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Analysis of Cyanide in Cassava with Different Storage Durations Using Visible Light Spectrophotometry and Ninhydrin Reagen

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Abstract

Cassava (*Manihot esculenta* Crantz) is the third most important staple food in Indonesia, after rice and corn. Although it offers numerous health benefits, cassava also contains potentially harmful compounds, such as cyanide. This study aimed to analyze cyanide levels in freshly harvested cassava and cassava stored for five days, using both qualitative and quantitative methods. The research gap identified is the limited data on how storaåge duration affects cyanide levels in cassava and its implications for food safety. To address this, the study used qualitative analysis with picrate paper and quantitative analysis through UV-Vis spectrophotometry, employing ninhydrin as the reagent. The qualitative results revealed that both freshly harvested cassava and cassava stored for five days tested positive for cyanide, as indicated by the formation of a red-brown color on the picrate paper. The quantitative analysis was preceded by method validation, which showed excellent performance, including linearity (r = 0.9982), detection limit (LoD = 1.33 µg/mL), quantification limit (LoQ = 4.43 µg/mL), recovery rate (98.7%), and relative standard deviation (RSD = 1.53%). The cyanide content in freshly harvested cassava was found to be 4.94 µg/g, while the content in cassava stored for five days was 7.16 µg/g. Both cassava samples were found to be safe for consumption, as their cyanide levels were below the safety threshold of 10 ppm. This study provides valuable insights into the safe consumption of cassava, even with storage, underlining the importance of proper handling.

Keywords: Cyanide, Cassava, UV-Vis Spectrophotometry, Ninhydrin Reagent

1. Introduction

Indonesia is a country rich in both animal and plant resources, with abundant natural resources that are crucial for human survival, especially in terms of food. Food is an essential element in fulfilling the basic needs of humans, providing energy for daily activities. One of the main sources of energy is carbohydrates, which are found in plants such as rice, corn, and tubers. Tubers, particularly cassava (*Manihot esculenta* Crantz), play a significant role in the Indonesian diet, serving as a vital carbohydrate source for many communities across the nation.

Cassava is an important global carbohydrate source and the third-largest staple food in Indonesia, after rice and corn. It contributes significantly to food diversification and serves as a raw material for the food industry. Its versatility makes it a critical part of the Indonesian food system (Sipayung and Ginting, 2020). However, despite its widespread use, cassava contains cyanide compounds, which can be toxic. The level of cyanide in cassava can vary, with varieties classified based on their hydrogen cyanide (HCN) content. Non-toxic cassava contains less than 50 mg/kg of HCN, moderately toxic varieties have between 50-100 mg/kg, and highly toxic cassava contains more than 100 mg/kg of HCN (Lumbantobing, Napitupulu, and Jura, 2020).

The World Health Organization (WHO) sets a safe limit of 10 ppm of total cyanide in cassava flour (Burns et al., 2012). Cyanide is produced when the cassava plant's cyanogenic glycosides are broken

down by enzymes in the cells, releasing hydrogen cyanide (HCN), which is highly toxic. This compound can disrupt cellular respiration, leading to respiratory issues and potentially causing illness or death when consumed (Purwati, Thuraidah, and Rakhmina, 2016). Notably, the cyanide content in cassava increases as the storage duration extends (Lumbantobing et al., 2020).

Cyanide is a group of compounds containing the cyano group (−C≡N) and is found in nature in various forms, including free cyanide, simple cyanides, cyanide complexes, and cyanide derivatives (Pitoi, 2015). There are several methods to analyze cyanide, each tailored for different cyanide groups, such as weak acid dissociable (WAD) cyanide, total cyanide, free cyanide, and reactive cyanide, often using techniques like distillation, silver nitrate titration, selective ion electrodes, or the USEPA test (Pitoi, 2015).

This study aimed to address the gap in understanding how the storage duration of cassava affects its cyanide content, particularly focusing on freshly harvested cassava and cassava stored for five days. The analysis of cyanide levels will be conducted using UV-Vis spectrophotometry, which provides a reliable means of measuring cyanide concentrations in cassava. This research is crucial for determining the safety of consuming cassava stored for varying periods. The goal is to assess the cyanide content in freshly harvested cassava and cassava stored for five days, using the UV-Vis spectrophotometric method, and to determine whether these levels remain within safe consumption limits.

2. Methods

Equipment

The following equipment was used in the study: analytical balance, beaker, volumetric flasks, stirring rods, test tubes, test tube racks, dropper pipettes, spatula, measuring cups, blender, Erlenmeyer flasks, Whatman filter paper, crucibles, and UV-Vis spectrophotometer.

Materials

The materials used in the study included freshly harvested cassava, cassava stored for five days, sodium carbonate, picric acid, NaOH, ninhydrin, KCN, and tartaric acid.

1. Sample Collection

The cassava used in this study was sourced from a local farm in Bandung, West Java, Indonesia.

2. Determination of Cyanide Content

The determination was carried out at the Herbarium Jatinangor, Taxonomy Laboratory, Department of Biology, FMIPA, Universitas Padjadjaran (UNPAD).

3. Sample Preparation

The cassava was first washed to remove dirt, then peeled. Afterward, the cassava was stored at room temperature for a specified duration before being processed into a fine paste.

Preparation of Solutions

1. To prepare a 100 ppm cyanide solution, 125 mg of KCN crystals (molecular weight = 65) were dissolved in distilled water, making a final volume of 50 mL. This solution was then used to prepare the cyanide solutions.

2. A 1% ninhydrin solution was prepared by dissolving 1 gram of ninhydrin in 100 mL of distilled water.
3. A 1 M NaOH solution was prepared by dissolving 4 grams of NaOH in 100 mL of distilled water.
4. Picric acid paper was prepared by dissolving 5 grams of sodium carbonate and 500 mg of picric acid in 100 mL of distilled water. Filter paper was cut to the required size and soaked in this solution for one hour, then dried.

Determination of Maximum Wavelength

The maximum wavelength for cyanide detection was determined using a potassium cyanide standard solution at a concentration of 35 ppm, prepared by diluting 1 mL of a 100 ppm KCN solution to a final volume of 25 mL. To this solution, 1 mL of ninhydrin and 1 mL of Na_2CO_3 were added. The pH was adjusted to 12 using 1 M NaOH, and the solution was then diluted to a final volume of 10 mL. The absorbance was measured using UV-Vis spectrophotometry in the range of 560–620 nm.

Preparation of Calibration Curve

Standard cyanide solutions were prepared at six different concentrations: 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm, and 35 ppm, by diluting the 100 ppm KCN stock solution. Each solution received 1 mL of ninhydrin and 1 mL of Na_2CO_3. The pH was adjusted to 12 using 1 M NaOH, and the volume was brought up to 25 mL with distilled water. These solutions were analyzed using UV-Vis spectrophotometry at the determined maximum wavelength of 584 nm.

Method Validation

1. *Accuracy Test*

   Seven grams of sample were ground, and 5 mL of sodium carbonate solution was added, followed by cyanide standard solutions at concentrations of 10 ppm, 15 ppm, and 20 ppm. Ninhydrin (1 mL) was added to each solution, and the final volume was brought to 25 mL. Absorbance was measured for each concentration three times to determine the recovery percentage.

2. *Precision Test*

   Precision was evaluated by measuring absorbance at three concentrations (15 ppm) with six repetitions for each. The standard deviation (SD) and relative standard deviation (RSD) were calculated to assess the precision.

3. *Linearity Test*

   The calibration curve was generated by plotting the absorbance values against the concentrations (10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm, and 35 ppm). Linear regression was performed to obtain the equation of the line ($y = bx + a$). The detection limit (LoD) and quantification limit (LoQ) were calculated using statistical analysis.

4. *Selectivity Test*

   Two solutions were prepared for selectivity testing:

- 1 mL of ninhydrin was pipetted into a 25 mL volumetric flask, and distilled water was added to the mark. The absorbance was measured from 300 to 700 nm.

- 6.25 mL of KCN stock solution was pipetted into a 25 mL volumetric flask, followed by 1 mL of ninhydrin and distilled water to the mark. The absorbance was measured in the same wavelength range.

Identification of Cyanide in Samples

The cyanide identification procedure followed Zulfadli (2017), with modifications. First, 10 g of the sample was weighed and ground, then 20 mL of distilled water was added and the mixture was allowed to sit for 10 minutes. After filtration, 1 mL of 5% tartaric acid was added, and the mixture was left for 5 minutes. Filter paper was then immersed in the solution for one hour, followed by drying. The filter paper was hung in an Erlenmeyer flask, sealed with a rubber stopper, and heated at 50°C for 15 minutes. The color change of the picrate paper was observed: a red-brown color indicated the presence of cyanide, while an unchanged color indicated a negative result.

Cyanide Quantification in Samples

Seven grams of sample were ground in a mortar and mixed with 5 mL of Na_2CO_3. The mixture was stirred for 5 minutes, and the filtrate was separated using a Buchner filter. To the filtrate, 1 mL of ninhydrin was added, and the pH was adjusted to 12 using 1 M NaOH. The final volume was brought to 10 mL with distilled water. The absorbance of the sample was then measured using UV-Vis spectrophotometry at the maximum wavelength determined earlier.

3. Result and Discussion

In this study, the cassava samples used were freshly harvested and those stored for five days, both obtained from the eastern region of Bandung. Before conducting the cyanide content analysis, plant identification was carried out at Universitas Padjadjaran (UNPAD) to confirm that the plant used was indeed cassava (*Manihot esculenta* Crantz). Sample preparation was performed to facilitate the analysis, with the samples being ground into a fine paste. During the preparation process, extraction was carried out using sodium carbonate. The methods used to analyze cyanide content in cassava included both qualitative and quantitative tests.

Qualitative Analysis

The qualitative analysis aimed to determine the presence of cyanide in the cassava samples (freshly harvested and stored for five days) by observing the color change on filter paper. The filter paper, initially yellow due to the presence of picric acid, would change to red, orange, or brown if cyanide was present. This test is based on the formation of a colored complex between cyanide and picric acid.

The analysis began by grinding 10 grams of cassava to ensure that the contained substances were released, allowing the cyanogenic glycosides in the cassava to produce cyanide (CN^-) that would later undergo hydrolysis. The ground sample was then macerated with 50 mL of distilled water for 10 minutes. The purpose of this maceration process was to dissolve the cyanide ions (CN^-) that had been released during the grinding process. The following reaction occurs during this stage:

$$\mathbf{CN^-(g) + H_2O(l) \rightarrow HCN(g) + OH^-(g)}$$

After maceration, 10% tartaric acid was added, which helps generate HCN gas. The HCN is produced when the hydrogen from tartaric acid reacts with cyanide ions in the solution. The reaction is as follows:

$$\mathbf{2CN^-(g) + 2H^+(g) \rightarrow 2HCN(g)}$$

Next, filter paper (approximately 10 cm in diameter) was prepared and immersed in saturated picric acid solution, which is yellow in color. The filter paper was then hung at the opening of the Erlenmeyer flask, which was moistened with an 8% Na_2CO_3 solution. This setup was designed to trap the HCN gas produced and allow it to react with the picric acid on the filter paper, forming a red-brown Na-picrosianate complex. The filter paper was positioned so that it did not come into direct contact with the liquid inside the Erlenmeyer flask.

The flask was sealed with the filter paper and then heated in a water bath at 50°C for 15 minutes. This heating step helps accelerate the reaction between HCN, picric acid, and Na_2CO_3. Afterward, the color change on the filter paper was observed. If the color shifted from yellow to red, orange, or brown, it indicated the presence of cyanide in the sample. The overall reaction that occurs in the qualitative analysis is as follows:

HCN(g) + Picric Acid → Na-picrosianate (Red-Brown)

Results of Qualitative Analysis

From the qualitative test results, the following color changes were observed, indicating the presence or absence of cyanide in the samples (Table 1).

Tabel 1. The color shifted of qualitative analysis

Sample	Color Before	Color After	Cyanide Content
Positive Control	Yellow	Red-Brown	+
Negative Control	Yellow	Yellow	-
Freshly Harvested Cassava	Yellow	Red-Brown	+
Cassava Stored for 5 Days	Yellow	Red-Brown	+

These observations confirm that cyanide was present in both the freshly harvested cassava and the cassava stored for five days, as indicated by the red-brown color change on the filter paper. In contrast, the negative control (without cyanide) remained yellow, while the positive control, containing cyanide, exhibited the characteristic red-brown color.

Maximum Absorption Wavelength

In this study, a 35 ppm standard solution was used to determine the maximum wavelength. The maximum wavelength is the wavelength at which the highest absorbance value is obtained. The wavelength range measured was between 560 and 620 nm.

The objective of determining the maximum wavelength is to identify the absorption range that provides the highest absorbance value for the cyanide standard solution dissolved in water. This absorbance was then measured using a UV-Vis spectrophotometer within the wavelength range of 560 to 620 nm. After conducting the measurements, the maximum wavelength was found to be 584 nm. This value indicates a slight shift from the maximum wavelength reported in the literature (Soares, 2013). Despite this shift, the result still meets the criteria for its intended use in analysis, confirming the validity of the measurement for cyanide detection in cassava samples.

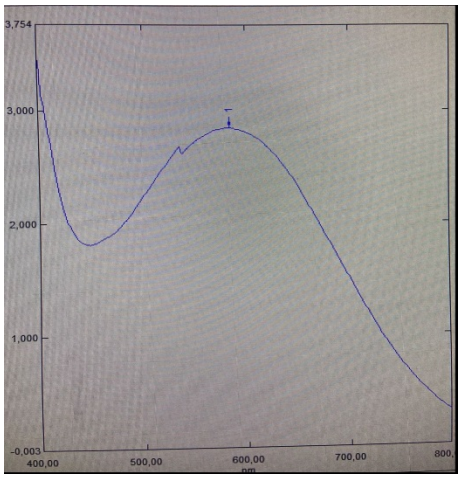

Figure 1. Spectrum of maximum wavelength for cyanide with ninhydrin reagent.

The slight deviation in the wavelength observed in this study may be attributed to experimental conditions, such as the specific concentration of reagents used or slight variations in the spectrophotometer calibration. Nevertheless, the results demonstrate that the method is reliable and suitable for the intended analytical purposes.

Method Validation

Method validation is essential to ensure that the parameters used in the research meet the required standards, providing accurate and reliable results. The validation process in this study included the evaluation of several key parameters: linearity, detection limit (LoD), quantification limit (LoQ), precision, and accuracy.

Calibration Curve

The calibration curve equation represents the relationship between the concentration (x-axis) and absorbance (y-axis) (Figure 2). The concentration values obtained during the experiment are plotted on the x-axis, while the corresponding absorbance values measured with the UV-Vis spectrophotometer are plotted on the y-axis. The linear regression equation derived from the calibration curve is given by:

$$y=0.0199x+0.1817$$

with a correlation coefficient (r) of 0.9982. The correlation coefficient (r) close to 1 indicates a strong linear relationship between the concentration and absorbance, meaning that the increase in absorbance is directly proportional to the increase in the concentration of cyanide. According to Sharger (1985), a correlation coefficient (r) of 0.9982 meets the acceptance criteria for a reliable and valid calibration curve.

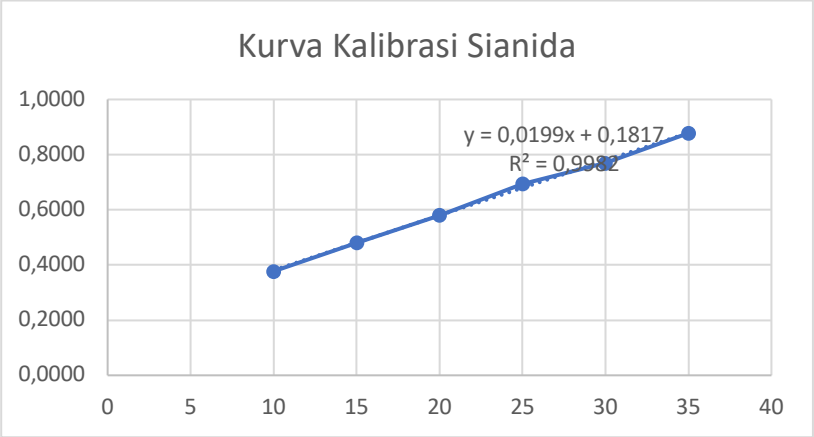

Figure 2. Calibration curve for cyanide.

This strong correlation suggests that the spectrophotometric method employed in this study is highly accurate and precise for quantifying cyanide concentrations in cassava samples, demonstrating the robustness of the analytical technique used. The calibration curve, with its high linearity, provides a reliable reference for determining cyanide content in cassava based on absorbance measurements.

From the linearity data of the cyanide standard calibration curve, the detection limit (LoD) and quantification limit (LoQ) were calculated. The detection limit is defined as the lowest concentration of cyanide that can be reliably detected, and the result obtained for LoD was 1.33 µg/mL. This value indicates the lowest concentration that can be detected in a sample, which is critical for determining the presence of cyanide at trace levels.

The quantification limit (LoQ) represents the lowest concentration at which cyanide can be quantified with confidence, and the result for LoQ was found to be 4.43 µg/mL. This value signifies the minimum concentration at which the cyanide content in cassava samples can be determined with sufficient accuracy.

Additionally, the coefficient of variation (CV) was calculated, yielding a value of 1.97%. Since the acceptable CV value is less than 5%, this indicates that the results of the analysis are precise and repeatable. This confirms that the method used in this study is not only capable of detecting and quantifying cyanide at low concentrations, but also produces consistent and reliable results.

Overall, the method validation results demonstrate that the analytical technique employed in this study meets the necessary criteria for accuracy, reliability, and sensitivity, making it suitable for the determination of cyanide levels in cassava samples.

Selectivity Test

The selectivity test was conducted to assess the ability of the reagent to measure a specific substance accurately and precisely, even in the presence of other potential components within the sample matrix (Susanti, 2010). This test is crucial to ensure that the reagent selectively reacts with cyanide without interference from other compounds that might be present in the cassava samples.

During the selectivity test, a shift in the maximum wavelength was observed, moving from the previously determined value to 600 nm (Figure 3). This shift indicated that the presence of other

compounds in the sample did not significantly affect the ability of the reagent to measure cyanide. The change in wavelength is important as it confirms that the reagent's response is primarily due to cyanide, with minimal interference from other substances in the sample.

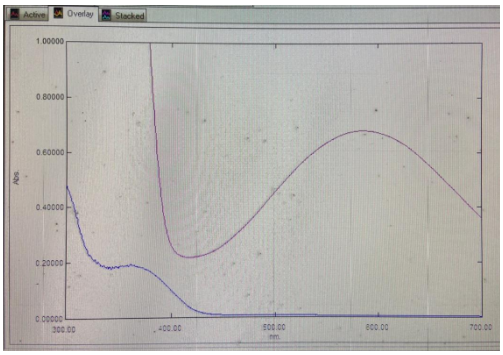

Figure 3. UV-Vis spectrum overlay for the selectivity test.

The UV-Vis spectrum overlay for the selectivity test (Figure 3) demonstrated that the reagent used in this study was capable of selectively detecting cyanide, even when other compounds were present in the sample. This result further validated the specificity of the analytical method, confirming its reliability for use in the cyanide analysis of cassava with different storage durations.

In summary, the selectivity test confirmed that the method was specific to cyanide detection, ensuring that the presence of other compounds did not compromise the accuracy of the results.

Accuracy

The next step in method validation was accuracy, which is expressed as the percent recovery of the added analyte. Accuracy can be determined using two methods: the spiked-placebo recovery method or the standard addition method, the latter of which was employed in this study.

In the standard addition method, 7 grams of cassava sample were finely ground, and 5 mL of sodium carbonate solution was added. Then, cyanide standards with concentrations of 10 ppm, 15 ppm, and 20 ppm were added to the sample. Afterward, 1 mL of ninhydrin was added to the solution, and the final volume was adjusted to 25 mL in a volumetric flask. Each concentration was measured three times to calculate the percent recovery.

The average percent recovery was calculated for each concentration, and the results were within the acceptable range of 80-120% (Harmita, 2004), which indicates that the method provides good accuracy. The table below presents the accuracy data for cyanide analysis, showing that the recovery values were consistent and met the criteria for method validation.

Table 1. Accuracy Results for Cyanide

Theoretical Concentration (ppm)	Measured (µg/mL)	% Recovery	Average % Recovery
10	14.69	93.8%	
	14.59	92.8%	92.6%
	14.44	91.3%	
15	20.12	98.7%	
	20.22	99.4%	99.1%
	20.17	99.1%	
20	26.10	103.9%	
	26.25	104.7%	104.4%
	26.20	104.4%	

The precision test aimed to determine the level of accuracy or validity of repeated analysis results under the same conditions (Harmita, 2004). To assess the precision, the standard addition method was repeated six times on the same day at a concentration of 15 ppm. The results indicated that the coefficient of variation (CV) did not exceed the established limit of <2%, which signifies that the method used demonstrated excellent precision and reliability.

Table 2. Interday Precision Results

No.	Concentration (µg/mL)	Day 1	Day 2	Day 3
1	15	20.92	21.42	20.86
2	15	21.32	21.77	21.72
3	15	21.62	22.02	21.82
4	15	21.52	21.57	21.87
5	15	22.02	21.37	22.12
6	15	21.77	21.92	21.64
Average		21.53	21.68	21.64
SD		0.3486	0.2451	0.3985
RSD		1.619	1.652	1.192

The accuracy and precision of cyanide measurements are presented in the tables below. The data from the interday precision test show consistent results across three days, with small variations in the measurements. Similarly, the results from the extraday precision test indicate minimal fluctuation in values over the three days, supporting the method's reliability.

Table 3. Extraday Precision Results

Day	Concentration (µg/mL)
1	21.531
2	21.682
3	21.648

The interday precision results show minimal variation across three consecutive days, with average values around 21.6 µg/mL (Table 2). The standard deviation (SD) ranged from 0.2451 to 0.3985, and the relative standard deviation (RSD) was consistently below 2%, indicating excellent precision. The extraday (Table 3) precision results also demonstrated stability, with the average concentration consistently near 21.6 µg/mL and a very low RSD of 0.2933. These findings confirm the method's high precision and reproducibility for cyanide analysis in cassava.

The determination of cyanide content was subsequently carried out on cassava samples harvested freshly and those stored for five days. Prior to the cyanide analysis, sample preparation was conducted to ensure the samples were ready for analysis using the visible light spectrophotometry method with ninhydrin reagent. The addition of ninhydrin was intended to form a complex that exhibited red and blue colors in a high pH environment (pH 12-14). Under these conditions, cyanide forms cyanide ions (CN^-), while the addition of sodium carbonate (Na_2CO_3) aimed to stabilize the cyanide compound, preventing it from easily evaporating.

Table 4. Cyanide Content in Cassava Samples

Sample	Concentration (µg/mL)	Weight (g)	Cyanide Content (µg/g)
Freshly harvested	1.476	4.958	4.945
	1.470	7.440	4.939
	1.470		4.939
Five days stored	2.1635	7.173	7.160
	2.1580	7.540	7.155
	2.1580		7.155

The cyanide content found in freshly harvested cassava from the Eastern Bandung area was 4.945 µg/g, which is within the permissible limit. In contrast, the cassava stored for five days showed a higher cyanide content of 7.16 µg/g. These results indicate an increase in cyanide levels after storage, suggesting that the duration of storage may influence the cyanide content in cassava.

4. Conclusion

Based on the results of the research on the analysis of cyanide in cassava with different storage durations using visible light spectrophotometry and ninhydrin reagen, it can be concluded that cassava from the Eastern Bandung area contains cyanide, both qualitatively and quantitatively. The cyanide content in freshly harvested cassava was found to be 4.945 µg/g, while cassava stored for five days showed a higher cyanide level of 7.16 µg/g. This suggests that the storage duration influences the cyanide content in cassava.

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