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, and on nitrate and nitrite level in spinach and mustard.

Methods: Nitrates identification was done using sulfanilic acid reagent and N-(1-naphthyl) ethylenediamine dihydrochloride. Identification of nitrate was done using reagent ferrous sulfate. Determination of nitrates 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 nitrate 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.

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 nitrate 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 considered the potential of nitrosamine formation from nitrite [4].

Previous research has shown a change in nitrite and nitrate contents in fresh and boiled vegetables. Nitrate and nitrate levels in vegetables have decreased, after boiling for 15 min. Nitrate contents 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 (CO₂), and water (H₂O) in pigmented plants with the help of solar energy [8]. Photosynthesis is closely related to the assimilation of nitrate, the formation of nitrate ions (NO₃⁻) into nitrite ions (NO₂⁻), ammonia (NH₃), 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 nitrate and nitrite 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, antipyrene, 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

Put the humus soil media in plastic bag 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=100 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 homogenously 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. Percentage of recovery can be calculated by the following formula:

Recovery percentage = (C_s-C_a)/(C_s)×100%

Information:
C_s = Analytical concentration in the sample before addition of standard
C_a = Analytical concentration in the sample after addition of standard
C_s = 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 = √Σ((X-X̅)^2)/(n-1))

Where
X = Level of substance in the sample
X̅ = 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        |
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].

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

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**Table 2: Level of nitrate and nitrate in spinach and mustard at different harvesting time**

| No. | Harvesting time | Harvesting day | Level (µg/g) | Mustard | Nitrate | Nitrate |
|-----|----------------|----------------|-------------|---------|---------|---------|
| 1   | Morning        | 20             | 51.91 ± 0.08 |         | 179.16 ± 0.12 |         |
|     |                | 23             | 56.13 ± 0.04 |         | 177.13 ± 0.18 |         |
|     |                | 26             | 53.08 ± 0.11 |         | 184.23 ± 0.03 | 30.29 ± 0.10 |
| 2   | Afternoon      | 20             | 73.16 ± 0.09 |         | 158.57 ± 0.98 | 67.58 ± 0.10 |
|     |                | 23             | 73.40 ± 0.59 |         | 164.19 ± 0.57 | 69.81 ± 0.10 |
|     |                | 26             | 76.63 ± 0.15 |         | 169.00 ± 1.77 | 70.89 ± 0.13 |
| 3   | Evening        | 20             | 64.41 ± 0.05 |         | 162.39 ± 0.16 | 69.81 ± 0.10 |
|     |                | 23             | 67.56 ± 0.10 |         | 171.45 ± 0.12 | 70.89 ± 0.13 |
|     |                | 26             | 69.81 ± 0.10 |         | 175.45 ± 0.13 | 70.89 ± 0.13 |

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

| No. | Recovery percentage | Spinach | Nitrate | Nitrate | Mustard | Nitrate | Nitrate |
|-----|---------------------|---------|---------|---------|---------|---------|---------|
| 1   | 98.39               | 98.50   | 102.26  | 97.27   | 98.98   | 100.16  | 1.15%   |
| 2   | 99.41               | 99.12   | 101.34  | 99.69   | 99.60   | 100.60  | 1.96%   |
| 3   | 99.20               | 100.10  | 101.84  | 98.27   | 98.60   | 99.96   | 1.85%   |
| 4   | 99.06               | 100.74  | 98.76   | 100.57  | 98.60   | 100.60  | 1.85%   |
| 5   | 98.69               | 101.45  | 99.87   | 102.54  | 99.60   | 100.60  | 1.96%   |
| 6   | 99.14               | 101.04  | 97.68   | 101.40  | 99.60   | 100.60  | 1.96%   |

RSD: Relative standard deviation

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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**
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.

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