

Evaluation of the Hydrocyanic Acid Level in Fresh Cassava Leaves: Case of the Experimental Field in the Ombella M'Poko in the Central African Republic

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Abstract

Cassava (*Manihot esculenta*) is a perennial shrub in the Euphorbiaceae family. Thus, the objectives assigned to this study are to evaluate the hydrogen cyanide content of the 58 accessions of cassava in the collection to Samba and specifically, to evaluate the content of hydrocyanic acid in fresh and dried cassava leaves. To determine the pH value in each accession and to assess the phytosanitary status of the accessions. Titrimetry was used to determine hydrocyanic acid. It is used as part of this work to determine the level of hydrocyanic acid contained in cassava leaves. The results made it possible to classify the accessions analysed into three groups: accessions with a low cyanide content whose concentrations vary from 13 mg to 49.33, accessions with a moderate concentration (50.33 mg to 98.33 mg) and highly concentrated accessions (100.66 mg to 144 mg). The pH value for the different is almost identical.

Keywords

Cassava, Hydrocyanic Acid, Titrimetry

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a plant native to Brazil (South America),

cultivated throughout the tropics for its fleshy roots rich in starch [1]. It feeds more than 700 million people and is cultivated in more than 105 countries worldwide [2] with a global production of 292 million tonnes in 2017 according to the FAO. Approximately 60% of cassava produced is intended for human consumption, 20% for animal feed and 10% is processed into secondary products [3]. The leaves are consumed as vegetables in many parts of Africa. In the Central African Republic, cassava production is 725,000 tonnes of cassava, or 2.9 million tonnes of fresh tuber, followed by yam 450,000 tonnes and maize 167,000 tonnes FAOSTAT, 2017. Despite its benefits, cassava is one of the most toxic food plants in the world. This toxicity is linked to the presence of two cyanogenic glucosides: linamarin (93%) and laurostralinalin (7%) [4]. These glucosides are not toxic in themselves but following enzymatic hydrolysis release hydrocyanic acid [5]. Many studies seem to establish a link between diet and the appearance of certain pathologies. One of the main explanatory factors would be repeated exposure to low doses of toxic contaminants via food. KONZO disease in Congo is caused by the consumption of cassava, which contains a high level of HCN [6]. The consumption of cassava poorly detoxified in hydrocyanic acid seems to play a role in the genesis of these neuropathies in tropical regions [7]. It feeds 800,000,000 people worldwide [8] (FASTAT 2017) (Poor processing of cassava, including poorly done retting, leaves a by-product containing cyanide unfit for consumption. Regular consumption of this type of product can generate a neuromyelopathy called konzo. It is a form of symmetrical, permanent spastic paraparesis with a sudden onset. It is attributed to exposure to cyanide resulting from a mono-diet of bitter cassava, which is not or poorly “toxic” [9], as well as a diet low in sulfur amino acids (Ministry of Health of Mozambique, 1984). KONZO presents as an irreversible neurological disorder affecting the upper part of the body, widespread in rural areas of sub-Saharan Africa, whose main crop consists of bitter varieties of cassava. Due to the dangers of chronic toxicity of this plant, there is talk of deploying more intensive efforts to obtain low and acceptable levels of cyanide. The first possibility of reducing the cyanide level in cassava lies in the selection of cultivars free or with low content of HCN [10]. It is difficult to find cyanide-free varieties, but studies will always continue to be done. Cassava varieties that produce less than 50mg/kg of HCN of peeled and fresh roots are considered harmless [11]. CAR is one of the countries where cassava is the staple food and its cultivation is known throughout the country. The leaves are eaten as a vegetable and the roots as the energy support. Since its introduction in the Central African Republic in the 1850s [12], many studies have been carried out on its improvement [13] (and on varietal selection [14]. An epidemiological survey confirmed the presence of KONZO disease in Health District No. 2 in 2009 [15]. Also, a study reported that during the rainy season, the demand for cassava chips is higher than the supply in most of the country. This deficit sometimes leads some processors to voluntarily shorten the processing time of cassava. As a result, the time for retting and drying operations is often shortened. This leads to risks of toxicity due to the persistence of cyanogenic derivatives in the chips. In order to guarantee food secu-

urity, it is a question of launching research on the toxicological aspect of this plant. We chose the CAR because cassava is a staple food for the Central African population, that is to say, it is consumed by more than 97% of the population with a daily consumption frequency of 2 times a day for the most part. This could constitute a health risk due to the presence of HCN.

This study can help identify accessions that have a high HCN content in order to consider their withdrawal via a food chain toxicity study. This is why, through this research project, we proposed to evaluate the HCN content in the different cassava accessions in circulation in the Central African Republic to identify varieties with high toxicity potential and to develop a consumer awareness campaign. The aim is to recommend cassava accessions that have a low hydrocyanic acid content to the population.

2. Research Questions

Do fresh leaves of cassava accessions have significant levels? Are they toxic? What impact can they have on humans? How can a significant release of hydrocyanic acid be achieved using an effective method?

2.1. General Objective

This study aims to evaluate the hydrocyanic acid content of the 58 cassava accessions in the Samba collection.

2.2. Specific Objectives

To assess the hydrocyanic acid content in fresh and dried cassava leaves.

Assess the phytosanitary status of the 58 accessions.

Determine the pH value in each accession.

Determine the correlation between cyanogenic acid content and pH.

3. Materials and Methods

3.1. Sample Collection

The cassava leaf samples used in this work were collected from the 58 cassava accessions in the LASBAD experimental site located in the Samba village, 20 km south of Bangui, whose geographical coordinates are as follows: This collection was set up in 2022 as part of a WAVE program project supported by Cassava cuttings from 58 accessions were collected in the diffN = 04°18'24.7, E = 018°25'32.7 and an Altitude = 380.

Materials used: In order to carry out the various analyses, we used the following materials:

- A pair of scissors for harvesting fresh leaves from cassava accessions;
- Large zipped bags for storing samples;
- GPS for coordinates;
- Two coolers for storing samples;
- A vehicle for collecting samples;

- Graduated test tubes;
 - Beakers;
 - Volumetric flasks;
 - Meyer Erlenmeyer flasks;
 - Mechanical and electrical scales;
 - From a MEXXI electric grinder;
 - pH meter;
 - From a Kjeldahl flask;
 - From a water heater;
 - From a thermometer;
- From a graduated burette.

3.2. Dosage of Hydrocyanic Acid

Titrimetry was used for the determination of hydrocyanic acid. It is a dosing technique used in analytical chemistry to determine the concentration of a chemical species in solution. It is retained in the context of this work in order to determine the level of hydrocyanic acid contained in cassava leaves. Thus the collected cassava leaves were crushed via the MEXXI Grinder 20 g of crushed leaves of each sample were placed in an 800 ml kjeldahl flask on which 200 ml of distilled water was added and left to macerate for 20 hours at room temperature; it was distilled using a steam entrainment device; 130ml of distillate was collected in 20 ml of the NaOH solution (0.5 g/20ml of water); it was distilled again; 100 ml of the solution was taken and 8 ml of 6 N NH₄OH and 2 ml of 5% KI were added; it was titrated with 0.02 N AgNO₃ in a burette; the turbidity of the solution indicated the end of the reaction.

➤ Hydrogen cyanide (mg/100g) = $1.08 \text{ Veq} * 2.5 * 100/\text{me}$.

➤ Veq = volume of AgNO₃ poured at equilibrium.

Me = mass of the sample.

3.3. Determination of pH

The importance of pH determination was to show the activity of linamarase which is optimal at pH = 6 - 7 and at a temperature of 55°C [16]. When the pH is close to 5, hydroxy nitrile (HNL) catalyzes the degradation of cyanohydrin into acetone and hydrocyanic acid (HCN), which is volatile and very toxic [17].

The pH determination was made using a reference pH meter (-Seven Compact; -Ph/Ion Meyer S220; -METTLER TOLEDO). The end of the pH meter topped with a bulb was dipped into the sample (crushed leaves, weighed 20 g in 200 ml of distilled water before and after maceration to ensure which value could respond to linamarase activity) for one minute. The pH value was automatically displayed on the screen of the device.

4. Results

4.1. Hydrocyanic Acid Dosage

The variation of hydrocyanic acid concentration according to different accessions, the accessions having low HCN content are 32 in number with the concentration

varying from 13 to 49.33 mg (**Table 1**). Those with moderately concentrated hydrocyanic acid concentration are 21 in number, the concentration of which varies from 50.33 mg to 98.33 mg. Thus, the variation in the hydrocyanic acid concentration of the highly concentrated accessions, which are 5 in number, varies from 100.66 mg to 144 mg.

Table 1. Variation in HCN content of accessions, measured pH value and severity index.

Accession number	Vernacular name	Severity index	Content in mg/100g	pH value
1	ICRA21 CUT	0	135.133333	6.20333333
2	MAMBERE24 BAO CUT	0	134.666667	6
3	BOUBOUROU 22 BAD CUT	0	67.3333333	6.33333333
4	BOMBEKITI 26 MBE	0	67	6.01
5	NDOUROU WALI 20 B1	0	27	6.46666667
6	BOMBEKITI23 BHO	0	27.3333333	6.03
7	TOGO WHITE 08 NGO	0	13	6.4
8	MEYA 57 YAN	0	27.6666667	6.06666667
9	RENDE 07 PAN	0	27.3333333	6.33333333
10	UNKNOWN 04	0	13.3333333	6.05
11	ICRA 25 LOB	0	27.3333333	6.2
12	VOUROU 10 PKANGABA	0	54.3333333	6.03
13	6 MONTHS 01 BEG	0	27.3333333	6.01333333
14	TOGO BLANC02 BEG	0	27	6.02666667
15	TOGO WHITE 12 NDA	0	54	6.01666667
16	GBALOUKO 45 YON	0	27.3333333	6.06666667
17	ICRA 17 GOOD	0	27.6666667	6.33333333
18	UNKNOWN 18	0	27.3333333	6.16666667
19	GABON 05 PAN	0	27.6666667	6.33333333
20	6 MONTHS 03 BEG	0	30	6
21	RENDE 06 PAN	0	43	6.23333333
22	UNKNOWN 9 NGO	0	37.3333333	6.1
23	TOGO WHITE 11 SAT	0	40.6666667	5.96666667
24	TOBGO WHITE 13 GOOD	0	61.3333333	6.13333333
25	TOGO WHITE 15 BOM	0	72.3333333	6.43333333
26	KJ 16 ROM	0	26.6666667	6.1
27	BOUBOUROU 19 BOK CUT	0	22.6666667	6.03333333
28	MAMBERE 27 BOU CUT	0	16	6.36666667
29	TAGBA 28 BOA	0	51.6666667	6

Continued

30	6 MONTHS 29 BOA.	0	44.3333333	6.16666667
31	ATTOU BOUKOKO 30	0	81	6.06666667
32	ICRA 14 GOOD	0	100.6666667	6.16666667
33	YALIPE 31 BOY	0	55.3333333	6.23333333
34	RENDER 32 BOD	0	71.6666667	6.03333333
35	MBOUMBA 33 BOGI	0	23.6666667	5.86666667
36	NIGERIA 34 BOD2	3	25.6666667	5.8
37	MAMBERE 35 BOZ	0	29.6666667	6.03333333
38	MOGBONDO 36 GBA	2	76.3333333	6.46666667
39	BAMBAKI 37 KPE	3	19.3333333	6.2
40	RETURN 38 KPO	0	96.6666667	6.03333333
41	NZETE YAMBONGO 48	0	80.3333333	6.00333333
42	UNKNOWN 39 GBO	0	84.6666667	6.26666667
43	NDETE. R 53	0	48.3333333	6.06666667
44	GABO 49 DAB	0	40	6.16666667
45	NGUETE 54 ZA	0	76	6.36666667
46	ABOUNDO 55	0	32	6
47	DON'T CARE 40 GOU	0	35.3333333	6.2
48	GBEBAKA 50	0	98.3333333	6
49	KJ 58 YAM	0	63.6666667	6.33333333
50	JPN 56 FAL	0	49.3333333	6.03333333
51	ICRA 51 WOT	0	103.333333	6
52	GABON 41 BEA	0	50.3333333	6.23333333
53	DOGBO 42 TON	0	144	5.93333333
54	NDENDELE 52 WAZ	0	31.6666667	6
55	GBALOUKO UM 43 YOU	0	18.3333333	6
56	BAKOTA 46 BO	0	68.6666667	6.26666667
57	KOUNDE 44 YOU	0	56.3333333	6.01666667
58	BAKOTA 47 BEL	0	74	6.06666667

According to the epidemiological evaluation of these different accessions, all accessions with a severity index of 0 are healthy, i.e. no mosaic symptoms. Then, those with a severity index of 2 indicate the spot covering half of the leaf blade, with the appearance of leaf deformations. Finally, those with a severity index of 3 indicate that the affected leaves are deformed, partially curled, with reduced vegetative apparatus.

The pH value measured overall is almost identical for all accessions with the Min value of 5.80 and the Max value of 6.47. The mean value is 6.13 with a standard deviation of 0.15 followed by the median 6.07 (Figure 1).

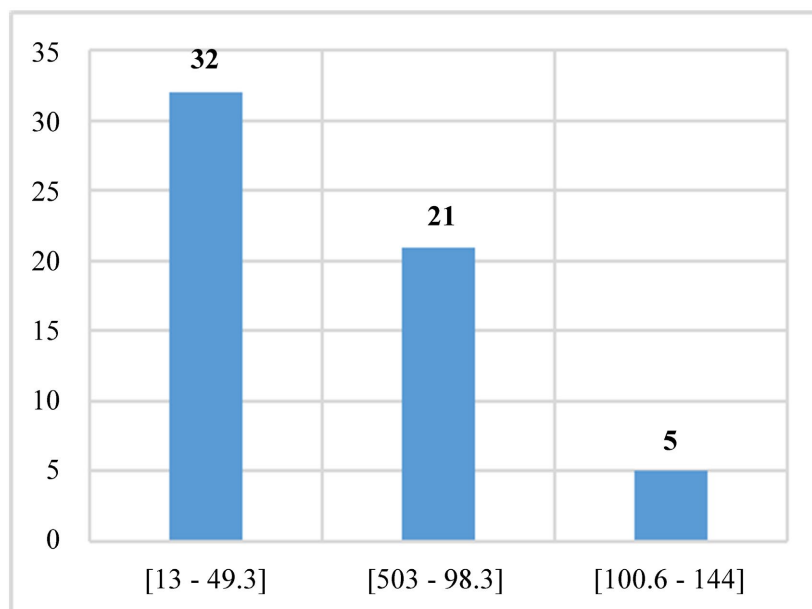


Figure 1. Distribution of cassava accessions according to HCN content.

4.2. Comparison Test between Low, Moderately and Highly Concentrated Accessions

The mean low HCN content is 29.4 (SD = 9.14) while the mean moderate HCN content is 69.6 (SD = 13.80). The results of the student t test comparison on the low and moderate HCN samples do not suggest a statistically significant difference in high HCN content ($t(31) = -11.7$; $p > 0.05$; 95% CI = $[-47.1; -33.2]$). The mean low HCN content is 29.4 (SD = 1.61) while the mean high HCN content is 123.6 (SD = 8.96). The results of the student t test comparison on the low and high HCN samples suggest a statistically significant difference in HCN content ($t(4) = -10.3$; $p < 0.05$).

The mean content of moderately concentrated HCN is 69.5 (SD = 3.01) while the mean content of concentrated HCN is 123.6 (SD = 8.96). The results of the student's t-test on the samples of moderately concentrated and concentrated HCN contents suggest a significant difference in HCN content ($t(4) = -5.7$; $p < 0.05$).

4.3. Correlation Test between HCN Contents and Measured pH Values

We performed a correlation test between HCN content and pH value of accessions to determine the direction (or sense) and strength of the possible correlation between these two variables. The results of the test below show that (i) HCN content and PH value of accessions is negatively correlated (cf $-0.05683993 < 0$) and (ii) there is no correlation between HCN content and PH value of accessions (cf absolute value of correlation coefficient less than 0.1).

4.4. Assessment of the Phytosanitary Status of the 58 Accessions

The results obtained show that the collection is in good phytosanitary condition,

as only 3 out of 58 plants showed symptoms of mosaic disease at severity 2 and 3, with an incidence of 5%. The cuttings were originally taken from asymptomatic plants. This may explain the good phytosanitary condition of the collection.

4.5. Determination of pH

The pH value measured overall is almost identical for all accessions, having the Minimum value of 5.80 and the Maximum value of 6.47. The mean value is 6.13 with a standard deviation of 0.15 followed by the median 6.07.

4.6. Hydrocyanic Acid Dosage

According to the rate of hydrocyanic acid evaluated in the framework of this work, the HCN content in the fresh leaves of these different accessions of cassava from the experimental field clearly showed the three different levels of content, namely: low content; moderately concentrated content and concentrated content [18].

5. Discussions

The evaluation of the rate of hydrocyanic acid in the fresh leaves of 58 cassava accessions, allowed to divide the accessions into three groups according to the HCN content: weakly (13 to 49, 33 mg), moderately (50.33 mg to 98.33 mg) and strongly (100.66 mg to 144 mg) These concentrations are mostly very high compared to the FAO threshold which is 10 mg HCN.kg⁻¹. That is to say, the regular consumption of the leaves of these accessions can generate a health problem linked to the high content of HCN. This leads us in the perspectives to evaluate the toxic potential of these accessions through an experiment which will be carried out on moose. In case of problem, the results will be published to the population so that appropriate solutions can be found.

Thus, the results of the student's t-test on the samples of low and moderately concentrated HCN contents do not suggest a statistically significant difference in high content ($t(31) = -11.7$; $p > 0.05$; 95% CI = [-47.1; -33.2]). That is, the low and moderately high average contents are almost negligible, unlike the results of the student's t-test on the samples of low and high HCN contents which suggest a statistically significant difference in HCN content ($t(4) = -10.3$; $p < 0.05$). Thus, the results of the student's t-test on the samples of moderately concentrated HCN and concentrated HCN contents suggest a significant difference in HCN content ($t(4) = -5.7$; $p < 0.05$). The results of the above test show that (i) the HCN content and the pH value of the accessions are negatively correlated (cf $-0.05683993 < 0$) and (ii) there is no correlation between the HCN content and the pH value of the accessions (cf absolute value of the correlation coefficient less than 0.1). Furthermore, hydrocyanic acid (HCN) is a volatile compound. It evaporates rapidly in air at temperatures above 28°C and dissolves easily in water. It can easily be lost during transportation, storage and analysis of samples. Cyanogen content of cassava leaves normally ranges between 300 and 1500 mg HCN /kg fresh weight [19]. These concentrations are higher than those obtained in this work. Cyanide con-

centration often varies widely between accessions or varieties and ecological and cultural conditions [20]. The essential substrates for the conversion of cyanide to thiocyanate are thiosulfate and 3-mercapto-pyruvate, derived mainly from the sulfur-containing amino acids cysteine, cystine, and methionine. Vitamin B12 in the form of hydroxycobalamin likely influences the conversion of cyanide to thiocyanate. Hydroxycobalamin has been reported to increase urinary excretion of thiocyanate in experimental animals ingesting small doses of cyanide [21] [22]. From 60 to 100 percent of injected cyanide in toxic concentrations is converted to thiocyanate within 20 hours, and enzymatic transformation accounts for more than 80 percent of cyanide detoxification [23]. Thiocyanate is widely distributed in body fluids, including saliva, and it can be readily detected. In healthy humans, a dynamic equilibrium between cyanide and thiocyanate is maintained. A low-protein diet, particularly one lacking sulfur-containing amino acids, may reduce the detoxification capacity and thus make a person more vulnerable to the toxic effect of cyanide [24]. Therefore, cyanogenic glycosides as such are not toxic, but once ingested by humans, they undergo decomposition to hydrocyanic acid by enzymes of the intestinal flora [25]. The dose of 1 mg HCN.kg⁻¹ of body weight is considered likely to cause acute poisoning in humans [26]. Other authors such as [27] have placed the minimum lethal dose between 0.5 and 3.5 mg HCN.kg⁻¹ of body weight. In animals, the lethal dose is 1 mg HCN.kg⁻¹ of body weight. The hydrocyanic acid content set by the FAO must be less than or at most equal to 10 mg HCN.kg⁻¹ of product [28].

Then, the average soil pH for optimal cassava growth and development ranges between 5.5 and 6.5. The pH obtained from the studied accessions ranges from 5.80 to 6.47. Which is within the range of soil pH required [29]. Soils with pH below 5.5 are acidic and can only support cassava growth if treated with lime. Incorporating lime in the soil preparation process helps normalize the soil pH before cassava cultivation. This is a common practice here in Nigeria. Cassava growth can be affected if there is a high salt concentration in the soil with pH as high as 7.8. [30].

Finally, the incidence of mosaic disease is 5%, which shows a good phytosanitary condition of the collection. The cuttings used to set up the collection were taken from asymptomatic plants, hence the importance of collecting cuttings from healthy plants before replanting to limit the spread of the disease [31] [32]. The collection plot was regularly cleaned, which may partly explain the good phytosanitary condition [33]. This collection constitutes an important source of healthy cassava material that can serve as a basis for a project to multiply and disseminate cuttings of good phytosanitary quality. Previous work has shown that the incidence of mosaic disease at the national level was 85% and that 79% of producers use contaminated cuttings [34].

From all the above, although cassava is an important crop and a source of food for many populations, it is essential to understand the health risks associated with its consumption. The toxins present in cassava can be dangerous if not properly

removed through proper preparation and cooking. It is, therefore, crucial to take the necessary precautions to avoid hydrocyanic acid poisoning. By opting for food alternatives and educating people about the risks associated with cassava, we can protect the health of those who depend on this vital crop. The major problem is the presence of cyanide in cassava, which is poisonous and must be removed before consumption. To date, there is no proper processing method to completely eliminate cyanide in the by-products.

6. Conclusion

This work has helped us to understand that the cyanide content in the leaves is higher than the FAO standard. This would represent a public health risk through regular consumption of cassava. The information from this work makes it possible to estimate the risks of exposure to cyanide linked to the consumption of cassava. This study requires in-depth work to have reliable conclusions. A work evaluation of toxic potential of the different accessions is necessary. The results will guide major future decisions on the issue to guarantee the health of consumers.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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