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EFFECTIVENESS OF VOLTAMMETRY AND UV-VIS SPECTROPHOTOMETRY METHODS IN ANALYZING NITRITE IN EDIBLE SWIFTLET NEST

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ABSTRACT

Edible Swiftlet Nest (ESN) is a swiftlet's saliva product that contains many beneficial compounds. However, improper washing leaves behind toxic nitrite content. To ensure its absence, sensitive and accurate methods are needed, such as UV-Vis spectrophotometry. However, long analysis time and complicated maintenance, limit its further use. In this research, cyclic voltammetry was developed as an alternative analysis method for nitrite content in ESN. The results show that the LOD and LOQ of this method are 0.658529 ppm and 1.995544 ppm, respectively. Compared to this value, analysis using UV-visible spectrophotometry shows lower LOD and LOQ values, respectively 0.061579 ppm and 0.186602 ppm.

Keywords: ESN, Nitrite, Cyclic Voltammetry, LOD, LOQ, UV-Visible Spectrophotometry

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INTRODUCTION

Edible Swiftlet Nest (ESN) is rich in nutritional compounds such as N-acetylneuraminate, galactosamine, glucosamine, fucose, and essential amino acids.[1] However, ESN also contains dangerous compounds, especially nitrites which have the risk of cancer.[2] Nitrite occurs naturally in ESN due to its habitat, which comes from decaying organic matter.[3] Based on China Food Standard (GB2760-2011), ESN nitrite should not exceed 30 ppm. The stringent nitrite regulations are the background to the need for sensitive and accurate methods for analysis. Several methods were developed for nitrite analysis, one of them is UV-Vis spectrophotometry and voltammetry. Waskito *et al.* (2023) analyzed nitrite levels in ESN using UV-Vis spectrophotometry.[4] This method is the most widely used because of its easy protocol. In line with this, the simple voltammetry protocol and rapid response make it a potential method for further development. This study aims to compare between voltammetry and UV-Vis spectrophotometry, to obtain the most sensitive method for analyzing nitrite in ESN.

EXPERIMENTAL

Material

The materials used were white ESN and water samples obtained from CV. Dwi Anugrah Surabaya; ascorbic acid (Cahaya Kimia, Indonesian), sulfanilamide (HiMedia Laboratories, India), NED (Merck, USA), $NaNO_2$ (Chemical Indonesia Multi Sentosa, Indonesian) and KCl (Merck, Germany). The instruments used were UV-Vis Spectrophotometry (Shimadzu UV-1800), and Voltammetry 797 VA Computrace (Metrohm).

ESN Preparation

ESN is washed with ascorbic acid following the "Dwi Anugrah Surabaya" procedure.

Nitrite Analysis Using Voltammetry

Optimization was performed for deposition time, scan rate, and potential. After that, a total of 1 g of ESN was ground, dissolved in 40 mL of distilled water, and heated at 50 °C for 20 minutes. A mixture of 20 mL

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of ESN sample solution and 5 mL of 3% KCl solution was prepared in the voltammeter container for nitrite analysis using voltammetry with a Cu SAE electrode under optimal parameters.

Nitrite Analysis Using UV-Vis Spectrophotometry

A total of 20 mL of distilled water was heated to 80 °C for 30 minutes to dissolve 0.5 g of ESN. The solution was centrifuged at 3500 rpm for 15 minutes. To this was added 1.25 mL of sulfanilamide and NED. The solution absorption was analyzed at 543 nm.

RESULTS AND DISCUSSION

Voltammetry Method

Deposition Time Optimization

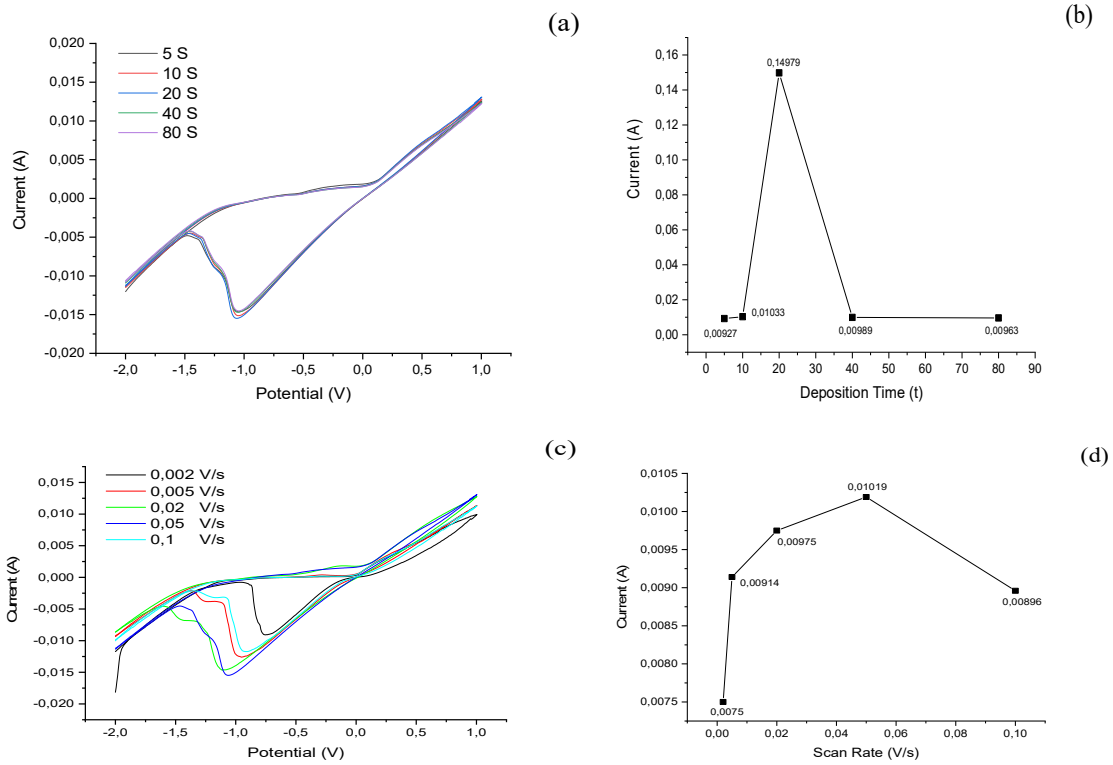
Based on Fig.-1(b), the deposition time of 20 s provides the highest increase in the current response of 0.149 A, indicating the optimal deposition time. This shows that the deposition time is 20 seconds because there is an increase in ions attached to the electrode surface, but approach to saturation point and exhaustion of the adsorption site on the electrode surface.[5,6]

Scan Rate Optimization

The scan rate controls how fast the electrode scans are for analyte ions in the applied potential. Based on Fig.-1(d), a scan rate of 0.05 V/s provides the highest current response of 0.01019 A, indicating the optimal scan rate. This is because a higher scan rate reduces the diffusion layer, so that the diffusion rate is faster than the reaction rate, facilitating optimal accumulation of analyte ions and charge transfer at the electrode surface, resulting in a higher current response.[5]

Potential Optimization

Cyclic voltammetry induces nitrite ion movement towards the electrode, capturing anodic and cathodic currents in initial and end potential scans. Based on Fig.-1(f) and 1(h), the 0.9 V initial and 1.3 V end potentials show the highest current responses, respectively 0.00673 A and 0.00757 A, indicating the optimal potential because optimal nitrite ionization occurs, leading to optimal deposition and accumulation of nitrite ions on the electrode surface.



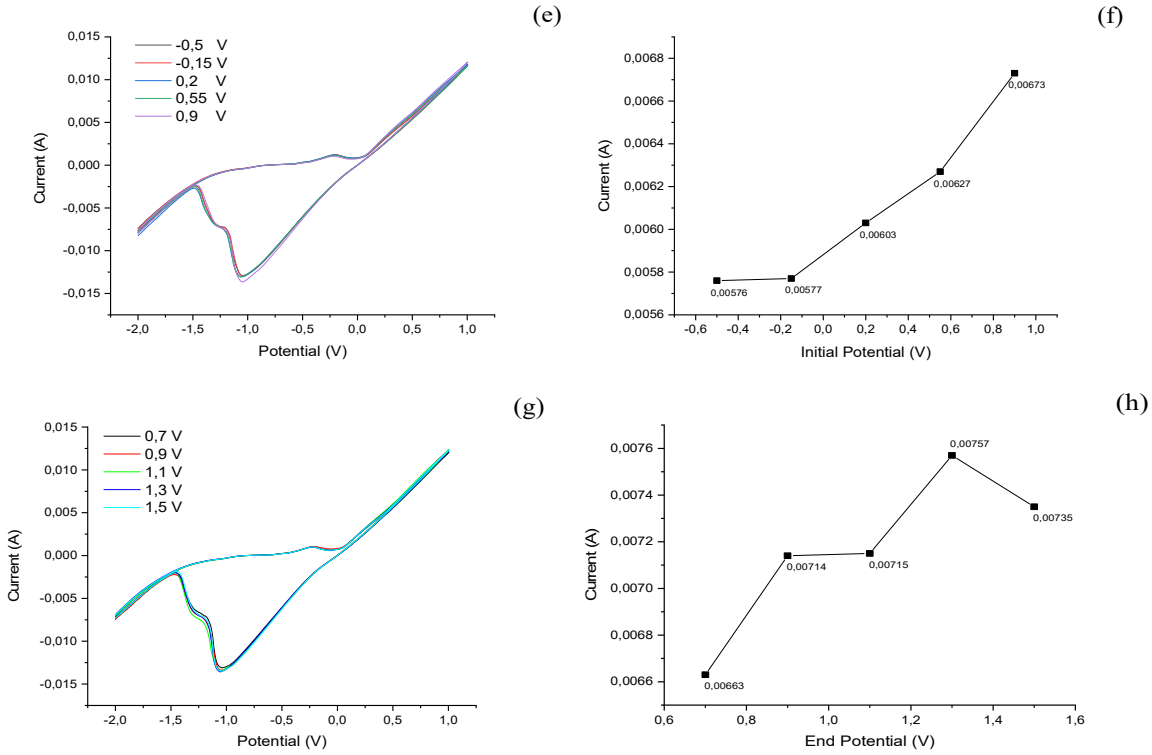


Fig.-1: Voltammogram of Various Deposition Times (a), Deposition Time vs i_{pc} Curve (b), Voltammogram of Various Scan Rates (c), Scan Rate vs i_{pc} Curve (d), Voltammogram of Various Initial Potentials (e), Initial Potential vs i_{pc} Curve (f), Voltammogram of Various End Potential (g), and End Potential vs i_{pc} Curve (h)

Analysis of Nitrite in ESN Using Voltammetry under Optimum Conditions

Nitrite in ESN was analyzed using cyclic voltammetry with Cu SAE under optimal parameters, aiming to determine the sensitivity of this method for ESN nitrite analysis. The first analysis was performed on nitrite standard solution with voltammogram results (Fig.-2(a)), which showed a linear correlation between current response (i_p) width and nitrite standard solution concentration, following the Randles-Sevcik equation where the i_p is linearly related to the analyte concentration. The i_{pc} data formed a strong linear relationship ($y = 10^{-5}x + 0.0094$, $R^2 = 0.9996$) between nitrite standard solution concentration and i_{pc} (Fig.-2(b)).

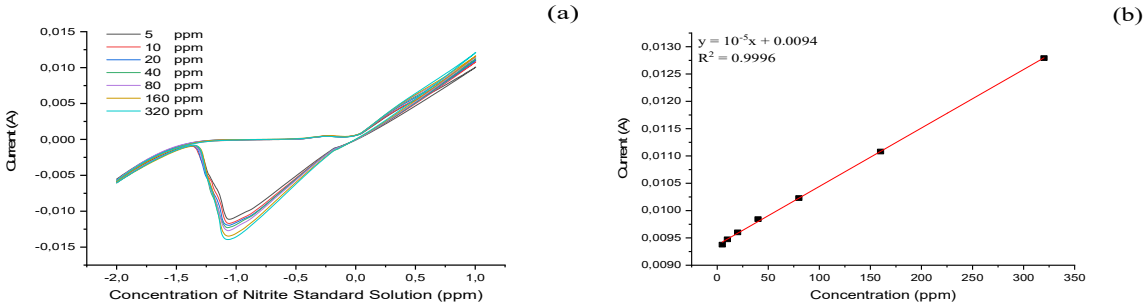


Fig.-2: Voltammogram of Concentrations of Nitrite Solution (a) Calibration Curve (b)

Based on the data (Table-1), the decrease in nitrite levels in ESN washed with ascorbic acid can be detected well using voltammetry from the high concentration (90.7018 ppm) to the lowest concentration (0.8602 ppm). This shows that voltammetry is sensitive for the analysis of ESN nitrite. The decrease in nitrite was significant to the increase in ascorbic acid levels. This is in line with research by Waskito *et al.* (2023) who reported that the higher the ascorbate concentration in ESN washing water, the higher the nitrite reduction.[4] Washing with 8% ascorbic acid resulted in 28.1664 ppm, complying China Food Safety Standard (GB2760-2011) requirement of less than 30 ppm. ESN washed with 12–20% ascorbic acid solution has nitrite levels

similar to 8%, indicating the 8% solution is optimal for reducing nitrite in ESN. Ascorbic acid as a reducing agent, supplies electrons to nitrite to produce nitric oxide, which is then oxidized into dehydroascorbic acid.[7]

Table-1: Results of ESN Nitrite Content Analysis Using Voltammetry

ESN Washing Treatment	i_{pc} (A)	Nitrite Levels (ppm)
Unwashed	0.010307018	90.7018
ESN + AA 4%	0.009967459	56.7459
ESN + AA 8%	0.009681664	28.1664
ESN + AA 12%	0.009656197	25.6197
ESN + AA 16%	0.009429825	2.9828
ESN + AA 20%	0.009408602	0.8602

UV-Vis Spectrophotometry Method

Nitrite in ESN was analyzed using UV-Vis spectrophotometry at 543 nm. The first analysis was performed on nitrite standard solution, which provides absorbance data that formed a strong linear relationship ($y = 0.59552x + 0.7909$, $R^2 = 0.9997$) between nitrite standard solution concentration and absorbance (Fig.-3). The calibration curve shows that higher nitrite concentrations lead to increased absorbance, following the Lambert-Beer Law, where the concentration of the irradiated solution is directly proportional to the absorbance.[8]

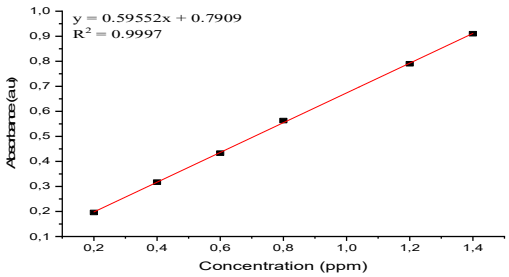


Fig.-3: Standard Nitrite Calibration Curve using UV-Vis Spectrophotometry

Table-2: Results of ESN Nitrite Content Analysis using UV-Vis Spectrophotometry

ESN Washing Treatment	Nitrite Levels (ppm)
ESN + AA 4%	0.2074
ESN + AA 8%	0.0999
ESN + AA 12%	0.0876
ESN + AA 16%	0.0552
ESN + AA 20%	0.0432

Data show that ascorbic acid reduces nitrite in ESN. The decrease of nitrite levels analyzed by UV-Vis spectrophotometry showed lower detection levels compared to voltammetry. Indicates that this method is more sensitive for analyzing nitrite levels in ESN.

Determination of Detection Limit and Quantification Limit

Data from cyclic voltammetry and UV-Vis spectrophotometry was processed to determine the Limit of Detection (LOD) and Limit of Quantification (LOQ), aiming to compare the sensitivity between the two analytical methods for ESN nitrite analysis. LOD is crucial for comparing and selecting the most efficient analytical method based on its detection capability for the lowest analyte concentration, while LOQ measures the smallest accurately measurable amount in the sample.[9] In the cyclic voltammetry, the LOD is 0.658529 ppm, and the LOQ of 1.995544 ppm. Meanwhile, the UV-Vis spectrophotometry LOD is 0.061579 ppm and LOQ is 0.186602 ppm. This shows that the UV-Vis spectrophotometry method has higher sensitivity than voltammetry for ESN nitrite analysis.

CONCLUSION

The optimization obtained optimal voltammetry parameters for nitrite analysis in ESN, namely a deposition time of 20 s, a scan rate of 0.05 V/s, and a potential of 0.9–1.3 V. This method obtained higher LOD and

LOQ values than UV-Vis spectrophotometry, indicated that the UV-Vis spectrophotometry method has higher sensitivity than voltammetry for ESN nitrite analysis.

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CONFLICT OF INTERESTS

No conflict of interest exists, the authors claim.

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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