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Determination of Nitrite Levels in Boiled White and Purple Cabbage Based on Boiling Time Variations

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Abstract. Cabbage is a type of vegetable that contains various vitamins such as vitamins A, C, and K. One of the chemical components found in cabbage is nitrite (NO_2). Nitrite is a nutrient formed naturally with concentrations depending on species variation, season, light, and fertilizer use. This study aims to determine the nitrite levels in boiled purple and white cabbage with varying boiling times using the UV-Vis spectrophotometric method. Additionally, this research evaluates whether the nitrite content in the boiled cabbage remains within the Acceptable Daily Intake (ADI). The extraction was carried out using distilled water with boiling time variations of 0, 5, 10, 15, and 20 minutes. Nitrite levels were determined using the UV-Vis spectrophotometric method. The nitrite content found in white cabbage for boiling durations of 0, 5, 10, 15, and 20 minutes were 1.672 ppm, 0.986 ppm, 1.037 ppm, 0.951 ppm, and 0.999 ppm, respectively. For purple cabbage, the levels were 1.627 ppm, <0.5 ppm, 0.624 ppm, 0.775 ppm, and <0.5 ppm, respectively. The nitrite contents in both white and purple cabbage were bellow the ADI value of 0.07 mg/kg weight, equivalent to 4.2 mg/day for a 60 kg adult, the potential cumulative nitrite intake from other dietary sources should also be considered.

Keywords : White cabbage; Purple cabbage; Nitrite levels; UV-Vis spectrophotometry.

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Introduction

Vegetables are a source of vitamins and minerals required by the human body in relatively small amounts but cannot be replaced by other food types. The Food and Agriculture Organization (FAO) recommends a regular vegetables intake of 75 g/capita/year, whereas the World Health Organization (WHO) suggest a daily intake of 400 grams of vegetables [1].

One of the commonly consumed and easily accessible commercial vegetables in both traditional and modern markets is cabbage (Brassica oleracea). Research conducted by Husaini et al. [2] in Medan revealed that cabbage is sold in both market types. The most widely consumed variety is white cabbage, due to its high vitamin content and easy availability [3].

Cabbage contains several essential vitamins such as A, C, and K, and is also rich in phytonutrients, which function as natural antioxidants. The primary minerals found in cabbage include potassium, calcium, phosphorus, sodium, and iron. Secondary metabolites in cabbage include flavonoids, indoles, phenols, distillation compounds, glutathione, and cellulose [4]. One notable component found in vegetables is nitrite (NO_2). Over 80–95% of dietary nitrite intake comes from vegetables, especially green leafy types such as lettuce, spinach, cabbage, carrots, rocket, beetroot, and radishes [5]. Post-harvest nitrite levels in vegetables can be affected by storage and processing methods. Food processing refers to converting raw ingredients into forms that are closer to being ready for consumption [6]. In common cooking practices, vegetables are typically boiled for 5, 10, 15, or 20 minutes [7].

According to research by Romsiah and Meidalena [8], boiling time affects the nitrite content in vegetables such as water spinach, celery, and broccoli. The nitrite levels increases with longer boiling times. reported that boiling celery increases nitrite levels to above the WHO recommended limit. Other studies also show that boiling time influences nitrite levels in carrots, where nitrite concentrations decrease significantly after 5 minutes of boiling but rise again with prolonged heating [7].

Nitrite is a product of nitrogen oxidation and is a naturally occurring compound in plants. Its concentration is influenced by factors such as fertilizer use, light intensity, harvest time, soil type, and plant growth conditions. Nitrite is considered a toxic chemical and exposure to high levels can cause symptoms such as paralysis, cyanosis, and even death [5]. Another harmful effect of nitrite is methemoglobinemia, in which nitrite oxidizes ferrous ions (Fe^{2+}) in hemoglobin to ferric ions (Fe^{3+}), forming methemoglobin that cannot bind oxygen. This condition reduces oxygen availability in the body and is especially dangerous for infants, leading to the so-called “blue baby syndrome.” In adults, nitrite exposure may also lead to the formation of carcinogenic compounds [9].

The FAO/WHO has established the Acceptable Daily Intake (ADI) for nitrite at 8 mg/day for a 60 kg adult. Nitrate present in vegetables may be reduced to nitrite through the action of nitrate reductase enzymes and other reducing agents [7, 10]. Based on these considerations, this study aims to determine the nitrite content in white and purple cabbage using the UV-Vis spectrophotometric method.

Experimental

Materials and instruments. The materials and instruments used in this study included white cabbage purchased from farmers in Belawan, Bondowoso, and purple cabbage from the same region. Chemical reagents included sulfanilamide (p.a. Merck), N-(1-naphthyl) ethylenediamine dihydrochloride (NED) (p.a. Merck), standard nitrite solution ($NaNO_2$), chloroform ($CHCl_3$), hydrochloric acid (HCl), and distilled water (aquadest), UV-Vis spectrophotometer (UV Shimadzu 1700), analytical balance, measuring cylinders (Pyrex), volumetric flasks (Pyrex), Erlenmeyer flasks (Pyrex), beakers (Pyrex), volumetric pipettes (Pyrex), glass funnels, aquadest bottle, glass stirring rods, stopwatch, Millipore filter paper, and Whatman filter paper.

Preparation of Reagent Solutions. Sulfanilamide solution: 1 g of sulfanilamide was dissolved in 10 mL of concentrated HCl and diluted with distilled water to a final volume of 100 mL [1]; NED dihydrochloride solution: 100 mg of NED dihydrochloride was dissolved in 100 mL of distilled water and stored in a dark bottle in a refrigerator [1]; Standard nitrite solution: Prepared from sodium nitrite ($NaNO_2$) according to standard procedures.

Preparation of Nitrite Standard Series (Calibration Curve). Nitrite standard solutions were prepared at concentrations of 0.5, 1.0, 1.5, 2.5, and

3.5 ppm. To each standard solution, 1 mL of sulfanilamide solution was added, shaken, and allowed to stand for 5 minutes. Then, 1 mL of NED solution was added, shaken again, and left for 10 minutes. Absorbance was measured immediately at 540 nm (not exceeding 2 hours after preparation). A calibration curve was constructed by plotting concentration against absorbance to derive a linear regression equation [2].

Measurement of Unboiled Cabbage Samples. Ten grams of cabbage sample were ground using a mortar, transferred to a 100 mL volumetric flask, and diluted with distilled water. The mixture was left to stand for 5 minutes and filtered. Fifty milliliters of the filtrate were reacted with 1 mL of sulfanilamide solution (5 minutes), followed by 1 mL of NED solution (5 minutes). The mixture was stirred and absorbance was measured at 540 nm [3].

Measurement of Boiled Cabbage Samples. Ten grams of each cabbage sample were placed in a beaker and filled with distilled water to a final volume of 100 mL. The samples were boiled for 5, 10, 15, and 20 minutes. After boiling, each sample was mashed and diluted again to 100 mL. Then, 50 mL of each extract was reacted with 1 mL of NED solution (5 minutes), followed by 1 mL of sulfanilamide solution (5 minutes). Absorbance was measured at 540 nm [3]. All measurements were performed in triplicate for each treatment to ensure statistical reliability.

Data analysis. The absorbance data from the nitrite standard series were used to generate a calibration curve and linear regression equation (Y = aX + b). The regression coefficient (R^2) was evaluated to ensure acceptance criteria (R^2 > 0.99). Nitrite concentrations were calculated using the equation 1.

$$K = \frac{Y \; x \; V \; x \; Fp}{Sample\; Weight} \qquad (1)$$

Where: Y = absorbance; K = nitrite concentration in the sample (µg/g); V = volume of the sample before dilution (mL); F_p= dilution factor.

Result and Discussion

The cabbage samples used in this study were white and purple cabbage, which had previously undergone botanical identification. According to Certificate No. 70/PL17.8/PG/2024, the white cabbage was identified as Brassica oleracea L., and Certificate No. 69/PL17.8/PG/2024 identified the purple cabbage as Brassica oleracea L. var. capitata f. rubra. Extraction was performed using distilled water with boiling time variations of 0, 5, 10, 15, and 20 minutes.

Based on the identification results, both white cabbage and purple cabbage were found to contain nitrite, either in raw form or after boiling. This was confirmed by the formation of a purple color resulting from the reaction between the sample, sulfanilic acid, and NED (N-(1-naphthyl) ethylenediamine dihydrochloride) [4, 5]. Nitrite content was determined using the UV-Vis spectrophotometric method due to its high sensitivity and ease of application [6]. The sample preparation followed the Griess method, which is based on a diazotization reaction: nitrite reacts with a primary aromatic amine under acidic conditions to form a diazonium salt. This salt then couples with a phenyl-containing compound to produce an azo dye (**Figure 1**) [7].

In this study, N-(1-naphthyl) ethylenediamine dihydrochloride (NED) served as the coupling reagent, while sulfanilic acid was used as the diazonium source. The reaction produced a reddish-purple azo dye. The intensity of this color is proportional to the nitrite concentration, thus enabling its quantification [8].

Before measuring nitrite levels in cabbage samples, the maximum absorption wavelength of sodium nitrite standard solution was determined at 540 nm (**Figure 2**). The calibration curve yielded the regression equation y = 0.2322x + 0.045 with an R^2 value of 0.9973 (**Table 1**; **Figure 3**).

Based on the measurements, nitrite content in 10 g of white cabbage without boiling was 16.72 mg/kg. For boiling durations of 5, 10, 15, and 20 minutes, the nitrite levels were 9.86 mg/kg, 10.37 mg/kg, 9.51 mg/kg, and 9.99 mg/kg, respectively. In purple cabbage, the nitrite contents were 16.27 mg/kg (raw), <0.05 mg/kg (5 min), 6.24 mg/kg (10 min), 7.75 mg/kg (15 min), and <0.05 mg/kg (20 min) (**Table 2**). The calibration curve showed good linearity (y = 0.2322x + 0.045, R^2 = 0.9973). Based on the practical quantification limit used in this study, the LOQ was considered to be 0.5 ppm, while the LOD was estimated to be approximately 0.15 ppm.

Nitrite levels in vegetables are known to be influenced by the boiling process through several mechanisms, one of which is the dissolution of

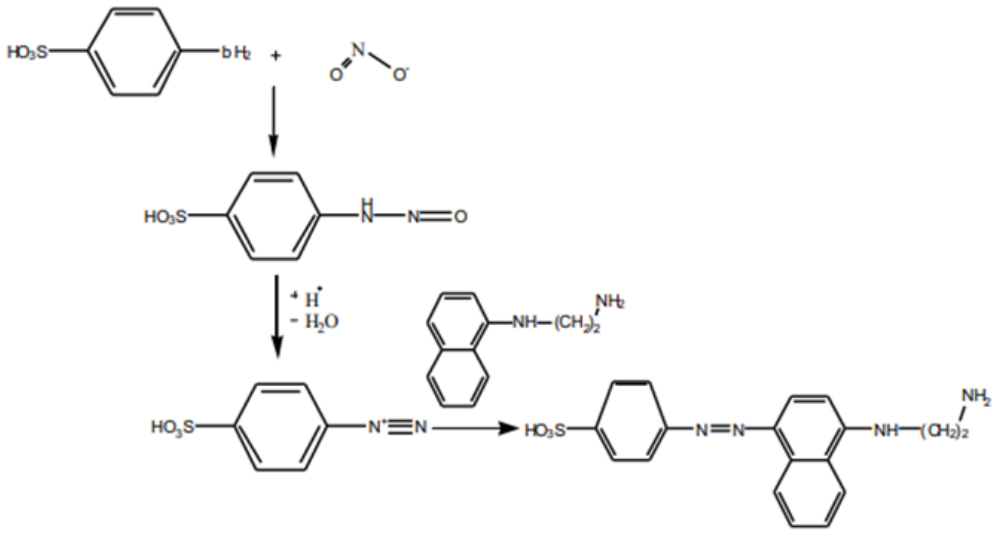

Figure 1. Griess reaction between sulfanilic acid under acidic conditions and α-naphthylamine [9]

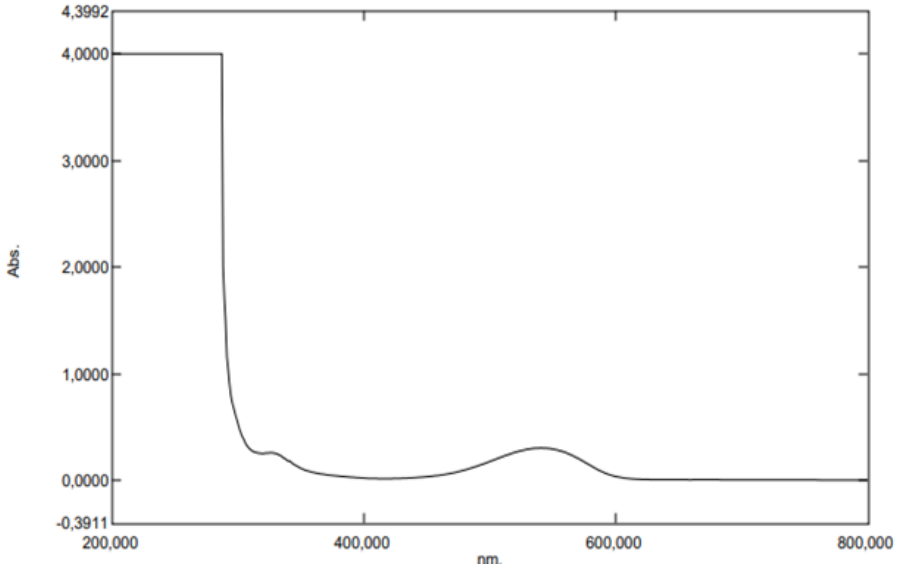

Figure 2. Maximum wavelength of sodium nitrite solution

chemical compounds, including nitrite, into water during heating. In general, prolonged boiling tends to reduce nitrite levels due to thermal degradation occurring during the process. However, the relationship between boiling time and nitrite concentration is not always linear. In the early stages of boiling, the disruption of vegetable cell structures can lead to the release of nitrite into the medium, resulting in a temporary increase in measured nitrite levels. Further degradation subsequently leads to a decrease in nitrite concentration. Several studies have reported such fluctuating patterns of nitrite changes in various vegetables, where the final nitrite content after heating is determined by the balance between nitrite release from tissues, chemical transformations, and thermal degradation [10-12].

Table 1. Absorbance of Nitrite Standard Solutions

Concentration (ppm)	Absorbance
0,5	0,157
1,0	0,287
1,5	0,399
2,5	0,602
3,5	0,87

The nitrite content in raw (unboiled) cabbage was higher due to nitrite's solubility in water. During boiling, nitrite leaches into the cooking water. Moreover, boiling may inactivate microorganisms that reduce nitrate to nitrite [11-13]. These findings align with those of Emawati et al.[14], who found that fresh spinach had higher nitrite levels than boiled spinach.

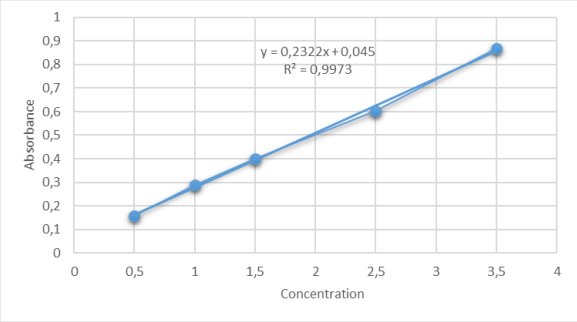

Figure 3. Nitrite Calibration Curve

According to the ADI (Acceptable Daily Intake), the maximum allowable nitrite intake is 0.07 mg/kg of body weight, equivalent to 4.2 mg/day for a 60 kg adult. If one consumes 100 g of white cabbage with a nitrite level of 16.72 mg/kg, the intake would be 1.672 mg/day, which remains within the safe range. Therefore, nitrite levels in both white and purple cabbage samples are considered safe for consumption, but the potential cumulative nitrite intake from other dietary sources should also be considered.

Effect of Boiling Time on Nitrite Levels. The research data were statistically analyzed using a Two-Way ANOVA test. Prior to this, a normality test was performed, showing a result of 0.653 ($p > 0.01$), indicating that the data were normally distributed.

ANOVA results showed that boiling time (0, 5, 10, 15, and 20 minutes) had a significant effect on nitrite levels, with a significance value of 0.000 ($p < 0.05$), meaning the effect was statistically significant (**Table 3**). Short boiling time may reduce nitrite levels due to leaching and thermal degradation, while prolonged boiling may lead to the oxidation of nitrate to nitrite, increasing nitrite content [15].

Conclusion

The nitrite content in unboiled white and purple cabbage was 16.72 mg/kg and 16.27 mg/kg, respectively. For white cabbage boiled for 5, 10, 15, and 20 minutes, the nitrite levels were 9.86 mg/kg, 10.37 mg/kg, 9.51 mg/kg, and 9.99 mg/kg, respectively. In purple cabbage boiled for 5, 10, 15, and 20 minutes, the nitrite levels were <0.5 ppm, 6.24 mg/kg, 7.75 mg/kg, and <0.5 ppm, respectively. Boiling time significantly affected the nitrite content in both white and purple cabbage. The variations in boiling duration (0, 5, 10, 15, and 20 minutes) influenced the nitrite levels detected in the samples.

Table 2. Nitrite Levels in White and Purple Cabbage Samples

Sample	Boiling Time (min)	Abs	Nitrite (ppm)	Nitrite Content (mg/kg)
White Cabbage	0	0.343	1,672	16,72
	5	0.182	0,986	9,86
	10	0.196	1,037	10,37
	15	0.176	0,951	9,51
	20	0.188	0,999	9,99
Purple Cabbage	0	0.333	1,627	16,27
	5	0.052	< 0,5	<0.05
	10	0.100	0,624	6,24
	15	0.135	0,775	7,75
	20	0.062	< 0,5	<0.05

Table 3. Two-Way ANOVA Summary (Tests of Between-Subjects Effects) Dependent Variable: nitrite content

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	859.217[a]	9	95.469	8.810E30	.000
Intercept	2255.587	1	2255.587	2.081E32	.000
waktu	533.152	4	133.288	1.230E31	.000
jenis	205.775	1	205.775	1.899E31	.000
waktu * jenis	120.290	4	30.073	2.775E30	.000
Error	2.167E-28	20	1.084E-29		
Total	3114.804	30			
Corrected Total	859.217	29			

[a] R Squared = 1.000 (Adjusted R Squared = 1.000)

Author Contributions

Each author involved in the preparation of this article made significant contributions through scientific discussions, research design, implementation, and manuscript preparation. E.F. was responsible for designing the study, developing the methodology, and coordinating the overall execution of the project. N.N. carried out the experiments and was responsible for data collection in the laboratory. B.M. performed data analysis, interpreted the results, and prepared the supporting figures and tables. I. contributed to the literature review, drafted the background section, and validated the research findings. M.R wrote the initial draft of the manuscript, revised the content, and ensured the manuscript adhered to the journal's formatting guidelines. All authors participated in scientific discussions and approved the final version of the manuscript prior to submission.

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