

## Chapter

# The Cyanogenic Potential of Certain Cassava Varieties in Uganda and Their Fermentation-Based Detoxification

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## Abstract

Cassava is the leading staple food in the developing world, providing an essential diet for about half a billion individuals. However, cassava contains significantly toxic compounds, the cyanogenic glycosides. Ingestion of such toxins in large quantities can lead to acute cyanide poisoning and may cause death in both humans and animals. Therefore, cassava may present a potential health risk to consumers. Information regarding the cyanogenic glycoside content is vital in averting health risks associated with cassava consumption. Accordingly, the seven most common local cultivars in Zombo district and six improved cultivars were grown and later characterized based on their cyanogenic potential. Additionally, the root tubers of *Nyar-udota* and *Nyar-papoga* were fermented to detoxify them from the cyanogens. The cyanogenic glycoside levels in the selected cultivars surpassed the critical value of 10 ppm established by the World Health Organization. The improved cassava had lower and moderately identical concentrations of HCN, unlike the local varieties. Cyanogenic contents were highest at 8-10 months. Fermentation led to substantial detoxification of the cyanogens, and the decrease varied with the fermentation period. In making choices for the cultivation and consumption of cassava, it is crucial to consider the cultivar, period of harvesting, and detoxification by fermentation.

**Keywords:** cassava, cyanogenic potential, cyanogenic glycosides, cyanide poisoning, detoxification, fermentation, food safety, food security

## 1. Introduction

Cassava produced by 105 countries is the basic food for more than 600 million people worldwide [1]. Cassava is a very important food source in the tropics, ranking third after rice and maize [2, 3]. It is presently one of Uganda's most vital food crops, ranking second to bananas in terms of the area it occupies, per capita consumption, and total production [1]. About 275 million tons of cassava were produced globally in 2018, with the largest producer being Africa (contributing 61.1% of the total), followed by Asia (29.0%), the Americas (9.8%), and Oceania (0.1%) [4]. In 2020, cassava production globally exceeded 302 million tons, with more than half of the

production recorded in Africa [5]. Nigeria is the world's leading cassava producer, producing 35 million metric tons. In comparison, Uganda's cassava production is about 5 million metric tons each year, and the traditional cassava growing regions in Uganda include the North, West Nile, and Eastern parts of the country [1].

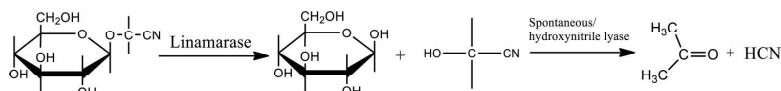
Cassava, a carbohydrate-rich crop, has many uses, including food for human consumption, animal feeds, fuel for producing biofuel & ethanol, and industrial raw material in making paper, citric acid, clothing, alcohol, medicine, and chemicals [4]. Cassava is easily grown and can produce better yields in good and even poor soils, subject to dry conditions. The roots are starchy and may be sweet or bitter, and the young leaves are a good source of protein [6]. Owing to the perceived agricultural advantages of cassava growing and the increasing demand for food as a result of population pressures, cassava usage has been extending to some parts of Africa and elsewhere where it was not formerly used [7]. Traditionally, cassava has been grown as a food security crop, a form of protection against drought and the failure of other staple crops. It is mainly planted in the first rainy season of the year rather than the second, and it is customarily intercropped with beans, maize, and sweet potatoes [8].

Africa's cassava production is mainly for domestic consumption. The cassava supports local food security as well as an economic activity for the farmers, with the main products being fresh cassava roots and processed cassava products [4]. In Uganda, cassava growing is mostly practiced by smallholder farmers covering 1–2 acres of land to ensure food security and generate income. However, there is an effort by the government of Uganda to encourage large-scale cassava production to cater for the ever-growing commercial uses of cassava in the baking industry, pharmaceutical industries, and the manufacture of paper board and starch, biofuel, and alcohol [1]. Most of the cassava is sold as dry cassava chips or cassava flour milled from the dried chips and as fresh cassava roots, especially in urban areas [1].

Unfortunately, all the cassava cultivars produce toxic compounds in the form of cyanogenic glycosides, such as linamarin and lotaustralin, in varying concentrations, ranging from around 10 mg/kg to over 500 mg/kg fresh weight basis [9]. The cyanogenic glycoside content in cassava roots is determined by the cultivar and the growth conditions [10]. These cyanogens are spread in all parts of the plant, with the highest amounts in the leaves and the root cortex (skin layer). The root parenchyma (interior) has comparatively smaller amounts of cyanogens. The so-called sweet cassava varieties have only a small amount of cyanogens in the parenchyma so that after peeling, these roots can be safely boiled and eaten [6]. Bitter cassava's bitter taste is primarily due to linamarin [11]. Cassava produces the two cyanogenic glycosides as a defence mechanism to prevent predator attacks.

The cyanogenic glycosides are nitrile-containing plant secondary compounds that produce cyanide (cyanogenesis) after their enzymatic breakdown. A cyanogenic glucoside is typically a D-glucose joined by a  $\beta$ -linkage to an acetone cyanohydrin derivative [12]. There are about 25 different types of cyanogenic glucosides; the only difference between them is the residual group attached to the end of the acetone cyanohydrin. Linamarin has a hydrogen atom, whilst lotaustralin has a methyl (-CH<sub>3</sub>) group.

Cassava produces the cyanogenic glucosides in a particular way. The first step is the conversion of L-valine into (Z)-2-methylpropanal oxime, which is catalysed by two similar cytochromes (P450s) which are encoded by the genes CYP79D1/D2 [13]. There are two of these genes since *M. esculenta* is an allopolyploid. In the next step (Z)-2-methylpropanal oxime reacts to acetone cyanohydrin, and in the final step, a glucose molecule is bound to the acetone derivative, forming linamarin.



**Figure 1.**  
Cyanogenesis in cassava.

The physiology and the biochemistry of cyanogenesis (**Figure 1**) in cassava have been well studied [14, 15]. Cyanogenesis in cassava starts when there is damage in the plant tissue. When the vacuole is ruptured, linamarin is released, and it is hydrolyzed by a cell wall-associated  $\beta$ -glycosidase, linamarase [14]. Linamarin hydrolyzes producing an unstable hydroxynitrile intermediate, acetone cyanohydrin, and glucose. Acetone cyanohydrin spontaneously decomposes to form acetone and HCN at pH >5.0 or temperatures >35°C. Acetone cyanohydrin can also be broken down by the enzyme hydroxynitrile lyase (HNL) [16–20].

In Africa, consumption of poorly processed cassava, specifically by nutritionally compromised individuals, has led to several cyanide-associated health disorders [21]. The severity of these disorders is dependent on the quantity of the cyanogens consumed, the frequency of cyanogen exposure, and the consumer's health. The presence of toxins in cassava presents a health risk because inadequate preparation of cassava can leave sufficient quantities of residual cyanide in cassava products. The consumption of cassava and its products containing significant amounts of cyanogens causes cyanide poisoning with symptoms of dizziness, vomiting, headache, stomach pains, diarrhoea, weakness, nausea, and occasionally death [22, 23]. The HCN is very poisonous because it binds to the  $\text{Fe}^{2+}$  in haemoglobin, forming cyanohaemoglobin [24]. As a result, there is impediment of the respiratory cycle because the binding affinity of cyanide is much higher than the equivalent binding affinity of oxygen.

Ingestion of cyanide from cassava aggravates goitre and cretinism in areas deficient in iodine [25] and is almost undoubtedly the cause of konzo in central, eastern, and southern Africa. Konzo is an irreversible paralysis of the legs that starts suddenly, occurring mostly in children and women of childbearing age [26–28]. Tropical ataxic neuropathy (TAN) is a chronic condition of gradual onset and occurs in older people who consume a monotonous cassava diet. It causes loss of vision, deafness, weakness, and ataxia of gait [29–31].

The body's major defence in countering cyanide's toxic effects is converting it to thiocyanate mediated by the enzyme rhodanese [32]. Therefore, individuals with low protein and in particular low cysteine intake in their diets are more vulnerable to cyanide poisoning since detoxifying cyanide to thiocyanate by rhodanese requires cysteine as a substrate [32]. In addition, a number of minor reactions help in the detoxification of ingested cyanide. Firstly, cystine can react directly with the cyanide forming 2-iminothiazolidine-4-carboxylic acid, which is excreted in the saliva and urine [33]. Secondly, a small amount of the cyanide may be converted into formic acid and then excreted in urine [33]. Thirdly, cyanide can react with hydroxycobalamin (vitamin B12) to form cyanocobalamin, which is excreted in the urine and bile. Reabsorption of cyanocobalamin may also occur by the intrinsic factor mechanism in the ileum, permitting effective recirculation of vitamin B12 [33]. Fourthly, methaemoglobin effectively competes with cytochrome oxidase for cyanide, and its formation from haemoglobin, effected by sodium nitrite or amyl nitrite, is exploited in the treatment of cyanide intoxication [33].

In Uganda and the West Nile sub-region in particular, excessive consumption of bitter cassava is responsible for disability amongst children. The region depends on cassava as

its primary food source. Dr. Tito Beyeza, an orthopaedic surgeon at Makerere University College of Health Sciences, reported that 10 out of 40 children who underwent surgery at Nebbi Hospital had cyanide, signifying a serious health hazard. He added that removing cyanide requires surgery, which is expensive. 'A surgery like this is ordinarily done in Mulago at Uganda Shillings 800,000', he said. The regional coordinator of the Uganda Society for Disabled Children, Mr. Stephen Eguma, also noted that several families are affected. 'It is an expensive disease to treat for our poor parents here. And they let the children just grow with the deformity and disability which affects the child's future' [34].

Nevertheless, in an attempt to deter thieves, animals, and pests, many farmers from cassava-growing countries oftentimes prefer the bitter varieties [35]. In some places, the more-toxic cassava varieties are a fallback resource (a 'food security crop') during famine [36]. Generally, higher cyanide content correlates with higher yields. During drought times, the cyanide content of both sweet and bitter cassava varieties rises [37]. Bitter cassava varieties are more readily available and cheaper during drought periods because they are more drought resistant. However, due to food shortages during drought, less time is sometimes available for the complete processing required, leaving sufficient quantities of the cyanogens in cassava [38].

Substantial reduction in the per capita cyanide intake could prevent the medical conditions caused by cyanide overload. It is, therefore, crucial to characterise cassava cultivars based on their cyanogenic potential so that cultivars with the lowest levels of toxins are recommended for household consumption. Also, to realise the full potential value of cassava, a lot has to be done at the processing level. Therefore, better and more effective processing methods, especially fermentation [39], have to be promoted and improved to reduce further the cyanide content in cassava flour to within acceptable limits (safe level) of 10 ppm, set by the World Health Organisation (WHO) [36].

## **2. Materials and methods**

### **2.1 Materials**

The materials used during this research included containers (basins), airtight polythene bags, a thermometer, a refrigerator, a distillation flask, a kitchen knife, a pH meter. Others included a reciprocating shaker, filter funnels, micro burette, 125 mL Erlenmeyer flasks, Filter paper (Whatman #42), disposable plastic vials, and distillation apparatus. The main reagents that were used during laboratory analysis were concentrated sulphuric acid, sodium hydroxide, potassium permanganate, 5% potassium iodide solution, 0.02 N silver nitrate, potassium dichromate, ferrous ammonium sulphate, and distilled water.

### **2.2 Sample acquisition**

The cassava varieties used were obtained from the same garden in Agure village, Palei-west ward, Zombo Town council in Zombo district, Uganda.

#### *2.2.1 Cultivation of cassava*

A plot of land measuring 20 × 7 m was cleared manually, tilled, and 13 ridges measuring 18 × 0.6 m were made. The spacing between the ridges was 0.5 m. Stems of six improved cultivars of cassava (NASE 03, NASE 09, NASE 14, NASE

19, TME 14, and TME 204) were obtained from National Agricultural Research Organisation (NARO) at Abii Farm, Arua district, whilst the seven local cassava cultivars ('*Bisimwenge*', '*Nyar-anderian*', '*Nyar-papoga*', '*Nyar-pamitu*', '*Nyar-matia*', '*Nyar-udota*', and '*Terengule*') were collected from local peasant farmers in Zombo district, Uganda.

The cuttings from each cultivar, measuring about 27 cm in length, were planted at about 45° on the crest of the ridges [40]. Care was taken to ensure that the buds were not inverted during planting in order to prevent delayed sprouting [40]. The planting distance was about 0.5 × 0.5 m. Weeding was done at 4, 8, and 12 weeks, respectively, after planting since the crop was planted as a sole crop [41].

### 2.2.2 Collection and preparation of cassava samples

For the determination of cyanogenic glycosides content variation with cassava age, the samples were collected and prepared monthly (on the 15th of each month) for cassava aged 7–13 months. For the comparative analysis of the cyanogenic glycosides content in the various cassava cultivars, the samples were obtained only at the age of 13 months.

Fresh cassava root samples were obtained directly from the garden using a hand hoe. The soils were removed and then the samples were transported home in polythene bags for preparation and then to the Government Analytical Laboratory for analysis. 40 g of each peeled and washed sample was mashed using a wooden pestle and mortar and weighed. The samples were then kept in a deep freezer at a temperature of - 4°C awaiting analysis within 24 hours.

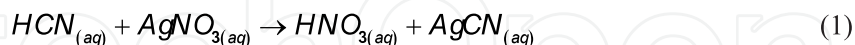
For heap fermentation, cassava roots grown for thirteen (13) months were got from the garden and the peels were removed using a kitchen knife. The peeled root tubers were subjected to partial sun drying at a temperature range of 28 to 40°C and at varied periods (0, 1, 2, 3, and 4 hours). The dried root tubers were heaped together on dry banana leaves with polythene sheets underneath and then covered with dry banana leaves, followed by black polythene sheets. The root tubers were heaped to enable terrestrial fermentation by the growth of moulds, and it was carried out in a grass-thatched hut having a clay floor to afford steady warmth. The period of fermentation was varied by withdrawing some of the heaped cassava after 2, 3, 4, 5, and 7 days for *Nyar-papoga*, and 0, 2, 3, 4, 6, 8, and 10 days for *Nyar-udota* variety.

The moulds from the fermented cassava roots were removed by scrapping them with a blunt kitchen knife. The cassava was pounded and then subjected to sun-drying for around 8 hours at a temperature ranging from 28 to 40°C. The dried cassava was then milled and analysed at the Government Analytical Laboratory. As a control, fresh tubers that were not fermented but sun-dried as well as a fresh tuber that was not dried, were milled and analysed.

### 2.3 Determination of level of cyanides in cassava

The standard method of FAO [42] was used to analyse the cassava samples at the Government Analytical Laboratory (GE058/07) in Kampala. Briefly, in order to set free all the bound hydrocyanic acid, the sample (10 to 20 g) was placed in a distillation flask, and distilled water (about 200 ml) was added and left to stand for two to 4 hours. The mixture was distilled with steam and 150–200 ml of distillate was collected in a solution of 0.5 g of sodium hydroxide in 20 ml of water. The distillate was then diluted to a volume of 250 ml.

To 100 ml of distillate was added 8 ml of 5% potassium iodide solution and titrated with 0.02 N silver nitrate (1 ml of 0.02 N silver nitrate corresponds to 1.08 mg of hydrocyanic acid) using a micro burette. The endpoint was shown by a faint but permanent turbidity, which was easily recognised, particularly against a black background. When all the cyanide ions have reacted with the silver ions, any excess silver ions react with the iodide ions giving a precipitate of silver iodide.



## 2.4 Data analysis

Graphs were generated using computer packages: SPSS 16 and Microsoft Excel from the results of laboratory analysis. Descriptive statistics for the overall HCN levels in each of the two cassava varieties (local and improved) were obtained. Student t test was used to compare the amount of HCN in the local and improved cassava varieties. Results were significant at 0.05 level. The experimental data were analysed using the two-way ANOVA for comparison of the effect of the duration of fermentation (days) and period of partial drying on the amount of hydrogen cyanide. The variation of HCN level with the duration of fermentation in *Nyar-udota* cassava cultivar was analysed using One-way ANOVA.

## 3. Results and discussions

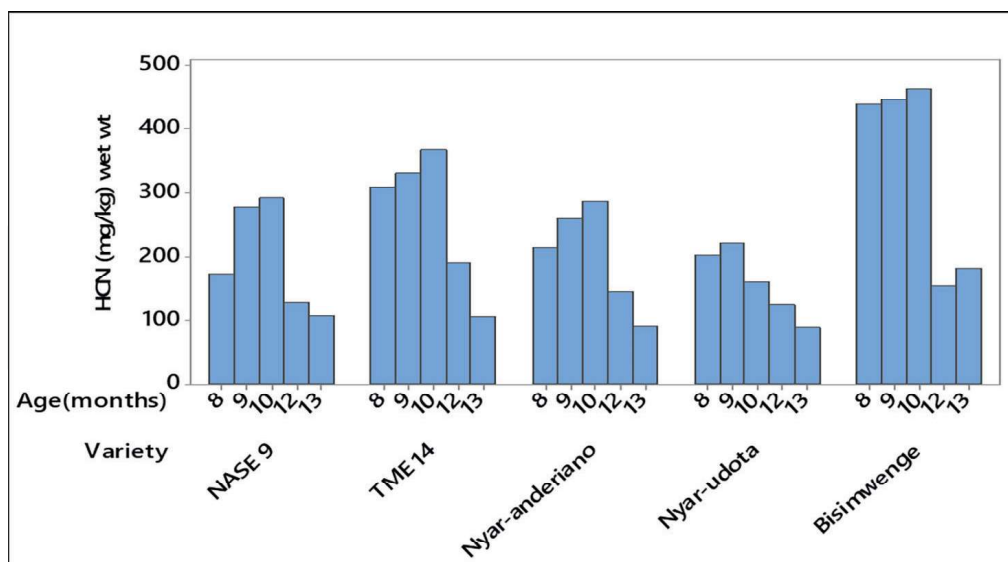
### 3.1 Effect of age of cassava on the levels of hydrogen cyanide

The effect of the age of cassava on the levels of hydrogen cyanide was studied, and the result showing the trend is shown in **Figure 2**.

The level of hydrogen cyanide generally showed an increasing pattern from the 8th month up to the 10th month for varieties (NASE 9, TME 14, *Nyar-anderiano*, and *Bisimwenge*). Then it started decreasing until the 13th month, except for *Bisimwenge*, which showed a slight increase from the 12th month up to the 13th month. For *Nyar-Udota*, there was an increase from the 8th month up to the 9th month, after which the level of hydrogen cyanide started decreasing until the 13th month. By the 13th month, *Bisimwenge* had the highest amount of hydrogen cyanide (181.48 mg/kg), followed by NASE 9 (109.33 mg/kg), TME 14 (105.60 mg/kg), *Nyar-anderiano* (90.00 mg/kg), and finally *Nyar-udota* (88.50 mg/kg) with the lowest amount of the hydrogen cyanide.

This trend could be attributed to the following two opposing factors:

Firstly, cyanogen synthesis, which is based on the expression of the gene CYP79D1/D2, takes place in the young shoots. After the synthesis, it is translocated to the roots [43]. This increases the level of cyanogen in the roots. Secondly, cyanogen re-assimilating based on the expression of the gene  $\beta$ -CAS, where they are exploited for the synthesis of amino acid [12] as well as the enzymes, linamarase and HNL, both of which take part in breaking down the cyanogens upon tissue rupture, based on their expression clustered together. Both cyanogen re-assimilation and the action of linamarase and HNL reduce the level of cyanogen in the roots.



**Figure 2.**  
 A graph showing the variation of levels of hydrogen cyanide (HCN) with age in five cassava cultivars (NASE 9, TME 14, 'Nyar-anderiano', 'Nyar-udota', and 'Bisimwenge').

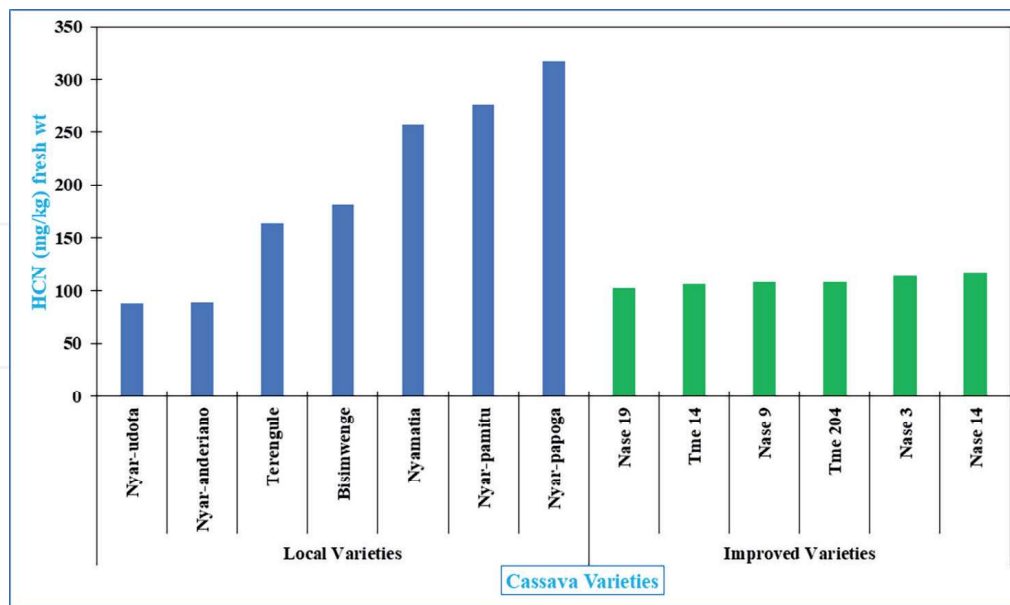
When cyanogen synthesis outweighed, there was an increasing trend of the cyanide level (8–10 months). During the tender age of cassava, cyanogen synthesis is enhanced because of the increased number of young shoots sprouting, which is responsible for the synthesis of the cyanogens. This led to an overall increase in the cyanogen level.

Meanwhile, when cyanogen re-assimilation and action of linamarase and HNL outweighed cyanogen synthesis, there was a decreasing trend in the graph (10–13 months) except for *Nyar-udota* where the decrease was from the 9th month as in **Figure 2**. As the cassava matures, the number of young shoots being produced reduces drastically, leading to a decrease in the amount of cyanogen synthesised, as the rate of cyanogen re-assimilation and action of linamarase and HNL remains fairly constant. Thus, overall, the level of the cyanogens was reduced.

### 3.2 Hydrogen cyanide levels in the cassava varieties at maturity

The levels of hydrogen cyanide found in the different cassava varieties at maturity (13 months) are presented in **Figure 3**.

The levels of the hydrogen cyanide increased in the order; *Nyar-udota* < *Nyar-anderiano* < NASE 19 < TME 14 < NASE 9 < TME 204 < NASE 3 < NASE 14 < *Terengule* < *Nyar-matia* < *Bisimwenge* < *Nyar-pamitu* < *Nyar-papoga*. In the improved cassava varieties, the HCN level was highest for NASE 14 (116.51 mg/kg) and lowest for NASE 19 (101.84 mg/kg). Amongst the local cassava varieties considered in this study, the cyanide levels in *Nyarudota* (88.5 mg/kg) and *Nyar-anderiano* (90.0 mg/kg) were the lowest, even much lower than for the improved varieties. This was in agreement with what was reported by Afoakwa et al. [44], who generally reported lower HCN in local varieties than in improved ones. The cyanide levels in the other four local cassava varieties were higher than those in the improved varieties (**Figure 3**), contrary to the findings of Afoakwa et al. [44]. Generally, the improved cassava varieties considered in this study have shown



**Figure 3.** A graph showing the levels of hydrogen cyanide (in mg/kg) in all the cassava varieties planted at maturity (13 months).

significantly lower levels of hydrogen cyanide (mean value = 108.75) than the local cultivars (mean value = 201.65) ( $t = 2.331$ ,  $p = 0.042$ ). Furthermore, the cyanide level variation in the improved varieties (standard deviation was 5.31) was much lower than in the local cultivars (standard deviation 89.00).

Generally, this trend could be attributed to the fact that in the improved cassava cultivars, the linamarase gene, which is responsible for the disintegration of the cyanogens, has higher transcriptional activity than the bitter cultivars.

It is also possible that there is more inhibition of the cytochrome gene expression that catalyses the first step in the synthesis of linamarin in the improved cassava varieties than the local ones. According to Sirtunga and Sayre [43], the linamarin content of cassava roots reduced by 99% in transgenic plants expressing the cytochrome P450 genes (CYP79D1 and CYP79D2) that catalyse the first step in the synthesis of linamarin.

Nonetheless, all the values lie within the cyanide range in cassava root parenchyma of 10–500 mg cyanide equivalents/kg dry weight [43, 45, 46].

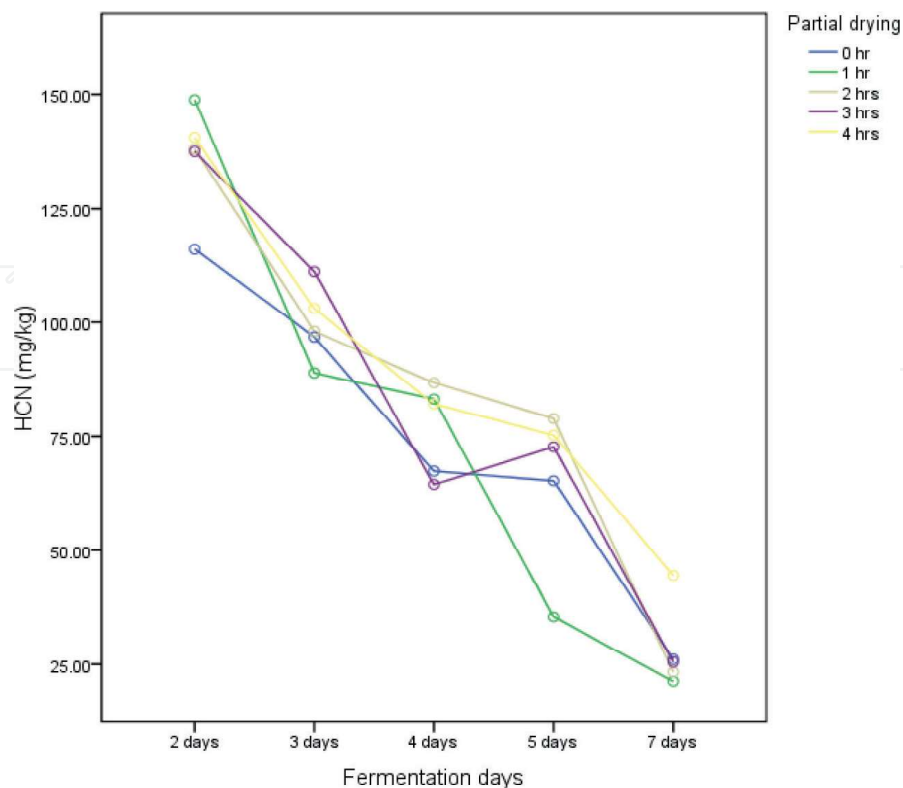
### 3.3 Variation of the level of hydrogen cyanide with fermentation days in

#### 3.3.1 Nyar-papoga cassava variety

The hydrogen cyanide level (mg/kg) dry weight was obtained for cassava samples subjected to varied hours of partial sun-drying and the number of days of fermentation. The trend is presented in the line graph in **Figure 4**.

The level of hydrogen cyanide was high after two (2) days of fermentation but kept reducing steadily until the seventh (7th) day of fermentation. The hydrogen cyanide level in the local cassava variety ('Nyar-papoga') varied significantly ( $F_{(4, 16)} = 62.48$ ,  $p = 1.49 \times 10^{-9}$ ) with the number of days of fermentation.





**Figure 4.** A graph showing the variation of hydrogen cyanide level in a local cassava variety (*Nyar-papoga*) with hours of partial drying and fermentation days.

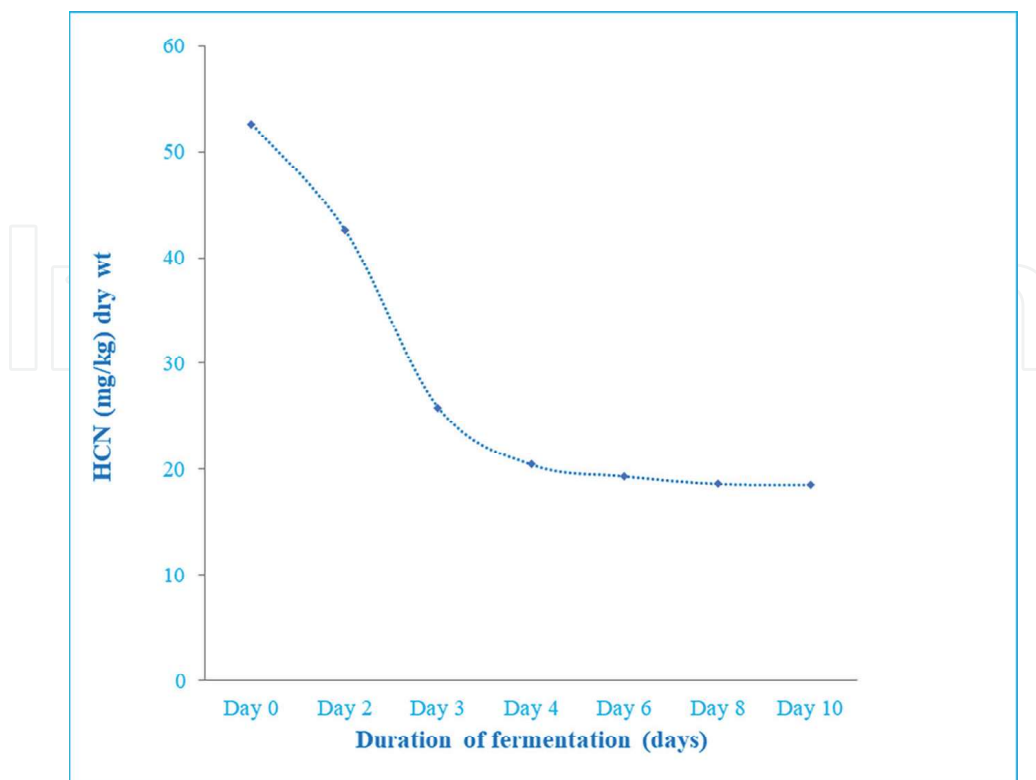
### 3.3.2 *Nyar-udota* cassava variety

The hydrogen cyanide level in *Nyar-udota* cassava variety that was subjected to fermentation for a varying number of days was determined. The result is presented in the line graph in **Figure 5**.

The level of hydrogen cyanide in the unfermented (Day 0) dried *Nyar-udota* cassava variety was the highest (52.63 mg/kg). The level of the hydrogen cyanide then decreased steadily with fermentation days until the fourth day. Thereafter, it remained fairly constant until the 10th day of fermentation (18.58 mg/kg), although there was only a slight decrease in the level of hydrogen cyanide. Generally, the hydrogen cyanide level reduced significantly ( $F_{(1, 12)} = 19.46$ ,  $p = 8.49 \times 10^{-4}$ ) with the period of fermentation, with a percentage reduction of about 65% on the 10th day of fermentation.

Fermentation probably causes more cells to rupture, easily bringing about contact between substrate cyanoglycosides and the enzymes, consequently leading to the breakdown of cyanoglycosides to release free HCN [39]. Moreover, heap fermentation generates heat that volatilizes free hydrogen cyanide [39]. However, Lambri et al. [47] and Bradbury [48] revealed that fermentation temperature was not significant because no consistent differences were exhibited between 30 and 35°C fermentation temperatures. However, the warmth generated by fermentation could progressively evaporate the free hydrogen cyanide, which is volatile at 25.7°C [39, 49].

According to Westby [49], the essential features of efficient processing of the cyanogens involve adequate tissue disruption to enable endogenous linamarase to



**Figure 5.** A graph showing the variation of hydrogen cyanide level with fermentation period in Nyar-udota cassava variety.

come into contact with linamarin and then favourable conditions for the breakdown of acetone cyanohydrin, or conditions that can facilitate spontaneous volatilisation of the compound. In the case of heap-fermented products, microbial growth reduces cyanide content by softening the cassava roots, which increases the contact between endogenous linamarin and linamarase [50].

A series of microorganisms in which the microbial groups, lactic acid bacteria (LAB), and yeasts predominate, characterise natural fermentation [51, 52]. The most frequent LAB species are *Lactobacillus manihotivorans* and *Lactobacillus plantarum* [53]. *L. manihotivorans* exists only during the first period of fermentation, when it may accelerate the rate of degrading starch [52], resulting into contact between linamarase and cyanogenic glycosides, thus, reducing the cyanide level as fermentation progresses. Meanwhile, *L. plantarum*, which is present during all the steps of the fermentative process, acidifies the substrate. Therefore, as the fermentation progresses, there is a gradual decrease in the number of microorganisms due to the increased acidity of the medium [54]. This slows down the fermentation process until it finally stops (**Figure 5**).

#### 4. Conclusions

Improved cassava varieties have lower levels of hydrogen cyanide, and the level does not significantly vary amongst them. The local cassava varieties considered have high levels of hydrogen cyanide except *Nyar-Udota* and *Nyar-anderiano* and there is

significant variation of the cyanide levels amongst them. Generally, the improved cassava varieties considered have lower hydrogen cyanide levels than the local ones.

The hydrogen cyanide levels in the cassava cultivars studied were found to be highest at the ages of 8–10 months.

Fermentation reduces the hydrogen cyanide level significantly, and the decrease varies with the fermentation period.

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## **Conflict of interest**

The authors declare no conflict of interest.

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