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Research article

# Exploring hydrogen cyanide variation in cassava leaves and effective removal strategies

# Exploración de la variación de cianuro de hidrógeno en hojas de yuca y estrategias efectivas de eliminación

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## ABSTRACT

Cassava leaves have significant nutritional potential, but their high content of cyanogenic glycosides, which are synthesized as a defense mechanism and release hydrogen cyanide (HCN), poses a challenge using leaves as a potential food. This study evaluated the variation of HCN content in cassava leaves from seven and five cultivars at two stages after planting, respectively (seven and nine months). HCN levels ranged from 1800 to 2761 mg $kg^{-1}$ (ppm) at seven months of age, with significant biological variation observed within cultivars. A reduction in HCN content was noted at nine months of age, with a maximum decrease of 74 % in one cultivar. Additionally, treatments involving crushing leaves followed boiling with NaHCO3 resulted in 90 % HCN reduction. This research highlights the importance of understanding HCN variability across cultivars and plant ages and suggests practical methods to reduce HCN content in leaves, making them a potential safer alternative nutritional source.

**Keywords:** Cyanogenic glycosides, cyanide removal, linamarin, *Manihot esculenta*, toxicity.

## RESUMEN

Las hojas de yuca presentan un potencial nutricional significativo; sin embargo, su alto contenido en glucósidos cianogénicos, los cuales son sintetizados como un mecanismo de defensa de la planta, liberan cianuro de hidrógeno (HCN). Esta situación plantea un desafío para su uso como fuente alimentaria potencial. Este estudio evaluó la variación en el contenido de HCN en hojas de yuca de siete y cinco cultivares en dos etapas de desarrollo post-siembra, respectivamente (siete y nueve meses). Los niveles de HCN variaron entre 1800 y 2761 mg $kg^{-1}$ (ppm) a los siete meses, observándose una variación biológica significativa dentro de los cultivares. Se observó una reducción en el contenido de HCN a los nueve meses, con una disminución máxima del 74 % en uno de los cultivares. Además, los tratamientos que involucraron triturado de las hojas seguido de ebullición con $NaHCO_3$ resultaron en una reducción del 90 % de HCN. Esta investigación resalta la importancia de comprender la variabilidad del HCN entre cultivares y edades de las plantas, y sugiere métodos prácticos para reducir su contenido, lo que podría convertir a las hojas de yuca en una fuente nutricional alternativa más segura.

**Palabras clave:** Eliminación de cianuro, glucósidos cianogénicos, linamarina, *Manihot esculenta*, toxicidad.

## INTRODUCTION

Cassava (*Manihot esculenta*) is a tuberous crop characterized by a high accumulation of starch in their roots. More than one third of world population depends on cassava as a source of calories (Ospina *et al.,* 2021). This plant is cultivated largely by smallholder farmers for consumption and commercialization, representing one of their main incomes. The crop performs quite well under drought conditions becoming important in the current crisis of climatic change (Díaz and López, 2021). Although cassava roots are rich in starch, their nutritional value is very low. In contrast, leaves have high values of protein, vitamins and elements (Latif and Müller, 2015). Cassava is characterized to produce cyanogenic glycosides (CNglcs), linamarin and lotaustralin, which are produced in all the plant, but the level is highly superior in leaves (Latif and Müller, 2015). CNglcs biosynthesis occurs in the leaves and then translocated to the roots (Mcmahon *et al.,* 2022). The predominant CNglc in cassava is linamarin, which is synthetized from valine through the activity of the enzymes CYP79D1 and CYP79D2, producing 2-methylpropanal oxime (Schmidt *et al.,* 2018). This oxime is converted into acetone cyanohydrin by action of the enzyme CYP71E7, and it is finally glycosylated by the UDP-enzymes glycosyltransferases UGT85K4-K5 to obtain linamarin (Mcmahon *et al.,* 2022).

Cassava foods prolonged exposure in humans can cause cyanide poisoning if products are eaten without processing correctly (Nyirenda, 2020). This occurs when cassava leaves or roots food are chewed activating the CNglcs hydrolysis reaction and releasing HCN (Sun *et al.,* 2018). CNglcs can be hydrolyzed producing Hydrogen Cyanide (HCN) predominantly by $\beta$-glycosidase (linamarase) and hydroxinitrile lysase enzymes (Mcmahon *et al.,* 2022). The toxicity of HCN in food chronically presents iodine deficiency disorder, tropical ataxic neuropathy (TAN) and paralytic (Konzo) (Okafor *et al.,* 2002). By contrast, acute toxicity effects can prevent correctly the electron transport chain in the mitochondria affecting the oxygen levels in the body tissues ending in coma or even in dead (Nyirenda, 2020).

Although is produced constantly, HCN varies ontogenetically, phenologically, and chronologically. For example, older leaves and roots have less cyanide (Alamu *et al.,* 2023). The environmental conditions have a strong impact in the biosynthesis and accumulation of CNglcs (Sun *et al.,* 2018). Several studies have reported an important variation in the CNglcs content between different cassava cultivars (Ospina *et al.,* 2024). However, the genetic basis of HCN variation is still unknown, although important efforts through Quantitative trait loci (QTL) identification and Genome-wide association studies (GWAS) have contributed to gain a better understand of the genetic basis and environmental factors affecting this trait (Gomez *et al.,* 2022). The variability of CNglcs content is high even between roots and leaves of the same plant (Echeverry-Solarte *et al.,* 2013). However, most of the studies provide only a single value for each cassava material and no SE or SD is reported.

The aim of this work was to evaluate the content of HCN for seven cassava cultivars at seven months of age and five cassava cultivars at nine months of age, measure the biological variation in HCN between three technical replicates were performed for each cultivar. Also, two low cost and easy reproducible techniques to remove HCN from two Colombian commercial varieties of cassava leaves were tested.

## MATERIAL AND METHODS

### Plant material and field establishment for HCN variation analysis

In total nine cassava Colombian cultivars from Amazonas (6), Antioquia (2) and Arauca (1) were planted in a farm localized in La Vega, Cundinamarca (lat 5°00´44.188" N, long 74°21´31.005" W, Colombian Andean region). This region is characterized by a bimodal behavior with a dry season (from December to March) and a rainy season (from April to November). Planting was conducted from stems cuttings obtained from healthy plants and were sown randomly with a planting density of 60 cm. The plants were weeded each month, and no fertilizers were applied. The experimental design was completely randomized, where three plants as biological replicates per cultivar were used.

### Sampling and processing

Stems were planted in August 2022 and samples were taken in March 2023. Sampling was conducted on March 2023 at midday with 26 °C and 65 % of relative humidity (HR), for seven cultivars (ANT02, ARA02, AMA31, AMA32, AMA36, AMA44 and AMA45) during the rainy season, and for five cultivars (AMA46, ARA02, AMA36, ANT01 and ANT02) in May 2023 at midday with 27 °C and 64 % HR at rainy season. Tender leaves, from three biological replicates per cultivar, generally the first fully expanded matured, were taken and stored in plastic bags. Immediately leaves were cleaned with sterile water. Total HCN was determined following the methodology described by Essers *et al*. (1993) and Baird *et al*. (2017). Briefly, technical replicates were established, consisting in three samples of 1 g each of tissue per biological replicate and cultivar. Each sample was homogenized for 2 min in a blender (Osterizer, model 4655, Colombia) in 50 mL of extraction buffer (0.1 M orthophosphoric acid in ethanol 25 %) and centrifuged for 10 min at 6000 RPM (Essers *et al.,* 1993; Ospina, 2018). Then in a glass vial, 100 µl of supernatant leaf extract was mixed with 400 µl of 0.1 M of phosphate buffer (pH 6.0) and 100 µl of linamarase enzyme was added and incubated for 15 min at 25 °C. Then 600 µL of sodium hydroxide 0.2 M was added and the mix incubated for 5 min at 25 °C. Then, 2.8 mL of phosphate buffer (pH 6.0)

0.1 M was added. The reaction was mixed with 100 µL of chloramine T, incubated for 5 min to finally, add 600 µL of the reagent isonicotinate/1,3-dimethyl barbiturate and incubate for 10 min. The absorbance was measured using a spectrophotometer UV-VIS (UNICO, model S-1200). To validate the linearity and reliability of the method, a calibration curve was established based on triplicate determinations of each standard concentration, employing KCN to generate HCN standards. HCN content was determined according to the following equation adapted from Essers *et al*. (1993) and Ospina *et al.,* (2021):

$$HCN\ (ppm) = \frac{\frac{m_{HCN}}{V_{total}}\ F_{d1}F_{d2}}{m_{DW}}$$

Where $m_{HCN}$:µg/mL of HCN read from the calibration curve, using absorbance readings at 440 nm, $V_{total}$: Final volume contained in the glass vial, $F_{d1}$:Dilution factor 1 ($V_{total}/V_{extract}$); $V_{extract}$: Volume of leaf extract used; $F_{d2}$:Dilution factor 2 ($V_{solvent}/V_{extract}$); $V_{solvent}$: Volume of extraction buffer used (50mL). $m_{DW}$: Leaf sample mass on a dry weight basis, in grams (g).

The coefficient of variation was calculated to the technical replicates results and mean and standard deviation of the measurements for each evaluated cassava cultivar at nine months of age were obtained. Additionally, an ANOVA was performed to assess the statistical differences within and between the HCN measurements of the cultivars. Regarding the cultivars, the HCN results obtained from plants harvested at seven months and nine months were compared.

### HCN Removal

Two methods encompassing boiling as thermal treatment and shredding/crushing as mechanical process techniques were applied on fresh cassava leaves respectively from the commercial varieties Venezolana and Punta de lanza following a comprehensive literature review, primarily focusing on the works of Montagnac *et al*. (2009) and Latif *et al*. (2019). In the first time each method was performed using crushing with mortar and pestle or manual shredding followed by boiling for ten minutes. Additionally, these methods were carried out again using a 0.4 % sodium bicarbonate (NaHCO3) instead of water in boiling treatment to compare the efficacy of HCN removal. HCN content on fresh and treated cassava leaves samples were analyzed to quantify the HCN suppressed by the same methodology performed by Essers *et al*. (1993).

**Table 1.** Concentration of HCN ppm (mg $kg^{-1}$) for three biological replicates in seven cassava cultivars at seven months after planting.

| Cultivar Code | R1 | R2 | R3 | Mean | Standard deviation |
|---|---|---|---|---|---|
| **ANT02** | 2151.00 | 648.00 | 2601.00 | 1800.00 | 1022.72 |
| **ARA02** | 1856.00 | 1786.00 | 2134.00 | 1925.33 | 184.07 |
| **AMA31** | 3830.00 | 790.00 | 1920.00 | 2180.00 | 1536.59 |
| **AMA32** | 2977.00 | 2072.00 | 929.00 | 1992.67 | 1026.30 |
| **AMA36** | 2142.00 | 2526.00 | 3617.00 | 2761.67 | 765.22 |
| **AMA44** | 1262.00 | 3932.00 | 2127.00 | 2440.33 | 1362.30 |
| **AMA45** | 1438.00 | 2190.00 | 1897.00 | 1841.67 | 379.04 |

## RESULTS

### HCN content at seven months after planting (MAP)

In first time the HCN content was measured in tender leaves from five cassava cultivars at 7seven months after planting (MAP). The HCN average in leaves ranged between 1800 (ANT02 cultivar) to 2761 ppm (AMA36 cultivar) (Fig. 1, Table 1). There was an important variation in HCN between different biological replicates within the same cultivar. Thus, for example, cultivar AMA31 HCN content varied between 790 ppm, being one of the lowest, and 3830 ppm, the highest observed (Table 1). In other cases, the differences between replicates were not so evident. The three individuals in the cassava cultivar ARA02 had a similar behavior. Even though this variation, due to the high standard deviation of the data, the ANOVA allowed to determinate that there were not significant differences in HCN between these seven cassava cultivars at seven MAP.

### HCN content at nine months after planting

To compare and evaluate the variation of the HCN content through the age of cassava, three of the previous cassava cultivars (ANT02, ARA02 and AMA36) were evaluated two months after (nine MAP). Two additional cassava cultivars were included at this time point. In addition, and to exclude the possibility that the variation observed between biological replicates observed previously was due to technical variation, three reads (technical replicates) were taken for each of these samples. Thus, three different individuals of each cultivar were classified as biological replicates (R1, R2, and R3), and for each biological replicate, three technical replicates (RT1, RT2, and RT3) were conducted.

In most cases values between replicates of the same individual were quite similar. For example, In (Table 2), the third individual of the cultivar ARA02 has values around 540 mg HCN $kg^{-1}$ (ppm) for its technical replicates. However, in other cases the variation was quite high. For example, the three technical replicates for individual 1 in cultivar AMA46. In this case, the HCN content varied from 462 ppm (AMA46) to 1186 ppm (ANT02).

Concerning the variation between technical replicates and based on the coefficient of variation (CV), the values predominantly tended to be below 20 % of variation.

However, in some cases the CV value, in at least one of a biological replicate, was the same value or higher than 20 %. To the cultivar AMA46 R1, the CV was 42 % evidencing specially in RT2 a high HCN content despite of use optimized conditions there may be various obtaining an atypical value in this study. Taking these results, it can be concluded that some of the biological variation observed can be explained only partially by the technical variation, but most of it corresponds to differences between individual plants.

Concerning the variation between replicates, it was observed that in most of the cases, two biological replicates showed a similar behavior which was quite different from the third sample. For example, the value for individuals one and two in the AMA46 cultivar was 376 and 310 respectively, but the mean for the individual three was almost double (699 ppm). A major intracultivar variation was observed for AMA36 with values ranging between 213 and 1633 HCN ppm. The high values of standard deviation support this observation (Fig. 2a). The ANOVA shows no significant differences in HCN between cultivars at nine MAP (Table 2).

For cultivars which data were obtained at seven and nine MAP a consistent and drastic diminution in HCN was observed (Fig. 2b). The most remarkable example was the cultivar AMA36 with a diminution of 74 % (2762 ppm at seven months versus 714 ppm at nine months). For the other two cultivars the reduction was around 34 and 47 %. The ANOVA allowed to detect significant differences in HCN between 7 and 9 MAP for cultivars ARA02 and AMA36 (Fig. 2b).

## HCN removal treatments

With the aim to evaluate different treatments to reduce or remove HCN, two different commercial cultivars localized in a farm in San Cayetano (Bolívar, Colombia) were selected. These plants were of seven MAP. The initial values of HCN were 2293 and 2030 ppm (mg $kg^{-1}$) for Venezolana and Punta de lanza respectively (Table 3). Two different methods to disrupt the tissue were tested (shredding and crushing) followed by boiling for ten minutes in water. For each of these, in boiling process was $NaHCO_3$ added or not. The shredding treatment removes 75% and 44 % of HCN for Venezolana and Punta de lanza respectively, while crushing remove 83 and 90 %. However, when $NaHCO_3$ is added to these treatments, a major percentage of elimination is observed, reaching a reduction of more than 90 % in crushing plus $NaHCO_3$ with HCN values of 216 and 169 ppm being very low (Table 3).

## DISCUSSION

In this study we evaluated the variation of HCN in different cassava cultivars and at two different times after planting (seven and nine MAP). Although the HCN content was different between cultivars, no significant differences were observed. A significant reduction in HCN content was observed at nine MAP compared with seven MAP being statistically different for two of three cassava cultivars compared. In addition, we could demonstrate a reduction of 90 % of HCN content after a treatment of crushing leaves followed by 10 minutes of boiling in water with $NaHCO_3$. This study provides important information concerning the variation in HCN not only in different cultivars but also during the age of the plant.

**Table 2.** Concentration of HCN ppm (mg $kg^{-1}$) for biological and technical replicates in five cassava cultivars at nine months after planting.

| Cultivar | Biological replicate | Technical replicate | | | Mean | Standard Deviation | Coefficient of variation | Biological replicate | |
|---|---|---|---|---|---|---|---|---|---|
| | | RT1 | RT2 | RT3 | | | | Mean | Standard deviation |
| AMA46 | R1 | 228.88 | 541.28 | 358.90 | 376.35 | 156.93 | 42% | 462.34 | 208.29 |
| | R2 | 261.13 | 430.23 | 241.08 | 310.81 | 103.90 | 33% | | |
| | R3 | 841.63 | 552.26 | 705.66 | 699.85 | 144.77 | 21% | | |
| ARA02 | R1 | 811.34 | 1568.49 | 1130.72 | 1170.18 | 380.11 | 32% | 1022.73 | 429.65 |
| | R2 | 1188.54 | 1361.99 | 1527.19 | 1359.24 | 169.34 | 12% | | |
| | R3 | 544.28 | 519.50 | 552.54 | 538.77 | 17.19 | 3% | | |
| AMA36 | R1 | 1984.17 | 1838.91 | 1078.53 | 1633.87 | 486.40 | 30% | 714.09 | 797.58 |
| | R2 | 345.38 | 269.75 | 268.90 | 294.68 | 43.91 | 15% | | |
| | R3 | 212.03 | 209.57 | 219.60 | 213.74 | 5.23 | 2% | | |
| ANT01 | R1 | 521.84 | 521.84 | 464.54 | 502.74 | 33.08 | 7% | 756.10 | 290.32 |
| | R2 | 635.13 | 725.99 | 716.86 | 692.66 | 50.03 | 7% | | |
| | R3 | 858.15 | 1289.15 | 1071.37 | 1072.89 | 215.50 | 20% | | |
| ANT02 | R1 | 1481.35 | 2245.78 | 1660.08 | 1795.74 | 399.87 | 22% | 1186.08 | 579.62 |
| | R2 | 891.19 | 1339.13 | 1130.94 | 1120.42 | 224.15 | 20% | | |
| | R3 | 610.36 | 739.48 | 576.45 | 642.09 | 86.03 | 13% | | |

**Table 3.** HCN concentration (mg/kg) in two commercial cassava varieties after cyanide-removal treatments, and analysis of variance (ANOVA) of mean HCN content across treatments.

| Commercial variety | NT | S | SB | C | CB |
|---|---|---|---|---|---|
| **Venezolana** | 2293.00 | 552.83 | 587.64 | 390.42 | 216.40 |
| **Punta de lanza** | 2030.02 | 1133.16 | 691.43 | 196.15 | 169.38 |

| Treatment | Mean | Standard deviation |
|---|---|---|
| **NT** | 2161.51 | 185.96 |
| **S** | 843.00 | 410.40 |
| **SB** | 639.50 | 73.40 |
| **C** | 293.30 | 137.32 |
| **CB** | 192.90 | 33.23 |

| | df | SS | F | *p* |
|---|---|---|---|---|
| **Treatments** | 4 | 5006498 | 27.405 | 0.001348 |
| **Residuals** | 5 | 228360 | | |

**Note:** The HCN values represent the mean concentration (mg/kg) from both cassava varieties (Punta de lanza and Venezolana), averaged prior to the statistical comparison across treatments. ANOVA was conducted to evaluate the effect of each treatment on overall HCN reduction. The treatments are defined as follows **(NT)** no treatment, **(S)** shredding, **(SB)** shredding and sodium bicarbonate, **(C)** crushing, **(CB)** crushing and sodium bicarbonate.

The HCN values obtained for cassava cultivars ranged from 1800 to 2700 ppm at seven MAP. These values are in the range obtained for cassava in previous studies. Thus, for example from a cassava collection of 178 genotypes evaluated, the average was 2298 ppm (Ospina *et al.,* 2021). Although the cultivars evaluated in this study showed different values in HCN content, no significant differences were detected between them. It is important to highlight the large biological variation observed between individuals of the same cultivar such as Ospina *et al.,* (2024) finding high variation seven genetic diversity groups. These biological variations can explain at least partially the difficulty in founding differences between cultivars. Several studies have determined the HCN content in cassava cultivars, but most of them did not report this biological variation. In most of these studies three leaves from three different plants were pooled together to quantify the HCN, making it impossible to determinate the biological variation. Thus, the values reported in several studies do not include standard deviation which can affect the real HCN content. In further studies it will be imperative to consider this important source of variation.

The HCN content is a trait which has been previously reported as affected considerably by environmental factors. For example, during the zenith, CNglcs levels drop dramatically, while during the hours of dusk their levels increase significatively (Schmidt *et al.,* 2018). All samples obtained in this study were taken at noon, which probably captured the lower levels of HCN, but the situation was identical for all the plants.

Cassava leaves potential can become an important alternative source of nutrition, however the high content of HCN has impaired their consumption and distribution. With the aim of evaluating the possibility to reduce the HCN content in commercial cultivars, two different treatments were used to disrupt the tissue, followed by a 10-minute boiling treatment with or without addition of $NaHCO_3$. These treatments were selected to ease the reproducibility of the method, ensuring a quality suitable for consumption according to the standard normativity, brevity at the operational level and the low cost. The cyanide removal in food products depends on activating hydrolysis to degrade linamarin into its final products HCN and acetone and subsequently applying heat to separate these volatile substances from food (Bradbury and Denton, 2014). Using these treatments, a reduction of 90 % of HCN was observed utilizing crushing and NaHCO3 (Table 3.). Previous studies have indicated that boiling can remove around 50 % of HCN (Montagnac *et al.,* 2009). On the other hand, $NaHCO_3$ helps the breakdown at the cellular level, which promotes the enzyme-substrate interaction favoring the cyanide reducing and can remove up to 70 % of HCN (Latif *et al.,* 2019). Finally, although it has been demonstrated that the boiling time favors the HCN removal, it can lead to protein denaturation when the leaves are exposed for a prolonged period (Montagnac *et al.,* 2009). For this reason, we selected a relatively short time of boiling to avoid the loss of nutritional compounds present in cassava leaves and found a significant HCN reduction in both studied cultivars.

According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), as cited in the Codex Alimentarius CXS 193-1995 (General Standard for Contaminants and Toxins in Food and Feed), a provisional maximum tolerable daily intake (PMTDI) of 0.02 mg HCN/kg body weight has been established on a wet weight basis, expressed as total cyanide (Codex Alimentarius, 2024). Furthermore, the Technical Guide for Cassava Starch Production and Analysis developed by FAO indicates that in special cases—when initial cyanide levels are relatively high—the residual cyanide content on a dry weight basis can exceed 100 ppm (mg HCN/kg of sample) (*Food and Agriculture Organization of the United Nations* [FAO], 2022); Ceballos and Ospina, 2003).

Based on the average values obtained from the cyanide removal treatments, a residual content of 65 mg HCN/kg on a wet weight basis was established. Accordingly, it can be inferred that a 70 kg adult could safely ingest up to 1.4 mg of HCN from cassava leaves per day, corresponding to approximately 21.5 g of cassava leaves daily, without posing a chronic health risk. (*This estimation is based solely on the experimental results obtained in this study*.)

Previous and more recent studies have employed biotechnological strategies to silence or eliminate the function or the activity of CYP enzymes involved in the key step during the biosynthesis of CNglcs (Gomez *et al.,* 2022).

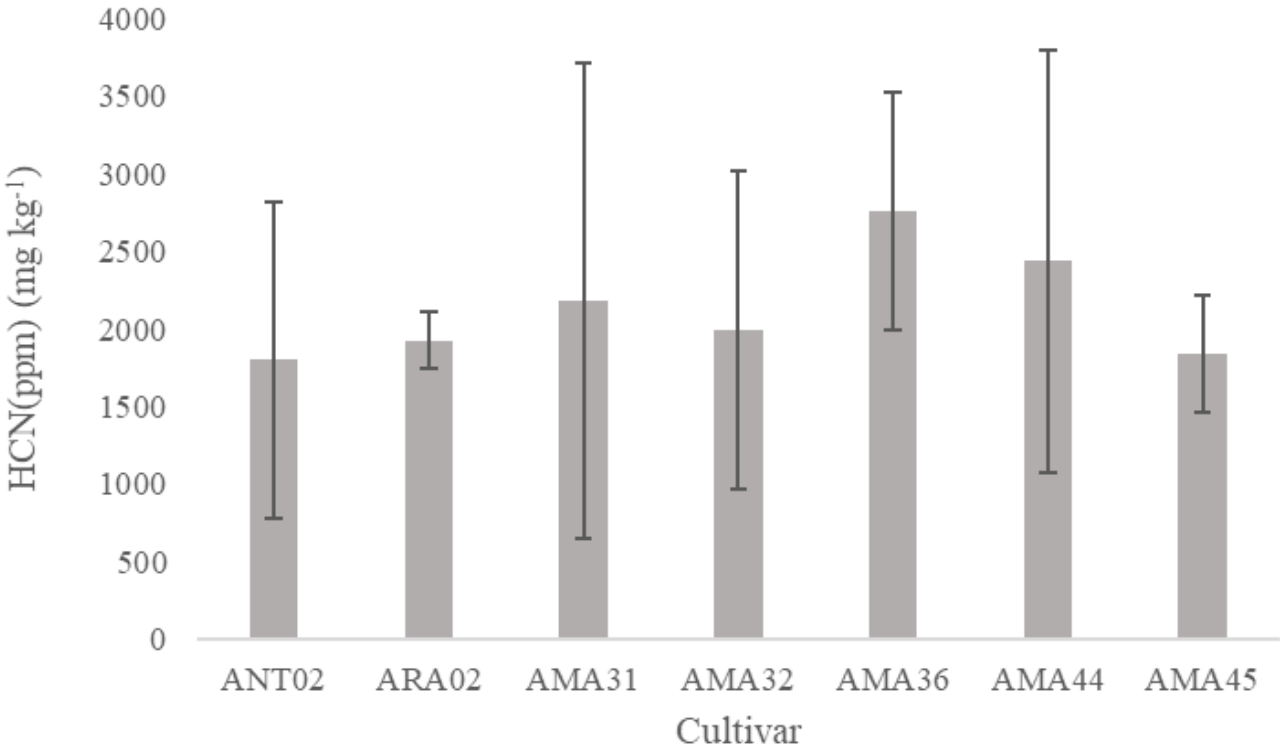


**Figure 1.** Concentration of HCN of seven cassava cultivars taken at seven months post-planting. Error bars indicate the standard deviation for three biological replicates. There are no statistically significant differences between the HCN values for the cassava cultivars (*p*-value 0.05).

However, it is important to notice these compounds are important during drought conditions (Mcmahon *et al.,* 2022). Cassava is considered one of the more resilient crops facing hydric stress and probably the reason is, at least partially, the presence of these CNglcs. Caution should be taken to eliminate completely the content of CNglcs which can compromise the adaptability of this crop. A more suitable strategy is to take advantage of the natural diversity of this crop to identify the cultivars with less content of HCN and use different and easy protocols to eliminate the HCN before the consumption. A better comprehension of the biology, dynamics and variability of the HCN in cassava leaves can guide better programs to make this waste product a real alternative source of nutrients for humanity.

## CONCLUSIONS

HCN content for seven cassava cultivars (MAP) ranged from 1800 to 2700 ppm, consistent with previous findings. Despite this range, no significant differences were detected between cultivars, likely due to the high biological variation within each cultivar, underscoring the need for future studies to account for this variability

For the study of technical replicates, it was observed that there is variation in the HCN content in the leaves of individuals belonging to the same cultivar, possibly due to environmental conditions or physiological responses that influence the increase or decrease of CNglcs in the studied individuals. However, it was evident that there are no differences in the variation of the mean values for HCN concentration between the analyzed cultivars.

The three cultivars used to compare HCN content at seven and nine MAP (ANT02, ARA02, and AMA36) showed a significant reduction, with the cultivar AMA36 experiencing a 74 % reduction in HCN content. Generally, the study found significant differences between the younger specimens (seven MAP) compared to the older ones (nine MAP).

Cassava leaves crushing treatment followed by ten minutes of boiling in water with NaHCO3 effectively reduced HCN content by 90 %. This finding is significant as it highlights a reliable method for cyanide reduction, while also providing valuable insights into HCN variation across different cultivars and plant ages.

## AUTHOR'S PARTICIPATION

CL, JS.: Study conception. IM.: Experimental work. IM, JS, DCh, CL.: Data analysis. IM, JS, DCh, CL.: Manuscript writing.

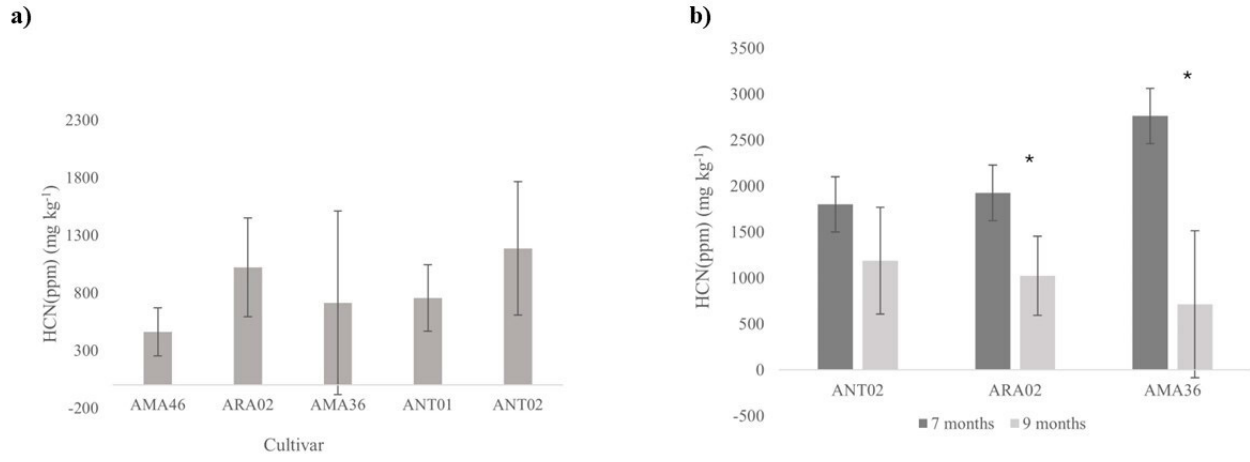


**Figure 2.** a) Concentration of HCN of five cassava cultivars taken at nine months post-planting. Error bars indicate the standard deviation for three biological replicates. There are no statistically significant differences between the HCN values for the cassava cultivars ($p$ value <0.05). b). Average concentration of HCN of three cassava cultivars taken at seven- and nine-months post-planting. Error bars indicate the standard deviation for three biological replicates. *Significant difference ($p$ value <0.05).

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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