



EFFECTIVENESS OF USING Zn POWDER AND SPONGY CADMIUM AS REDUCTOR ON FREE NITRITE AND NITRATE ANALYSIS METHOD VALIDATION IN BREAD SAMPLES BY UV-VIS SPECTROPHOTOMETRY

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ABSTRACT

This research had been carried out on the analysis of nitrite and nitrate in bread by UV-Vis spectrophotometry through a diazotization reaction. The research began by determining the optimum conditions for nitrite analysis while nitrate needs to be reduced using Zn powder or spongy cadmium so it can be analyzed by UV-Vis spectrophotometry. Ionic interference could affect nitrite analysis using UV-Vis spectrophotometry, then both nitrite and nitrate analysis methods were validated based on the method validation parameters. The diazotization reaction produced an azo compound which have λ_{max} 546 nm. The validation results showed that the linearity of nitrite and nitrate has $R^2 = 0.9990$ in the range $0-1 \text{ mg L}^{-1}$ with a molar absorptivity value for nitrite without a reduction process was $3.37 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$, meanwhile $2.91 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $3.83 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ was shown for the reduced nitrite of Zn powder and spongy cadmium. The detection limits were 4.29×10^{-3} , 9.34×10^{-3} , and $7.58 \times 10^{-3} \text{ mg L}^{-1}$. The %RSD results were 0.31-1.83% with recovery percentage of nitrite and nitrate in the range of 83.2-104.4%. The strongest ionic interference was shown by Fe^{2+} and Fe^{3+} ions at concentrations $< 10 \text{ mg L}^{-1}$. WHO has confirmed that the daily intake for nitrate that is acceptable to be consumed by human is 0-3.7 mg/kg.

Keyword: nitrite, spongy cadmium, UV-Vis spectrophotometry, Zn powder, validation method

INTRODUCTION

Wheat is one of the main food ingredients besides rice. Improvement of soil quality for wheat growth can be done by adding nitrogen fertilizer. Uncontrolled use of nitrogen fertilizers can lead to increased concentrations of nitrite and nitrate in wheat crops. Bread is one of the most famous food products processed from wheat. Processing wheat into bread requires water as a solvent for all the raw materials for making bread to become a

compact dough. Setiowati explained that drinking water contains high enough nitrate so that the use of wheat and water allows the accumulation of nitrite and nitrate in bread [1].

Nitrite and nitrate can have a negative impact on health. Some of the potential that could be caused by the dangers of nitrite include cardiovascular disease, hypertension, diabetes, and methemoglobin [2]. Based on the danger posed by nitrite and nitrate contamination in bread, it is necessary to

analyze the content of nitrite and nitrate in bread. Nitrite and nitrate analysis methods included the high performance liquid chromatography (HPLC) method conducted by Kodamatani [3]. This method has advantages of being sensitive and selective but requires expensive operational costs. Merusi conducted an analysis of nitrite and nitrate using the capillary electrophoresis method. This method had the advantage that the analysis is fast but requires complicated column preparation [4].

A simple, sensitive, and fast method in the analysis process is required in routine analysis of nitrite and nitrate. One method that meets the criteria. That is UV-Vis Spectrophotometry. Analysis of nitrite and nitrate using UV-Vis spectrophotometric method is based on the formation of azo compounds. Azo compounds are formed from the diazotation reaction between nitrite ions and amine compounds primary aromatics to form diazonium salts which are then coupled with derivatives of benzene, naphthalene, or some other heterocyclic compounds [5].

The nitrite analysis method using UV-Vis spectrophotometry has been modified, including the use of amine compounds. The compound that is usually used is sulfanilamide (NEDA) but the use of Griess reagent is less sensitive and causes a carcinogenic effect. Another Griess reagent is dapsone which is more sensitive than sulfanilamide reagent. Nagaraja conducted an analysis of nitrate in water using dapsone reagent (4,4-diamino diphenyl sulfone) with *n*-naphthol [6]. The maximum wavelength obtained is 540 nm with a linearity produced at 0.05-0.8 $\frac{mg}{L}$ and a sensitivity of 0.243 $\frac{L}{mol\ cm}$, but the use of these reagents can experience ionic interference

such as Co^{2+} , Ag^+ , Ni^{2+} , Zn^{2+} , CO_3^{2-} , PO_4^{3-} and WO_4^{2-} which can interfere with nitrite analysis. O-aminobenzoic acid (OABA)/NEDA reagent has also been used as a reagent and has better tolerance for ionic interference [7]. The use of OABA turned out to be considered less sensitive because the position of the carboxyl and amine groups were close together, causing steric barriers that could affect the process of forming azo compounds. Therefore, in this study, a reagent which is the position isomer of OABA is used, namely p-aminobenzoate (PABA) coupled with NEDA.

Nitrates cannot be analyzed directly using the UV-Vis Spectrophotometry method. Wang stated that VCl_3 can reduce nitrate to nitrite effectively and is not toxic but VCl_3 is expensive [8]. Zn powder can be used to reduce nitrate, but it allows a further reduction process to occur [1]. The use of a cadmium column is more effective for reducing nitrate but can cause suspended substances in the test solution, thereby reducing the flow rate of the reduction column [9].

In this study, the analysis of nitrite and nitrate in samples of bread by UV-Vis spectrophotometry will be carried out using PABA reagent coupled with NEDA to form azo compounds. Nitrate must first be reduced to nitrite in order to be analyzed using a UV-Vis spectrophotometer. The use of Zn powder and spongy cadmium as reductor will be compared to the effectiveness of the reduction process respectively. The presence of ions can interfere with the analysis of nitrite using the UV-Vis spectrophotometric method. Therefore, it is necessary to determine the level of ions that cause minimal disturbance to the nitrite analysis, then the analytical method is

validated to ensure that the analytical method has performance parameters that are able to overcome analytical problems. The analytical method that has been validated can then be applied to the analysis of nitrite and nitrate in the sample.

METHODS

1. Tools and Materials

The tools used for this experiment were a set of glassware, centrifuge, shaker, and UV-Vis spectrophotometer. Meanwhile the materials used are NaNO_2 , KNO_3 , PABA, NEDA, HCl, Zn powder, Zn rod, $\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$, NaCO_3 , $\text{Na}_2\text{C}_2\text{O}_4$, K_2SO_4 , $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$, $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (Pro analysis Merck).

2. Procedure

a. Optimization of nitrite analysis conditions

A total of 5.0 mL of 0.500 mg L^{-1} NaNO_2 solution was reacted with 3.5 mL of 3.65×10^{-4} M PABA and 2.0 mL of 2M HCl, then let the solution sits for 20 minutes. The benzene diazonium ion formed was coupled with 3.0 mL of 1.93×10^{-4} M NEDA. Then leave the solution to stand in the dark before measuring the absorbance at the maximum wavelength. The optimization carried out includes determining the maximum wavelength, the time of formation of the benzenediazonium ion, the time of formation and stability of the azo compound, the mole fraction of PABA and NEDA, the concentration of HCl and the pH buffer solution.

b. Optimization of nitrate reduction conditions using reductor

1) Zn powder

A total of 10.0 mL of 0.400 mg L^{-1} KNO_3 solution was reacted with 5 mL of ammonia buffer solution at pH 11 and 0.100 g of Zn powder. The mixture was stirred using a stirrer and centrifuged at 4000 rpm for 10 minutes. Optimization of nitrate reduction using Zn powder includes reducing temperature, reducing time, pH buffer, and weight of Zn powder.

2) Spongy cadmium

A total of 10.0 mL of 0.400 mg L^{-1} KNO_3 solution was reacted with 3.0 mL of ammonia buffer pH 8 and 0.300 g of spongy cadmium, then shaken for 60 minutes. The optimization includes reduction time, pH buffer, and weight of spongy cadmium.

3) Determination of anion and cation tolerance limits on interference of nitrite analysis

A total of 5.0 mL of 0.500 mg L^{-1} NaNO_2 solution was reacted with 2.0 mL of 1M HCl solution, 1.0 mL of 7.29×10^{-4} M PABA, and 2.0 mL of anion or cation solution in a 25 mL volumetric flask. Then leave the solution for 5 minutes before adding 1.0 mL of NEDA 7.72×10^{-4} M and the pH 3 buffer acetate to the limit mark. .

4) Validation of nitrite analysis method

The nitrite analysis method using UV-Vis spectrophotometry was validated using validation parameters such as linearity, sensitivity, detection limit, quantitation limit, precision, and accuracy.

5) Analysis of nitrite and nitrate in bread samples

Analysis of nitrite and nitrate content in bread samples was carried out using the

spiking method (standard addition). To each sample was added a standard solution with a concentration of 0.200, 0.400, and 0.600 mg L⁻¹. Then treated according to the nitrite and nitrate analysis procedure. The measurement results are plotted on a standard addition curve so that the concentration of nitrite and nitrate in the sample can be known.

RESULTS AND DISCUSSION

1. Optimization of Nitrite Analysis Conditions

Analysis of nitrite using UV-Vis spectrophotometric method is based on the diazotization reaction. The reaction is a reaction between nitrite ions with primary or secondary amine compounds in an acidic environment to form benzenediazonium ions. The benzenediazonium ion is then coupled with a benzene derivative to produce a purplish-pink azo compound [7].

a. Maximum wavelength

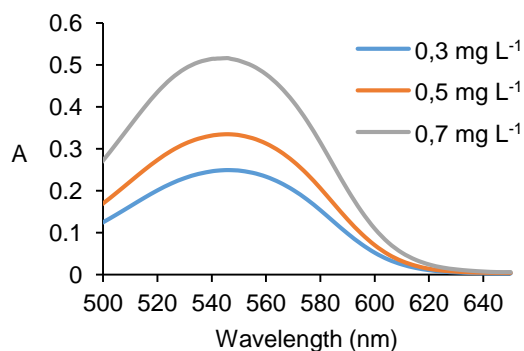


Figure 1. UV-Vis spectra of azo compounds at various concentrations

Figure 1 showed that the absorbance of azo compounds increases as the concentration of nitrite solution increases. The three concentrations of nitrite solution used had a maximum wavelength of 546 nm. The wavelength at the maximum absorption

corresponds to the characteristic absorption of colored compounds produced by electronic transitions from $\pi - \pi^*$ and $n - \pi^*$ from electrons in azo compounds [10].

The wavelength obtained from this study is influenced by the structure of the azo compound produced from the reaction of nitrite ion with PABA coupled with NEDA. The maximum wavelength of azo compounds obtained is not much different from the maximum wavelength of azo compounds produced from the reaction of nitrite ions with OABA coupled with NEDA which is 550 nm [7]. This difference can be caused by differences in the position of the amine group substituent in the OABA compound which is located in the ortho position while in the PABA compound it is located in the para position.

b. The reaction time of the formation of benzenediazonium ion

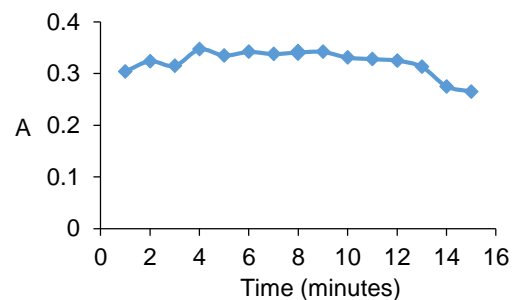


Figure 1. The relationship between the reaction time for the formation of benzenediazonium ions with azo compounds

The benzenediazonium ion was formed from the reaction between nitrite ions and PABA in an acidic environment. The effect of reaction time for the formation of benzenediazonium ion is presented in Figure 2. When the reaction time was more than 9

minutes, there is a decrease in absorbance due to the benzenediazonium ion formed in the solution phase having a short stability time so that it is easy to deform and decompose [11]. Therefore, the benzenediazonium ion that has been formed must be reacted immediately with reagents coupling so as not to decompose. The reaction time for the formation of benzenediazonium ions in this study faster than the diazotation reaction using OABA which is 20 minutes [7]. This time difference is due to the amine group in OABA is in the ortho position which allows there to be steric hindrance of the carboxyl group in the second step reaction. Thus, the formation of benzenediazonium ions takes longer.

c. Azo compound stability time

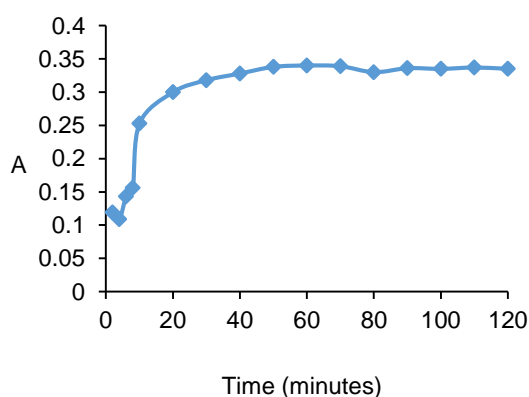


Figure 2. UV-Vis spectra of azo compounds at various concentrations

Based on Figure 3, Azo compounds had a stability time of 40-120 minutes for its absorbance measurement. Figure 3 shows that the absorbance of azo compounds increased with increasing curing time, which was 2-20 minutes. This is because the more benzenediazonium that formed and react with NEDA to form azo compound. The relatively constant absorbance occurred at

40-120 times indicating that the reaction between nitrite ions and PABA which produced benzenediazonium ions and the coupling reaction of benzenediazonium ions with NEDA had been maximized or the reagents used had reacted. The stability time of azo compounds from this study was longer than the stability time of azo compounds produced between nitrite ion and OABA/NEDA which is stable between 25-45 minutes [7].

d. HCl concentration effect

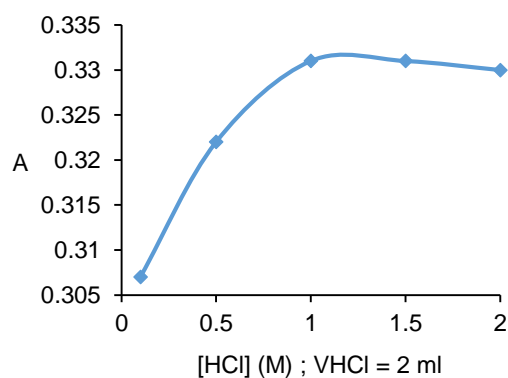


Figure 3. Effect of HCl concentration on the absorbance of azo compounds

Based on Figure 4, it is known that the optimum formation of nitric acid occurs at the use of 1 M HCl concentration and a volume of 2 mL while the use of HCl with a concentration of more than 1 M can interfere with the formation of nitric acid so that less benzenediazonium ions are formed. Under acidic conditions, azo compounds are in equilibrium between azo compounds and hydrazones. Therefore, the more HCl solution used, the protonation of the N atom in the azo compound will shift so that the equilibrium will shift towards the formation of hydrazones. This equilibrium shift will result

in a decrease in the number of azo compounds in the system [12].

e. Mol fraction of PABA and NEDA

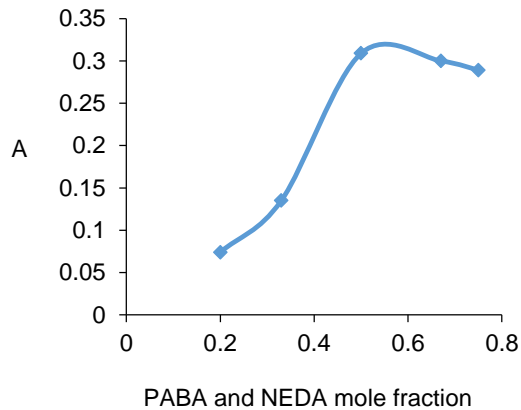


Figure 4. Absorbance of azo solution as a function of mole fraction of PABA and NEDA

Based on Figure 5, The mole fraction of PABA and NEDA which gives the maximum absorbance is 0.5 so that the stoichiometry of the reaction occurs when the number of moles for standard solutions of nitrite, PABA and NEDA is equal to 1.09×10^{-7} ; 7.34×10^{-5} dan 7.71×10^{-6} . It could be seen that the stoichiometry of the diazotization reaction occurs when the mole ratio of PABA and NEDA is 1:1 and corresponds to the mechanism of the diazotation reaction. At this stage, 1 mole of PABA will produce 1 mole of benzenediazonium ion which will be coupled by 1 mole of NEDA. The mechanism of the diazotation reaction consists of three steps, namely the formation of in situ nitric acid and nitroso groups, the formation of benzenediazonium ions and the coupling reaction.

f. Effect of pH buffer

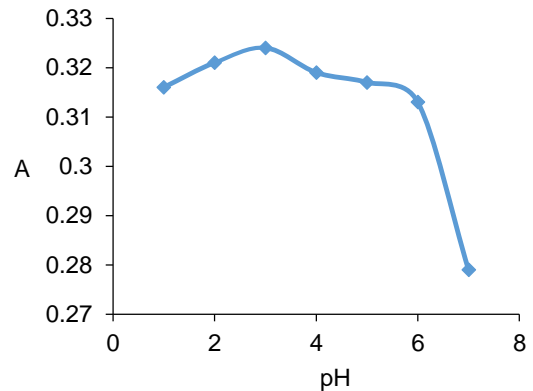


Figure 5. Effect of pH buffer on the absorbance of azo compounds

Based on Figure 6, it can be seen that the absorption of azo compounds increased at pH 1-3 and decreased at pH 4-7. The low absorbance at pH 1 and 2 was caused by the reaction conditions being too acidic, causing protonation of the azo group, while the decrease in absorbance at pH 4-7 could be due to the decreasing concentration of acid, the azo compound experienced a change in the actual pH. A very significant decrease occurred in the addition of buffer pH 7, which was caused by not fulfilling the coupling reaction conditions using NEDA due to a significant change in the pH of the solution. The optimum pH obtained in this study was in accordance with the optimum pH, the use of a buffer pH 3 can maintain the actual pH of the azo solution, which is between 1.24-1.28 [1]. The results of the optimization of the effect of pH on the absorbance of azo compound is presented in Figure 6.

g. Ionic interference to nitrite analysis

According to Narayana and Kenchaiah, the acceptable ion tolerance limit in nitrite

analysis was that which does not cause a change in absorbance > 5% [13].

Table 1. Ionic tolerance limits for nitrite analysis.

Ion Variety	Ionic tolerance limits (mg L ⁻¹)
CO ₃ ²⁻	< 500
C ₂ O ₄ ²⁻	< 500
SO ₄ ²⁻	> 10000
Mg ²⁺	< 10000
Ca ²⁺	> 10000
Zn ²⁺	> 10000
Fe ²⁺	< 10
Fe ³⁺	< 5

Table.1 showed that the most disturbing ions in nitrite analysis were Fe²⁺ and Fe³⁺ because they are able to reduce nitrite to NO, so that at low concentrations, it can reduce the absorbance of azo compounds > 5%. Metal cations can interfere with nitrite analysis by reacting with the azo group (-N=N-) to form other complex compounds. Meanwhile, anions can interfere with nitrite analysis by accelerating the degradation process of azo compounds [14]. In this research, for ions such as CO₃²⁻,

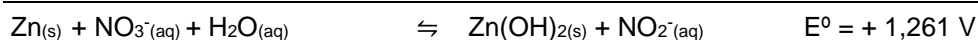
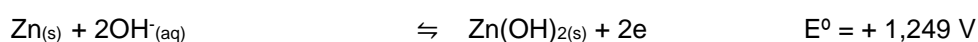
C₂O₄²⁻, SO₄²⁻, Ca²⁺, Zn²⁺, and Mg²⁺ are not very disturbing because these could be due to the fact that ions have higher standard reduction potential than nitrite ion, but these still lower than Fe²⁺ and Fe³⁺, so that these ions can only reduce absorbance in large concentrations [1].

2. Optimization of Nitrate Reduction Conditions Using Zn Powder and Spongy Cadmium as A Reductor

Analysis of nitrate was carried out by reducing it to nitrite and then reacting the resulting nitrite with PABA and NEDA to form azo compound.

a. Optimization of nitrate reduction conditions using Zn powder

Reduction of nitrate to nitrite by Zn powder occurred when Zn transfers electrons directly to nitrate to convert nitrate to nitrite which occurs on the surface of Zn followed by the release of nitrite because it has a low affinity for the surface of Zn. The reaction of nitrate reduction by Zn powder is presented in the following equation [15].



1) Effect of reduction temperatur

Based on Figure 7, the optimum absorbance of azo compounds was at a temperature of 50°C. This is because the heating process provides energy to the system so that the Zn powder is more easily oxidized and causes nitrate to be more easily reduced to nitrite. Research by Kumar using Mg(0) reducer showed that based on nitrate

reduction, it increased with increasing temperature to reach activation energy [16]. Absorbance decreased at a temperature of 60-70°C. This was due to the fact that in alkaline conditions and high temperatures, nitrate can undergo further reduction to NH₃ gas which is the main production of nitrate reduction in alkaline conditions in addition to forming nitrite [17].

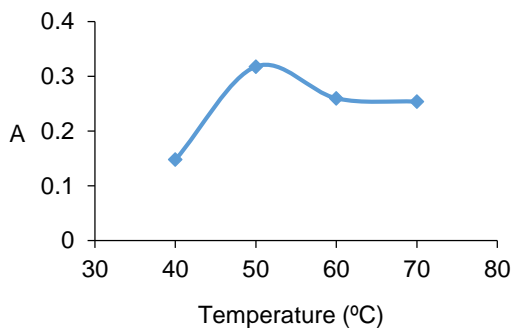


Figure 6. Effect of nitrate reduction temperature.

2) Effect of buffer pH

The use of ammonia buffers optimum at pH 11 due to mass transfer of OH⁻ ions from ammonia buffer pH 11 was sufficient to cause Zn powder to be oxidized to Zn(OH)₂ so that nitrate was reduced to nitrite. The effect of buffer pH on the absorbance of azo compounds is presented in Figure 8. The decrease in the reduction of nitrate to nitrite was due to the more Zn(OH)₂ formed, thus blocking the interaction between nitrate and Zn powder. The results of this study are in accordance with Kumar who used Mg(0) as a reducing agent which also decreased at too alkaline pH due to the more formation of Mg(OH)⁻² and blocking the interaction of nitrate with Mg(0) [16].

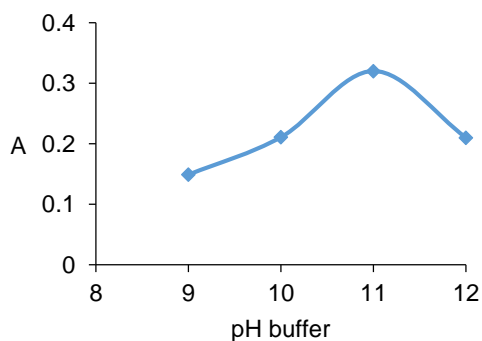


Figure 7. Effect of pH buffer on the absorbance of azo compound

3) Effect of reduction Time

Based on Figure 9 shows the optimum reduction time at 5 minutes in accordance with Merino [18]. The increase in absorbance of azo compounds showed that more nitrate was reduced to nitrite because at that time Zn powder could donate enough electrons to reduce nitrate and did not undergo further reduction. The decrease in absorbance occurred at a stirring time of 10 minutes caused by the length of contact time between nitrate and Zn powder, allowing nitrite to undergo further reduction to produce NO gas because Zn powder has a good surface for the occurrence of electron transfer events in the solution phase [19].

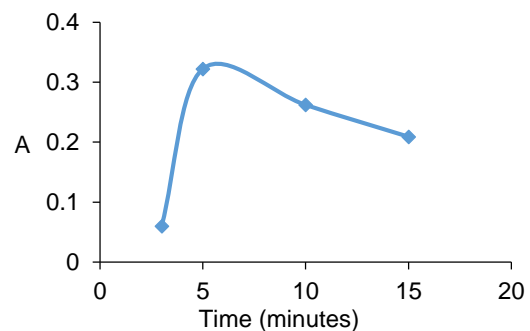


Figure 8. Effect of reduction time on the absorbance of azo compound

4) Effect of Zn powder Weights

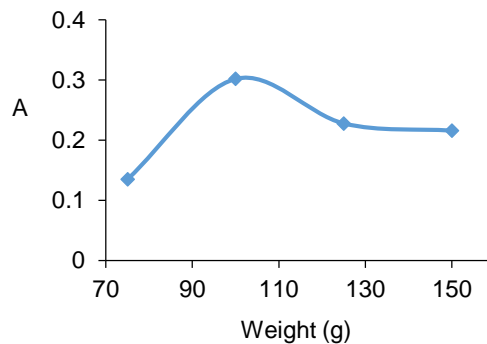


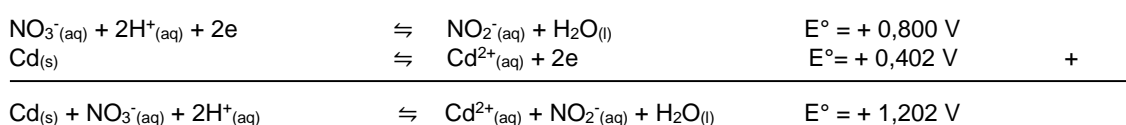
Figure 9. Effect of Zn powder weight on the absorbance of azo compound

Based on Figure 10 the reduction in the reduction of nitrate to nitrite on the addition of excess Zn powder is due to the more Zn powder added, the more electrons are donated so that it allows the formation of more $Zn(OH)_2$ so that it interferes with the reduction process. Kumar and Chakraborty explain that more nitrite was produced than NH_3 gas when less $Mg(0)$ was used [16]. Nitrat can be reduced to nitrite and NH_3 depends on the ability of $Mg(0)$ to donate electrons. The electron donating ability of $Mg(0)$ increases with increasing weight $Mg(0)$

is used. Nitrite produced more than NH_3 when $Mg(0)$ is used less, while NH_3 will produced more if the amount of $Mg(0)$ used during the reaction is greater.

b. Optimization of nitrate reduction conditions using spongy cadmium

Spongy cadmium is more effective than cadmium column because the reduction process occurs more evenly so that nitrate can be reduced to nitrite maximally [20]. The reaction that occurs during the reduction of nitrate to nitrite by spongy cadmium is presented in the following equation:



1) Effect of Buffer pH

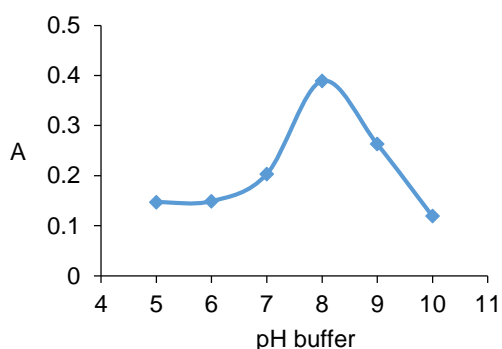


Figure 10. Effect of buffer pH on the absorbance of azo compound

Based on Figure 11 it can be seen that the increase in nitrite absorbance occurs when the addition of pH buffer 5 and the optimum is at pH buffer 8. The increase in absorbance indicates that more nitrate is reduced in acidic conditions. Meanwhile the decrease in absorbance may be due to the formation of $Cd(OH)_2$ deposits which can interfere with the nitrate reduction process.

This is in accordance with the research of Nikonorov and Belyanskaya, where nitrate reduction using Cd at a pH of more than 8 can reduce absorbance due to the formation of $Cd(OH)_2$ [21].

2) Effect of reduction Time

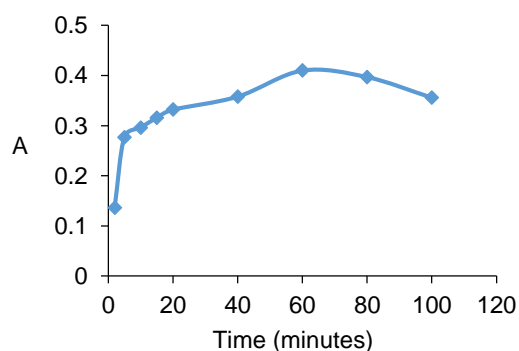


Figure 11. Effect of reduction time on the absorbance of azo compound

Based on Figure 12, the reduction of nitrate to nitrite is optimum with a reduction of about 60-90 minutes. This shows that the longer the contact time between nitrate and

spongy cadmium, the more nitrite produced . According to the results of Jones research, reduction of nitrate to nitrite was optimum with a reduction time of about 60-90 minutes because less than 60 minutes can produce imperfect reduction [9].

3) Effect of the spongy cadmium weight

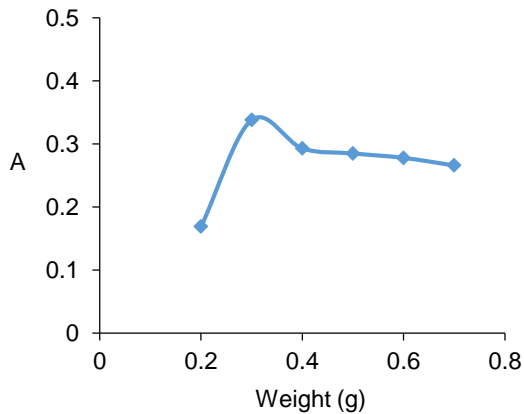


Figure 12. Effect of spongy cadmium weight on the absorbance of azo compound

Based on Figure 13, the absorbance of azo compounds increased with the addition of spongy cadmium. The decrease in absorbance occurred when the addition of spongy cadmium was 0.400 g and the decrease was relatively stable until the addition of 0.700 g was caused by the formation of Cd(OH)₂.

3. Validation of Nitrite and Nitrate Analysis Method

The correlation between absorbance and standard concentrations of nitrite and nitrate is presented in Figures 14,15, and 16. Based on the results of the study, it was shown that the nitrite analysis method without a reduction process and the nitrite reduced by nitrate using Zn powder and spongy cadmium had linearity in the range of 0-1 mg L⁻¹ with a correlation coefficient (R²) ≥ 0.9990. The linearity of the nitrite analysis method in the

range of 0-1 mg L⁻¹ has complied with Lambert-Beer law.

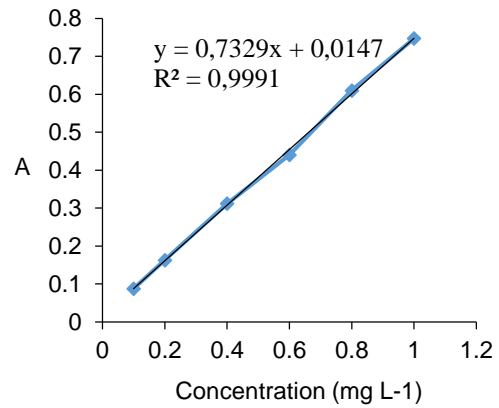


Figure 13. Nitrite calibration curve without reduction process

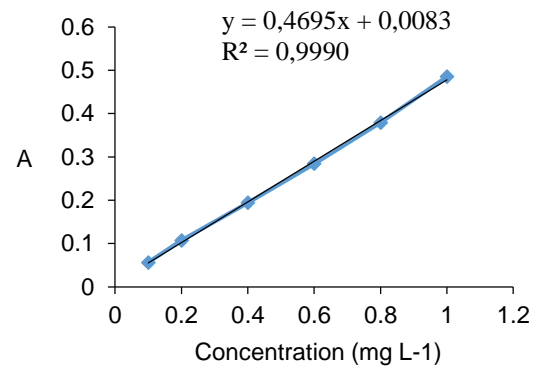


Figure 14. The reduced nitrite calibration curve using Zn powder

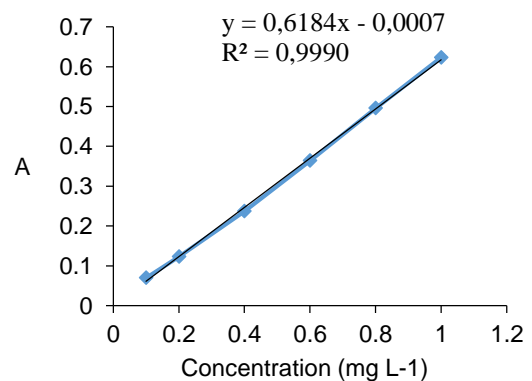


Figure 15. The reduced nitrite calibration curve using spongy cadmium

The sensitivity of the method was determined from the slope value of the

calibration curve equation which is expressed as the molar absorptivity (ϵ). The yield of nitrite is $3.37 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$. The value of nitrite that was reduced using Zn powder was $2.91 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and the value of nitrite that was reduced using spongy cadmium was $3.83 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$.

The nitrite detection limit value obtained was $4.29 \times 10^{-3} \text{ mg L}^{-1}$, meanwhile the detection limit value for nitrite reduced using Zn powder and spongy cadmium was 9.34×10^{-3} and $7.58 \times 10^{-3} \text{ mg L}^{-1}$. The detection limit of nitrite analysis obtained in this study was higher than that of nitrite analysis using dapsona/ α -napson which had a detection limit of $1.12 \times 10^{-3} \text{ mg L}^{-1}$ [7].

The precision of the nitrite analysis method is expressed as repeatability and is measured as Relative Standard Deviation (RSD). According to Gonzales, the criteria for an acceptable RSD value based on analyte concentration 0-1 mg L^{-1} is 16% [22]. Based on the results of this study, it was shown that the analysis of nitrite and nitrate using PABA and NEDA had %RSD in the range of 0.31-1.83%. The RSD value that has been reported

for nitrite analysis using sulfanilamide in surface water samples is 1.10% [18].

The accuracy of the analytical method is expressed in percent recovery (% recovery) and is determined using the spiking method [23]. %recovery for nitrite without reduction process, nitrite reduced using Zn powder and nitrite reduced using spongy cadmium ranged from 84.9-101.4%; 83.2-96.4% and 90.9-104.4%. The amount of % recovery in this study is acceptable because % recovery meets the acceptance criteria by Miller and Miller that % recovery close to 100% reduces the possibility of bias [23].

4. Analysis of Free Nitrite and Nitrate in Bread samples

To determine the content of nitrite and nitrate in pancakes and bread, measurements were made using the spiking method. Each analyzed sample was added with a standard solution of nitrite and nitrate with known concentrations. The results of the calculation of the content of nitrite and nitrate in pancakes and bread are presented in Table 2.

Table 2. The content of nitrite and nitrate in the sample

Sample	[Nitrite] ^(a) (mg kg^{-1})	[Nitrate] ^(b) (mg kg^{-1})	[Nitrate] ^(c) (mg kg^{-1})
Bread 1	0.162	0.778	0.825
Bread 2	0.197	0.819	1.069
Bread 3	0.178	0.762	0.765

Notes: (a) No reduction process, (b) reduction using Zn powder and (c) reduction using spongy cadmium

Based on Table 2, it can be seen that the content of nitrite and nitrate in pancakes and bread is very low when compared to the food safety quality standard according to the PERMENKES RI No. 1168/Menkes/Per/X/1999 which states the maximum limit of nitrite in food is 125 mg kg^{-1} [24].

CONCLUSION

Analysis of nitrite and nitrate by UV-Vis spectrophotometry through the reaction between PABA and nitrite in an acidic environment coupled with NEDA produced azo compounds with λ_{max} 546 nm. The use

of spongy cadmium as a reductor was more optimal because it has better sensitivity, detection limit, and % recovery than Zn powder. Only Fe²⁺ and Fe³⁺ ions had the greatest level of interference because they can reduce absorbance 5% with a concentration of 10 mg L⁻¹. The analysis method of nitrite and nitrate using PABA/NEDA reagent by UV-Vis spectrophotometry had linearity, sensitivity, detection limit, quantization limit, precision and accuracy that meet the criteria for acceptance of the method validation. The results of the analysis in bread showed that the content of nitrite and nitrate still can be accepted for daily consume.

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