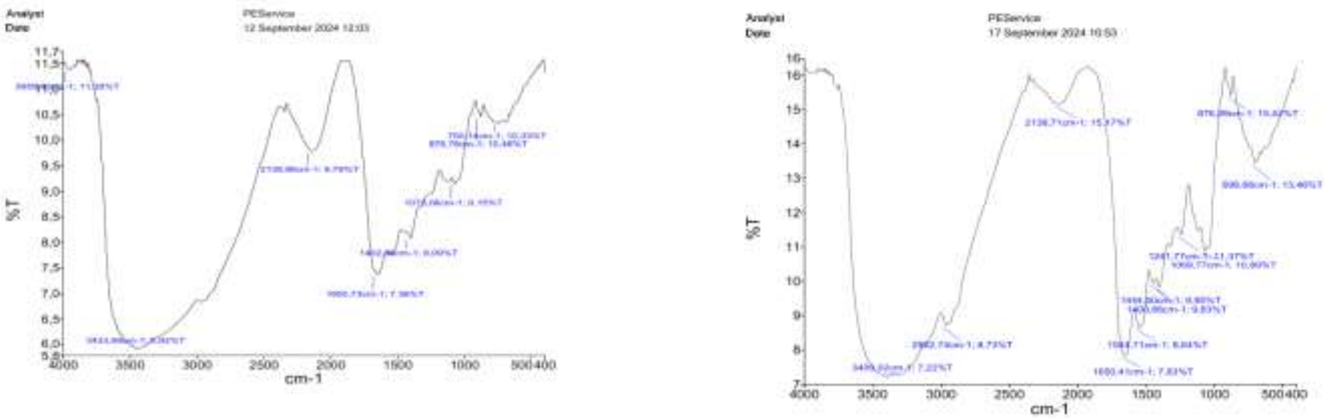
Figure 6. FTIR Spectra: a) Edible bird's nest sample Central Lombok; b) Edible bird's nest sample West Lombok; c) Edible bird's nest sample East Lombok

In FTIR spectra, transmittance is inversely proportional to the concentration of functional groups that absorb infrared radiation. The higher the concentration of a particular functional group in a sample, the greater the absorption of infrared light that occurs, which will decrease the transmittance value at the wavelength corresponding to the functional group (Giechaskiel & Clairotte, 2021). In Figure 6(b) it can be seen that the FTIR spectrum in the 1400 cm-1 area shows an increase in the %T value compared to Figure 6 (a) before treatment using alkali solvents. This indicates that there is a reduction in the concentration of Nitrite in the sample. The same thing also occurs in Figure 7 where there is an increase in %T after treatment using Oxygen Water solvents Figures 7(a) and 7(b). This is because the Nitro functional group bound to Edible Bird's Nest is dissolved in the solution, thus reducing the concentration of the Nitro group in the Edible Bird's Nest.

Biochemical Test of Bird's Nest

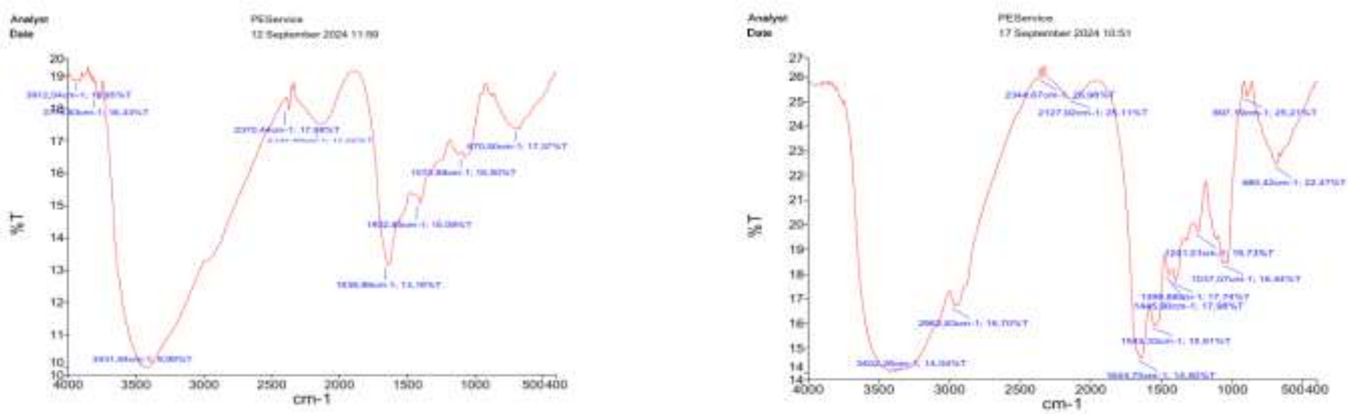
Edible Bird's Nest Macronutrient Test Results

Based on the Macronutrient test that has been conducted on swallow nest samples, namely Edible Bird's Nest Central Lombok, Edible Bird's Nest West Lombok and Edible Bird's Nest East Lombok, it shows positive results for the macronutrient test content, namely carbohydrate test, protein test and lipid test. This shows that Edible Bird's Nest has a high nutritional content as previously studied (Hamzah et al., 2013; Marcone, 2005; Ibrahim et al., 2021; Nasir et al., 2021). The test result data is presented in Table 2.



(a) (b)

Figure 7. Spektra FTIR edible bird's nest sample Central Lombok: a) Sample conditions before treatment, b) Optimum conditions for nitrite level reduction with 30 minutes contact time using alkali solvents



(a) (b)

Figure 8. FTIR Spectra of edible bird's nest sample Central Lombok: a) Sample condition before treatment, b) Optimum condition for nitrite level reduction with 15 minute contact time using oxygen water solvent

Table 2. Edible Bird's Nest Macronutrient Test Results

Materials	Carbohydrate		Protein	Lipids
Test	Benedict Test	Molisch Test	Biuret Test	Salkowsky
EBN1	+	+	+	+
EBN2	+	+	+	+
EBN3	++	++	+	+

Based on the results of phytochemical tests of swallow nest samples, namely Edible Bird's Nest Central Lombok, Edible Bird's Nest West Lombok and Edible Bird's Nest East Lombok, showed positive results for the polyphenol compound test content but negative for the alkaloid content test.

Table 3. Phytochemical Test Results

Materials Test	Fenolat/Polifenol Test	Alkaloid Test
EBN 1	+	-
EBN 2	+	-
EBN 3	+	-

Note: EBN 1 = Edible Bird's Nest Central Lombok; EBN 2 = Edible Bird's Nest West Lombok; EBN 3= Edible Bird's Nest East Lombok.

The results are presented in Table 3 Phenolate/polyphenol compounds are compounds that tend to dissolve in water because they bind to glucose as glycosides have a role as antioxidants that can counteract the formation of free radicals and prevent premature aging, lower blood glucose levels and prevent cancer. The presence of this polyphenol compound makes Edible Bird's Nest widely used as an ingredient for cosmetic products.

Conclusion

From the results of the research that has been done, the reduction of nitrite levels in Edible Bird's Nest using alkali solution and Oxygen Water was successful. Where the highest reduction of nitrite levels using alkali solvent with pH 7.5 Edible Bird's Nest Central Lombok, East Lombok, and West Lombok are as follows: 87.88%; 96.26%; and 94.59% with optimum contact time at 30 minutes. While the highest reduction of nitrite levels using Oxygen Water solution Edible Bird's Nest Central Lombok, East Lombok, and West Lombok are as follows:

92.51%; 98.5%; and 97.85% with optimum contact time at 15 minutes. This is also reinforced by FTIR spectra data from the optimum conditions for reducing nitrite levels where the characteristics of the FTIR spectra in the 1400 cm-1 wave number region have a %T value after treatment> to %T before treatment. The macronutrient test results show that Edible Bird's Nest contains carbohydrates, proteins and lipids. While the phytochemical test results show that Edible Bird's Nest contains phenolic compounds.

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Author Contributions
All authors have made a significant contribution to the development of this scientific work. The first author was responsible for conducting the research and preparing the scientific work. The second and third author provided guidance, direction, and advice throughout the research and preparation of the scientific work.

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Conflicts of Interest
The authors declare no conflict of interest in the publication of this scientific article.

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