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Analysis of Nitrite and Nitrate in Vegetables in Medan City

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ABSTRACT

Vegetables are the main sources of nitrate and nitrite in food. The presence of nitrate and nitrite at high level may cause negative impact on health, because nitrite and nitrate when reduced to nitrite, may react with alkyl amine or amide to form carcinogenic nitrosamine. The purpose of this research was to analyze nitrite and nitrate in several vegetables in Medan City. Samples were obtained from Berastagi Supermarket. Nitrite levels were analyzed by visible spectrophotometry method at maximum wavelength of 540 nm after derivatization with sulfanilic acid and N-(1-naphthyl) ethylenediamine dihydrochloride in acetic acid solution. Nitrate was assayed as nitrite after nitrate reduced into nitrite by Zn in acidic condition. The condition for nitrate reduction was optimized using concentrated HCl, HCl 1 N, and without the addition of HCl. The results showed that the nitrate reduction process with Zn using HCl 1 N was found to be the optimal condition. The results showed that levels of nitrite and nitrate in analyzed samples varied significantly. Spinach contained the highest levels of nitrite (24.681 mg/kg) while watercress contained the lowest levels of nitrate (4.662 mg/kg). However spinach contained the highest levels of nitrate (103.73 mg/kg) while kale contained the lowest levels of nitrate (9.68 mg/kg). The results of this study indicated that the nitrate levels are higher than nitrite levels in analyzed vegetables.

Keywords: Vegetables, Nitrite, Nitrate, Visible spectrophotometry

INTRODUCTION

Nitrite and nitrate are widely available in nature, in soil, water, and foods especially vegetables [1]. Nitrite may react with secondary or tertiary amines to form carcinogenic nitrosamines which may increase the risk of gastrointestinal cancer [1-3]. In addition, negative effects of nitrite and nitrate may cause symptoms of blue skin (cyanosis), shortness of breath, nausea, vomiting, shock, hyperparathyroidism, children polyuria, hypertension, and methemoglobinemia especially in infants. Death can occur if the levels of methemoglobinemia reached 70% in infants [4-5].

Vegetables are the major source of nitrite and nitrate intake from food. Nitrite and nitrate are deliberately added to some foods such as processed meats as preservatives and colouring agents. Nitrite and nitrate are also present in drinking water, processed meats such as canned meat, and sausage [5-8].

Several studies reported that the nitrate content in vegetables vary widely from 1 to 10.000 mg/kg. The levels of nitrite and nitrate are various not only depending on the type of vegetables, but also affected by many factors including environment factors such as atmospheric humidity, temperature, irradiance, and agricultural practices such as fertilization and nitrogen doses, availability of other nutrients, herbicides, and storage condition [6,9].

The intake permitted (Acceptable Daily Intake=ADI) by FAO/WHO is 220 mg of nitrate and 8 mg of nitrite per day for adult with the weight of 60 kg. Therefore, the amount of nitrite and nitrate present in vegetables is important to be considered due to their negative impact on health. Therefore, it is necessary to investigate the average intake of nitrite and nitrate from vegetables most commonly consumed by people in Medan City. The purpose of this study was to analyze the levels of nitrite and nitrate in vegetables most commonly consumed by the people in Medan City. Nitrite was analyzed by visible spectrophotometry method using Griess reagent. Nitrate was determined as nitrite after reduction into nitrite [3,6,10-13]. This study started with the optimization of nitrate reduction into nitrite using Zn powder with the different acidic condition.

MATERIALS AND METHOD

Chemicals

Chemicals used were pro analysis grade product of E. Merck (Germany) including; N-(1-naftil) Ethylenediamine Dihydrochloride (NED), sodium nitrite, sulfanilic acid, glacial acetic acid, hydrochloric acid, antipyrin, ferrous sulfate, zincum powder, sodium nitrate.

Instruments

Instruments used in this study were spectrophotometer UV-Vis (UV-1800 Shimadzu), analytical balance (Boeco Germany), water bath, filter paper, thermometer and glass wares.

Preparation of reagents

Acetic solution 15% was prepared by dissolving 75 ml glacial acetic acid and diluted with distilled water to make 500 ml. NED solution was made by dissolving 0.350 g N-(1-naftil) etylenediamin dihydrochloride in 250 ml acetic acid solution 15%, filtered and stored in brown bottle. Sulfanilic acid solution was prepared by dissolving 0.850 g sulfanilic acid in 250 ml acetic solution 15%, filtered and stored in brown bottle [14].

Sodium nitrate was dried at 110°C for one hr, then cooled in a desiccator. Weighed 100 mg sodium nitrate, and then transferred into 100 ml volumetric flask and dissolved in distilled water, and then made to 100 ml (C=1000 µg/ml) (Solution I). From this solution 1 ml was pipetted into 100 ml volumetric flask and added distilled water to make 100 ml (C=10 µg/ml) (Solution II).

The amount of 100 mg sodium nitrite was weighed and transferred into 100 ml volumetric flask and dissolved in distilled water, and then made to volume (C=1000 µg/ml) (Solution III). From this solution 1 ml was pipetted into 100 ml volumetric flask and added distilled water to make 100 ml (C=10 µg/ml) (Solution IV) [6].

Samples

The vegetables analyzed in this research were spinach (*Amaranthus tricolor* L.), lettuce (*Lactuca sativa* L.), Bok choy (*Brassica rapa* L.), and chinese kale (*Brassica oleracea* var. *alboglabra*), watercress (*Nasturtium officinale*), sweet mustard (*Brassicca rapa chinensis*), bitter mustard (*Brassica juncea*), Spinacia (*Spinacia oleracea* L.), red lettuce (*Lactuca sativa* var. *Crispa* L.), kale (*Ipomea aquatica*) vegetables obtained from Berastagi Supermarket in Medan.

Identification of nitrite and nitrate

Some amount of grounded (± 10 g) sample of each vegetable was transferred in to beaker glass, added distilled water, heated on water bath and shaken for a little while, then cooled and filtered. The supernatant was taken and placed in a test tube for nitrite and nitrate identification. Presence of nitrite was identified with sulfanilic acid and NED solution and allowed for a little while. The appearance of violet color was to indicate the presence of nitrite. The presence of nitrate was conducted by adding several drops ferrous sulfate solution and then added slowly few drops of concentrated sulfuric acid through the inner wall of the test tube. The presence of nitrate was indicated by the formation of chocolate ring [5,8,14].

Determination of absorbance curve of nitrite standard solution

4 ml of standard solution of nitrite (C=10.0 µg/ml) was transferred into 50 ml volumetric flask, added 2.5 ml sulfanilic acid solution and shaken, after 5 min 2.5 ml NED reagent was added and made to volume with distilled water and homogenized (C=0.8 µg/ml). Absorbance was measured at wave length of 400-800 nm. Then, absorbance and wave length was plotted to construct absorbance curve. Wave length of maximum absorbance was determined from the absorbance curve [6].

Absorbance stability of derivatized nitrite to determine working time

Four (4) ml of standard solution of nitrite (C=10.0 µg/ml) was transferred into volumetric flask of 50 ml, to which 2.5 ml of sulfanilic acid and stirred. After 5 min, 2.5 ml NED reagent was added and distilled water was added to make 50 ml. Absorbance was measured at wave-length of maximum absorbance obtained from absorbance curve (540 nm), and stability of absorbance was determined by observing absorbance at every minute for 1 hr. The absorbance was found to be relatively stable within 6 min in 7-12 min [6].

Determination of calibration curve

Standard solution of nitrite (C=10.0 µg/ml) of different volume (0.5,1,2,3,4 dan 5 ml) were transferred into separated volumetric flasks of 25 ml, then 2.5 ml sulfanilic acid reagent added and stirred to homogenize. After 5 min, 2.5 ml NED reagent was added, then distilled water was added to make volume of 25 ml and homogenized. The series of concentration of prepared solutions were of 0.1 µg/ml, 0.2 µg/ml, 0.4 µg/ml, 0.8 µg/ml, 1.0 µg/ml. Absorbance of each solution was measured at wave-length of 540 nm within 7 min. Calibration curve was made by plotting absorbance versus concentration of each solution. From the graph obtained, then linearity of regression equation and correlation coefficient were calculated ($Y=aX+b$) [6].

Effect of acidic condition to reduce nitrate into nitrite

5 ml of standard solution of nitrate (C=10.0 µg/ml) was transferred into volumetric flask of 50 ml. Then, 0.1 g Zn and 1 ml of different concentrations of HCl (concentrated HCl, HCl 1 N, without addition HCl) allowed to stand for 10 min (to reduce nitrate

to nitrite for nitrate quantification) were added and then 2.5 ml sulfanilic acid reagent was added and stirred. After 5 mins, 2.5 ml reagent NED was added, then distilled water was added to make 50 ml, then homogenized. Absorbance was measured at wave-length of 540 nm within 7 min time. The highest absorbance obtained was the optimum condition to reduce nitrate into nitrite. Then, the effect of the acidic condition to reduce of nitrate to nitrite was used to analyze nitrate in samples.

Determination of nitrite and nitrate in vegetables

Determination of the nitrite in vegetables was performed with procedure described [12]. Around 10 g grounded sample transferred into 250 ml beaker glass. Then, hot distilled water ($\pm 80^{\circ}\text{C}$) was added to make volume of 150 ml. Homogenized by stirring and heated on water-bath for 15 min while stirring. Allowed to cool and then transferred quantitatively into volumetric flask of 250 ml. Added distilled water to make volume of 250 ml, then filtered. About 10 ml first filtrate was discarded, the following filtrate was collected. The filtrate obtained was used firstly for nitrite and then for nitrate determination. For nitrite; 10 ml of filtrate transferred into a volumetric flask of 50 ml, then 2.5 ml sulfanilic acid reagent was added and stirred. Then after 5 mins, 2.5 ml reagent NED was added, and then distilled water was added to make 50 ml, and then homogenized. Absorbance was measured at wave-length of 540 nm after period of 7-12 min time. For nitrate; 10 ml of filtrate and transferred into volumetric flask of 50 ml, then 0.1 g Zn and HCl 1 N allowed to stand for 10 min (to reduce nitrate to nitrite for nitrate quantification) were added, then 2.5 ml sulfanilic acid reagent was added and stirred. After 5 min, 2.5 ml reagent NED was added, then distilled water was added to make 50 ml, then homogenized. Absorbance was measured at wave-length of 540 nm within 7 mins time. The result was the total nitrite that was originally present and nitrite from reduction of nitrate. Level of nitrite was calculated using from the regression equation:

$$Y=aX+b.$$

Nitrite concentration was calculated:

$$C=\frac{x \times V \times Fp}{\text{sample weight (g)}}$$

Notes:

Y=Absorbance

C=Concentration of nitrite in sample ($\mu\text{g/g}$)

X=Nitrite concentration in diluted sample solution calculated from regression equation ($\mu\text{g/ml}$)

V=Volume of sample solution before dilution (ml)

Fp=Dilution factor

Concentration of nitrite from the reduction of nitrate into nitrite=concentration of total nitrite after reduction–concentration of nitrite before reduction.

$$\text{Nitrate concentration}=\text{Nitrate concentration after reduction} \times \frac{\text{MW nitrate}}{\text{MW nitrate}}$$

RESULTS AND DISCUSSION

Identification of nitrite and nitrate in samples

Based on the result from the qualitative identification using sulfanilic acid and NED reagents indicated by the appearance of violet color to prove that all samples contained nitrite. The identification reaction was also confirmed with antipyrine in dilute hydrochloric resulted in the formation of green color. Identification of nitrate in samples was conducted using ferrous sulfate and concentrated sulfuric acid which resulted in brown ring indicating the presence of nitrate in samples [5,8,14].

The wave-length of maximum absorption

The wave-length at which maximum absorption occurred was determined by measuring absorption of a solution with concentration of 10 $\mu\text{g/ml}$. Absorption was measured at a range of wave length from 400 nm through 800 nm. Absorbance curve of nitrite derivative can be seen in Figure 1.

Maximum absorption was found to be 540 nm which is similar to that value previously reported [12]. Maximum absorption observed was then used to determine working time for analysis of nitrite and nitrate in samples.

Working time of measurement

Working time of measurement for nitrite and nitrate was determined to know the period of time with-in which the absorbance of solution still remains stable. Absorbance of nitrite derivative with *Griess* reagent can be seen in Figure 2.

Absorbance of 0.8 $\mu\text{g/ml}$ solution was measured at wavelength of 540 nm for 60 min. It was found that the absorbance was stable within 7-12 min which is called working time. This working time was used in analysis procedure.

Calibration curve

By plotting absorbance versus concentration of each solution then obtained graph, then linearity of regression equation and

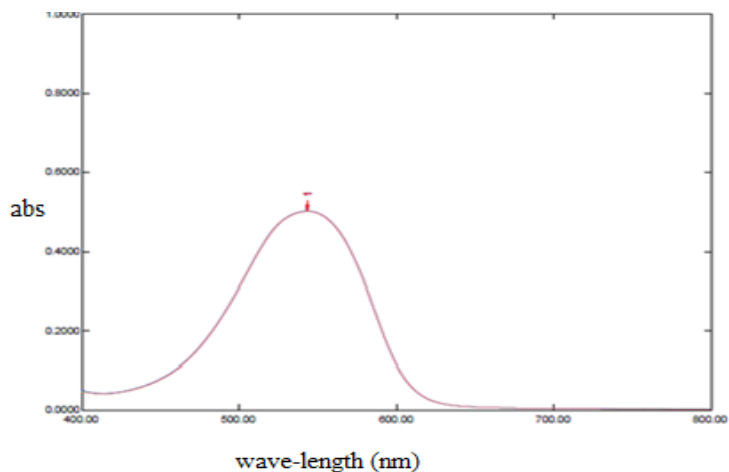


Figure 1: Absorbance curve of nitrite derivative

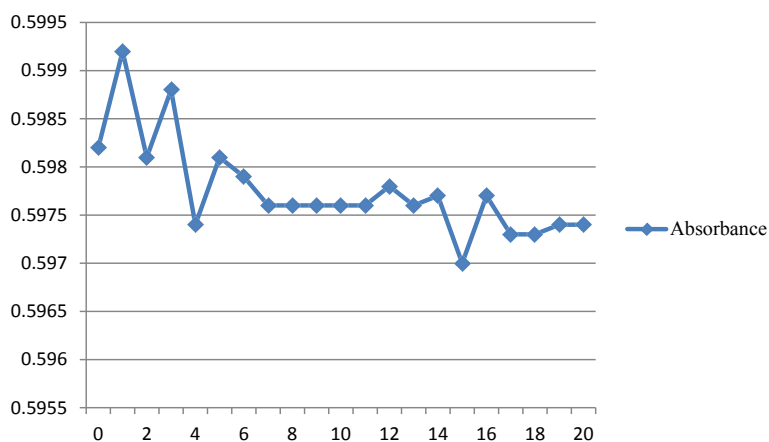


Figure 2: Absorbance of nitrite derivative with time

Correlation coefficient were calculated ($r=0.999$). This is to indicate that correlation between concentration and absorbance is high. The calibration curve can be seen in Figure 3.

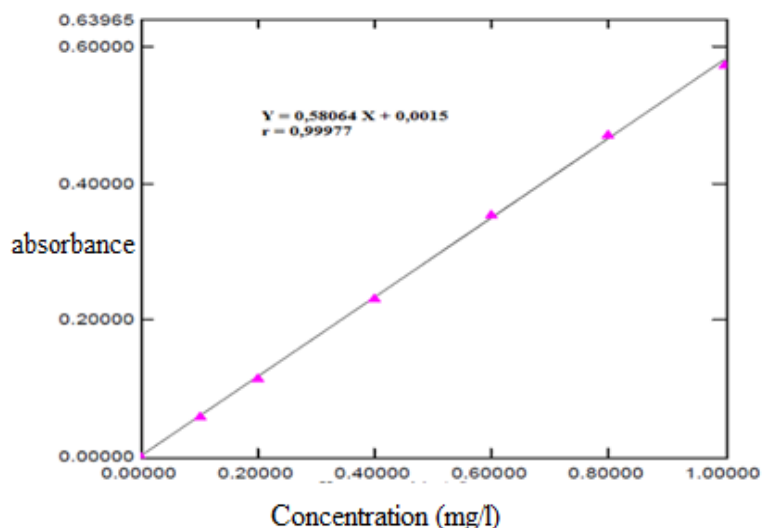


Figure 3: Calibration curve of nitrite derivative

From calibration curve was known that there is linearity between concentration and absorbance. Regression equation obtained is $Y=0.58064 \times +0.0015$ with coefficient correlation (r) of 0.99977 where $r>0.999$.

Effect of acidic condition to reduce nitrate into nitrite

The effect of acidic condition to reduce nitrate into nitrite was indicated by absorbance measured at 540 nm of nitrite after derivatization with Griess reagent. The result of this experiment can be seen in Table 1.

Table 1: Effect of acidic condition to reduce nitrate into nitrite

Replicate	Absorbance of Derivatized Nitrite with <i>Griess</i> Reagent with Different HCl Concentration		
	HCl (concentrated)	HCl (1 N)	Without HCl
1	0.0060	0.0127	0.0059
2	0.0059	0.0169	0.0057
3	0.0033	0.0189	0.0061
4	0.0022	0.0182	0.0068
5	0.0023	0.0183	0.0059
	$\bar{X} = 0.0039$	$\bar{X} = 0.017$	$\bar{X} = 0.00608$

From the Table 1. it can be concluded that the absorbance produced using dilute hydrochloric acid (1 N) was found to be the best and stable compared with concentrated HCl and without the addition of acid solution. This is to indicate that acidic condition 1 N HCl is the optimum acidic condition to reduce nitrate into nitrite.

Nitrite and nitrate levels in analyzed vegetables

The levels of nitrite and nitrate in the samples are presented in Table 2 and Figure 4, can be seen that spinach and other vegetables samples contained significant different levels of nitrite and nitrate. Of the samples analyzed, spinach contained the highest levels of nitrate (103.729 mg/kg) while kale contained the lowest levels of nitrate (9.681 mg/kg). Spinacia contained the highest levels of nitrite (24.681 mg/kg), while watercress contained the lowest levels of nitrite (4.622 mg/kg).

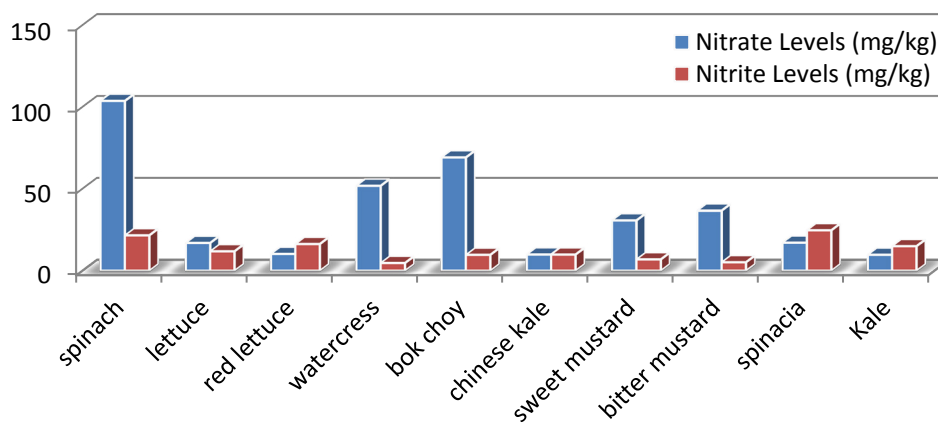


Figure 4: Levels of nitrite and nitrate in the vegetable samples

Table 2: Levels of nitrite and nitrate in the analyzed vegetables

No.	Vegetables	Nitrite Levels (mg/kg)	Nitrate Levels (mg/kg)
1	Spinach	21.589 ± 0.4706	103.729 ± 2.887
2	Lettuce	11.823 ± 0.0408	16.949 ± 1.139
3	Red Lettuce	16.275 ± 0.285	10.215 ± 1.161
4	Watercress	4.662 ± 0.057	51.830 ± 0.292
5	Bok Choy	9.657 ± 0.084	69.219 ± 1.281
6	Chinese Kale	9.810 ± 0.049	9.855 ± 1.029
7	Sweet Mustard	6.868 ± 0.097	30.739 ± 1.996
8	Bitter Mustard	5.117 ± 0.112	36.636 ± 2.508
9	Spinacia	24.681 ± 0.172	17.103 ± 1.137
10	Kale	14.960 ± 0.052	9.681 ± 1.281

Note: data is the mean of six replicates

From the data above, six vegetables; spinach, bokchoy, watercress, sweet mustard, bittermustard, and lettuce contained nitrate levels higher than nitrite levels. This could be due to fact more than 90% nitrogen absorbed in plants in the form of nitrate, since nitrate is the main nutrient for plant growth. In addition, the higher nitrate concentration is associated with the effect of light intensity [13]. But in other four vegetables; red lettuce, chinese kale, spinach, and kale contained nitrite levels were higher than nitrate levels. This is could be influenced by agricultural practices such as nitrogen doses and fertilization, availability of other nutrients, the use of herbicide, and storage condition [6,9]. Similar result was also reported by previous study that the nitrate content in several vegetables vary widely ranges from 1-10.000 mg/kg. Nitrite or nitrate levels in vegetables vary greatly from one to another. These different were affected by many factors including season, light, temperature, storage condition, the type of fertilizers, and processing, etc. [3,8,9,15-18].

High levels of nitrite and nitrate in vegetables, especially spinach, bok coy, sweet mustard, bitter mustard greens, watercress, and spinacia are very of great concern. Because high intake of nitrite and nitrate may cause negative impact for health, since nitrite may

react with alkyl amine to form nitrosamine. This reaction may occur in acidic condition in stomach. In addition, nitrate present in vegetables can be reduced to nitrite in gastrointestinal tract by bacteria. Moreover, the high level of nitrate in vegetables can be reduced into nitrite during processing and hence nitrite level could exceed/above Acceptable Daily Intake (ADI) made by WHO (8 mg/day) [7,9,10].

CONCLUSION

Spinach contained the highest level of nitrate in analyzed vegetables (103.729 mg/kg) while kale contained the lowest levels of nitrate (9.681 mg/kg). Spinach also contained the highest levels of nitrite (24.681 mg/kg), while watercress contained the lowest level of nitrite (4.622 mg/kg). In the samples analyzed, nitrite levels range from 5-25 mg/kg, while nitrate levels ranged from 9-104 mg/kg. Further study on factors affecting the nitrate and nitrite level in vegetables is necessary to investigate.

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