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Research Article

EFFECT OF HARVESTING TIME AT MORNING, AFTERNOON, AND EVENING ON NITRATE AND NITRITE LEVEL IN SPINACH (*AMARANTHUS TRICOLOR* L.) AND MUSTARD (*BRASSICA RAPA* L.)

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ABSTRACT

Objective: The objective of this study is to determine the effect of harvesting time at morning, afternoon, and evening on nitrate and nitrite level in spinach and mustard.

Methods: Nitrite identification was done using sulfanilic acid reagent and N-(1-naphthyl) ethylenediamine dihydrochloride. Identification of nitrate was done using reagent ferrous sulfate. Determination of nitrites level was performed visible spectrophotometry using N-(1-naphthyl) ethylenediamine dihydrochloride at maximum wavelength of 540 nm. Nitrate determination is taken with the same method but started with reduction process from nitrate into nitrite using Zn powder in acid condition and then measured as nitrite.

Results: Research result shows that there is a change of nitrate and nitrite level from the spinach harvested at morning, afternoon, and evening.

Conclusion: Spinach and mustard are better harvested in the morning because it contains nitrite less than in spinach picked afternoon and evening. Level of nitrite increases from morning to afternoon and decreases from afternoon to evening. However, the level of nitrate decreases from morning to afternoon and increases from afternoon to evening.

Keywords: Harvesting time, Nitrate, Nitrite, Spinach, Mustard.

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INTRODUCTION

Vegetables have been used since thousands of years ago to be consumed and processed naturally without using any mixture. The content of nutrients such as vitamins and minerals most sourced from vegetables [1]. One type of vegetables that many people favored are spinach and mustard because it has a soft texture, easy in the can, the price is relatively cheap, and how the cultivation is easy. Spinach and mustard are very good for children, especially babies. For babies, spinach and mustard usually mix with steam rice. Spinach and mustard also speed up the healing process for the sick [2].

Vegetables are rich in mineral salts such as calcium, phosphorus, and iron. Spinach also contains several vitamins, such as Vitamins A, B, C, and E. Vegetables also contain nitrite and nitrate that are harmful to the body [3]. The amount of intake allowed (FAO/WHO acceptable daily intake = ADI) for 60 kg body weight is 80 mg nitrite and 220 mg of nitrate. Consuming lots of vegetables is highly recommended, but considering the very high nitrate content in vegetables, it is considering the potential of nitrosamine formation from nitrite [4].

Previous research has shown a change in nitrite and nitrate contents in fresh and boiled vegetables. Nitrite and nitrate levels in vegetables have decreased, after boiling for 15 min. Nitrite levels increased from storage time, while for nitrate content decreased [5]. Vegetables with urea fertilizer have a higher nitrite and nitrate levels when compared with vegetables that without urea fertilizer [6].

The nitrate content in the plant is influenced by soil treatment, the amount and time of nitrogen fertilizer application, temperature, light intensity, harvest time, plant diseases, canning, and storage process [7]. The intensity of light is very influential on the rate of photosynthesis. The rate of photosynthesis will increase with the increase of light intensity, thus affecting the nitrate and nitrite levels in plants (Lakitan, 1995). Photosynthesis is the process of synthesis of sugars (carbohydrates) from inorganic materials, carbon dioxide (CO_2), and water (H_2O) in pigmented plants with the help of solar energy [8]. Photosynthesis is closely related to the assimilation of nitrate, the formation of nitrate ions (NO^{3-}) into nitrite ions (NO^{2-}), ammonia (NH_3), and amino acids [9]. The purpose of this study was to determine the effect of harvesting time (morning, afternoon, and evening) on nitrite and nitrate levels in spinach (*Amaranthus tricolor* L.) and mustard (*Brassica rapa* L.).

MATERIALS AND METHODS

Methods

The method used in this study is an experimental research method and is used to determine the nitrite and nitrate levels in spinach and mustard that have been harvested in the morning, afternoon, and evening. Samples were planted by researchers with harvest age of 20th day, 23rd day, and 26th day. Sampling was done purposive, without comparing the sample from one place to another place, because the sample is considered homogeneous [10].

Materials

The materials used in this study were pro-analytical qualities of the E-Merck production, i.e., sodium nitrite, sulfanilic acid, N-(1-naphthyl) ethylenediamine dihydrochloride, glacial acetic acid, dilute hydrochloric acid, antipyrine, ferrous sulfate, concentrated sulfuric acid, and zinc powder.

Tools

The tools used in this research are one unit of ultraviolet-visible spectrophotometer (Shimadzu), analytical balance (Boeco), glassware (Iwaki), water bath, filter paper, parchment paper, tissue, rubber ball, spatula, thermometer, test tube, tube clamp, stir bar, mortar, and pestle.

Spinach and mustard planting procedures

Putted humus soil media in plastic bag left for 1 week. After 1 week of spinach and mustard seeds, 3 seeds were distributed in the separated plastic bag, watered in the afternoon daily, and harvested at 20th day, 23rd day, and 26th day.

Preparation of reagents
Acetic acid solution of 15% v/v was prepared by diluting 75 mL glacial acetic acid with distilled water into 500 mL volumetric flask.

N-(1-naphthyl) ethylenediamine dihydrochloride solution was prepared by dissolving and diluting 0.35 g N-(1-naphthyl) ethylenediamine dihydrochloride with acetic acid solution of 15% v/v into 250 mL volumetric flask, filtered with filter paper, and stored in an amber bottle.

Sulfanilic acid solution was prepared by dissolving and diluting 0.85 g of sulfanilic acid with acetic acid solution of 15% v/v into 250 mL volumetric flask, filtered with filter paper, and stored in an amber bottle.

Ferrous sulfate solution was prepared by dissolving and diluting 2.8 g of ferrous sulfate with freshly boiled water and cooled.

Preparation of nitrite stock solution
100 mg of sodium nitrite powder inserted into a 100 mL volumetric flask and dissolved in distilled water, then diluted to the marking line (C=1000 µg/mL). Added 1 mL of standard solution and put into a 100 mL volumetric flask, diluted with distilled water until the mark line, and homogenized (C=10 µg/mL).

Preparation of maximum wavelength and operating time of standard nitrite
4 mL of standard nitrite solution (C=10 µg/mL) was added into a 50 mL volumetric flask, added 2.5 mL of sulfanilic acid reagent, shaken to homogeneous, allowed to stand for 5 min, added 2.5 mL of N-(1-naphthyl) ethylenediamine dihydrochloride, diluted with distilled water until the mark line, homogenized, measured the absorbance at a wavelength of 400–800 nm with a distilled water blank for determination of maximum wavelength, and measured the absorbance at maximum wavelength per minute for 60 min with a distilled water blank for determination of operating time (C=0.8 µg/mL).

Linearity, limit of detection, and limit of quantitation
Respectively, 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 6 mL, and 7 mL of standard nitric solution (C=10 µg/mL) was added into a 50 mL volumetric flask, added 2.5 mL of sulfanilic acid reagent, shaken to homogeneous, allowed to stand for 5 min, added 2.5 mL of N-(1-naphthyl) ethylenediamine dihydrochloride, diluted with distilled water until the mark line, homogenized, measured the absorbance at maximum length and operating time with a distilled water blank (0.2 µg/mL, 0.4 µg/ml, 0.6 µg/mL, 0.8 µg/mL, 1.0 µg/mL, 1.2 µg/mL, and 1.4 µg/mL), and calculated correlation coefficient, regression coefficient, and regression line equation.

Determination of nitrite levels in spinach and mustard
10 g of sample was put into a 250 mL beaker glass, added 150 mL of hot distilled water (±80°C), stirred to homogeneity, and heated over a water bath for 15 min while stirring. The mixture was cooled to room temperature, transferred into a 250 mL volumetric flask, added distilled water until the mark line, shaken until homogeneously mixed, filtered, and discarded first 25 mL of filtrate, followed by subsequent filtrate. Added 5 mL filtrate, put into a 50 mL volumetric flask, added 2.5 mL of sulfanilic acid reagent, shaken to homogeneous, allowed to stand for 5 min, added 2.5 mL of N-(1-naphthyl) ethylenediamine dihydrochloride, diluted with distilled water until the mark line, homogenized, and measured the absorbance at maximum length and operating time with a distilled water blank. The concentration of nitrite in the sample can be calculated by the regression equation, and the level of nitrite in the sample can be calculated according to the dilution factor.

Determination of nitrate levels in spinach and mustard
10 g of sample was put into a 250 mL beaker glass, added 150 mL of hot distilled water (±80°C), stirred to homogeneity, and heated over a water bath for 15 min while stirring. The mixture was cooled to room temperature, transferred into a 250 mL volumetric flask, added distilled water until the mark line, shaken until homogeneously mixed, filtered, and discarded first 25 mL of filtrate, followed by subsequent filtrate. Added 5 mL filtrate, put into a 50 mL volumetric flask, added 0.1 g of zinc powder in a hydrochloric acid solution, stirred for 10 min, added 2.5 mL of sulfanilic acid reagent, shaken to homogeneous, allowed to stand for 5 min, added 2.5 mL of N-(1-naphthyl) ethylenediamine dihydrochloride, diluted with distilled water until the mark line, homogenized, and measured the absorbance at maximum length and operating time with a distilled water blank. The concentration of nitrate in the sample can be calculated by the regression equation, and the level of nitrate in the sample can be calculated according to the dilution factor. The nitrate level is the level of nitrite after reduction minus the level of nitrite before reduction.

Accuracy and precision
The nitrite and nitrate recovery test can be done by adding the standard solution to the sample and then analyzed by the same treatment in the sample [11]. Percentage of recovery can be calculated by the following formula:

Recovery percentage = $(C_A-C_B)/(C_C)\times100\%$

Information:
C_A = Analytical concentration in the sample after addition of standard
C_B = Analytical concentration in the sample before addition of standard
C_C = The concentration of standard added to the sample

Precision test based on nitrite and nitrate recovery results determined standard deviation (SD) of nitrite and nitrate. To calculate the SD and relative SD (RSD), the following formula was used (Sudjana, 2005):

SD = √((X–X)^2)/(n–1))

RSD = SD/X×100%

Where
X = Level of substance in the sample
X = The average level of substance in the sample
N = Number of repetitions
SD = Standard deviation
RSD = Relative standard deviation.

RESULTS AND DISCUSSION

Maximum wavelength and operating time
Determination of maximum absorbance curve was done at wavelength 400–800 nm. Measurements of nitrite absorbance were performed at a concentration of 0.8 µg/mL. Nitrite maximum absorbance was done at wavelength 540 nm. The wavelength is equal to the maximum wavelength of nitrite according to Hess, which is 540 nm [12]. The determination of nitrite operating time was performed to determine the time at which the compound had the most stable absorbance color value when measured by visible light spectrophotometry. The determination of nitrite operating time was carried out at a concentration of 0.8 µg/mL measured every minute for 60 min. Obtained the most stable time is between 8 and 10 min with a concentration of 0.8 µg/mL where at that time the absorbance does not change.

Linearity, limit of detection, and limit of quantitation
The calibration curve is a series of standard solutions of the substances to be analyzed with various concentrations measured, then made a

Table 1: Linearity data of nitrite

No	X (concentration)	Y (absorption)
1	0.2	0.10407
2	0.4	0.20817
3	0.6	0.31244
4	0.8	0.41699
5	1.0	0.52319
6	1.2	0.62474
7	1.4	0.72516

Table 2: Level of nitrite and nitrate in spinach and mustard at diiferent harvesting time

No.	Harvesting time	Harvesting day	Level (μg/g)			
			Spinach		Mustard	
			Nitrite	Nitrate	Nitrite	Nitrate
1	Morning	20	51.91±0.08	179.16±0.12	23.87±0.67	52.36±1.10
		23	56.13±0.04	177.13±0.18	25.89±0.56	53.57±0.99
		26	53.08±0.17	184.23±0.03	28.39±1.40	56.50±1.19
2	Afternoon	20	73.16±0.09	158.57±0.98	36.46±0.91	24.21±3.93
		23	73.40±0.59	164.19±0.57	39.50±6.51	30.14±3.74
		26	76.63±0.15	169.00±1.77	42.08±1.74	31.50±3.63
3	Evening	20	64.41±0.05	162.39±0.16	32.29±2.96	33.67±6.96
		23	67.58±0.10	171.45±0.12	34.81±2.46	35.50±3.62
		26	69.81±0.10	175.45±0.13	40.08±4.31	41.25±5.91

Table 3: Recovery percentage and RSD of nitrite and nitrate in spinach and mustard

No.	Recovery percentage			
	Spinach		Mustard	
	Nitrite	Nitrate	Nitrite	Nitrate
1	98.39	98.50	102.26	97.27
2	99.41	99.12	101.34	99.69
3	99.20	100.10	101.84	98.27
4	99.06	100.74	98.76	100.57
5	98.69	101.45	99.87	102.54
6	99.14	101.04	97.68	101.40

RSD: Relative standard deviation

curve which is the relationship between absorbance and concentration. If the Lambert–Beer law is met, then the calibration curve is a straight line. The concentration used for making this calibration curve is 0.2–1.4 μg/mL. Regression equation obtained is Y=0.59614X+0.00959 with a correlation coefficient (r) equal to 0.99999. The value of r>0.99 indicates a linear correlation between X and Y [13]. Limit detection and quantitation limits are calculated from the regression equation obtained from the calibration curve. The limits of detection of nitrate and nitrite were 0.03796 mg/kg, while the limit of quantitation of nitrite and nitrate was 0.1265 mg/kg (Table 1 and Fig. 1).

Nitrite and nitrate levels in spinach and mustard

The sample used is spinach and mustard. Vegetables are harvested in the morning, afternoon and evening. The prepared samples were measured at a wavelength of 540 nm between 8 and 10 min. Level of nitrite and nitrate in spinach and mustard at different harvested time can be seen in Table 2.

The results showed that spinach and mustard samples contain higher nitrate content than nitrite content. This is because more than 90% of nitrogen is absorbed by plants in the form of nitrate. Nitrate is a major nutrient for plant growth. This is also due to the nitrogen cycle in the event of nitrification, where ammonia is oxidized to nitrite and the nitrite is oxidized to nitrate. Based on the effect of harvesting day, there is an increase of nitrite and nitrate level, where the longer the harvesting time, the higher the nitrite and nitrate level, this is probably because of the abundance of nitrogen absorbed by the crop as the time of harvesting and other supporting factors such as moisture, soil, weather, light intensity, plant disease or insect damage [14].

From the results obtained, nitrate levels are higher in the morning but lower in the afternoon and evening, while nitrite levels are higher in the afternoon and evening, but lower in the morning. Nitrite concentration increases during the day due to metabolic processes. Nitrate reductase can be induced by nitrate, light, and glucose. Nitrate is metabolized in plants by the enzyme nitrate reductase. This nitrate reductase enzyme is useful for converting nitrate to nitrite [14]. According to Maynard *et al*., increased nitrate concentration associated with the intensity of light because of the light needed to activate the enzyme nitrate reductase. The higher the intensity of sunlight, the higher the activity of nitrate reductase enzyme. Enzyme nitrate reductase is needed to convert nitrate into nitrite. This is also because during the day there is a process of photosynthesis that produces a reducing sugar that is glucose that can reduce nitrate to nitrite [15].

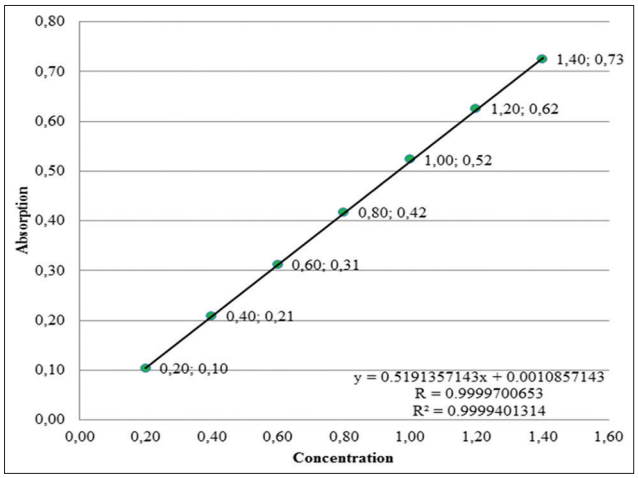

Fig. 1: Linearity curve of nitrite

Accuracy and precision

The accuracy test with the percentage parameter of recovery was performed using a spinach and mustard samples in the afternoon picking on the 23rd day. The standard addition method is accomplished by adding a certain amount of raw solution to the sample. Then, the solution was measured uptake at a wavelength of 540 nm. Table 3 shows the recovery percentage and RSD of nitrite and nitrate in spinach and mustard.

Based on Table 3 obtained, the average recovery percentage of nitrite and nitrate in spinach is 98.98% and 100.16% with RSD of 0.37% and 1.15%, while in mustard, it is 100.60% and 99.96% with the RSD of 1.85% and 1.96%. The recovered percentage shows good accuracy and precision for determination of nitrite and nitrate in the sample. The accuracy test result of the recovery percentage test meets the requirement, with recovery percentage between 80 and 120%. The precision test results of the RSD test meet the requirement, with RSD <2% [16].

CONCLUSION

There was a difference of nitrite and nitrate levels in spinach and mustard harvested on the 20th day, 23rd day, and 26th day. There was a difference in nitrite and nitrate levels harvested in the morning, afternoon, and evening. The highest levels of nitrite contained in spinach and mustard

were during the afternoon and evening, while the highest levels of nitrate contained in spinach and mustard were during the morning.

AUTHORS CONTRIBUTION

This work was carried out in collaboration between all authors. Nahitma Ginting designed the research, Jansen Silalahi, Tuty Roida Pardede, and Sudarmi supervise the research, Nerdy did the research work, collected the data, and analyzed the data.

CONFLICT OF INTERESTS

The Authors declare that they have no conflicts of interests.

REFERENCES

1. Dias JS. Nutritional quality and health benefits of vegetables. Food Nutr Sci 2012;3:1354-74.
2. Saparinto C. Grow Your Own Vegetables. Yogyakarta: Lily Publisher; 2013.
3. Akbar R. Various Pharmacies Plant Live Around us. Jakarta: One Books; 2015.
4. Silalahi J. Problem of nitrite and nitrate in food. Med J Ked Ind 2005;31:460-1.
5. Amri B. Effect of boiling and storage time on nitrate and nitrite levels on spinach (*Amaranthus tricolor* L.). Medan: Fakultas Farmasi; 2016.
6. Asmaradhani D. Effect of fertilization on nitrate and nitrite level on kale (*Ipomoea reptana* poir). Medan: Fakultas Farmasi; 2016.
7. Keeton JT. History of Nitrate an Nitrite in food. New York: Humana Press; 2011.
8. Murthy R, Dougherty PM, Zarnoch SJ, Allen HL. Effects of carbon dioxide, fertilization, and irrigation on photosynthetic capacity of loblolly pine trees. Tree Physiol 1996;16:537-46.
9. Latifa IC, Anggarwulan E. Nitrogen content, nitrate reductase activity, and biomass of kimpul (*Xanthosoma sagittifolium*) on shade and nitrogen fertilizer variation. Nusantara Biosci 2009;1:65-71.
10. Sudjana N. Statistic Method. 6th ed. Bandung: Tarsito; 2005.
11. Harmita H. Method Validation Implementation Guidelines and Calculations. Jakarta: Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Indonesia; 2004.
12. Hess J. Association Official Methods of Analytical Chemists. 17th ed. Virginia: AOAC Inc.; 2000.
13. Nerdy, Putra ED, Tjahjono DH. Development and validationof high performance liquid chromatography mass spectrometry method for determination of rifampicin, isoniazidand pyrazinamide from tablet preparation. Int J Pharmtech Res 2014;6:1647-64.
14. Hill MJ. Nitrates and Nitrites From Food and Water in Relation to Human Disease. Cambridge: Woodhead Publishing Limited; 1996.
15. Maynard DN, Barker AV, Minotti PL, Peck NH. Nitrate Accumulation in Vegetables. J Adv Agron 1976;28:71-118.
16. Nerdy. Development and validation of ultraviolet spectrophotometric method for determination of mebhydrolin napadisylate in tablet preparations. Asian J Pharm Clin Res 2017;10:367-72.
17. Kamboj A, Sidana P, and Jain UK. Development and Validation of UV Spectroscopic Methods for Simultaneous Estimation of Salbutamol Sulphate and Doxophylline In Combined Solid Dosage Form. Int J Pharm Pharm Res 2017;9(6):117-122.
18. Jeewantha HA, Ivanovich SA, and Mihailovich KP. Validated Spectrophotometric Method for The Estimation of Vincristine and Vinblastine. Int J Pharm Pharm Res 2017;9(4):78-86.