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Short Communication

Effect of Different Variety and Maturity on Hydrocyanic Acid (HCN) and Protein Concentration of on Cassava (*Manihot esculanta*, Crantz) Leaves

Sarah Idris[1], Rosnah Shamsudin[1,2]*, Mohd Zuhair Mohd Nor[2], Mohd Noriznan Mokhtar[2], Siti Salwa Abd Gani[1]

[1]Halal Products Research Institute, Universiti Putra Malaysia, Serdang, Selangor, 43400, Malaysia, gs46354@student.upm.edu.my, rosnahs@upm.edu.my, ssalwaag@upm.edu.my

[2] Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, Serdang, Selangor, 43400, Malaysia, rosnahs@upm.edu.my, zuhair@upm.edu.my, noriznan@upm.edu.my

*Corresponding author: Rosnah Shamsudin Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, Serdang, Selangor, 43400, Malaysia; rosnahs@upm.edu.my

Abstract: There is a demand and a promising market for cassava (*Manihot esculanta*, Crantz) leaves as a supplementary animal feed because of their nutritional value and availability throughout the year. The hydrocyanic (HCN) and protein concentrations were determined for two local varieties, white and pulut, with two different maturities. A two-way ANOVA was conducted to Tukey's multiple ranges to observe the significant difference at $p < 0.05$. The results showed that maturity had a statistically significant effect on HCN concentration. Additionally, there was a significant interaction between variety and maturity, specifying that any differences in variety were dependent upon the cassava leaves' maturity and that any differences between young and mature were dependent upon which variety they were. The variety and maturity of cassava leaves had a significant effect on the concentration of protein. The results showed a considerable impact on variety and a substantial effect on maturity on the protein concentration of cassava leaves. In summary, the young pulut had the highest protein concentration. However, the cyanide concentration in all samples was above the safe level set by World Health Organization (WHO), indicating that animals should not consume cassava leaves without adequately removing the toxic compound.

Keywords: cassava leaves; cyanide concentration; maturity; protein concentration; variety

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1. Introduction

Cassava (*Manihot esculenta*, Crantz) is a tropical woody shrub in the Euphorbiaceae family, also known as yucca, manioc, tapioca and in Malaysia is known as 'ubi kayu' (Jamil

& Bujang, 2016). One of the advantages of the cassava plant is it can tolerate drought and survive in low-fertility acidic soils (Ubwa et al., 2015). The tuber is used to make cassava chips and starch, while the aerial portions (leaves and stems), with a proportion of 9.0–1.5% of the total weight of the plant, are usually left in the field or utilized as animal feed after harvesting (Chaiareekitwat et al., 2022; S. Idris et al., 2021). Cassava leaf contains three times more crude protein than other cassava plant parts (S. Idris et al., 2023).Therefore, it was acceptable for protein-energy ruminant feed. The cassava plant is categorized into two types: (a) those with or without branching at the top and (b) those that spread, as shown in Figure 1. In Asian nations, including Malaysia, Indonesia, and sub-Saharan Africa, tender leaves are edible as a traditional vegetable commonly served with rice.

White Pulut

Figure 1. (a) White cassava plant (branching at top) ; (b) Pulut cassava plant (spreading).

It is crucial to evaluate the protein content of cassava leaves because it has been known that green vegetables are the lowest-priced source of protein. Since cassava leaf has three times more crude protein than various cassava plants' components, it can be used as a ruminant protein source (Idris et al., 2021). Another study reported the crude protein content of cassava leaf was 17.7–38.1% D.M. (Awoyinka et al., 1995). Crude protein levels in Malaysian cassava cultivars ranged from 21.51 to 30.31% D.M. (Jamil & Bujang, 2016). Since the root of the cassava contains low protein, the leaves should receive better attention as human food and animal feed.

The presence of cyanogen glucosides throughout the cassava plant and the leaves are six to 20 times higher than in the root (Ekpo & Baridia, 2020; Jamil & Bujang, 2016; Eleazu & Eleazu, 2012). It determines the bitterness of the root and other parts of the plant, and it is vital as a defence mechanism against insect herbivores (da Silva Santos et al., 2020; Roslim et al., 2016). The variety, age, soil, geographic location, and environmental factors affect how much cyanogen glucosides are synthesized (Nwokoro et al., 2010; Ubwa et al., 2015). Cassava is divided into two types, sweet and bitter, based on the concentration of cyanogen glucosides in the root (Domínguez et al., 1983). The bitter type contains 50 mg HCN/Kg and

above (wet basis), while the sweet type has less than this amount (Ojiambo et al., 2017; Pendak, 2011).

Sweet cassava matures 6–12 months after planting (MAP) and loses quality if not harvested on time, whilst bitter cassava matures 10–14 MAP, becoming fibrous if not harvested on time (Pendak, 2011). Previous research has shown that HCN is synthesised at the cassava plant shoot apex and transported to the root (Chaiareekitwat et al., 2022; Latif & Müller, 2015). Therefore, the concentration of cyanogen glucosides in cassava leaves is highest at the top and decreases as it descends. Cyanogen glucosides are hydrolysed by endogenous enzymes when the plant tissue is damaged during harvesting, by herbivore chewing, food preparation or within the digestive system to sugar and alpha-hydroxyanisole (Ubwa et al., 2015). Alpha-hydroxyanisole has undergone an intramolecular reaction to release the toxic HCN gas (Barry Nestel & Reginald MacIntyre, 1973; Müller-Schwarze, 2009). HCN inhibits cytochrome oxidase, eventually leading to death, as shown in Figure 2. The tissues readily absorb the HCN through the bloodstream because of their tiny size and low charge density (da Silva Santos et al., 2020).

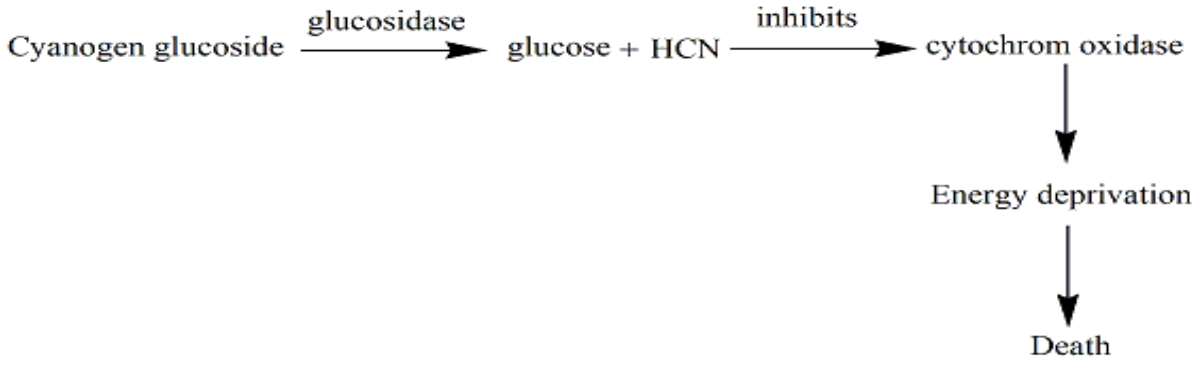

Figure 2. Mechanism of action of cyanogens (according to Makkar *et al*., 2007)**.**

WHO recommends the maximum lethal dose of HCN in food taken orally is 10 mg HCN/kg of body weight, which is equal to 10 ppm (Ojiambo et al., 2017; Ubwa et al., 2015; Umuhozariho et al., 2014). Cassava leaf is hardly used fresh because it contains HCN, which is poisonous to livestock. It is usually processed by combining sun-drying with chopping and wilting until the level of HCN in the hay or dried meal is safe for animals (Ravindran, 2017; Wanapat, 2009). However, ruminants are more vulnerable to HCN poisoning than non-ruminants (Ajayi & Joseph, 2019).

Although few studies investigated the HCN content and protein concentration of cassava leaves from Malaysia, these studies only cover a particular variety and different maturity stages. Therefore, a survey of HCN and protein using the Bradford method is needed as a reference. Thus, this paper investigated the HCN and protein concentration of two cassava varieties with different maturities and selected the best sample to be processed as an animal feed source, which contained the lowest HCN and highest protein concentration.

2. Materials and Methods

2.1 Sample Collection and Preparation

One kilogram of cassava leaves from white and pulut varieties, as shown in Table 1, were collected from a local planter in Banting, Malaysia. The samples were collected on the same day to obtain samples with the same ages at six MAPs. To describe young and mature leaves, respectively, leaves were randomly selected from the defined heights from the plants' top (tender shoot apex) and bottom (woody stem). The leaves were harvested, bagged in plastic, and preserved in a polystyrene box with ice during transportation because the protein and cyanide content is thermolabile. Therefore, the samples must be kept between 0 and 4°C. The samples were cleaned, and each sample was randomly taken. The leaves were cut into small pieces and were frozen in liquid nitrogen. Then, the sample was ground using a kitchen blender. For storage, it was packed in zip-lock bags and stored at -80°C for further analysis (Chaiareekitwat et al., 2022).

2.2 HCN Analysis Based on Reduction of Picrate

A flat-bottomed screw-capped glass bottle was filled with a 20 mg sample and 1 ml of the 0.2 M phosphate buffer (pH 8). A picrate paper attached to a plastic strip was glued on the screw cap of the bottle above the solution and immediately closed. The bottle was shaken slowly to mix the sample with the buffer. The bottle was incubated at room temperature (30 ± 2°C) for 16 hr. Later, the picrate paper was then taken out and submerged for 30 minutes in 5 mL of distilled water. The colourless filter paper was removed, and the picrate solution was boiled for 5 minutes in a water bath. The solution was centrifuged at 4000 rpm for 5 min. The absorbance of the coloured solution was measured against the reagent blank (0 ml of sample, 1 ml of buffer and 5 ml of picrate solution) at 510 nm. Subsequently, HCN concentration for each sample was determined from the calibration curve with the linear regression (R^2) was 0.9666 and the linear equation was $y = 0.0262x + 0.0668$, wherein y was an absorbance value for sample and x was a yield of extracted HCN for samples that proportional to the potassium cyanide (KCN) concentration.

Table 1. Variety of cassava leaf samples.

Variety	Shapes	Description
White		The colour of the leaf is green The colour of the petiole is green The shape of the lobes is lanceolate

Variety	Shapes	Description
Pulut		The colour of the leaf is green The colour of the petiole is reddish-green The shape of lobes is linear

2.3 Protein Analysis Based On the Bradford Method

The Bradford method, which analyses the protein-dye complex of a sample, is based on the absorbance shift seen in an acidic solution of the dye Coomassie® Brilliant Blue G-250. In 1 ml of extraction buffer containing 0.05 M tris base, 0.1% ascorbic acid, 0.1% cysteine hydrochloride, 1% polyethylene glycol, 0.15% citric acid (monohydrate), and 0.008% 2-mercaptoethanol in distilled water. During homogenisation, 0.05 g of the antioxidant polyvinyl polypyrollidone (PVPP) is added to each sample. Homogenates are transferred to 2 ml Eppendorf tubes and centrifuged for 20 minutes at 12000 rpm at 4°C. A supernatant of 0.1 ml was collected, and 3 ml of Bradford reagent was added. The mixture was swirled gently to avoid any formation of foam and incubated for 5 minutes. The protein content of cassava leaves was measured in this study using a Multiskan GO microplate spectrophotometer (Thermo Scientific 1510) at 595 nm in comparison against a blank (buffer plus Bradford reagent). The protein concentration in each cassava leaf was expressed in ppm using the bovine serum albumin (BSA) calibration curve as a standard protein. The linear regression (R^2) for the calibration curve was 0.9932. Subsequently, protein concentration for each extracted sample was determined from the linear equation of y=0.0004x + 0.0511, wherein y was absorbance value for sample and x was a yield of extracted protein for samples proportional to BSA concentration.

2.4 Statistical Analysis

Two independent variables (varieties of cassava plant and maturity of leaves) influenced dependent variables (HCN and protein concentration) were analyzed using IBM SPSS Statistics for Windows (Version 26.0, IBM Corp, Armonk, New York, USA). A two-way analysis of variance (ANOVA) at a 95% confidence interval was carried out to test differences between the means of more than two groups. Tukey's multiple comparison tests identified the significant difference between means. Finally, the interaction between the one independent variable effects on the other independent variable was analyzed.

3. Results and Discussions

Table 2 demonstrates the amount of HCN and protein concentration in cassava leaf samples according to the KCN and BSA calibration curves. The cassava leaves of different maturity and variety had different HCN and protein values. The findings indicated that the HCN varied significantly ($p < 0.05$) among different maturity from the white variety. The

HCN value ranged from 48.02 to 93.21 ppm. This finding is consistent with that of Jamil and Bujang (2016), who found that the cyanide content in cassava leaves in Malaysia was 88.67–147.2 ppm. The young white variety demonstrated the highest HCN concentration, followed by the mature pulut, young pulut and mature white cassava leaves. There is a significant difference for young white cassava leaves from other samples at $p < 0.05$. The significant difference could be due to the different leaf positions representing leaf maturity. The youngest leaves are collected from the shoot apex (top position), and the matured leaves are located at the bottom position, resulting in different amounts of HCN content. According to Ravindran (1992), the cyanide concentration in cassava leaves was highest at the top position and declined towards the lower position. Generally, young cassava leaves contain the highest level of HCN and will decrease to 50–70% in mature leaves (Nambisan & Sundaresan, 1994).

Table 2. Tukey's multiple comparison tests on variety and maturity.

Variety	Maturity	HCN (ppm)	Protein (ppm)
White	young	93.21±2.65 [a]	413.90±98.90 [b]
	mature	48.02±2.38 [b]	453.00±198.00 [b]
Pulut	young	67.10±0.81 [b]	860.6±167.60 [a]
	mature	66.34±11.82 [b]	636.4±38.40 [a, b]

The final value was expressed as the average ± standard deviation. Means with the same letter are not significantly different at $p < 0.05$ using the Tukey test.

Furthermore, the results showed that HCN concentration in all cassava leaves exceeded the sample's lethal dose intake of 0 ppm (Jamil & Bujang, 2016; Latif & Müller, 2015). Therefore, the leaves should be detoxified before consumption because they are highly poisonous. The presence of HCN in all study leaves confirmed earlier reports that all cassava cultivars contain a cyanogenic glucoside in wide disparities according to varieties(C. Domínguez et al., 1983). At a temperature above 25.6°C, hydrolysis of cyanogen glycosides will release cyanide gas, which may cause dizziness, headache, fatigue and sometimes lead to death (Latif & Müller, 2015).

The results also revealed that the protein concentration demonstrated a significant difference between young white cassava leaves and others, ranging from 413.9 to 860.6 ppm. The young pulut exhibited the highest protein content; the lowest value was found in young white cassava leaves. The protein concentration was significantly higher ($p < 0.05$) for young pulut cassava leaves than the other samples. On a fresh basis, protein content was reported to be 4.0–9.6%, while dry basis content had higher crude protein at 20.6–36.4% (Latif & Müller, 2015). Therefore, the young variety is more suitable than the white variety for using cassava leaves as a protein source.

Two-way ANOVA was conducted to investigate two main effects (variety and maturity) on cassava leaves' HCN and protein concentration, as shown in Tables 3 and 4. As shown in Table 2, the two-way interaction showed that the leaf maturity and interaction

(variety × maturity) statistically significantly affected the HCN concentration at $p < 0.05$. From Table 4, there were significant effects of variety such that pulut had higher protein concentration than white cassava leaves. The main impact on maturity was also substantial, as the young contained higher protein concentrations than mature cassava leaves.

Table 3. The two-way ANOVA test is used to determine the effect of the variety and maturity of cassava leaf on HCN concentration.

Source	df	Sum of squares	Mean square	*F*-value	*p*-value
Variety	1	30.10	30.10	0.787	0.43
Maturity	1	1054.66	1054.66	27.58	0.01
Variety × Maturity	1	986.90	986.90	25.81	0.01
Error	4	152.97	38.24		

R squared = 93.1% (adjusted *R* squared = 88.0%)

df – degree of freedom; dependent variable –HCN content

Table 4. The two-way ANOVA test of the effect of variety and maturity of cassava leaf on protein concentration.

Source	df	Sum of squares	Mean square	*F*-value	*p*-value
Variety	1	175824.50	175824.50	22.98	0.01
Maturity	1	208012.50	208012.50	27.19	0.01
Variety × Maturity	1	5940.50	5940.50	0.78	0.43
Error	4	30602.00	7650.50		

R squared = 92.7% (adjusted *R* squared = 87.3%)

df – degree of freedom; dependent variable –protein concentration

5. Conclusions

This study has shown that cassava leaves are rich in protein. However, the amount of cyanide in cassava leaves showed that animals should not consume cassava leaves without adequately removing the toxic compound. A variation of protein and HCN content is found in different varieties and maturity of cassava leaves. The highest protein content was found in young pulut cassava leaves, while the lowest HCN content was in mature white cassava leaves. The effect of maturity significantly affects the HCN content of cassava leaves. The results could be preliminary data on nutrient objectives of sources related to cassava leaves that can be used as a reference for future research.

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experiments, and collected and analyzed the result data. All authors have read and agreed to the published version of the manuscript.

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References

Ajayi, M. O., & Joseph, E. (2019). Characteristics of ensiled cassava leaves and maize stover as dry season feed for ruminants. *Nigerian Journal of Animal Production*, *46*(4), 273–281.

Awoyinka, A. F., Abegunde, V. O., & Adewusi, S. R. A. (1995). Nutrient content of young cassava leaves and assessment of their acceptance as a green vegetable in Nigeria. *Plant Foods for Human Nutrition*, *47*(1), 21–28. https://doi.org/10.1007/BF01088163.

Barry Nestel, & Reginald MacIntyre. (1973). Chronic Cassava Toxicity. *Proceedings of an Interdisciplinary Workshop*, 89–96.

Chaiareekitwat, S., Latif, S., Mahayothee, B., *et al*. (2022). Protein composition, chlorophyll, carotenoids, and cyanide content of cassava leaves (Manihot esculenta Crantz) as influenced by cultivar, plant age, and leaf position. *Food Chemistry*, *372*, 131173. https://doi.org/10.1016/j.foodchem.2021.131173

da Silva Santos, B. R., Requião Silva, E. F., Minho, L. A. C., *et al*. (2020). Evaluation of the nutritional composition in effect of processing cassava leaves (Manihot esculenta) using multivariate analysis techniques. *Microchemical Journal*. https://doi.org/10.1016/j.microc.2019.104271

Domínguez, C. E., Fuentes de Piedrahíta, C. L., Ceballos, L. F., *et al*. (1983). *Morphology of the Casava Plant*. Centro Internacional de Agricultura Tropical (CIAT).

Ekpo, U. A., & Baridia, D. F. (2020). Effect of processing on the chemical and anti-nutritional properties of cassava leaves (sweet and bitter varieties). *ARC Journal of Nutrition and Growth*, *6*(2), 6–12. https://doi.org/10.20431/2455-2550.0602002

Eleazu, C. O., & Eleazu, K. C. (2012). Determination of the proximate composition, total carotenoid, reducing sugars and residual cyanide levels of flours of 6 new yellow and white cassava (*Manihot esculenta* Crantz) varieties. *American Journal of Food Technology*, *7*(10), 642–649. https://doi.org/10.3923/ajft.2012.642.649

Idris, S. B., Shamsudin, R., Mohd Nor, M. Z., *et al*. (2021). Proximate composition of different parts of white cassava (*Manihot esculenta* Crantz) plant as a ruminant feed. *Advances in Agricultural and Food Research Journal*, *2*(1), 1–9. https://doi.org/10.36877/aafrj.a0000181

Idris, S., Shamsudin, R., Mohd Nor, M. Z., *et al*. (2023). Hydrocyanic acid, protein concentration, and phytochemical compounds of pulut and white varieties in young and matured cassava (*Manihot*

esculenta, Crantz). *Pertanika Journal of Science and Technology*, *32*(1). https://doi.org/10.47836/pjst.32.1.04

Jamil, S. S., & Bujang, A. (2016). Nutrient and antinutrient composition of different variety of cassava (Manihot esculenta Crantz) leaves. *Jurnal Teknologi*, *78*(6–6), 59–63. https://doi.org/10.11113/jt.v78.9024

Latif, S., & Müller, J. (2015). Potential of cassava leaves in human nutrition: A review. *Trends in Food Science & Technology*, *44*(2), 147–158.

Müller-Schwarze, D. (2009). Hands-On Chemical Ecology. In *Springer*.

Nwokoro, O., Ogbonna, J. C., & Okpala, G. N. (2010). Simple picrate method for the determination of cyanide in cassava flour. *Bio-Research*, *7*(2), 502–504. https://doi.org/10.4314/br.v7i2.56582

Ojiambo, O. C., Nawiri, M. P., & Masika, E. (2017). Reduction of cyanide levels in sweet cassava leaves grown in Busia County, Kenya based on different processing methods. *Food Research*, *1*(3), 97–102.

Pendak, Y. (2011). *Baloi : Sweet-type tapioca variety suitable for planting on acid sulphate soil*. Retrieved from http://myagric.upm.edu.my/id/eprint/12062

Ravindran, V. (2017). Utilization of cassava (*Manihot esculenta crantz*) leaves in animal nutrition. *Journal of the National Science Foundation of Sri Lanka*, *21*(1).

Roslim, D. I., Herman, Sofyanti, N., *et al*. (2016). Characteristics of 22 cassava (*Manihot esculenta Crantz*) genotypes from Riau Province, Indonesia. *Sabrao Journal of Breeding and Genetics*, *48*(2), 110–119.

Ubwa, S. T., Otache, M. A., Igbum, G. O., *et al*. (2015). Determination of cyanide content in three sweet cassava cultivars in three local government areas of Benue State, Nigeria. *Food and Nutrition Sciences*, *06*(12), 1078–1085. https://doi.org/10.4236/fns.2015.612112

Umuhozariho, M. G., Shayo N, B., Msuya, J. M., *et al*. (2014). Cyanide and selected nutrients content of different preparations of leaves from three cassava species. *African Journal of Food Science*, *8*(3), 122–129. https://doi.org/10.5897/ajfs2013.1100

Wanapat, M. (2009). Potential uses of local feed resources for ruminants. *Tropical Animal Health and Production*, *41*(7), 1035–1049. https://doi.org/10.1007/s11250-008-9270-y