

Assessment of cyanide content in cassava (*Manihot Esculenta Crantz*) varieties and derived products from Senegal

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Abstract: We investigated cyanide levels in four cassava varieties and some derived products from Senegal. The study was conducted on fresh tubers by sampling in three longitudinal positions (1/4; 1/2; 3/4) and four radials (A, B, C and D). In addition, the sampling of derived products was carried out by product types (chip dried, graded, unpressed and pressed attiéke, gari and flour). Cyanide contents were determined using differential pulse polarography. Results showed that whatever the variety considered, there was no significant difference between the total HCN content in cassava slices (1/4; 1/2; 3/4). Similarly, results from different layers (A, B, C and D) did not highlight the radial variability of the total HCN content. We showed a variable cyanide content (mg HCN/kg fresh material) in cassava varieties: 104.3 ± 3.9 ; 171.6 ± 5.4 ; 231.2 ± 10.2 ; 270.8 ± 12.0 for Kombo, Nigeria, Soya and Gniargui respectively. The detoxification processes (attiéke, gari and flour) indicated a reduction rate in excess of 80% and may be effective to reduce the cyanide content in cassava root down to a tolerable level (< 50 mg/kg).

Keywords: Cassava Root, Varieties, Cyanide, Derived Products, Detoxification

1. Introduction

Cassava (*Manihot esculenta Crantz*) is an extensively cultivated tuber crop and a staple food for millions of people in the tropical regions of Africa, Latin America and Asia. Globally, in terms of annual production, it is the fifth most important food crop after maize, rice, wheat and potato [19]. While starchy tuberous roots are the main food source, the young leaves, which are high in protein, are also consumed, particularly in Africa [1, 30, 32]. Despite all the usefulness of cassava, its use as a food source is limited by its perishability, its low protein content and its potential toxicity [37, 39]. Cassava contains two cyanogenic glucosides, linamarin and a small amount of lotaustralin, which are catalytically hydrolyzed to release toxic hydrogen cyanide (HCN) when the plant tissue is crushed [5, 31]. Several varieties of cassava have been identified and grouped into bitter and sweet depending on the quantity of linamarin in the tuber. The consumption of cassava and its derived products which contain large

amounts of HCN may be responsible for such visible manifestations as goiter and cretinism [10, 3, 16], tropical ataxic neuropathy [34] and Konzo [26].

In some countries such as Côte d'Ivoire, Benin and Nigeria, people have quickly recognized the need to transform cassava into derived products (Cossettes, Chikwangue, Fufu, Gari, Attiéke, Tapioca [8, 2] whose gain is usually higher than the fresh roots. The processing methods used traditionally are sun drying, soaking and fermentation followed by cooking. These processing methods could lead to reduce the cyanide content in cassava products [7, 13, 35, 36] to improve its palatability and convert it into a storable form.

It is worth indicating that, in Senegal, the level of cassava consumption is very low. In this country, it is generally consumed as vegetable in the daily meals of households [17]. In addition, a virtual lack of processing units and inadequacy of cassava processing equipment are noted [24]. With its potential for processing, cassava could be an alternative to imported food in order to diversify food and ensure food safety [21] in Senegal.

The aim of this study was to estimate the rate of cyanide in four cassava varieties from Senegal and assess some cassava detoxification processes.

2. Materials and Methods

2.1. Experimental Design

This study was conducted on 4 cassava varieties from Senegal: Soya, kombo, Gniargui and TMS 30572 (origin from Nigerian). We established two batches. Each batch contained three tubers per variety from the department of Tivaouane, one of the three departments of Thies regions along the western part of Senegal.

Fresh cassava from the first batch were planted in July 2009, harvested 20 months following planting and immediately sent to Belgium within 48 hours by DHL for analysis.

The Soya cassava varieties from the second batch were planted in July 2010, harvested 9 months after planting, packed in plastic bags, stored at -60°C until July 2011 and sent to Belgium by ITA employees for analysis.

Cassava-derived products such as grated, pressed and unpressed cassava, chips, attieke, gari and flour were also used.

2.2. Preparation of Derived Products

The preparation of derived products was performed at the Institute of Food Technology (ITA) in Senegal. The different products are:

Cassava chips:

Fresh cassava was peeled manually with kitchen knife, washed, cut into small pieces and dried.

Cassava flour:

For cassava flour, the tubers were peeled, washed, grated and pressed to extract the remaining juice. After pressing, the cassava compact blocks were grinded and sieved into flour to remove any waste, then they were sun-dried to reach a moisture content of 12 percent.

Gari:

Fresh cassava was peeled, washed, grated into small pieces and fermented for two days. The product obtained was then pressed. The pulp was grinded, sieved and roasted with a gari roasting pan up to reach a moisture content of 10.5 percent.

Attieke:

The fresh cassava roots were peeled, washed, cut into pieces and milled. The paste obtained was fermented, normally for approximately 12 hrs, by mixing with a traditionally prepared inoculum, comprising cooked cassava that was anaerobically fermented for 36 hrs at ambient temperature. The paste thus formed was pressed to remove the juice. The mashed cake was then crumbled, sieved and processed into semolina that was partially sun-dried, cleaned and finally steam-cooked into attieke-semolina.

2.3. Samples Preparation and Cyanide Measurements

Samples preparation and cyanide measurements were performed at the laboratory of analytical chemistry at Gembloux Agro-Bio Tech, University of Liege, in Belgium.

2.3.1. Samples Preparation

Sampling of Cassava Tubers

Upon receipt, fresh cassava tubers were immediately stored at -20°C to avoid enzymatic hydrolysis of cyanogenic glycosides. After 24 hours of storage, they were sampled.

Slices (1/4, 1/2, 3/4) of Samples for Each Variety

The desired position (1/4, 1/2, 3/4) was observed on varieties of tubers (Fig. 1). Using a sharp knife, 1 cm-thick slices were taken at this position and then cut into small pieces. These pieces were immediately immersed into polypropylene bags containing liquid nitrogen for about 1 minute and then grinded with a small grinder (IKA). The powder obtained was put in small bottles that were labeled, sealed and stored at -20°C . We proceeded in the same way for all varieties.

Layers (A, B, C and D) of Samples for Each Variety

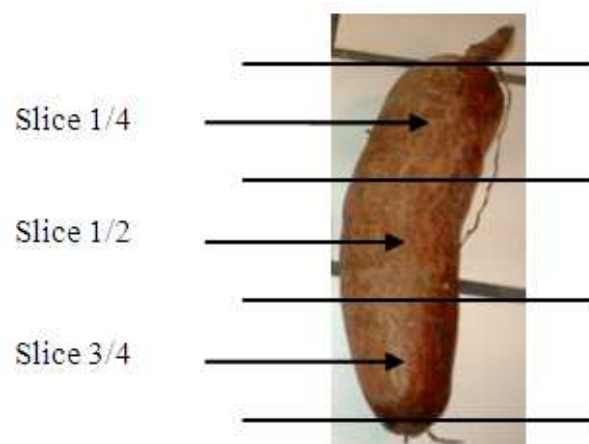


Figure 1: Positions of different slices on cassava root. Slice 1/4 (Head); Slice 1/2 (Middle) and Slice 3/4 (Tail).

Cassava is composed of several layers. The tissues in the layers were separated into four parts (Fig. 2): the cortex (part A) and B-C- and D-part tissues. The cortex could easily be removed from parenchymal tissue by hand. The periderm on the cortex was discarded. B- part tissue was a white parenchymal tissue which was 2 to 3 mm thick next to the cortex. C- part tissue was a yellow parenchymal tissue, 7 to 8 mm thick, next to B- part tissue. D- part tissue, 16 to 18 mm thick, was the remaining core tissue. Each separated tissue fraction was weighed. The entire fraction was used for analysis. While the various layers were formed as above one by one, for the samples from the first batch, they were simultaneously formed for those from the second batch.

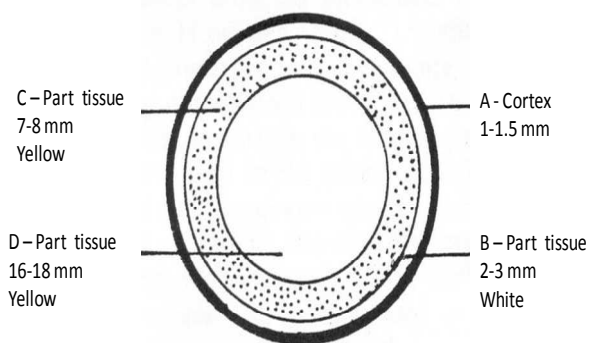


Figure 2: Scheme of the various tissues in cassava root: A- Cortex; B- Part tissue (White); C and D- Part tissue (Yellow)

Sampling of Derived Products

Such wet derived products as attieke grated, pressed and unpressed were immediately stored at -20°C , whereas others adequately dry derived products were kept in the dark.

2.3.2. Cyanide Measurements

Total Cyanide Contents in Cassava Roots

The measurements of total cyanide contents in cassava roots were performed according to the auto-enzymatic method [6].

The sample (1g) of finely ground cassava was incubated in 20 ml acetate buffer (pH 5.5) at 37°C for 18 hours. After incubation, the samples were cooled at room temperature (20°C) and then, 4 ml of NaOH were added. The cassava samples were placed into a distillation flask round bottom containing distilled water. The flask was heated at 100°C in a digital temperature-controlled dry bath and its central neck was connected to the condenser glass directly attached to two bubbler in series containing each 10 ml of 0.1 M K_2CO_3 . After, 10 ml of H_2SO_4 were added to the flask by one of the two side necks to facilitate evaporation of HCN in sample. In the second lateral neck, a very thin tube was inserted, directly immersed into the solution and allowing the entry of air.

The air flow was controlled by vacuum pump placed after the second bubbler. During distillation, the HCN released was constantly carried through the glass condenser and trapped in the first bubbler. The second is considered as a control measure. After 15 minutes, the solution of the first bubbler was collected in a flask (50 ml) and made to volume with 0.1 M K_2CO_3 . The anion CN^- was measured using a polarograph (Metrohm E 506 with Stand Polarecord 663 VA Stand) in differential pulse mode. Oxygen dissolved in the solution was removed by bubbling nitrogen (inert gas) for 5 minutes. Finally, the registration of the polarogram was done in an unstirred solution.

Residual Cyanide Contents in Cassava-Derived Products

The residual cyanide contents in cassava-derived

products measurements were performed according to the method described above with slight modification by adding the commercial linamarase.

Cyanide Content calculation

The polarogram height peaks measurements were expressed as μA . Ion CN^- concentration (C) was calculated relative to the reference solution.

Cyanide content (T) (mg HCN/kg matter).

$$T = \frac{C * 50}{\text{Control sample}}$$

2.4. Statistical Analysis

All values are reported as means \pm SEM. Analysis of variance (ANOVA) with two classification criteria, combined model mixt and fisher test for $\alpha = 0.01$ [14] were used to express the variation of cyanide contents in cassava. Statistical calculations were performed (on the entire data set) using MINITAB. Where a significant effect was detected, a paired Student's t-test was used to determine levels of statistical significance between groups.

3. Results and Discussion

3.1. Total Cyanide Contents in Cassava Slices (1/4, 1/2, 3/4) and Varieties

Statistical analysis showed that:

-For fixed principal factor (slice type), Fisher calculated ($F_{\text{cal}} < F_{\text{re}}$), we accept H_0 . Total cyanide contents in cassava slices showed no significant difference;

-For the random accessory factor (variety type), $F_{\text{cal}} > F_{\text{re}}$, we reject H_0 . Total cyanide contents in cassava varieties were significantly different.

The total cyanide contents in different slices and cassava varieties from the first batch are presented in table 1. Result showed the significant difference between cassava varieties ($p < 0.05$). Total cyanide contents did not differ in each slice. Kombo variety presented the lowest total cyanide content in each slice. The significant difference between total cyanide contents in cassava varieties obtained in this study is consistent with the patterns reported by [15], [21] and [4] who found a significant difference between HCN concentrations in cassava varieties. Based on total cyanide contents, cassava can be classified into three groups as follows: sweet varieties (< 50 mg/kg), intermediate varieties (50-100 mg/kg) and bitter varieties (> 100 mg/kg) [9, 38, 39, 33]. In view of our results, we could say that the cassava varieties studied would be bitter, due to their high level of cyanide contents. The reduction of cyanide content would be important to make these products agreeable for consumption.

Table 1: Total cyanide contents in slices and cassava root (1st batch) by variety

Type of sample	Kombo	Nigeria	Soya	Gniargui	Significance <i>p</i>
Slice 1/4	93.5±3.7 ^d	182.9±8.4 ^c	210.1±6.9 ^b	228.7±2.4 ^a	**
Slice 1/2	105.1±3.2 ^d	156.7±1.7 ^c	218.4±6.3 ^b	291.1±3.5 ^a	*
Slice 3/4	114.1±0.6 ^d	175.0±4.1 ^c	264.9±3.1 ^b	292.5±5.5 ^a	**
Cassava root	104.2±3.9 ^d	171.6±5.4 ^c	231.2±10.2 ^b	270.8±12.0 ^a	**

Total cyanide content is expressed in mg HCN/kg of fresh matter

a,b,c,d Means in the same a row with different superscript letters are significantly different (P<0.05)

** p<0.01; * p<0.05

3.2. Total Cyanide Contents in Layers (A, B, C, D) of Cassava Roots

Table 2 presents total cyanide contents in the cortex, B, C and D part tissue in the first batch of cassava roots. We identified a significant difference between the layers (p<0.05). The total cyanide contents found in the different layers (A-B-C-D) were lower than those in slice (1/4; 1/2; 3/4). This difference could be explained in terms of the difference in cutting method to obtain layers and slices for sampling. We established that the total cyanide content was high in the cortex and decreased sharply towards the center of the root in each variety. These results are complied with those achieved by [29, 25] who showed that along the proximal to the distal end, the cyanide content decreases sharply. Our results suggest that it is safe to remove or exclude the cortex portion before eating the root.

Table 2: Total cyanide contents in different layers of cassava root (1st batch) by variety.

Type of sample	Kombo	Nigeria	Soya	Gniargui
A- Cortex	24.0±3.7 ^a	40.8±5.1 ^a	52.9±3.4 ^a	105.6±6.7 ^a
B- Part tissue	7.9±0.5 ^b	11.1±2.8 ^b	9.5±0.6 ^b	23.3±1.2 ^b
C- Part tissue	4.3±1.1 ^c	8.9±1.3 ^b	9.4±3.3 ^b	9.7±1.3 ^c
D- Part tissue	1.2±0.1 ^d	5.3±1.8 ^c	3.9±1.1 ^c	8.3±0.3 ^c

Total cyanide content is expressed in mg HCN/kg of fresh matter: A- Cortex; B- Part tissue (White). C and D- Part tissue (Yellow).

a,b,c Means with different superscripts in the same column are significantly different (P<0.05)

3.3. Comparison of Total Cyanide Contents in Layers of Cassava Root of Soya Varieties

Total cyanide contents in layers of cassava roots of Soya varieties from the first and second batch are showed in Table 3.

Table 3: Comparison of total cyanide contents in layers of cassava root of Soya varieties

Type of sample	Soya 1 st batch	Soya 2 nd batch	Significance <i>p</i>
A- Cortex	52.9±3.4	446.9±22.7	**
B- Part tissue	9.5±0.6	361.8±6.1	**
C- Part tissue	9.4±3.3	348.2±12.4	**
D- Part tissue	3.9±1.1	405.4±4.6	**

Total cyanide content is expressed in mg HCN/kg of fresh matter: A- Cortex; B- Part tissue (White); C and D- Part tissue (Yellow)

** p<0.01: significant

Results showed that the total cyanide contents for the second batch were higher than those attained with the first batch, whatever the layers considered (p<0.01). This difference could firstly be explained by the sampling method and secondly by the harvest period and the age of the tubers. For Soya of second batch, the sampling method adopted leads to greatly reduced exposure time for the layers, which would limit the hydrolysis of cyanogenic compounds. As for cassava age, results attained in this study were similar to those reached by [22, 21] in other varieties. These authors showed that cyanide contents in cassava root decreases with age. Furthermore, in Thies region, June to October corresponds to high rainfall and November to May corresponds to low rainfall. Harvesting second batch samples in June 2011 (the relatively dry period recorded just before harvesting) could explain the high cyanide contents. Our results are in agreement with those of [12, 13, 18] who showed the high cyanide contents when rainfall is low and low cyanide contents when rainfall is high.

3.4. Cyanide Contents in Cassava Roots and some Derived Products

Total and residual cyanide contents in four cassava varieties and some derived products were presented in Figures 1, 2, 3 and 4 for Kombo, Nigeria, Soya and Gniargui respectively. Our results showed that cyanide concentration decreases in cassava products compared to cassava fresh roots, whatever the varieties considered. We

established a reduction rate of HCN approximately at 15%, 70% and 80% in cassava chips grated, unpressed and pressed respectively (Fig 1, Fig 2, Fig 3 and Fig 4). For the finished derived products, results revealed a reduction rate about 85% for attieke and above 95% in dehydrated products (gari, flour). Residual cyanide contents in cassava chips obtained in this study were similar to those of [11]. By contrast, our results were higher than those achieved by [27] which vary between 1 and 10 mg/kg. This difference could be explained by the use of sweet varieties by these authors and the improved processing methods to produce cassava chips. The low residual cyanide found in attieke, gari and flour could be explained by the degradation of

cyanogenic compounds through unit operations such as grating, fermentation, pressing, drying and cooking [7, 13, 28, 35, 36, 33]. Moreover, the lower HCN contents found in gari than in attieke could be explained by the fact that gari was obtained through dry cooking while attieke was obtained by steam cooking. Further studies could be conducted to confirm these hypotheses. Residual cyanide contents in the cassava-derived products studied were lower than 50 mg/kg. As the lethal dose for humans is 50 mg/kg, we might think that the processing methods of attieke, gari and flour could be effective to reduce the amount of HCN down to tolerable levels.

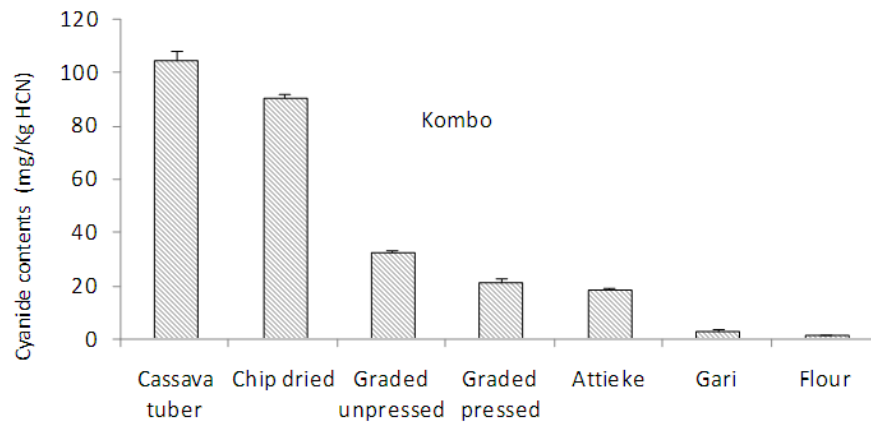


Figure 3: Cyanide contents in cassava root of Kombo variety and some derived products

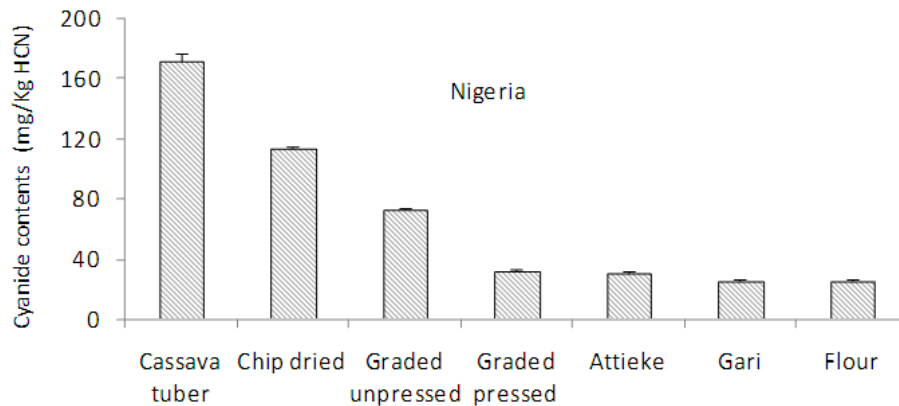


Figure 4: Cyanide contents in cassava root of Nigeria variety and some derived products

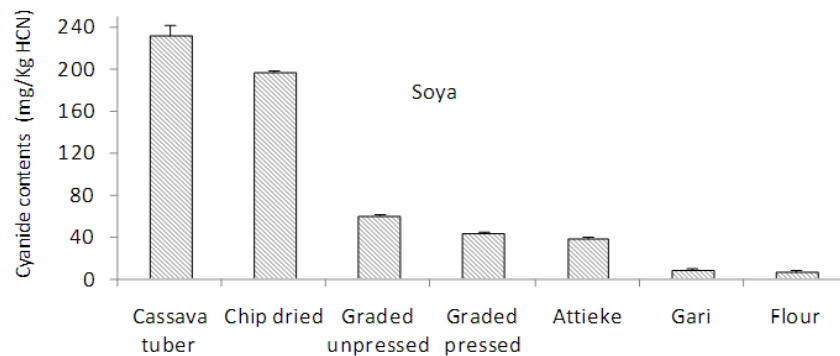


Figure 5: Cyanide contents in cassava root of Soya variety and some derived products

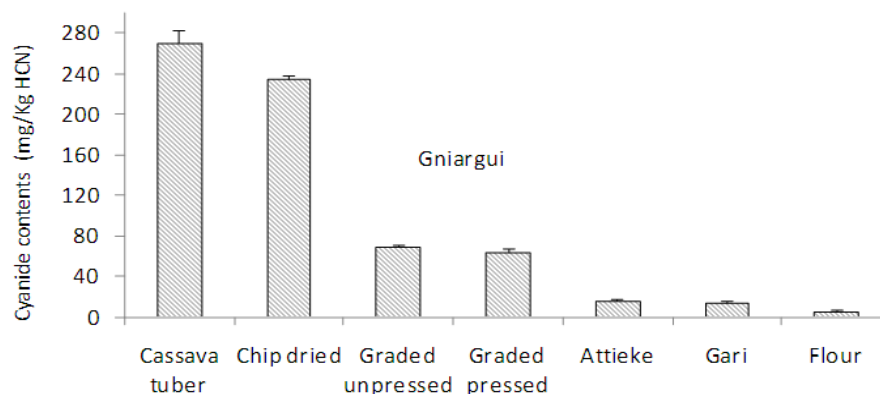


Figure 6: Cyanide contents in cassava root of Gniargui variety and some derived products

4. Conclusion

This study revealed that the total cyanide contents in cassava roots were higher than 100 mg HCN/kg and these cyanide contents varied in cassava varieties from Senegal. We established that the methods of cassava processing were able to reduce total cyanide contents in cassava roots. Moreover, gari and cassava flour prepared from Kombo and Soya varieties showed the residual cyanide content below the level of 10 mg HCN/kg that is considered safe [20]. These results may be used on a household and cottage-industry to ensure food safety.

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