Bamboo Activated Charcoal on Phytochemical Substances and Quality of Cassava Leaves *(Manihot Utilissima)*

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Abstract. The study aimed to evaluate the effect of activated charcoal on phytochemical substances and the quality of cassava leaves. The research consisted of two stages, with the first stage, using a completely randomized design to determine the optimal treatment of activated charcoal content and soaking time on cassava leaves. The second stage evaluated the quality of the cassava leaves using the best treatment from the first stage. The study found that bamboo-activated charcoal levels of 2%, 4%, and 6% with a soaking time of 36 hours reduced the phytochemical substances of cassava leaves (HCN, tannin, and flavonoids). Additionally, bamboo-activated charcoal levels improved the quality of cassava leaves at the 2% level ($P<0.05$), as seen by an increase in crude protein (32.895%), crude fiber (13.915%), soluble protein (10.620%), in vitro dry matter digestibility (70,055%), and in vitro organic digestibility (67,538%).

1 Introduction

In Indonesia, cassava (*Manihot utilissima*) is a significant crop because it grows easily even in marginal locations [1]. [2], average harvest areaof cassava in Indonesia is 849.30 thousand ha, which is equivalent to 3.79% of the total world harvest area. Cassava leaves can be harvested every three months, with a yield of 11.786 kg^{-1} ha^{-1} dry matter (DM) [3], which represents about 10–40% of the cassava plant [4]. Cassava leaves are rich in nutrients such as 20% crude protein [5], and carotene, beta-carotene, calcium, iron, phosphorus, magnesium, vitamins A, B1, and are also used as an anthelmintic in ruminants [6]. In addition, cassava leaves are a source of lactic acid bacteria and probiotics for livestock [7].

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Cassava leaves are known to be high in protein, vitamins, and minerals, and can be a great source of nutrition for livestock. It is important to remember that cassava leaves also contain tannin [9] and cyanide (HCN) [8], however flavonoids are always present together with tannins [10]. The three substances can be toxic if consumed by livestock in high doses, so cassava leaves must first undergo feed processing in order to be used as animal feed.

One effective feed processing to reduce HCN, tannin and flavonoid levels is through the soaking process. HCN and flavonoid substance is unstable in water, so it will dissolve in water [11], and is easily released into the air. Therefore, tannin is a polyphenolic compound that can dissolve in water or strong acids, so soaking can effective method to remove HCN from cassava leaves [12].

Activated charcoal has many functions including being able to absorb harmful substances. It is considered an effective adsorbent [13, 14], and is widely used for its easy, cheap, and eco-friendly properties [15]. Compared to other materials, activated charcoal has a low cost, high attractiveness, lightweight, flexibility, durability, and four times the absorption rate with a surface area ten times wider. Bamboo activated charcoal produces a high adsorption rate that exceeds standards, and is included in the commercial activated charcoal group and has a much higher adsorption rate when compared to activated charcoal made from mangrove charcoal and coconut shells [13]. Bamboo activated charcoal contains 5% water content, 6% volatile matter content, 7,33% ash content, 81,67% carbon content, iodine uptake of 1091,426 mg g^{-1}, and methylene blue uptake capacity of 198,724 mg g^{-1}, the surface area of activated charcoal is 737,74 m^2 g^{-1} and the surface acidity is 0.5122 mmol g^{-1} as well as the presence of O-H, carbonyl, alkyne and ester functional groups [16]. [17], stated that activated bamboo charcoal has a water content of 3,05%, ash 3,67%, an activated charcoal surface area of 745,21 m^2 g^{-1} and a methylene blue absorption capacity of 250 mg g^{-1}. How activated charcoal works in adsorbing HCN and tannin as seen in Figure 1.

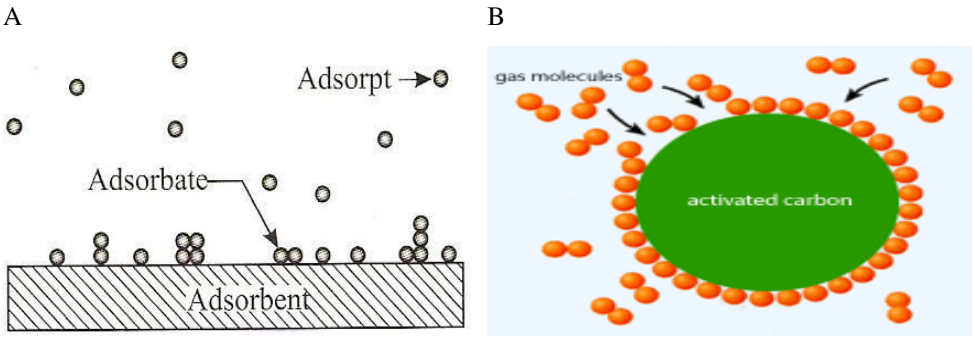

Figure 1. Adsorption Process (A) and Adsorption Process with Activated Charcoal (B)

Based on the background above, it is necessary to see what extent the effectiveness of activated bamboo charcoal with soaking time can affect the phytochemical content and quality of cassava leaves. It is hoped that this will be very useful for alternative livestock feed in the future.

2 Materials and Methods

2.1 Material

There were two stages of the research. The stages carried out in this study were the preparation of cassava leaves, chopping, treatment, washing, drying, HCN, tannin, and flavonoid analysis, selection of the best treatment, and quality analysis of cassava leaves. The activated bamboo charcoal used in the research was obtained using method charcoal for versatile applications [18] (Figure 2).

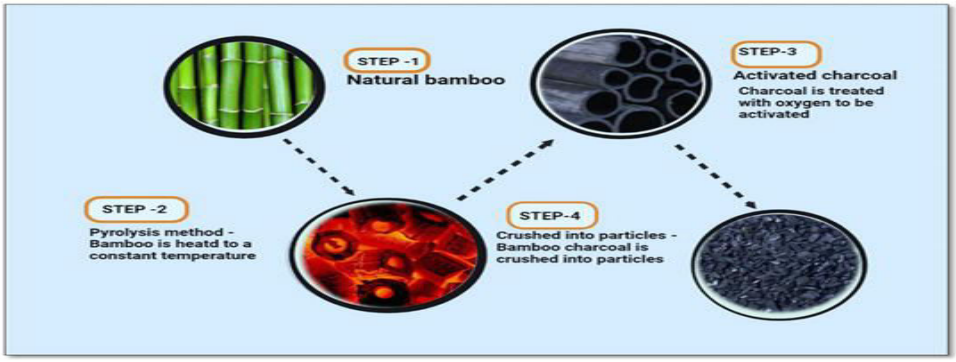

Figure 2. Process for Making Bamboo Activated Charcoal

2.2 Methods

There were two stage this research. One stage was to evaluation of the capacity of activated charcoal to reduce HCN, tannin and flavonoids levels in cassava leaves (*Manihot utilissima*). The second stage was to the quality and digestibility of cassava leaves *(Manihot utilissima) in-vitro.*

2.2.1 stage 1 : Evaluation of the capacity of activated charcoal to reduce HCN, tannin and flavonoids levels in cassava leaves (Manihot utilissima)

A completely randomized factorial design (CRD factorial) utilizing three replications and two factor was the initial stage of the research design. The research was conducted at the Integrated Biotechnology Laboratory, Animal Husbandry Faculty, Hasanuddin University. The treatments consisted of :

- Factor A : Activated charcoal level
  - A1 : 0% activated charcoal level
  - A2 : 2% activated charcoal level
  - A3 : 4% activated charcoal level
  - A4 : 6% activated charcoal level
- Factor B : Soaking time
  - B1 : 0 hours soaking time
  - B2 : 12 hours soaking time
  - B3 : 24 hours soaking time
  - B4 : 36 hours soaking time

2.2.2 stage 2: Quality and digestibility of cassava leaves (Manihot utilissima) in-vitro

The best outcome from the first stage of treatment, the second stage. The greatest reduction in HCN, tannin, and flavonoid levels is used to determine which treatment is most effective. A CRD with four treatments and six duplicates was used in this treatments.

The second stage research at the Dairy Livestock Laboratory, Faculty of Animal Husbandry, Bogor Agricultural University. The treatments consisted of :

P1 : Control
P2 : 2% activated charcoal content with 36 hours soaking time (A2B4)
P3 : 4% activated charcoal content with a soaking time of 36 hours (A3B4)
P4 : 6% activated charcoal content with a soaking time of 36 hours (A4B4)

2.3 Parameters

2.3.1 Stage 1: Evaluation of the capacity of activated charcoal to reduce HCN, tannin and flavonoids levels in cassava leaves (Manihot utilissima)

2.3.1.1 HCN level

The research using steam distillation to determine the HCN content [19]. 10–20 g of sample placed in a Kjeldahl flask. Adding 100 ml of distilled water, let it soak for two hours. After that, distill it with an 100 ml of distilled water. Twenty milliliters of 5% NaOH were added to an Erlenmeyer flask before the distillate was collected. Following the distillate's 150 ml volume, 5 ml of 5% KI and 8 ml of NH4OH were added, use 0.02 N AgNO3 to titrate until it turns hazy. Formula to the HCN content showed in equation 1 :

$$\% \square\square\square = \frac{Titration\ volume\ x\ 0{,}54}{sample\ weight\ (g)} \square\ 100\% \qquad (1)$$

2.3.1.2 Tannin dan Flavonoid Level

Tannin and flavonoid content using atomic absorption spectrophotometry [20]. ± 0.5 g sampel was put in to a 50 ml measuring flask. Flush the line with hot water, then wait for it to cool. After shaking, strain and pipette 0.5 milliliter of the filtrate. Add 0.5 saturated sodium carbonate solution, 0.25 ml of Folin, and 4.5 ml of distilled water. After homogenizing, let stand for half an hour in a dark area. measured using a spectrophotometer set to 680 nm in wavelength. Make a standard and blank curve with the help of tannin/flavonoid acid.

2.3.2 Stage 2 : Quality and digestibility of cassava leaves (Manihot utilissima) in-vitro

2.3.2.1Crude protein and crude fiber

Using proximate analysis, determine crude protein and crude fiber [21]. Proximat analysis proximate analysis in accordance with AOAC 2005 standards. The micro Kjeldahl method was used to determine the amounts of proteins. The purpose of this analysis is to determine protein depending on the nitrogen being converted to ammonia and the carbon in the components being oxidized. Next, ammonia and extra acid combine to make annonium sulfate. Ammonia is vaporized for internal absorption of boric acid solution once the solution turns basic. Titration of HCL yields the amount of nitrogen present. How to apply the Kjeldahl method to determine protein levels. Titration, distillation, and destruction are the three main components of Kjeldahl's protein analysis methods. When the crude fiber

ignites under certain circumstances, the sample that is left behind after digestion with 1.25% H2SO4 and 1.25% NaOH solns is lost.

2.3.2.2 Soluble Protein

Soluble protein using Lowry method [22]. A scale tube containing 7.5 milliliters of distilled water were added to 1.5 grams of the sample, homogenized with centrifuged for 15 minutes, and the precipitate and supernatant were then separated. One milliliter of a 10% TCA solution was added to two milliliters of supernatant. Centrifuging the mixture for fifteen minutes separated the precipitate and solution. sample added 0.1 ml of TCA, 1.9 ml of distilled water and 2.5 ml of Lowry's reagent, the mixture was allowed to stand at room temperature for ten minutes. The folim reagent was added, and the mixture was incubated for 30 minutes to produce a blue hue. Spectrophotometer using a standard solution of bovine serum albumine (BSA) at a wavelength of 600 nm.

2.3.2.3 Digestibility: Dry matter and organic

Digestibility dry matter (IVDM) and organic (IVOD) as determined by the in vitro method [23]. There are two primary phases to the approach. First step: at 38°C and in the dark, a tiny (0–5 g) sample of dry fodder is anaerobically digested by rumen microbes. Step 2 : In vitro implementation (Step 1 : Rumen fluid digestion, Step 2: Pepsin digestion). Can be calculate: After weighing one gram of residue and is put in a cup with a known weight. Bake for 24 hours at 100–105°C, or until the weight remains constant. Allow to cool in a desiccator for about 15 minutes. To determine the IVDM, it is weighed, and for IVOD, it is baked at 600° C for ± 8 hours.

2.4 Data analysis

The collected data were subjected Analysis of Variance (ANOVA) using the SPSS version 24.0 software, with a 95% confidence interval. If there was a significant differences between the treatments, further analysis was conducted using the Duncan test in significance level of 0.05.

3 Results

3.1 Stage 1 : Evaluation of the capacity of activated charcoal to reduce HCN, tannin and flavonoids levels in cassava leaves *(Manihot utilissima)*

Activated charcoal levels of 2%, 4%, 6% and soaking time of 0,12,24,36 hours on cassava leaves showed a significant effect ($P<0.05$) to reduce HCN, tannin and flavonoids levels in cassava leaves.

Table 1 presents that the higher level of activated charcoal, the lower the level of phytochemical substances in cassava leaves. Meanwhile, the average levels of HCN and flavonoids using DMRT analysis did not show significant differences ($P>0.05$) in factors A2, A3 and A4. Apart from that, the average tannin content of DMRT results also showed that factors A1, A2, A3, and A4 had the same impact ($P>0.05$). Levels of HCN, tannin and flavonoids in factor B had a real effect ($P<0.05$), based on variance analysis findings. The longer the soaking time, the lower the levels of HCN, tannin and flavonoids. However, on average A2, A3, and A4 did not have a significant effect ($P>0.05$) on tannin levels even though there was a decrease.

The interaction between factors A and B in the first stage of the study had a positive effect on reducing HCN, tannin, and flavonoid levels in cassava leaves. The treatment with the highest decrease in HCN levels was A4B4, which showed a 63.76% reduction. Tannin levels decreased by 77.78%, and flavonoid levels decreased by 64.94%. These findings suggest that the use of activated charcoal can effectively reduce the levels of these phytochemical substances in cassava leaves.

Table 1. Levels of HCN, Tannin, and Flavonoid in Cassava Leaves *(Manihot utilissima)*

Factor A: Activated charcoal level (%)	**Factor B: Soaking Time (hours)**			
	1 (0)	**2 (12)**	**3 (24)**	**4 (36)**
HCN (g)				
1 (0)	0.075^{h}	0.066^{efg}	0.052^{cde}	0.047^{cd}
2 (2)	0.074^{gh}	0.059^{dg}	0.051^{cde}	0.039^{ab}
3 (4)	0.071^{fgh}	0.057^{def}	0.051^{cde}	0.036^{ab}
4 (6)	0.054^{cde}	0.052^{cde}	0.046^{cd}	0.028^{a}
Tannin (g)				
1 (0)	23.400^{d}	7.550^{a}	7.033^{a}	7.200^{a}
2 (2)	20.800^{b}	6.800^{a}	6.300^{a}	5.700^{a}
3 (4)	21.100^{b}	6.783^{a}	6.400^{a}	5.300^{a}
4 (6)	22.567^{bc}	6.300^{a}	5.450^{a}	5.200^{a}
Flavonoid (g)				
1 (0)	0.887^{e}	0.880^{e}	0.803^{e}	0.540^{d}
2 (2)	0.583^{d}	0.573^{d}	0.510^{cd}	0.373^{ab}
3 (4)	0.583^{d}	0.540^{d}	0.510^{cd}	0.357^{ab}
4 (6)	0.533^{d}	0.470^{cd}	0.450^{cd}	0.313^{a}

Notes : Different sguperscripts in the same line or colom show significant differences ($p<0.05$).

3.2 Stage 2 : *Quality and digestibility of cassava leaves (Manihot utilissima) in-vitro*

Activated charcoal levels of 2%, 4%, 6% and soaking time of 36 hours on cassava leaves showed a noteworthy impact ($P<0.05$) on the quality and digestibility.

Table 2. Crude protein, Crude fiber, Soluble protein and Dry matter digestibility and Organic Digestiibility *in-vitro*

Treatment, %	**P1**	**P2**	**P3**	**P4**	**SE**
Crude protein	30.560^{a}	32.895^{c}	31.810^{b}	31.208^{ab}	0.309
Crude fiber	18.488^{c}	13.915^{a}	17.140^{b}	18.167^{c}	0.258
Soluble protein	7.998^{c}	10.620^{d}	7.110^{b}	5.155^{a}	0.197
Dry matter digestibility	50.465^{b}	70.055^{c}	49.623^{b}	38.631^{a}	0.426
Organic digestibility	48.647^{b}	67.538^{c}	47.672^{b}	36.332^{a}	0.391

Notes: Different superscripts in the same line show significant differences ($p<0.05$).

Table 2 presents that, the treatment had a significant impact to the levels of protein and crude fiber, according to the analysis of variance (P<0.05). The activated charcoal P2 treatment had the highest average crude protein and the lowest crude fiber. Further testing with DMRT showed that the levels of activated charcoal P1, P3, and P4 on crude protein levels, and P1 and P4 on crude fiber levels were not significantly different (P>0.05). Additionally, increasing levels of activated charcoal resulted in lower crude protein levels and higher crude fiber levels.

The treatments had significant effects on the levels of soluble protein, IVDM and IVOD, as the analysis of variance showed (P<0.05). The highest average values for soluble protein, IVDM and IVOD were found in P1. Further testing with DMRT revealed that the average IVDM and IVOD values for P1 and P3 were not significantly different (P>0.05) when activated charcoal was used.

4 Discussion

4.1 Stage 1 : Evaluation of the capacity of activated charcoal to reduce HCN, tannin and flavonoids levels in cassava leaves *(Manihot utilissima)*

HCN levels also decreased with increasing soaking time. This is due to the reaction of HCN with water. HCN in cassava leaves is 95% linamarin and 5% lotaustralin. Linamarin when reacting with water (H_2O), then with the help of the linamarase enzyme it will be broken down into acetone and hydrogen cyanide. This linamarin reaction consists of several stages, starting from the separation of glucose and some cyanohydrin, then with the help of β-glycosidase and cyaohydrin separated into carbonyl compounds and hydrogen cyanide. This process causes the structure of cassava leaves to become soft and water seeps into the leaf structure. In the leaf structure, water breaks down or decomposes hydrogen cyanide from cyanogenic glycoside bonds into ammonium formate and amorphous substances, so that hydrogen cyanide comes out of the cassava leaves. During soaking, a diffusion process occurs, followed by the breakdown or decomposition of HCN, and finally, [24] and [25], state that HCN is unstable in water and can be removed by soaking treatment or by chlorination with certain materials.

Activated charcoal can weaken tannin properties. This is due to the nature of activated charcoal which can stimulate changes in the tannin compound group, so that tannin turns into weak tannin. [26], activated charcoal can also slow down the rate of tannin because with increasing contact time in the adsorption process, the number of active tannin sites decreases, so that the tannin content in the material decreases.Tannin substances are soaked with water, the extraction process occurs, and tannin is separated from the solid matrix into the liquid or soaking water. Tannins are a group of polyphenolic compounds that are soluble in water or strong acids [12].

The decrease in flavonoid levels is also caused by flavonoids that can dissolve in water during soaking. The chemical structure of flavonoids generally contains hydroxyl groups (-OH) that can interact with water through hydrogen bonds, thus making them soluble in polar solvents such as water. After soaking, the flavonoid component sustains structural damage, which results drop in flavonoid levels [27]. Polyphenolic substances derived from flavonoids will decompose and dissolve in water when structural damage occurs [28].

When HCN, tannin, and flavonoids come out of cassava leaves, active charcoal immediately adsorbs them and sticks to the pores of the active charcoal, so that the levels of HCN, tannin, and flavonoids in the water decrease. When the levels are reduced in water, the water can again remodel the remaining substances in the cassava leaves, so that the

more active charcoal levels, the lower the levels of HCN, tannin, and flavonoids in cassava leaves. Activated charcoal can also neutralize HCN, which is acidic, and can inhibit the oxidation rate of toxins and neutralize acids [29,30].

4.2 Stage 2 : Quality and digestibility of cassava leaves *(Manihot utilissima)* in-vitro

Crude protein levels fall and crude fiber levels rise with increasing levels of activated charcoal treatment. Because of its vast surface area and pore structure, activated charcoal can absorb certain materials, including protein from animal feed. This causes the higher the level of activated charcoal, the crude protein level in the feed decreases. This shows that the use of activated charcoal as an adsorbent cannot be used in high levels. In this study, the level of activated charcoal that can still maintain the quality value of cassava leaves is 2%. The surface area of activated charcoal ranges from 300-3500 m^2 g^{-1} and with an internal pore structure that causes activated charcoal to have properties as a gas adsorbent and chemical compounds, acids and bases [31]. Excessive use of activated charcoal has the potential to reduce the nutritional content of the feed, due to the nature of activated charcoal which can absorb the nutritional content of the feed [32,33].

Nutrients can be disturbed in the digestion process due to the higher crude fiber level, resulting in a lower average digestibility value despite having a high crude protein level. [34], feed that contains high protein but also high crude fiber level, the protein will not be digested properly. IVDM and IVOD are affected by the levels of crude protein and crude fiber. When crude protein levels are high, digestibility is increased, and when crude fiber levels are low, digestibility is also increased. The digestibility of organic matter from a feed material is also caused by the content of crude fiber [35,36], and some of the crude protein [37]. High levels of crude fiber feed will result in increased energy loss through feces (fecal energy), especially feed containing high BETN [38]. VIOD value is closely related to the VIDM value, because DM consists of organic and inorganic materials. A decrease in the VIDM value will result in a decrease in the VIOD value. [39], the decrease in organic matter content in the fermentation process is due to the breakdown of organic matter (especially carbohydrates) which is used as source of energy for the growth and activity of microorganisms.

5 Conclusion

The utilization of bamboo-activated charcoal levels can be effective in reducing the phytochemical substances of cassava leaves such as HCN, tannin, and flavonoids. The reduction can be achieved by soaking the cassava leaves a solution containing 2%, 4%, or 6% bamboo activated charcoal for 36 hours. Improvements in the quality of cassava leaves, levels of soluble protein, crude protein, crude fiber, IVDM and IVOD occurred when treated with 2% activated bamboo charcoal levels.

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