



Analysis of Cyanide Contents in Cassava Leaves (*Manihot esculenta* Crantz) Based on Boiling Time with Formation of Hydrindantin Complex by Using UV-Vis Spectrophotometry

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Abstract

*This study aimed to determine cyanide contents in cassava leaves (*Manihot esculenta* Crantz) based on boiling time with the formation of hydrindantin complex using UV-Vis spectrophotometry. The variation of boiling time was 0, 5, 10, and 15 minutes respectively. The cyanide content was prepared by extracting the sample using water for 2 hours. Then the sample was distilled to obtain a distillate that containing cyanide. Determination of cyanide was carried out by reacting samples containing cyanide with 1% ninhydrin and 0.5% Na₂CO₃, then adding 1 M NaOH to form blue hydrindantin compounds in an alkaline condition. Blue hydrindantin compounds formed were analyzed by spectrophotometry at a maximum wavelength of 590 nm. The results showed that cyanide contents in cassava leaves based on boiling time 0, 5, 10, and 15 minutes respectively were 20.73 ppm, 13.92 ppm, 9.18 ppm, and 3.38 ppm. The longer the cassava leaves were boiled, the better it was to be consumed because it will significantly reduce cyanide contents.*

Keywords: Cassava leaves, cyanide, hydrindantin, UV-Vis spectrophotometry

Introduction

Vegetables are one of the basic human needs which are a source of nutrition for the body. One type of vegetable that is consumed by many people is cassava leaves. Cassava leaves are a vegetable that has a high nutritional content. The nutritional content in cassava leaves includes protein, carbohydrates, phosphorus, calcium, and others (Direktorat Gizi Departemen Kesehatan RI, 1992). However, apart from the high nutritional content, cassava leaves also contain toxins that can endanger the health of the body, such as thiocyanate (SCN⁻), which can inhibit iodine absorption. The thiocyanate content in cassava leaves is 0.010 ppm (Lubis & Jumirah, 2013). Cassava leaves also contain cyanide toxins. The cyanide content in cassava leaves is high enough to reach 136 mg/kg of dry matter of cassava leaves (Rukmana, 1997).

This cyanide, when consumed in large doses, will cause rapid breathing, chills, headaches, nausea, seizures, weakness. This poisoning system then raises pressure from the inhalation system and, if not helped, will cause death. Depending on the amount of cyanide, it can cause illness and death (Yatno et al., 2015). The normal dose of cyanide acid in humans is 0.5 - 3.5 mg/kg body weight (Winarno, 2002).

Cyanide levels in cassava leaves must be reduced or eliminated to make them safe for

consumption. Cyanide levels can be reduced through several processing methods such as slicing, peeling, soaking, washing, fermentation, heating (boiling, steaming), drying, and canning (Venagaya et al., 2017). Cyanide quickly evaporates due to heat and dissolves easily in water (Winarno, 2002).

Processing of cassava leaves in the community is by boiling it first. During the boiling process, the cyanide will dissolve in the cooking water, so it is recommended that the remaining water from the boiled cassava leaves be discarded. Some people do not know that cassava leaves contain cyanide so that it can trigger poisoning if improper processing.

Several months ago, there was news that one of the residents' livestock was poisoned due to consuming the remaining boiled cassava leaves. One of the victims who came from Meko Village, Poso Regency, said that he boiled cassava leaves which he would process into cooking. Then the remaining boiled water is given to the livestock, with the hope that the boiled water will have a good impact on the livestock. After that, the animal suddenly died. It is suspected that the animal was poisoned by cyanide originating from the cassava leaves itself. Gomez (1985) stated that the maximum limit of cyanide content that is safe for livestock is 100 mg per kg of dry feed ingredients. According to Basri, et al. (2015) that cyanide levels that can cause death are

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above the threshold of 2.4 mg/kg body weight of goats or sheep.

This study chose a boiling as a treatment. The use of the boiling process is because it has advantages, including the process is fast, relatively easy, and can be done at low cost. Besides, boiling is also one of the processing processes of cassava leaves commonly used by the community.

The cyanide content in cassava leaves can be determined by analysis using ninhydrin as an alkaline reagent. This method provides an easy and accurate step for cyanide determination using ninhydrin as the single and inexpensive reagent. This method is sensitive, generally free of disturbance from intruding species, and does not require heating or extraction (Nagaraja et al., 2002). This cyanide determination uses an instrument, namely a UV-Vis spectrophotometer. The advantage of this method is that it can determine very small levels of substances, the results obtained are quite accurate, the process is fast, this method of analysis takes easy and simple steps.

In this paper, we give preliminary results for the analysis of cyanide levels in cassava leaves was carried out based on the boiling time and the formation of hydrindantin complex using UV-Vis spectrophotometry.

Methods

The tools used in this research are a set of distillation tools, a container (basin), beaker, test tube, Erlenmeyer, digital balance, UV-Vis spectrophotometer, electric bath, stirring rod, dropper pipette, measuring cup, measuring flask, stopwatch, spatula, filter paper, funnel, spray bottle, mortar, and pestle.

The materials used in this study were cassava leaf extract, 1 M NaOH solution (Merck), KCN solids (Merck), 0.5% Na₂CO₃ solution (Merck), 1% ninhydrin solution (Merck), distilled water, and tap water.

Sample Preparation

Samples (cassava leaves) are picked in the morning and then washed thoroughly. Furthermore, the water was heated to boiling, then the sample was added and boiled alternately according to the predetermined length of time, namely 0, 5, 10, and 15 minutes. After reaching the specified time, the sample is removed and drained. After that, the sample is mashed (Kurnia et al., 2012).

Cassava Leaf Extraction

The sample extraction in this study was using the distillation method. Cassava leaves that have been mashed are weighed using a digital balance of 20 grams. Then put it in 250 mL Erlenmeyer, and add 100 mL of distilled water into the Erlenmeyer, then stir. After that, cover the Erlenmeyer using aluminum foil, then let it sit for 2 hours. After that, the sample is put into a distillation flask and distilled for 1 hour (Kurnia et al., 2012).

Cyanide Analysis

The quantitative analysis of flavonoids in this study used the UV-Vis spectrophotometric method with the formation of hydrindantin complexes. Put five mL of cassava leaf extract into the test tube. Put 1 mL of 1% ninhydrin solution and two mL of 0.5% Na₂CO₃ and let it stand for about two minutes to give the red complexion, then add 11 drops of 1 M NaOH to form a blue color let stand for 10 minutes. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 590 nm (Kusumaningtyas et al., 2015).

Results and Discussion

Sample Preparation

Sample preparation in this study is by boiling the sample based on the length of time that has been. The purpose of boiling is to reduce cyanide levels in cassava leaves. Heating is able to evaporate larger amounts of cyanide with increasing boiling time (Putra et al., 2009). Then the sample is crushed. The goal is to expand the surface of the sample, so that the contact between the sample and the solvent is maximized.

Sample Extraction

Extraction is a way to separate a mixture of several substances into components. In the extraction process, it is crucial to choose a suitable solvent (Malik et al., 2014).

The extraction in this study used a water solvent because cyanide is easily soluble in water. This method is easy to do and uses simple tools, namely by immersing the refined sample in a water solvent. The samples were immersed for two hours. The aim is that all of the chemical components in cassava leaves can be attracted to all and so that the extraction process occurs optimally. In this immersion process, the linamarin compound will hydrolyze (react with water) and form cyanide acid which is soluble in water. Linamarin, if hydrolyzed, will form cyanide acid, which is soluble in water and volatile (Hutami et al., 2014). Then proceed with the distillation stage. The distillation stage aims to separate the desired substance, namely cyanide, from other components (Kurnia et al., 2012).

Cyanide Analysis

Cyanide is a chemical compound consisting of a 'cyano group' (C≡N), which consists of triple carbon atoms bonded to a nitrogen atom. Cyanide is a deadly chemical poison that can take many forms. Cyanide can be a colorless gas, such as hydrogen cyanide (HCN) or cyanogenic chloride (CNCl), or a crystalline form such as sodium cyanide (NaCN) or potassium cyanide (KCN) (Hlaing et al., 2011). Cyanide is found in various plants and is distributed in parts such as seeds, followed by fruit, leaves, stems, and roots (Smith & Mudder, 1991).

Cyanide analysis in cassava leaves was carried out by forming a hydrindantin complex, using

ninhydrin as a reagent in an alkaline environment (Nagaraja et al., 2002). The resulting hydrindantin complex turns red when 0.5% Na_2CO_3 is added. Then the solution was added with 11 drops of 1 M NaOH solution to form a blue color. NaOH functions as a reagent to condition the pH of the formation of hydrindantin complexes because the hydrindanthine complex has a different color over a certain pH range (Cahyani et al., 2015). Hydrindantin blue is formed spontaneously and is stable in 0-30 minutes. After 30 minutes, the hydrindantin color will fade. Thus, 5 minutes was chosen as the optimum time for cyanide measurement (Zulfah et al., 2015).

Measurement of cyanide levels in cassava leaf extract (*Manihot exculenta* Crantz) using a UV-Vis

spectrophotometric instrument. Measurement of sample levels of cassava leaves using a wavelength of 590 nm is the wavelength of the complementary color of the measured solution (blue). The complementary color absorbed by the hydrindantin complex is orange (Kusumaningtyas et al., 2015).

Based on the results of research that has been done regarding the analysis of cyanide levels in cassava leaves (*Manihot esculenta* Crantz), the results of the study are presented in Table 1.

Based on Table 1, it can be seen that the decrease in cyanide levels in cassava leaves at each boiling time. An illustration of an increase in carbohydrate levels can be seen in Figure 1.

Table 1. Results of cyanide analysis in cassava leaves based on boiling time

Boiling Time (Minutes)	Cyanide Content (ppm)
0	20.73
5	13.92
10	9.18
15	3.38

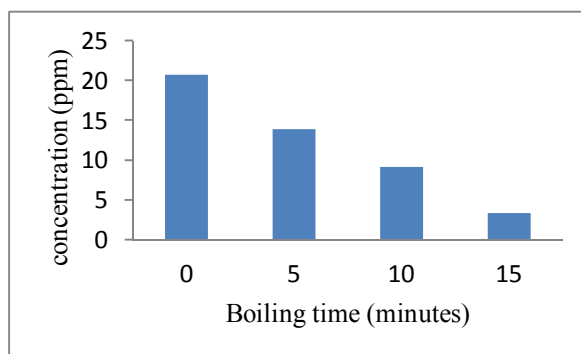


Figure 1. Graph of the relationship between boiling time and cyanide concentration

Based on the data in Figure 1, it can be seen that the longer the boiling time, the more cyanide content in cassava leaves decreases.

The decrease in cyanide is due to the dissolution of cyanide in boiling water, and when the remaining boiling water is removed (drained), the cyanide is wasted so that the cyanide content in cassava leaves will decrease (Venagaya et al., 2017). Heating (boiling) is able to deactivate the linamarase enzyme so that cyanide breakdown from linamarin does not occur and is able to evaporate larger amounts of cyanide with increasing boiling time. The higher the temperature and the length of the heating time, the more the cyanide content decreases because cyanide is easily soluble in water and quickly evaporates due to heat (Yatno et al., 2015).

Cassava leaves contain two cyanogenic glucosides, namely linamarin and lotaustralin (Cereda & Mattos, 1996). If the cassava leaves are crushed, sliced or chewed, causing damage to the cell walls of the tissue, the two compounds are in

contact with linamarase and oxygen enzymes to produce glucose, acetone, and cyanide acid (McMahon et al., 1995).

Conclusions

Based on the research that has been done regarding the analysis of cyanide levels in cassava leaves based on boiling time, the highest cyanide content in cassava leaves is found at 0 minutes or without boiling, namely, 20.73 ppm, while the lowest is at 15 minutes boiling time is 3.38 ppm.

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